

A

ACETOGENS

Kirsten Küsel¹, Harold L. Drake²

¹Friedrich Schiller University Jena, Jena, Germany

²University of Bayreuth, Bayreuth, Germany

Synonyms

Homoacetogens

Definition

Acetogens are defined as anaerobic prokaryotes that use the acetyl-CoA pathway for the (a) reductive synthesis of the acetyl moiety of acetyl-CoA from CO₂, (b) conservation of energy, and (c) assimilation of CO₂ into biomass.

Introduction

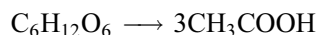
Acetogens utilize the acetyl-CoA “Wood–Ljungdahl” pathway as a terminal electron-accepting, energy-conserving, CO₂-fixing process. The reductive synthesis of acetate from CO₂ differentiates acetogens from organisms that synthesize acetate by other metabolic processes. Although the production of acetate as a sole reduced end product is the classic hallmark of acetogens, the production of acetate is not a part of the definition, because the acetogen might not form acetate in situ or when cultured in the laboratory.

The first acetogen, *Clostridium aceticum*, was isolated from soil by the Dutch microbiologist K. T. Wieringa in 1936. This spore-forming, mesophilic bacterium was shown to grow at the expense of H₂–CO₂ and synthesizes acetate according to the following stoichiometry (Wieringa, 1939–1940):



In 1936, this reaction constituted a unique mechanism for the fixation of CO₂. Unfortunately, the culture of

C. aceticum was lost and no further work was done until it was re-isolated in the early 1980s (Braun et al., 1981). In 1942, F. E. Fontaine and coworkers isolated the second acetogen, *Clostridium thermoaceticum* (later reclassified as *Moorella thermoacetica*), a spore-forming, thermophilic bacterium that catalyzed the near stoichiometric conversion of glucose to acetate (Fontaine et al., 1942):

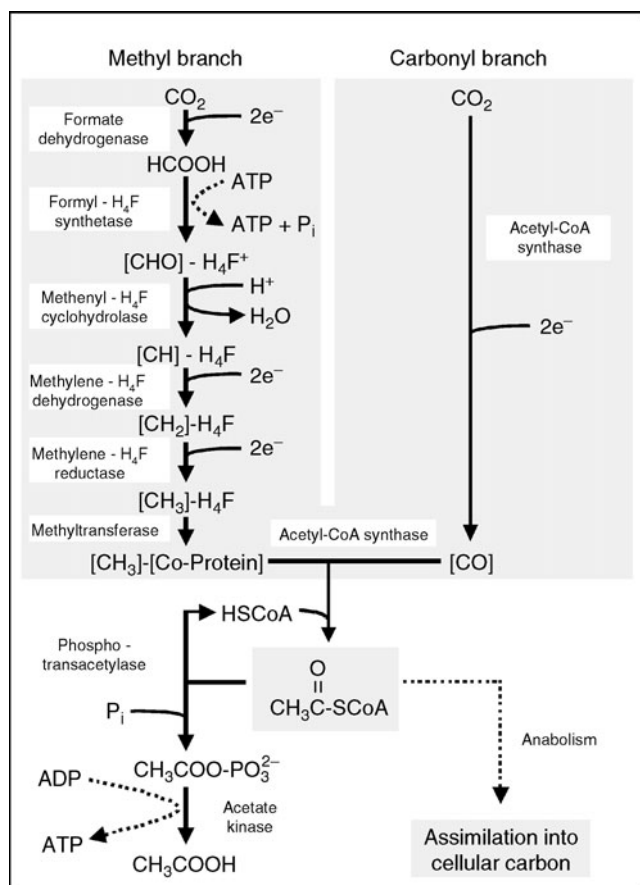


C. thermoaceticum became the most extensively studied acetogen and was used to resolve the enzymology of the acetyl-CoA pathway in the laboratories of two biochemists, H. G. Wood and L. G. Ljungdahl (Drake and Daniel, 2004).

The demonstration that ¹⁴CO₂ was incorporated equally into both carbons of acetate by *C. thermoaceticum* was the first ¹⁴C-study in biology (Barker and Kamen, 1945). The synthesis of acetate from two molecules of CO₂ was later confirmed by Wood using ¹³CO₂ and mass spectrometry (Wood, 1952). Both studies demonstrated that acetogens featured a new autotrophic mechanism for the fixation of CO₂. The acetyl-CoA pathway, also referred to as the Wood–Ljungdahl pathway, is now recognized as a fundamental component of the global carbon cycle, important to primary production in subsurface-ecosystems, and essential to the metabolic potentials of not only acetogens but also methanogens, sulfate-reducers, and anammox bacteria (Wood and Ljungdahl, 1991; Drake, 1994; Schouten et al., 2004).

The acetyl-CoA Wood–Ljungdahl pathway

The acetyl-CoA pathway is a reductive, linear, “one-carbon” process (Figure 1) in contrast to cyclic CO₂-fixing processes (Calvin cycle, reductive tricarboxylic acid cycle, and hydroxypropionate cycle). The two branches of the acetyl-CoA pathway merge at the synthesis of acetyl-CoA that is converted to either acetate or



Acetogens, Figure 1 The acetyl-CoA “Wood-Ljungdahl” pathway. H_4F , tetrahydrofolate; HSCoA , coenzyme A; P_i , inorganic phosphate; e^- , electron; co-protein, corrinoid enzyme; ATP, adenosine-5'-triphosphate. The pathway is from Müller et al. (2004) and used with kind permission of Horizon Bioscience.

assimilated into biomass (Drake, 1994; Drake et al., 2006). Acetyl-CoA synthase (ACS) not only catalyzes the reduction of CO_2 to CO and the synthesis of acetyl-CoA but also oxidizes CO to CO_2 . Thus, the acetyl-CoA synthase is also termed CO dehydrogenase (CODH). Energy conservation occurs during the reductive synthesis of acetate by substrate-level phosphorylation and chemiosmotic processes. For each glucose that is converted to three acetates, four molecules of ATP are produced by substrate-level phosphorylation (ATP_{SLP}). However, when growth occurs under autotrophic conditions, there is no net gain in ATP_{SLP} and growth is dependent upon translocation of protons or sodium ions.

The relatively simple features of the linear acetyl-CoA pathway and the fact that metals and metal sulfides do the biochemical work of CO_2 fixation in the key enzymes of the pathway (i.e., CODH and ACS) suggest that the acetyl-CoA pathway might have been the first autotrophic and early respiratory process on earth and, thus, important in the evolution of life (Wood, 1991; Miyakawa et al.,

2002; Russel and Martin, 2004). It has been proposed that biochemistry started when marine CO_2 from volcanoes and hydrothermal H_2 met at a hydrothermal vent rich in metal sulfides, where an analogue of the exergonic acetyl-CoA pathway catalyzed the synthesis of organic precursors to fuel primordial biochemical reactions.

Phylogenetic diversity of acetogens

Over 100 acetogenic bacterial species have been isolated to date from very diverse habitats, including marine and freshwater sediments; salt lake soda deposits; tundra, forest, and agricultural soils; the subsurface; fecal material; and marine and salt marsh plants (Drake et al., 2006, 2008). Acetogens display extreme genetic diversity, having genomic G + C contents that vary between 22 mol% (*Clostridium ljungdahli*) to 62 mol% (*Holophaga foetida*). Acetogens have been assigned to 22 different bacterial genera including *Acetivibrio*, *Acetobacterium*, *Acetohalobium*, *Acetonebium*, *Bryantella*, “*Butyrivibrio*,” *Caloramator*, *Clostridium*, *Eubacterium*, *Holophaga*, *Moorella*, *Natroninella*, *Natronincola*, *Oxobacter*, *Ruminococcus*, *Sporomusa*, *Syntrophococcus*, *Tindallia*, *Thermoacetogenium*, *Thermoanaerobacter*, and *Treponema* (the name in quotation marks has not been validated). The diverse habitat range of acetogens demonstrates that they are adapted to a broad range of in situ conditions. Many acetogens are sporeformers (e.g., Byrner et al., 2000), a feature that aids in surviving unfavorable in situ conditions, and some acetogens have connecting filaments (Küsel et al., 2001) that might facilitate communication. The usage of the acetyl-CoA pathway for autotrophic assimilation of carbon and acetate utilization by methanogens suggests that Archaea also can grow via acetogenesis. Indeed, the methanogen *Methanosarcina acetivorans* C2A uses the acetyl-CoA pathway to convert carbon monoxide to both acetate and methane, that is, methane is not the sole reduced end product of this methanogen (Rother and Metcalf, 2004; Lessner et al., 2006). Similarly, the archaea *Archaeoglobus fulgidus* VC16 can grow via CO-dependent acetogenesis (Henstra et al., 2008).

Only some acetogenic genera, like *Moorella* and *Sporomusa*, are monophyletic, but many acetogens are phylogenetically dispersed within genera that contain non-acetogenic species. Thus, the phylogenetic position of 16S rRNA gene sequences is usually inadequate for resolving the functional identity of a potential acetogen. To date, only some highly specific 16S rRNA-based probes and primers have been designed to target subsets of acetogenic taxa (Küsel et al., 1999). Molecular approaches based on the analysis of functional genes central to the acetyl-CoA pathway (e.g., a gene for formyltetrahydrofolate synthetase) are still limited due to problems of probe specificity (Leapheart et al., 2003).

Functional diversity of acetogens

Acetogens utilize a wide variety of electron donors and electron acceptors and can engage alternative terminal

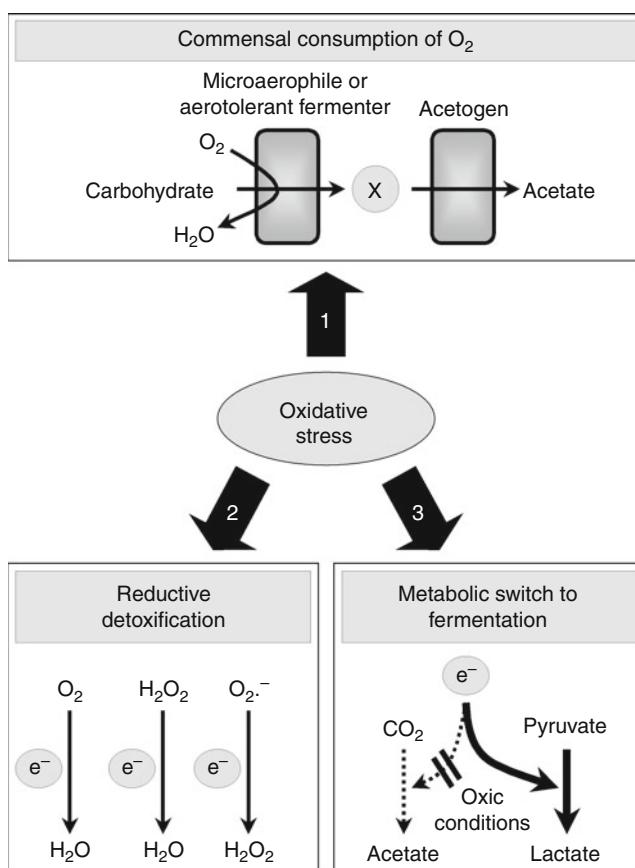
electron-accepting processes when challenged with O_2 (Drake et al., 2006). Many acetogens can utilize one or more terminal electron-accepting processes in addition to acetogenesis. For example, nitrate is a preferred electron acceptor for *M. thermoacetica* and is dissimilated to nitrite and ammonium (Seifritz et al., 1993), and perchlorate is a preferred terminal electron acceptor for *Moorella perchloratireducens* (Balk et al., 2008). The engagement of diverse redox couples enables acetogens to form junction points within and between biological cycles at the ecosystem level (Drake and Küsel, 2005). Acetogens are known to use the following alternative terminal electron acceptors that yield diverse end products listed below:

Fumarate $\rightarrow$ succinate
 Methoxylated phenylacrylates $\rightarrow$ methoxylated phenyl propionates
 Nitrate $\rightarrow$ nitrite
 Nitrite $\rightarrow$ ammonium
 Thiosulfate $\rightarrow$ sulfide
 Dimethylsulfoxide $\rightarrow$ dimethylsulfide
 Perchlorate $\rightarrow$ chloride
 Chlorate $\rightarrow$ chloride
 Pyruvate $\rightarrow$ lactate
 Acetaldehyde $\rightarrow$ ethanol
 Protons $\rightarrow$ hydrogen gas

CO , H_2 , carbohydrates, alcohols, carboxylic acids, dicarboxylic acids, aldehydes, substituent groups of various aromatic compounds, and certain halogenated substrates are examples of substrates that acetogens can oxidize. Although hexoses or pentoses are utilized by most of the acetogens isolated to date, none of these isolates appear to be able to degrade high molecular weight polymers, such as cellulose and lignin. Most acetogens have the capacity to produce more than acetate as their sole reduced end product, and this capacity is dependent on the availability of both reductant and terminal electron acceptors, including CO_2 . Thus, referring to acetogens as homoacetogens is usually a misnomer.

In anoxic habitats, acetogens compete with primary fermentors for monomeric compounds that are derived from the initial breakdown of cellulose and lignin and with secondary fermentors for fermentation products. Thermodynamic considerations suggest that acetogenesis should not be a highly competitive microbial process. Nonetheless, acetogens can outcompete methanogens for H_2 in freshwater sediments with low pH and low temperature and in the hindgut of certain termites (Conrad et al., 1989; Breznak and Kane, 1990; Drake et al., 2006). Microbially produced acetate can provide up to 100% of the energy requirement of wood-feeding termites. The attachment of H_2 -consuming acetogenic spirochetes to H_2 -producing protozoa provides H_2 concentrations above the known H_2 -threshold values for acetogens.

Another ecological advantage might be the low oxygen sensitivity of certain acetogens (Figure 2). Acetogens have been classically referred to as obligate, if not strict, anaerobes, and many enzymes central to acetogenesis are



Acetogens, Figure 2 Mechanisms by which acetogens cope with oxidative stress. X, products (e.g., H_2 , formate, lactate) that are derived from the partial oxidation of carbohydrates (in some cases, short-chain polymers [e.g., stachyose] that are not substrates for the acetogen); e^- , electron. (Modified from Müller et al., 2004, and used with kind permission from Horizon Bioscience.)

extremely sensitive to O_2 . However, acetogens have been isolated from oxic habitats, like soils and the rhizosphere of macrophytes that release O_2 through their roots, indicating that such acetogenic species must cope with periods of oxidative stress. Acetogens contain numerous enzymes that can reductively remove O_2 and its toxic by-products (e.g., superoxide and peroxide), when the concentration of O_2 is relatively low (Drake et al., 2006). These enzymes include peroxidase, NADH-oxidase, rubredoxin oxidoreductase (a superoxide reductase), rubrerythrin (a peroxidase), superoxide dismutase, catalase, and cytochrome *bd* oxidase (Das et al., 2001, 2005; Küsel et al., 2001). Acetogens can shift the flow of reductant away from the acetyl-CoA pathway to alternative terminal electron-accepting processes that are less sensitive to O_2 and operate at higher redox potentials than the very low standard redox potential of the CO_2 /acetate half-cell reaction (-290 mV). For example, *Clostridium glycolicum* RD-1 (isolated from seagrass roots) is an aerotolerant acetogen that switches from acetogenesis to

classic fermentation in response to O₂ (Küsel et al., 2001). Acetogens can also form symbiotic relationships with O₂-consuming microaerophiles and aerotolerant fermenters (Göbner et al., 1999). The microaerophile can be a fermentative non-acetogen that has the capacity to consume O₂ and forms fermentation products (e.g., lactate, formate, and H₂) that can be used by the acetogen for acetogenesis. Such interactions can protect acetogens from oxidative stress and form trophic linkages in habitats with fluctuating redox conditions.

Conclusions

It has been estimated that approximately 10¹² kg of acetate is synthesized per year via H₂-driven acetogenesis in sediments and the hindgut of termites (Breznak and Kane, 1990), a number that is fivefold greater than the annual amount of methane produced via the methanogenic reduction of CO₂. Such estimates accentuate the potential importance of acetogens and acetogenesis to the global carbon cycle. However, in situ acetate turnover measurements are complicated and acetogens catalyze a large number of redox reactions, suggesting that their in situ activities are not restricted to acetogenesis. Thus, although acetate forms an important trophic link in a wide variety of ecosystems, in situ information on acetogens and their activities are often theoretical.

Bibliography

- Andreesen, J. R., and Ljungdahl, L. G., 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. *The Journal of Bacteriology*, **116**, 867–873.
- Balk, M., van Gelder, T., Weelink, S. A., and Stams, A. J. M., 2008. (Per)chlorate reduction by the thermophilic bacterium *Moorella perchloratireducens* sp. nov., isolated from underground gas storage. *Applied and Environmental Microbiology*, **74**, 403–409.
- Barker, H. A., and Kamen, M. D., 1945. Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. *Proceedings of the National Academy of Sciences of the United States of America*, **31**, 219–225.
- Braun, M., Mayer, F., and Gottschalk, G., 1981. *Clostridium acetium* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Archives of Microbiology*, **128**, 288–293.
- Breznak, J. A., and Kane, M. D., 1990. Microbial H₂/CO₂ acetogenesis in animal guts: nature and nutritional significance. *FEMS Microbiology Reviews*, **7**, 309–313.
- Byer, D. E., Rainey, F. A., and Wiegel, J., 2000. Novel strains of *Moorella thermoacetica* form unusually heat-resistant spores. *Archives of Microbiology*, **174**, 334–339.
- Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., Mayer, H. P., and Schütz, H., 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiology Ecology*, **62**, 285–294.
- Das, A., Coulter, E. D., Kurtz, D. M., and Ljungdahl, L. G., 2001. Five-gene cluster in *Clostridium thermoaceticum* consisting of two divergent operons encoding rubredoxin oxidoreductase-rubredoxin and rubrerythrin-type A flavoprotein-high-molecular-weight rubredoxin. *The Journal of Bacteriology*, **183**, 1560–1567.
- Das, A., Silaghi-Dumitrescu, R., Ljungdahl, L. G., and Kurtz, D. M., Jr., 2005. Cytochrome *bd* oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *The Journal of Bacteriology*, **187**, 2020–2029.
- Drake, H. L., 1994. Acetogenesis, acetogenic bacteria, and the acetyl-CoA “Wood/Ljungdahl” pathway: past and current perspectives. In Drake, H. L. (ed.), *Acetogenesis*. New York: Chapman & Hall, pp. 3–60.
- Drake, H. L., and Daniel, S. L., 2004. Physiology of the thermophilic acetogen *Moorella thermoacetica*. *Research in Microbiology*, **155**, 869–883.
- Drake, H. L., and Küsel, K., 2005. Acetogenic clostridia. In Dürre, P. (ed.), *Handbook on Clostridia*. Boca Raton, FL: CRC Press, pp. 719–746.
- Drake, H. L., Küsel, K., and Matthies, C., 2006. Acetogenic prokaryotes. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes*, Vol. 2. New York: Springer-Verlag, pp. 354–420.
- Drake, H. L., Göbner, A. S., and Daniel, S. L., 2008. Old acetogens, new light. *Annals New York Academy of Sciences*, **1125**, 100–128.
- Fontaine, F. E., Peterson, W. H., McCoy, E., and Johnson, M. J., 1942. A new type of glucose fermentation by *Clostridium thermoaceticum* n. sp. *The Journal of Bacteriology*, **43**, 701–715.
- Göbner, A. S., Devereux, R., Ohnemüller, N., Acker, G., Stackebrandt, E., and Drake, H. L., 1999. *Thermicanus aegyptius* gen. nov., sp. nov., isolated from oxic soil, a fermentative microaerophile that grows commensally with the thermophilic acetogen *Moorella thermoacetica*. *Applied and Environmental Microbiology*, **65**, 5124–5133.
- Henstra, A. M., Dijkema, C., and Stams, A. J. M., 2008. *Archaeoglobus fulgidus* couples CO oxidation to sulfate reduction and acetogenesis with transient formate accumulation. *Environmental Microbiology*, **9**, 1836–1841.
- Küsel, K., Pinkart, H. C., Drake, H. L., and Devereux, R., 1999. Acetogenic and sulfate-reducing bacteria inhabiting the rhizoplane and deeper cortex cells of the sea grass *Halodule wrightii*. *Applied and Environmental Microbiology*, **65**, 5117–5123.
- Küsel, K., Karnholz, A., Trinkwalter, T., Devereux, R., Acker, G., and Drake, H. L., 2001. Physiological ecology of *Clostridium glycolicum* RD-1, an aerotolerant acetogen isolated from sea grass roots. *Applied and Environmental Microbiology*, **67**, 4734–4741.
- Leaphart, A. B., Friez, M. J., and Lovell, C. R., 2003. Formyltetrahydrofolate synthetase sequences from salt marsh plant roots reveal a diversity of acetogenic bacteria and other bacterial functional groups. *Applied and Environmental Microbiology*, **69**, 693–696.
- Lessner, D. J., Li, L., Li, Q., Rejtar, T., Andreev, V. P., Reichlen, M., Hill, K., Moran, J. J., Karger, B. L., and Ferry, J. G., 2006. An unconventional pathway for reduction of CO₂ to methane in CO-grown *Methanosarcina acetivorans* revealed by proteomics. *Proceedings of the National Academy of Science of the United States of America*, **103**, 17921–17926.
- Ljungdahl, L. G., 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annual Reviews of Microbiology*, **40**, 415–450.
- Miyakawa, S., Yamanashi, H., Kobayashi, K., Cleaves, H. J., and Miller, S. L., 2002. Prebiotic synthesis from CO atmospheres: implications for the origins of life. *Proceedings of the National Academy of Science of the United States of America*, **99**, 14628–14631.
- Müller, V., Inkamp, F., Rauwolf, A., Küsel, K., and Drake, H. L., 2004. Molecular and cellular biology of acetogenic bacteria. In Nakano, M., and Zuber, P. (eds.), *Strict and Facultative Anaerobes: Medical and Environmental Aspects*. Norfolk, UK: Horizon Scientific Press, pp. 251–281.

- Rother, M., and Metcalf, W. W., 2004. Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. *Proceedings of the National Academy of Science of the United States of America*, **101**, 16929–16934.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Schouten, S., Strous, M., Kuypers, M. M. M., Irene, W., Rijpstra, C., Baas, M., Schubert, C. J., Jetten, M. S. M., and Damste, J. S. S., 2004. Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria. *Applied and Environmental Microbiology*, **70**, 3785–3788.
- Seifritz, C., Daniel, S. L., Gößner, A., and Drake, H. L., 1993. Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. *The Journal of Bacteriology*, **175**, 8008–8013.
- Wieringa, K. T., 1939–1940. The Formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. *Antonie Van Leeuwenhoek Journal of Microbiology and Serology*, **6**, 251–262.
- Wood, H. G. 1952. A study of carbon dioxide fixation by mass determination on the types of C^{13} -acetate. *Journal of Biological Chemistry*, **194**, 905–931.
- Wood, H. G. 1991. Life with CO or CO_2 and H_2 as a source of carbon and energy. *Journal of the Federation of American Societies for Experimental Biology*, **5**, 156–163.
- Wood, H. G., and Ljungdahl, L. G., 1991. Autotrophic character of acetogenic bacteria. In Shively, J. M., and Barton, L. L. (eds.), *Variations in Autotrophic Life*. San Diego, CA: Academic, pp. 201–250.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Bacteria](#)
[Carbon \(Organic, Cycling\)](#)
[Fermentation](#)
[Hydrogen](#)
[Methanogens](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)

ACID ROCK DRAINAGE

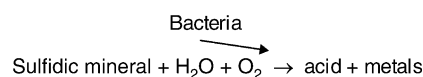
Lesley A. Warren
 McMaster University, Hamilton, ON, Canada

Synonyms

Acid mine drainage; AMD; ARD

Definition

Acid mine and acid rock drainage (AMD/ARD) refer to the extremely acidic ($pH < 3$), metal-rich waters that are derived from the weathering of sulfidic minerals when exposed to air, water, and microorganisms ([Figure 1](#); Nordstrom and Alpers, 1999; Bond et al., 2000):



Acid Rock Drainage, Figure 1 An AMD stream at a nickel-copper mine in northern Ontario, Canada. The orange and red colours in the stream are Fe-oxyhydroxide and Fe-sulphate mineral precipitates.

Environmental significance

AMD/ARD is considered to be the most important and widespread global-mining-industry-related pollution problem (Rowe et al., 2007). The main characteristics of ARD/AMD are (1) low pH, (2) high concentrations of dissolved heavy metals, and (3) high concentrations of sulfate (SO_4^{2-} , Tsukanoto et al., 2004). Total dissolved metal concentrations as high as 200,000 mg/L and dissolved SO_4^{2-} as high as 760,000 mg/L have been reported to be associated with AMD (Nordstrom et al., 2000).

AMD/ARD generation

AMD is generated through a combination of chemical and biological (microorganism) processes by which sulfidic minerals are converted to sulfates and iron oxyhydroxides

- Rother, M., and Metcalf, W. W., 2004. Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. *Proceedings of the National Academy of Science of the United States of America*, **101**, 16929–16934.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Schouten, S., Strous, M., Kuypers, M. M. M., Irene, W., Rijpstra, C., Baas, M., Schubert, C. J., Jetten, M. S. M., and Damste, J. S. S., 2004. Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria. *Applied and Environmental Microbiology*, **70**, 3785–3788.
- Seifritz, C., Daniel, S. L., Gößner, A., and Drake, H. L., 1993. Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. *The Journal of Bacteriology*, **175**, 8008–8013.
- Wieringa, K. T., 1939–1940. The Formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. *Antonie Van Leeuwenhoek Journal of Microbiology and Serology*, **6**, 251–262.
- Wood, H. G. 1952. A study of carbon dioxide fixation by mass determination on the types of C¹³-acetate. *Journal of Biological Chemistry*, **194**, 905–931.
- Wood, H. G. 1991. Life with CO or CO₂ and H₂ as a source of carbon and energy. *Journal of the Federation of American Societies for Experimental Biology*, **5**, 156–163.
- Wood, H. G., and Ljungdahl, L. G., 1991. Autotrophic character of acetogenic bacteria. In Shively, J. M., and Barton, L. L. (eds.), *Variations in Autotrophic Life*. San Diego, CA: Academic, pp. 201–250.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Bacteria](#)
[Carbon \(Organic, Cycling\)](#)
[Fermentation](#)
[Hydrogen](#)
[Methanogens](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)



Acid Rock Drainage, Figure 1 An AMD stream at a nickel-copper mine in northern Ontario, Canada. The orange and red colours in the stream are Fe-oxyhydroxide and Fe-sulphate mineral precipitates.

ACID ROCK DRAINAGE

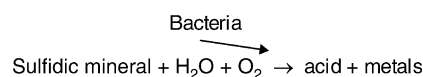
Lesley A. Warren
 McMaster University, Hamilton, ON, Canada

Synonyms

Acid mine drainage; AMD; ARD

Definition

Acid mine and acid rock drainage (AMD/ARD) refer to the extremely acidic (pH < 3), metal-rich waters that are derived from the weathering of sulfidic minerals when exposed to air, water, and microorganisms ([Figure 1](#); Nordstrom and Alpers, [1999](#); Bond et al., [2000](#)):



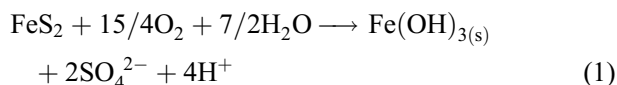
Environmental significance

AMD/ARD is considered to be the most important and widespread global-mining-industry-related pollution problem (Rowe et al., [2007](#)). The main characteristics of ARD/AMD are (1) low pH, (2) high concentrations of dissolved heavy metals, and (3) high concentrations of sulfate (SO₄²⁻, Tsukanoto et al., [2004](#)). Total dissolved metal concentrations as high as 200,000 mg/L and dissolved SO₄²⁻ as high as 760,000 mg/L have been reported to be associated with AMD (Nordstrom et al., [2000](#)).

AMD/ARD generation

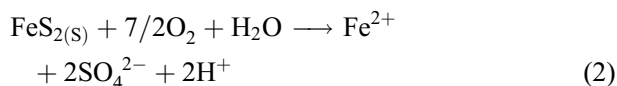
AMD is generated through a combination of chemical and biological (microorganism) processes by which sulfidic minerals are converted to sulfates and iron oxyhydroxides

when exposed to water and oxygen (see [Sulfide Mineral Oxidation](#)). The overall oxidative dissolution of pyrite (FeS_2), a widespread common mine constituent, and associated with AMD generation is given by [Equation 1](#):

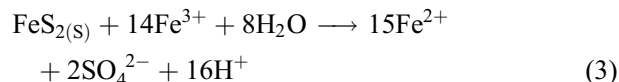


This process progressively increases water acidity, resulting in the mobilization of metals from mine wastes. AMD generation is typically amplified when the reactions involved in the overall process, described in [Equation 1](#), are catalyzed by aerobic Fe- and S-oxidizing bacteria, such as *Acidithiobacilli* (formerly *Thiobacilli*, e.g., *Acidithiobacillus ferrooxidans*; see [Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#); [Sulfide Mineral Oxidation](#)). However, factors such as levels of microorganism activity, pH, sulfide mineral area, crystallography, type of sulfide mineral, temperature, and oxygen concentration all interactively influence the rate of AMD generation (Berghorn and Hunzeker, 2001).

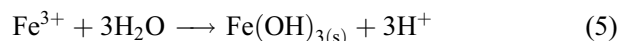
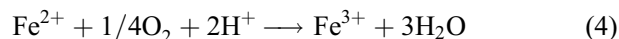
[Figure 2](#) provides a schematic illustrating the overall AMD generation process. Initiation at neutral pH occurs through the release of ferrous iron (Fe^{2+}) and acid (H^+) into solution ([Equation 2](#)):



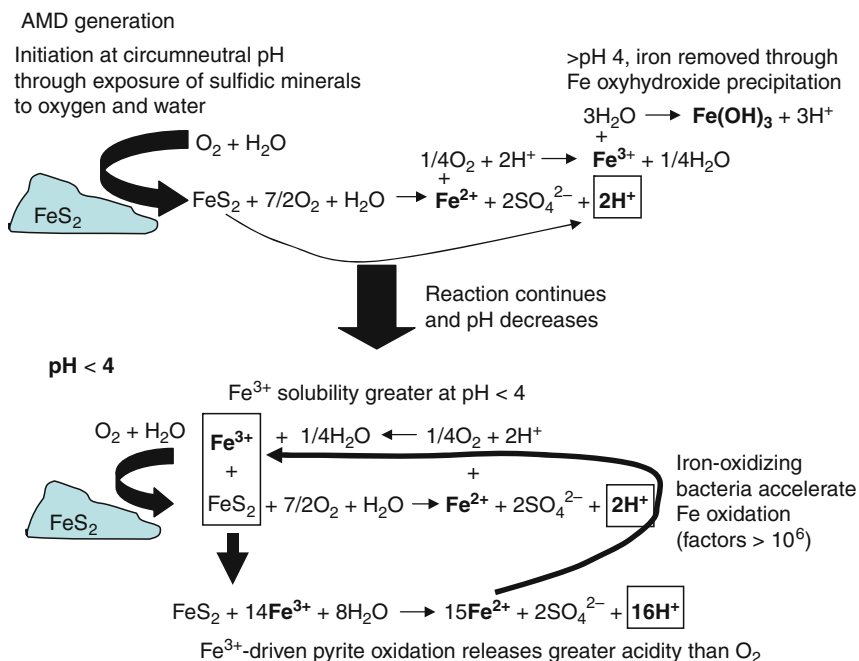
At pH values <4 , increased solubility of ferric iron promotes Fe^{3+} pyrite oxidation ([Equation 3](#)) at far greater rates than those associated with O_2 oxidation:



At pH values greater than 4, O_2 -driven oxidation of ferrous iron to ferric iron ([Equation 4](#)) results in Fe oxyhydroxide formation ([Equation 5](#)) and iron removal through precipitation:



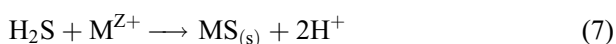
In addition, as the pH decreases below 4, iron-oxidizing bacteria, many of which are adapted to pH values of 2–4, substantively increase rates of Fe oxidation ([Equation 4](#)) by factors greater than 10^6 . For these reasons, the oxidation of Fe^{2+} to Fe^{3+} is often referred to as the rate-determining step in the acid generation process. Thus, once the system reaches pH values less than 4, a self-perpetuating cycle of Fe^{3+} -driven oxidation of pyrite and regeneration of Fe^{3+} through Fe oxidizers is initiated, producing more acidity and Fe^{2+} until either ferric iron or the pyrite waste is depleted ([Figure 2](#)).



Acid Rock Drainage, Figure 2 A schematic of AMD generation based on FeS_2 , indicating the relative importance of O_2 vs Fe^{3+} -driven oxidation rates and the role of microorganisms as a function of pH. At low pH, Fe^{3+} -driven pyrite oxidation releases greater acidity than O_2 -driven pyrite oxidation, which is more favourable at circumneutral pH values.

Treatment of acid mine drainage

AMD waters must be treated to remove metals and raise the pH before they are discharged to a receiving environment (Neculita et al., 2007). Numerous approaches, often characterized as active or passive, exist to treat AMD. Active processes refer to operations that require a relatively high degree of management and continuous input of consumables, while passive processes theoretically require minimal management and other costs once they are established (Johnson, 2006). The most widespread method is active treatment involving the addition of a chemical neutralizing agent or base (i.e., lime or Ca oxide, CaCO_3 , Na/Mg carbonates, Na/Mg hydroxides) to increase water pH and precipitate metals as hydroxides and carbonates. Over the last 20 years, there have been considerable new developments for AMD treatment including constructed wetlands (Johnson and Hallberg, 2002), bioreactors, and permeable reactive barriers (Blowes et al., 2000), which take advantage of the increasing knowledge of microbially driven reactions. Much of the biotechnology developments rely on sulfate-reducing bacteria (SRB) to treat AMD. Fundamentally, bioreactors use SRB to drive sulfide precipitation, removing metals from the AMD solution (Equations 6 and 7):



where M^{Z+} is a cationic metal such as Cu or Ni.

In addition, SRB will drive alkalinity generation (Equation 6) (Koschorreck and Tittel, 2007). However, all emerging treatment approaches are challenged with regard to long-term efficiency. In large part, this reflects the challenges in identifying the complex and dynamic reactions involved in the overall AMD process. The exact mechanisms and controls involved in reactions as well as an understanding and identification of the microbial flora required to efficiently drive desired reactions for bioreactors over the long-term have not yet been elucidated (Edwards et al., 2000; Bernier and Warren, 2005; Bernier and Warren, 2007). In particular, growing evidence of the importance of consortia or mixed microorganism communities in driving AMD-associated processes underscores the need to more fully investigate the interactive roles of bacteria, system pH, $[\text{Fe}]$, $[\text{O}_2]$, and other [metals] involved in the process to narrow the gap in determining effective long-term strategies to mitigate AMD.

Summary

AMD/ARD refer to metal-rich acidic waters that are generated through the exposure of sulfidic minerals in mine wastes to water, oxygen, and microorganisms. These discharges are a significant global pollution issue associated with mining activities. Both sulfur- and iron-oxidizing bacteria are involved with Fe-oxidizing bacteria playing a key role in the increased rates of AMD generation compared to abiotic (nonbiologically stimulated oxidation) sulfidic wastes. AMD must be treated for the high levels of acidity and metals before it can be released to the

environment. New treatment strategies are emerging that include some combination of chemical and biological approaches; however, all are challenged with regard to long-term efficacy. New research evaluating the roles of environmentally mixed communities of microorganisms demonstrates the opportunities to harness environmentally occurring microorganisms to combat AMD.

Bibliography

- Berghom, G. H., and Hunzeker, G. R., 2001. *Passive Treatment Alternatives for Remediating Abandoned-Mine Drainage*. New York: Wiley, pp. 111–127.
- Bernier, L., and Warren, L. A., 2005. Acidity generation in a mine tailings lake. *Geobiology*, **3**, 115–133.
- Bernier, L., and Warren, L. A., 2007. Geochemical diversity in S processes mediated by culture-adapted and environmental enrichments of *Acidithiobacillus* spp. *Geochimica et Cosmochimica Acta*, **71**, 5684–5697.
- Blowes, D. W., Ptacek, C. J., Benner, S. G., McRae, C. W. T., Bennett, T. A., and Puls, R. W., 2000. Treatment of inorganic contaminants using permeable reactive barriers. *Journal of Contaminant Hydrogeology*, **45**, 123–137.
- Bond, P. L., Druschel, G. K., and Banfield, J. K., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Edwards, K. J., Bond, P. L., Druschel, G. K., McGuire, M. M., Hamers, R. J., and Banfield, J. F., 2000. Geochemical and biological aspects of sulfide mineral dissolution: lessons from iron mountain, California. *Chemical Geology*, **169**, 383–397.
- Johnson, D. B., 2006. Biohydrometallurgy and the environment: intimate and important interplay. *Hydrometallurgy*, **83**, 153–166.
- Johnson, D. B., and Hallberg, K. B., 2002. Pitfalls of passive mine water treatment. *Reviews in Environmental Sciences and Biotechnology*, **1**, 335–343.
- Koschorreck, M., and Tittel, J., 2007. Natural alkalinity generation in neutral lakes affected by acid mine drainage. *Journal of Environmental Quality*, **36**, 1163–1171.
- Neculita, C.-M., Zagury, G. J., and Bussiere, B., 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. *Journal of Environmental Quality*, **36**, 1–16.
- Nordstrom, D. K., and Alpers, C. N., 1999. Geochemistry of acid mine waters. In Plumlee G. S., and Logsdon, M. J. (eds.), *The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques, and Health Issues*. Littleton, CO: The Society of Economic Geologists, pp. 133–1160.
- Nordstrom, D. K., and Southam, G., 1997. The geomicrobiology of acid mine drainage. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals*. Washington, DC: Reviews in Mineralogy Mineralogical Society of America, pp. 361–390.
- Nordstrom, D. K., Alpers, C. N., Ptacek, C. J., and Blowes, D. W., 2000. Negative pH and extremely acidic mine waters from Iron Mountain, California. *Environmental Science and Technology*, **34**, 254–258.
- Rowe, O. F., Sanchez-Espana, J., Hallberg, K. B., and Johnson, D. B., 2007. Microbial communities and geochemical dynamics in an extremely acidic, metal-rich stream at an abandoned sulfide mine (Huelva, Spain) underpinned by two functional primary production systems. *Environmental Microbiology*, **9**, 1761–1771.
- Tsukamoto, T. K., Killion, H. A., and Miller, G. C., 2004. Column experiments for microbiological treatment of acid mine drainage: low temperature, low-pH and matrix investigation. *Water Research*, **38**, 1405–1418.

Cross-references

Biomining (Mineral Bioleaching, Mineral Biooxidation)
Copper
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Iron Sulfide Formation
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation

ACIDOPHILES

“Acidophiles” are organisms thriving in environments below pH 5. For details, see entries “*Extreme Environments*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Hot Springs and Geysers*,” *Hydrothermal Environments (Marine)* and “*Acid Rock Drainage*.”

ACRITARCHS

Acritarchs are organic-walled acid-resistant microfossils known from the Proterozoic and throughout the Phanerozoic. The position of these microfossils is still uncertain. A number of acritarch genera have been assigned to Green Algae; others are considered to bear some resemblance to the cysts of dinoflagellates. For more information, please refer to entries “Algae, Eukaryotic” and “Protozoa.”

AEROBIC METABOLISM

Heribert Cypionka
University of Oldenburg, Oldenburg, Germany

Synonyms

Aerobic respiration; Oxygen metabolism

Definition

Aerobic metabolism comprises the reduction of molecular oxygen as electron acceptor of aerobic respiration, its use as cosubstrate in the degradation of certain compounds, and reactions leading to the detoxification of partially reduced oxygen species.

Introduction

Life has evolved in the absence of molecular oxygen (see Chapter *Early Earth*). Therefore, it is not surprising that basic metabolic pathways involved in growth and cell division (like DNA replication or protein synthesis) do not depend on the presence of molecular O₂. The evolution of the oxygen-producing (oxygenic) phototrophs dramatically changed the situation of living organisms. Molecular oxygen raised the redox potential of the environment and enabled microorganisms to respire with a much higher energy yield than before. Although oxygen is involved in

a limited number of reactions only, its presence or absence has fundamental impact on biogeochemical processes.

Aerobic degradation of organic matter

Aerobic respiration consumes a major part of the photosynthetically produced O₂ and about 90% of the organic matter (see Chapter *Carbon Cycle*). Aerobic (oxygen-metabolizing) organisms normally oxidize their substrates completely to CO₂. In most cases, O₂ is not directly involved in this process. Substrate oxidation generates reduced electron carriers by means of the respiratory chain. Oxygen is reduced as the last step of the respiration process.

Respiratory chain

Respiration is a membrane-bound process that generates a chemiosmotic proton gradient across the membrane (Mitchell, 1966). Respiratory chains are composed of series of enzymes, which are organized in multienzyme complexes and use cofactors that allow the stepwise transport of reducing equivalents to oxygen (Figure 1). In eukaryotes (plants, animals, fungi) it takes place within the mitochondria, which are assumed to have evolved from endosymbiotic bacteria. In prokaryotes (Bacteria, Archaea), the respiratory chain is located in the cytoplasmic membrane. The respiratory chain of the bacterium *Paracoccus denitrificans* appears to have many similarities to the mitochondrial type (Harold, 1986). However, many bacteria have variations compared to the mitochondrial type presented in Figure 1.

Energetics and ATP conservation

Most reducing equivalents are fed into the respiration process via NADH₂, which has a standard redox potential (E_0') of -0.32 V. The terminal electron-accepting redox pair O₂/H₂O has redox potential of $+0.82$ V. Thus, the usable redox potential is about 1.14 V. From this redox potential one can calculate a standard $\Delta G_0'$ of -440 kJ per mol of O₂. Assuming that ATP conservation requires about -75 kJ per mol, the conservation of 5 to 6 ATP per O₂ reduced is possible. As a consequence of the high ATP yield, aerobic organisms can form more biomass from the same amount of substrate than anaerobic organisms.

Use of oxygen as cosubstrate

Molecular oxygen is used as a cosubstrate in some catabolic and anabolic reactions (Lengeler et al., 1999). The enzymes involved are called mono- or di-oxygenases, depending on the number of oxygen atoms transferred to their substrate. Oxygenases are used by aerobes to activate certain substrates that do not have functional groups that allow a simple degradation. They catalyze the initial hydroxylation of hydrocarbons to yield alcohols that can easily be metabolized further. They are also involved in the ring cleavage of aromatic compounds and in the first oxidation step of ammonia oxydation. Anaerobic organisms are either unable to oxidize the substrates activated

- Trichet, J., and Defarge, C., 1995. Non-biologically supported organomineralization. *Bulletin de l'Institut Océanographique Monaco*, **14**, 203–236.
- Urry, L. A., Hamilton, P. C., Killian, C. E., and Wilt, F. H., 2000. Expression of spicule matrix proteins in the sea urchin embryo during normal and experimentally altered spiculogenesis. *Developmental Biology*, **225**, 201–213.
- Vermeij, G., 1989. The origin of skeletons. *PALAIOS*, **4**, 585–589.
- Weiner, S., and Dove, P., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–29.
- Weiss, I. M., Kaufmann, S., Mann, K., and Fritz, M., 2000. Purification and characterization of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevis*. *Biochemical and Biophysical Research Communications*, **267**, 17–21.
- Weiss, I. M., Gohring, W., Fritz, M., and Mann, K., 2001. Perlustrin, a *Haliotis laevis* (Abalone) nacre protein, is homologous to the insulin-like growth factor binding protein N-terminal module of vertebrates. *Biochemical and Biophysical Research Communications*, **285**, 244–249.
- Wilt, F. H., Killian, C. E., and Livingston, B. T., 2003. Development of calcareous skeletal elements in invertebrates. *Differentiation*, **71**, 237–250.
- Yan, Z., Jing, G., Gong, N., Li, C., Zhou, Y., Xie, L., and Zhang, R., 2007. N40, a novel nonacidic matrix protein from pearl oyster nacre, facilitates nucleation of aragonite in vitro. *Biomacromolecules*, **8**, 3597–3601.
- Yano, M., Nagai, K., Morimoto, K., and Miyamoto, H., 2007. A novel nacre protein N19 in the pearl oyster *Pinctada fucata*. *Biochemical and Biophysical Research Communications*, **362**, 158–163.
- Zhu, X., Mahairas, G., Illies, M., Cameron, R. A., Davidson, E. H., and Ettensohn, C. A., 2001. A large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo. *Development*, **128**, 2615–2627.

Cross-references

Animal Skeletons, Advent
 Calcium Biogeochemistry
 Carbonate Environments
 Critical Intervals in Earth History
 Ediacaran Biota
 Microbial Biomineralization
 Organomineralization
 Reefs
 Soda Ocean Hypothesis
 Sponges (Porifera) and Sponge Microbes
 Trace Fossils: Neoproterozoic

ANIMAL SKELETONS, ADVENT

Guoxiang Li, Maoyan Zhu, Zhe Chen
 Chinese Academy of Sciences, Nanjing, China

Synonyms

Early biomineralization event; Small shelly fossils

Definition

Animal skeleton. Biologically, an animal skeleton is a hard or rigid framework that could provide protection and

support in many types of animals. Most of the animal skeletons are mineralized, but some of them may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, excluding hydroskeletons. The “skeletons” of resistant organic biopolymers are not included herein.

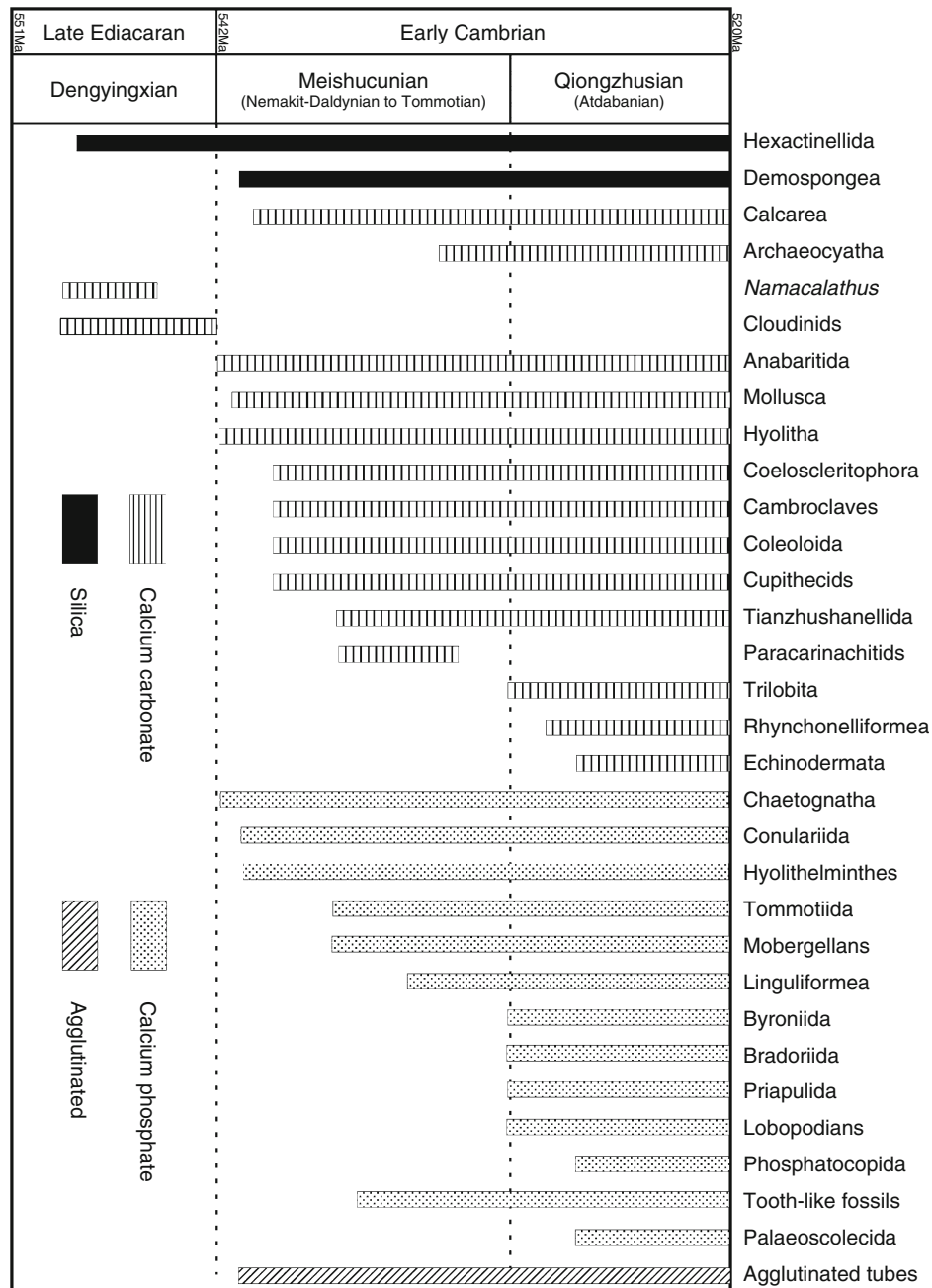
Exoskeleton. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites.

Endoskeleton. Endoskeletons are internal hard parts (typical of many vertebrates). Most of the endoskeletons are generally surrounded by skin and musculature.

Small shelly fossils (SSF or SSFs). A non-taxonomic term was first used by Matthews and Missarzhevsky in the title of their review paper in 1975 to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites.

Introduction

Skeletal biomineralization (skeletonization) was part of the initial diversification of early animals. The fossil record provides an important clue to the origin of biomineralization in the animal phyla (Kirschvink and Hagadorn, 2000). The earliest occurrence of animal skeletons was in the late Ediacaran, e.g., *Cloudina*, *Sinotubulites*, and *Namapoikia*, although the generic diversity was quite low and the biomineralization of these calcareous skeletons was not well controlled biologically (Grant, 1990). The early Cambrian (542–525 Ma) saw the largest burst of diversified animal skeletons, represented in the fossil record by poriferans, cnidarians, many extant bilaterian phyla, and some problematic taxa (Figure 1) (Bengtson, 2004). In terms of structural and mineralogical diversity of animal skeletons, the early Cambrian represents a quantum jump (Bengtson and Conway Morris, 1992). The abrupt advent of diversified skeletal fossils not only has been taken as a major piece of evidence for the Cambrian radiation but also was envisioned the biomineralization event. Since most of these skeletal fossils are typically small in size, and lots of them represent disarticulated sclerites and are enigmatic with respect to their biological affinities, they are commonly referred to as “small shelly fossils (SSFs)” in the literature (Matthews and Missarzhevsky, 1975; Qian, 1999). But some of the early skeletal fossils, such as the sponge-like archaeocyaths, trilobites, and bivalved arthropods, are rarely described under this nontaxonomic term, while some fossils described under the term of SSFs, such as embryo fossils and cnidarian polyps, may have been originally composed of resistant organic membranes or tubes without biomineralization, and the skeleton-like appearance is mainly due to the secondary phosphatization.



Animal Skeletons, Advent, Figure 1 Distribution of major skeletal animal taxa (biomineralized and agglutinated) from the late Ediacaran to the early Cambrian.

Mineralogy of early animal skeletons

Animals use a variety of minerals to fabricate their skeletons (Lowenstam and Weiner, 1989). However, there are only three principal classes of minerals being widely used by organisms, including calcium carbonates, calcium phosphates, and silica. Calcium, carbonate, and phosphate ions are abundant in the oceans, and are ready for use in skeletons (Kirschvink and Hagadorn, 2000), despite

skeletogenesis being a biologically controlled, complex process. Animals rarely switched their mineralogy after their first skeletogenesis, indicating that, for most lineages, environment does not strongly influence skeleton composition, although the selection of skeletal mineralogy during the first acquisition of skeletons in a clade may be somewhat related to ambient seawater chemistry (Knoll, 2003; Porter, 2007; Zhuravlev and Wood, 2008).

It is in most cases not apparent to identify the original minerals of early fossil skeletons because of diagenetic alterations. Especially, there was a very significant phosphogenic event around the Ediacaran-Cambrian transition (Cook and Shergold, 1984), and thus secondary phosphatization of early skeletal fossils was quite common (Qian and Bengtson, 1989). The original composition of fossil skeletons can be learnt by inference and comparative analysis. The fossil record shows that early animals mainly used calcium carbonates, apatites, and silica to build their skeletons (Figure 1).

Several polymorphs of calcium carbonates are the major skeleton-forming minerals, including calcite (high-Mg calcite, low-Mg calcite), aragonite, and vaterite. Vaterite is an extremely unstable polymorph of CaCO_3 and is rarely used as a biomineral. Aragonite is an unstable mineral and it is easily transformed to low-Mg calcite during diagenesis; hence, the original mineralogy of early calcareous fossils is difficult to identify in many cases (Bengtson and Conway Morris, 1992). The earliest animal skeletons during the late Ediacaran-Tommotian interval were composed exclusively of aragonite and high-Mg calcite, being consistent with an aragonite sea (Porter, 2007; Zhuravlev and Wood, 2008). Since the early Atdabanian, there occurred many clades of animals using low-Mg calcite to make skeletons, implying the onset of a calcite sea.

Apatite is another major mineral for early animals to make skeletons (Figure 1), and it is diagenetically the most stable among the major skeleton-building minerals (Bengtson and Conway Morris, 1992). Phosphatic skeletons are usually a combination of phosphate and organic material and hence are called organo-phosphatic. Comparative study indicates that phosphatic skeletons in the

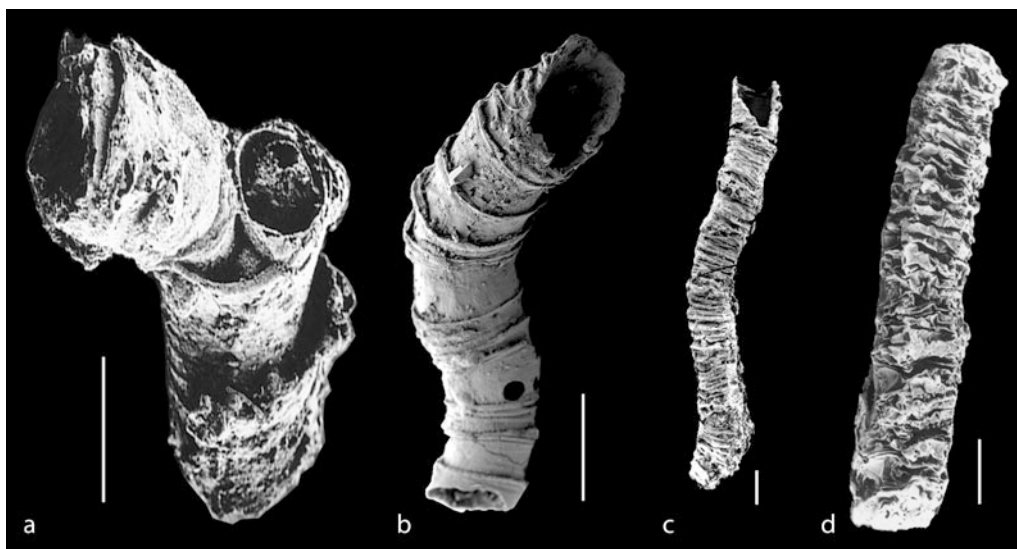
earliest metazoans appear to have been more widespread than today (Runnegar and Bengtson, 1990). The early phosphatic skeletal metazoans include brachiopods (Linguliformea), cnidarians (hyolithelminths, byroniids, and conulariids), bradoriids, lobopodian, protoconodonts, and conodont-like fossils, tomotiids, etc. The high diversity of phosphatic skeletons is somewhat related to wide distribution of phosphate deposits (phosphogenesis event) in various parts of the world during the early Cambrian (Cook and Shergold, 1984).

Siliceous (opal) skeletons differ in distribution from those made of CaCO_3 since silica biomineralization is limited to intracellular precipitation. Therefore, in animals, siliceous skeletons are limited to sponge spicules and other minor occurrences (Bengtson and Conway Morris, 1992). Opal has been used by sponges (including Demospongia and Hexactinellida) to build their skeletons since the late Ediacaran (Gehling and Rigby, 1996). Biogenic opal is unstable and it usually recrystallizes to quartz during diagenesis.

Agglutinated conchs are occasionally found in the Lower Cambrian rocks such as *Salterella*, *Volborthella*, and *Platysolenites*. Both *Salterella* and *Volborthella* are metazoans (Yochelson and Kisselev, 2003), but their biological affinities are uncertain. They have a calcareous sheath partially filled with detrital grains, agglutinating into laminations. *Platysolenites* represents an agglutinating foraminiferan (Glaessner, 1963).

Morphological variety of early animal skeletons

The morpho-types of the animal skeletons in the late Ediacaran are relatively few. They are mainly represented by weakly mineralized calcareous tubes (e.g., *Cloudina*,



Animal Skeletons, Advent, Figure 2 Examples of late Ediacaran skeletal fossils. (a, b) *Cloudina*, an enigmatic tubular fossil, possibly related to annelids; (c, d) *Sinotubulites*, a tubular fossil of unknown affinity. All from the Dengying Formation in southern Shaanxi, China. Scale bars = 500 μm .



Animal Skeletons, Advent, Figure 3 Examples of early Cambrian isolated sclerite fossils. (a) *Sinosachites*, a sclerite of halkieriids; (b1, b2) *Halkieria*, cultrate and palmate sclerites; (c) *Rhombocorniculum*, a tooth-like sclerite; (d) *Protohertzina*, a tooth-like sclerite of protocondonts (Chaetognatha). (e) *Scoponodus*, a sclerite of unknown affinity; (f1, f2) *Siphogonuchites*, sclerites of dorsal and ventral-lateral views; (g) *Microdictyon*, a net-like plate; (h) *Cambroclavus*, a nail-like sclerite of unknown affinity; (i) *Archiasterella*, a sclerite of cancelloriids. (j1, j2) *Tannuolina*, mitral and sellate sclerites (tommotiids); (k) *Lapworthella*, a sclerite (tommotiids). All from the Lower Cambrian of South China. (d–f2: After Qian and Bengtson, 1989). Scale bars = 100 μm , except in j2 = 500 μm .

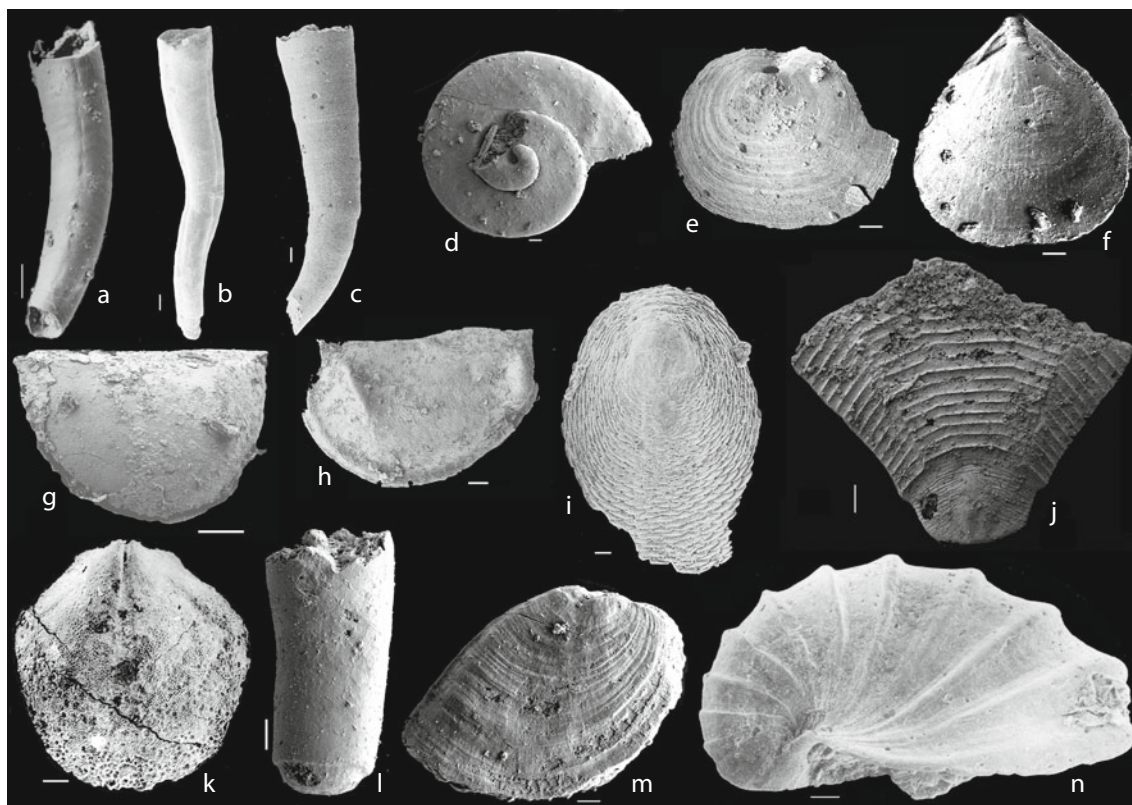
Sinotubulites) (Figure 2) (Hua et al., 2005), modular skeletons (e.g., *Namapoikia*) (Wood et al., 2002), sponge spicules (Brasier et al., 1997) or a reticulating net of spicules (e.g., *Paleophragmodictya*) (Gehling and Rigby, 1996), and goblet-shaped *Namacalathus* (Grotzinger et al., 2000).

Various types of the animal skeletons abruptly occurred in the early Cambrian. Using the theoretical “Skeleton Space” concept, Thomas et al. (2000) made an analysis of Cambrian animal skeletons. Their analysis showed that about 50% of skeletal design elements recognized among living and extinct marine metazoans were exploited in the early Cambrian (Tommotian) and about 80% exploited in the middle Cambrian Burgess Shale animals. The early Cambrian animal skeletons include univalved (Figure 4d, i, n) and bivalved shells (Figure 4e, f), arthropod carapaces (Figure 4g, h), diverse tubes (Figure 4a–c, l), spicules, diverse external sclerites (Figure 3), tooth-like structures (Figure 3c–e), calcareous massive reinforcements (e.g., archeocyathans), echinoderm ossicles, etc.

Late Ediacaran skeletal metazoans

Prior to the Cambrian, the evidence for animal life was almost the soft-bodied Ediacaran fauna and the advent of skeletal animals had been considered to be in the lowermost Cambrian until Germs (1972) described the first Precambrian skeletal fossil *Cloudina* from the Nama Group in Namibia. Since then, biomineralized fossil assemblages have been widely found in the latest Ediacaran over the world, although the biodiversity is very low. Up to now, there are only several skeletal fossil genera being discovered from the Upper Ediacaran rocks, including *Cloudina*, *Sinotubulites*, *Namapoikia*, *Namacalathus*, and *Paleophragmodictya*. Most genera became extinct at the base of the Cambrian, suggesting that a mass extinction occurred at the latest Ediacaran (Amthor et al., 2003).

The worldwide distributed *Cloudina* is a calcareous conical fossil consisting of funnel-like, apically flaring cones nested with one another (Figure 2a, b). The zoological affinities of *Cloudina* are uncertain, but occasional evidence for budding supports either a cnidarian interpretation



Animal Skeletons, Advent, Figure 4 Examples of early Cambrian tube-dwelling and shell-bearing animals. (a) *Torellella*, a tube-like fossil of unknown affinity; (b) *Anabarites*, a tube-like fossil of cnidarian. (c) *Conotheca*, a hyolith; (d) *Aldanella*, a possible gastropod; (e) A ventral valve of *Eohardrotreta*, acrotretid brachiopods; (f) A ventral valve of *Palaeobolus*, lingulid brachiopods; (g) *Dabashanella*, a phosphatocopid; (h) *Kunmingella*, a bradoriid; (i) *Canopoconus*, a possible mollusk; (j) *Hexangulaconularia*, a conulariid (Cnidaria); (k) *Lathamella*, a stem-group brachiopod; (l) *Cupitthea*, a tube-like fossil of unknown affinity; (m) *Watsonella*, a rostroconch (Mollusca); (n) *Archaeospira*, a gastropod. All from the Lower Cambrian of South China (b, c, i, n: After Qian and Bengtson, 1989). Scale bars = 100 μ m.

(Grant, 1990) or an annelid relation (Hua et al., 2005). The *Cloudina* tubes contain the earliest evidence of predatorial borings (Figure 2b). The enigmatic *Sinotubulites* (Figure 2c, d) is a cylindrical fossil, and its tube is composed of several thin layers and characterized by “tube-in-tube” structure (Chen et al., 2007). It has been only documented from the Dengying Formation in South China and co-occurred with *Cloudina*. The irregular wrinkle ornamentation of outer layer shows the *Sinotubulites* tubes being plastic, implying that the tube wall was dominantly organic with aragonitic mineralization (Chen et al., 2007).

Namapoikia is a late Ediacaran, fully biomineralized metazoan from the Nama Group, Namibia, and it is up to 1 m in diameter and bears a complex and robust calcareous skeleton (Wood et al., 2002). It resembles chaetid sponges or simple colonial cnidarians. *Namacalathus* is another calcified animal from the late Ediacaran, and it is goblet shaped up to 2 cm across (Grotzinger et al., 2000). It is more likely to be a cnidarian than a sponge. Both *Namapoikia* and *Namacalathus* co-occur with *Cloudina*.

Hexactinellid sponges have also been found in the late Ediacaran, including the body fossil *Paleophragmodictya*

(with a reticulating net of spicules) (Gehling and Rigby, 1996) as well as clusters of siliceous spicules (Brasier et al., 1997).

Early Cambrian skeletal metazoans

The great watershed in skeletal biomineralization was the early Cambrian, where the diversity of skeletal animals increases nearly exponentially over the interval. Compared with the limited diversity of skeletal fossils in the Ediacaran, the Lower Cambrian is marked by an extraordinary burst of skeletal fossils. Most skeletal metazoans (extant and extinct) appeared for the first time during the early Cambrian (Figure 1). Among these skeletal fossils, many “skeletons” were not integrated and consisted of numerous discrete sclerites, e.g., cambroclaves, tomotiids, halkieriids, cancelloriids, etc. (Figure 3).

Although the advent of animal skeletons in the early Cambrian is quite abrupt or explosive in relation to the long geologic history, the first appearances of different clades in the early Cambrian are sequential (Li et al., 2007). Anabaritids, hyoliths, maikhanellids (mollusks),

and protoconodonts (chaetognaths) first occurred in the early Meishucunian, coeloscleritophorans, tommotiids in the middle Meishucunian, while crown group brachiopods, skeletal arthropods, and lobopods first occurred in the early Qiongzhusian (Figure 1). The skeletal fossil record shows that the clades occurring in the pre-trilobitic interval (Meishucunian) include many extant phyla such as Porifera, Cnidaria, Annelida, Mollusca, Chaetognatha, Brachiopoda (Lingulids), and a number of extinct taxa such as Hyolitha, stem-group brachiopods, tommotiids, coeloscleritophorans, cambroclaves, coleoloids, paracarinachitids, etc. The pre-trilobitic skeletal fossils were dominated by helcionellids (Mollusca), hyoliths, other problematic sclerites, cap-shaped fossils, and tube-like fossils. They are distinctively different from the “Cambrian Evolutionary Fauna,” and were christened the “Tommotian Fauna” (Sepkoski, 1992).

In the Qiongzhusian (Atdabanian) stage, besides many extinct clades, nearly all extant skeletal invertebrate phyla occurred (except Byrozoa with first occurrence in the early Ordovician). The dominant and characteristic skeletal fossils of this interval include arthropods, brachiopods, and echinoderms, representing an initial stage of the “Cambrian Evolutionary Fauna.”

Summary

The earliest skeletal fossils came from the late Ediacaran rocks, but the early Cambrian witnessed the largest burst of animal skeletons. The abrupt advent of diverse skeletal fossils is an important part of the Cambrian explosion, and biomineralization of animal hard parts provides many advantages for metazoan diversification. Although the trigger for this early biomineralization event has been a subject of extensive debate in literature, it should be an integrating result of extrinsic and intrinsic (biological) factors. The fossil record also exhibits that, along with the rapid diversification of metazoans in the early Cambrian, the metazoans progressively exploited more ecological space. Most of the pre-trilobitic skeletal faunas came from the shallow-water deposits, but during the Qiongzhusian, there already existed a distinct differentiation of shallow water and deep water habitats: a strong linkage between the occurrences of the skeletal fossils and sedimentary environments.

Bibliography

Amthor, J. E., Grotzinger, J. P., Schröder, S., Bowring, S. A., Ramezani, J., Martin, M. W., and Matter, A., 2003. Extinction of *Cloudina* and *Namacalathus* at the Precambrian-Cambrian boundary in Oman. *Geology*, **37**, 431–434.

Bengtson, S., 2004. Early skeletal fossils. In Lipps, J. H., and Waggoner, B. M. (eds.), *Neoproterozoic-Cambrian Biological Revolutions. Paleontological Society Papers*, **10**, 67–78.

Bengtson, S., and Conway Morris, S., 1992. Early radiation of biomineralizing phyla. In Lipps, J. H., and Signor, P. W. (eds.), *Origin and Early Evolution of the Metazoa*. New York and London: Plenum Press, pp. 447–481.

Brasier, M., Green, O., and Shields, G., 1997. Ediacarian sponge spicule clusters from southwestern Mongolia and the origins of the Cambrian fauna. *Geology*, **25**, 303–306.

Chen, Z., Bengtson, S., Zhou, C. M., Hua, H., and Yue, Z., 2007. Tube structure and original composition of *Sinotubulites*: shelly fossils from the late Neoproterozoic in southern Shaanxi, China. *Lethaia*, **41**, 37–45.

Cook, P. J., and Shergold, J. H., 1984. Phosphorous, phosphorites and skeletal evolution at the Precambrian-Cambrian boundary. *Nature*, **308**, 231–236.

Gehling, J. G., and Rigby, K. J., 1996. Long expected sponges from the Neoproterozoic Ediacara fauna of South Australia. *Journal of Paleontology*, **70**, 185–195.

Germis, G. J. B., 1972. New shelly fossils from Nama Group, South west Africa. *American Journal of Science*, **272**, 752–761.

Glaessner, M. F., 1963. Major trends in the evolution of the Foraminifera. In von Koenigswald, G. H. R. (ed.), *Evolutionary Trends in Foraminifera*. Amsterdam: Elsevier, pp. 9–24.

Grant, S. W. F., 1990. Shell structure and distribution of *Cloudina*, a potential index fossil for the terminal Proterozoic. *American Journal of Science*, **290A**, 261–294.

Grotzinger, J. P., Watters, W. A., and Knoll, A. H., 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology*, **26**, 334–359.

Hua, H., Chen, Z., Yuan, X. L., Zhang, L. Y., and Xiao, S. H., 2005. Skeletogenesis and asexual reproduction in the earliest biomineralizing animal *Cloudina*. *Geology*, **33**, 277–280.

Kirschvink, J. L., and Hagadorn, J. W., 2000. A grand unified theory of biomineralization. In Bäuerlein, E. (ed.), *The Biomineralisation of Nano- and Micro-Structures*. Weinheim: Wiley-VCH Verlag, pp. 139–150.

Knoll, A. H., 2003. Biomineralization and Evolutionary History. *Reviews in Mineralogy and Geochemistry*, **54**, 329–355.

Li, G. X., Steiner, M., Zhu, X. J., Yang, A. H., Wang, H. F., and Erdtmann, B.-D., 2007. Early Cambrian metazoan fossil record of South China: Generic diversity and radiation patterns. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 229–249.

Lowenstam, H. A., and Weiner, S., 1989. *On biomineralization*. Oxford: Oxford University Press.

Matthews, S. C., and Missarzhevsky, V. V., 1975. Small shelly fossils of late Precambrian and early Cambrian age: a review of recent work. *Journal of the Geological Society*, **131**, 289–304.

Porter, S. M., 2007. Seawater chemistry and early carbonate biomineralization. *Science*, **316**, 1302.

Qian, Y., 1999. *Taxonomy and Biostratigraphy of Small Shelly Fossils in China*. Beijing: Science Press. (In Chinese with English abstract).

Qian, Y., and Bengtson, S., 1989. Palaeontology and biostratigraphy of the Early Cambrian Meishucunian Stage in Yunnan Province, South China. *Fossils and Strata*, **24**, 1–156.

Runnegar, B., and Bengtson, S., 1990. Origin of hard parts—early skeletal fossils. In Briggs, D. E. G., and Crowther, P. R. (eds.), *Palaeobiology—A Synthesis*. Oxford: Blackwell, pp. 17–30.

Sepkoski, J. J. (Jr.), 1992. Proterozoic-Early Cambrian diversification of metazoans and metaphytes. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 553–561.

Thomas, R. D., Shearman, R. M., and Stewart, G. W., 2000. Evolutionary exploitation of design options by the first animals with hard skeletons. *Science*, **288**, 1239–1242.

Wood, R. A., Grotzinger, J. P., and Dickson, J. A. D., 2002. Proterozoic Modular Biomineralized Metazoan from the Nama Group, Namibia. *Science*, **296**, 2383–2386.

- Yochelson, E. L., and Kisselev, G. N., 2003. Early Cambrian *Salterella* and *Volborthella* (Phylum Agmata) re-evaluated. *Lethaia*, **36**, 8–20.
- Zhuravlev, A. Yu., and Wood, R. A., 2008. Eve of biomineralization: Controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Animal Biocalcification, Evolution
 Breakup of Rodinia
 Ediacaran Biota
 Gondwanaland, Formation
 Origins of the Metazoa
 Trace Fossils: Neoproterozoic

ARCHAEA

Volker Thiel
 University of Göttingen, Göttingen, Germany

Synonyms

Archaeobacteria (term abandoned)

Definition

The Archaea are single-celled or filamentous prokaryotes that constitute the third phylogenetic domain of life, besides the Bacteria and the Eukarya. The word “Archaea” (singular archaeum, archaeon) is derived from the Greek word for “the old ones”.

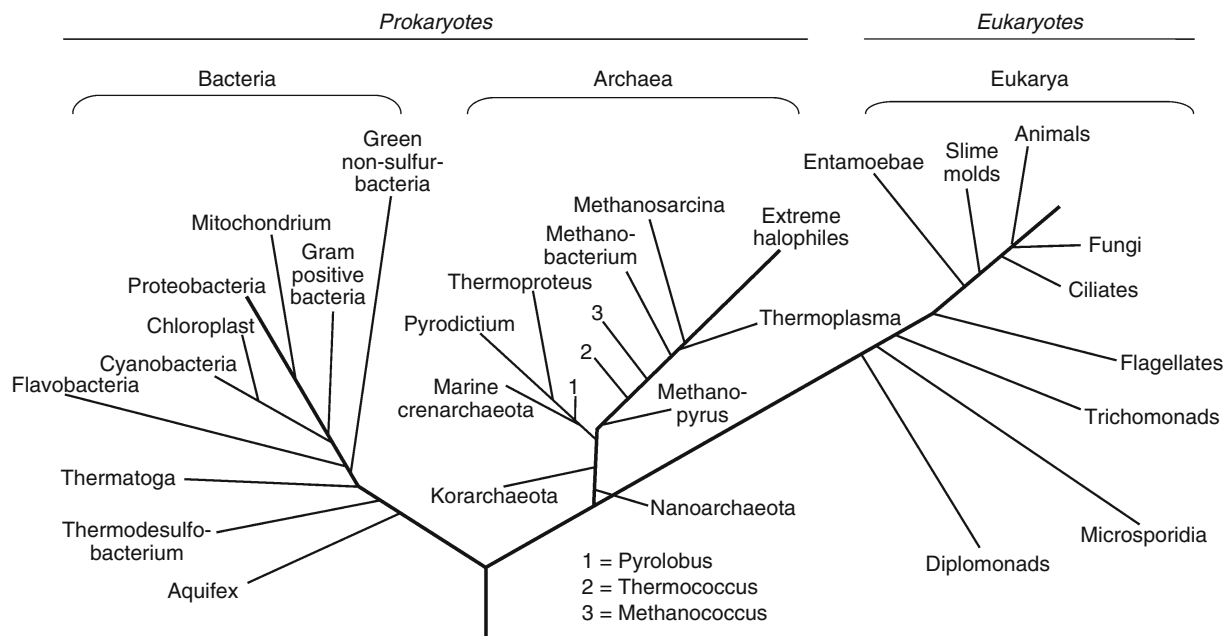
History

The discovery of the Archaea dates back to 1976 when Carl Woese, at his laboratory at Illinois University, compared prokaryotic small subunit ribosomal RNA sequences using oligonucleotide catalogs (Woese, 2007). Woese recognized *Methanobacterium thermoautotrophicum* as the first member of a fundamentally distinct group of prokaryotes that clustered away from all other bacteria. Consequently, Woese and Fox (1977) established the concept of two separate prokaryotic “urkingdoms,” Eubacteria and Archaeobacteria. Later, the term “Archaeobacteria” was changed to “Archaea” to emphasize the fundamental differences between both groups. Based on these discoveries, Woese and his coworkers proposed the now-accepted division of life into the three domains of Archaea, Bacteria, and Eukarya (Figure 1; Woese et al., 1990).

Specific traits

Like the Bacteria, most Archaea show a cell size between 0.5 and 2 μm and lack a cell nucleus, internal membranes, and organelles. However, a number of traits distinguish the Archaea from members of the other domains of life:

1. The genetic machinery of Archaea is different from Bacteria and Eukarya. The distinctive archaeal “signature” contains more than 1,000 archaeal protein-encoding genes. These signature genes account for a significant portion (9–15%) of archaeal genomic DNA (Graham et al., 2000). The archaeal RNA polymerase, an enzyme producing RNA chains from DNA gene templates, consists of eight (methanogens



Archaea, Figure 1 The phylogenetic tree of life. (Redrawn after Madigan and Martinko, 2006.)

Cross-references

Biomining (Mineral Bioleaching, Mineral Biooxidation)
Copper
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Iron Sulfide Formation
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation

ACIDOPHILES

“Acidophiles” are organisms thriving in environments below pH 5. For details, see entries “*Extreme Environments*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Hot Springs and Geysers*,” *Hydrothermal Environments (Marine)* and “*Acid Rock Drainage*.”

ACRITARCHS

Acritarchs are organic-walled acid-resistant microfossils known from the Proterozoic and throughout the Phanerozoic. The position of these microfossils is still uncertain. A number of acritarch genera have been assigned to Green Algae; others are considered to bear some resemblance to the cysts of dinoflagellates. For more information, please refer to entries “Algae, Eukaryotic” and “Protozoa.”

AEROBIC METABOLISM

Heribert Cypionka
University of Oldenburg, Oldenburg, Germany

Synonyms

Aerobic respiration; Oxygen metabolism

Definition

Aerobic metabolism comprises the reduction of molecular oxygen as electron acceptor of aerobic respiration, its use as cosubstrate in the degradation of certain compounds, and reactions leading to the detoxification of partially reduced oxygen species.

Introduction

Life has evolved in the absence of molecular oxygen (see Chapter *Early Earth*). Therefore, it is not surprising that basic metabolic pathways involved in growth and cell division (like DNA replication or protein synthesis) do not depend on the presence of molecular O₂. The evolution of the oxygen-producing (oxygenic) phototrophs dramatically changed the situation of living organisms. Molecular oxygen raised the redox potential of the environment and enabled microorganisms to respire with a much higher energy yield than before. Although oxygen is involved in

a limited number of reactions only, its presence or absence has fundamental impact on biogeochemical processes.

Aerobic degradation of organic matter

Aerobic respiration consumes a major part of the photosynthetically produced O₂ and about 90% of the organic matter (see Chapter *Carbon Cycle*). Aerobic (oxygen-metabolizing) organisms normally oxidize their substrates completely to CO₂. In most cases, O₂ is not directly involved in this process. Substrate oxidation generates reduced electron carriers by means of the respiratory chain. Oxygen is reduced as the last step of the respiration process.

Respiratory chain

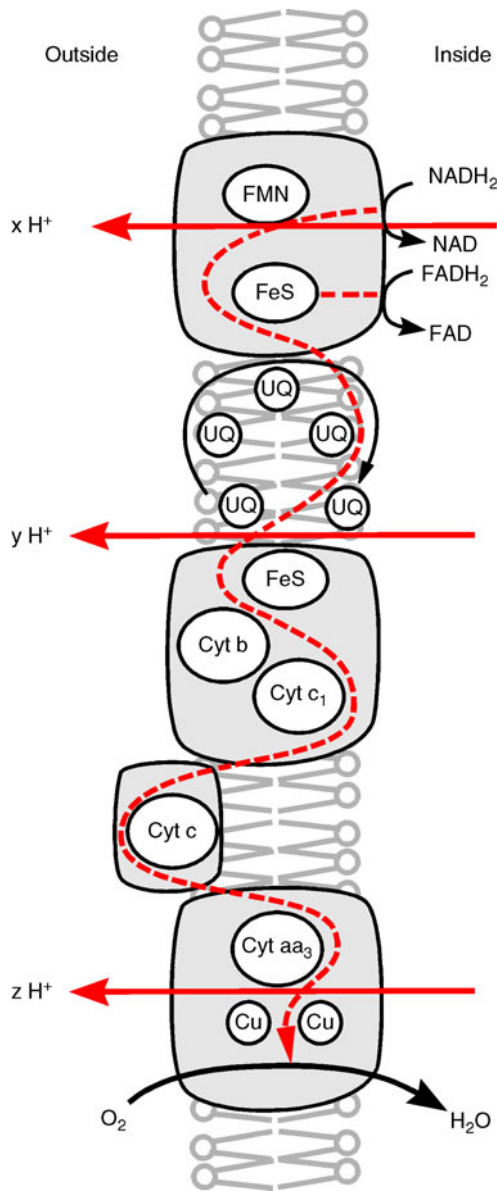
Respiration is a membrane-bound process that generates a chemiosmotic proton gradient across the membrane (Mitchell, 1966). Respiratory chains are composed of series of enzymes, which are organized in multienzyme complexes and use cofactors that allow the stepwise transport of reducing equivalents to oxygen (Figure 1). In eukaryotes (plants, animals, fungi) it takes place within the mitochondria, which are assumed to have evolved from endosymbiotic bacteria. In prokaryotes (Bacteria, Archaea), the respiratory chain is located in the cytoplasmic membrane. The respiratory chain of the bacterium *Paracoccus denitrificans* appears to have many similarities to the mitochondrial type (Harold, 1986). However, many bacteria have variations compared to the mitochondrial type presented in Figure 1.

Energetics and ATP conservation

Most reducing equivalents are fed into the respiration process via NADH₂, which has a standard redox potential (E_0') of -0.32 V. The terminal electron-accepting redox pair O₂/H₂O has redox potential of $+0.82$ V. Thus, the usable redox potential is about 1.14 V. From this redox potential one can calculate a standard $\Delta G_0'$ of -440 kJ per mol of O₂. Assuming that ATP conservation requires about -75 kJ per mol, the conservation of 5 to 6 ATP per O₂ reduced is possible. As a consequence of the high ATP yield, aerobic organisms can form more biomass from the same amount of substrate than anaerobic organisms.

Use of oxygen as cosubstrate

Molecular oxygen is used as a cosubstrate in some catabolic and anabolic reactions (Lengeler et al., 1999). The enzymes involved are called mono- or di-oxygenases, depending on the number of oxygen atoms transferred to their substrate. Oxygenases are used by aerobes to activate certain substrates that do not have functional groups that allow a simple degradation. They catalyze the initial hydroxylation of hydrocarbons to yield alcohols that can easily be metabolized further. They are also involved in the ring cleavage of aromatic compounds and in the first oxidation step of ammonia oxydation. Anaerobic organisms are either unable to oxidize the substrates activated



Aerobic Metabolism, Figure 1 Principle of respiratory electron transport and vectorial proton translocation. The first proton translocating complex (with FMN and FeS as prosthetic groups) channels electrons to the quinones (UQ), which perform a cycle, again coupled to proton translocation. Electrons move on to the cytochrome bc_1 -complex, via cytochrome c to the copper-containing terminal cytochrome oxidase (Cyt aa_3). O_2 is reduced in the last step only. The ATP synthesis coupled to reentry of the translocated protons is independent of the electron transport process. (From Cypionka, 2006.)

by means of oxygenases or possess different degradation pathways.

Anabolic reactions making use of molecular oxygen are the biosynthesis pathways of tetrapyrroles, ubiquinones, pyrimidines, sterols, and unsaturated fatty acids. Again,

either the products are absent in strict anaerobes (e.g., sterols), or anaerobes have found alternative pathways for their biosynthesis.

Toxic forms of oxygen and detoxification

Aerobic metabolism inevitably generates small amounts of partially reduced toxic oxygen species (Lengeler et al., 1999). These are the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$). These species can be formed via nonenzymatic one-electron reduction steps (e.g., with Fe^{2+} , quinols, or H_2S) or by nonspecific reactions with reduced flavoproteins. Aerobic bacteria can remove toxic oxygen species by nonenzymatic reactions (e.g., with glutathione) or by means of enzymes: Superoxide dismutase (SOD) converts superoxide to H_2O_2 , and catalyze dismutates H_2O_2 to water and O_2 . These enzymes often lack in strict anaerobes, which might explain their sensitivity toward oxygen.

Distribution of the aerobic lifestyle

Many microorganisms can thrive in oxic (oxygen-containing) as well as in anoxic (free of oxygen) environments. Those are classified as facultative aerobes (or facultative anaerobes). This means that they can rely on aerobic respiration as well as on respiration with other electron acceptors or on fermentative energy conservation. Groups of strict anaerobes comprise methanogens, sulfate reducers, and Clostridia. Even in strict anoxic (oxygen-free) environments facultative aerobes can be found (Süß et al., 2006).

Summary

Aerobic respiration is the most effective way of energy conservation. It is used to oxidize the major part of the organic matter on Earth. During aerobic respiration oxygen is reduced to water. In some cases, O_2 is used by oxygenases for the hydroxylation of inert substrates. Aerobic organisms have enzymatic and nonenzymatic mechanisms to detoxify partially reduced oxygen species such as superoxide or hydrogen peroxide.

Bibliography

- Cypionka, H., 2006. *Grundlagen der Mikrobiologie*. Heidelberg: Springer.
- Harold, F. M., 1986. *The Vital Force: A Study of Bioenergetics*. New York: Freeman and Company.
- Lengeler, J. W., Drews, G., and Schlegel, H. G., 1999. *Biology of the Prokaryotes*. Stuttgart: Thieme.
- Mitchell, P., 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biological Reviews of the Cambridge Philosophical Society*, **41**, 445–502.
- Süß, J., Schubert, K., Sass, H., Cypionka, H., Overmann, J., and Engelen, B., 2006. Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. *Environmental Microbiology*, **8**, 1753–1763.

Cross-references

Anaerobic Transformation Processes, Microbiology
 Archaea
 Bacteria
 Carbon (Organic, Degradation)
 Critical Intervals in Earth History

ALGAE (EUKARYOTIC)

Thomas Friedl¹, Nicole Brinkmann¹, Kathrin I. Mohr^{1,2}

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Infection Research, Braunschweig, Germany

Algae (eukaryotic)

Eukaryotic algae are a collection of extremely diverse, nonrelated organisms that perform photosynthesis in plastids, permanent organelles of green, brown, or bluish colors derived from endosymbiosis. In contrast to plants, algae do not form embryos.

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids (Keeling, 2004). Many authors even include the prokaryotic cyanobacteria into the algae, because they exhibit a life-style rather similar to their eukaryotic counterparts and often share the same habitat with eukaryotic algae. Cyanobacteria form the origin of plastids (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Plastids are the organelles of plants and eukaryotic algae that harbor photosynthesis and synthesize many chemical compounds also important for other biochemical pathways (e.g., aromatic amino acids, heme, isoprenoids, and fatty acids); nonphotosynthetic plastids are known from plants and many algal groups (Williams and Keeling, 2003). Recently, from strategies of synthesizing various information, that is, mainly DNA sequence analyses of concatenated genes as well as the incorporation of discrete characters such as insertions, deletions, and gene fusion events, and consideration of morphology and biochemistry, a phylogeny of eukaryotes has emerged which consists of five “supergroups” (Keeling, 2004). Algae are scattered among four of the five major eukaryotic groups. The “Plantae” comprises exclusively eukaryotes with plastids derived from primary endosymbiosis, that is, they are bound by two membranes, derived from the inner and outer membranes of a cyanobacterium which was transformed into an organelle through uptake and retention by the host cell followed by the loss of much of its genome (Keeling, 2004). The Plantae are formed by three distinct but related lineages, the viridiplantae (which comprises all green algae and land plants or embryophytes), the rhodophytes (red algae), and the glaucophytes. The “Chromalveolates” comprises large and abundant algal groups such as the heterokont algae (e.g., diatoms and brown algae), the haptophytes,

the alveolates (which include the dinoflagellates and apicomplexans), and the cryptomonads. Only a single lineage of each of the “Excavates” and “Rhizaria” includes algae, the euglenoids and chlorarachniophytes. Except for the Plantae, all other eukaryotic algal lineages acquired their plastids through secondary endosymbiosis (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Secondary plastids are surrounded by four or three membranes which resulted from phagocytosis of a primary alga, which was either a red alga or a green alga. In secondary plastids of both red and green origin, the primary algal nucleus is highly reduced (in the cryptomonads and chlorarachniophytes) or more commonly lost altogether (e.g., in the heterokonts and haptophytes; Keeling, 2004).

Green algae (Plantae supergroup)

The green algae are photosynthetic eukaryotes with double membrane-bound plastids (derived from primary endosymbiosis), stacked thylakoids, and the chlorophylls *a* and *b*. Their other accessory pigments are the carotenoids, beta-carotene, and xanthophylls. The main reserve polysaccharide is starch which is deposited inside the plastid (Lewis and McCourt, 2004; Pröschold and Leliaert, 2007). A few genera (e.g., *Prototheca*) contain non-photosynthetic plastids which lost their pigments secondarily. Apart from plastid-associated features, a unique stellate structure linking nine pairs of microtubules in the flagellar base of those green algae that are motile or produce motile stages is characteristic, as well as cell walls composed of cellulose which is present in most green algae (Graham et al., 2009). Most of these characters are shared with embryophytes (land plants) and, therefore, the green algae are difficult to define to the exclusion of the latter. The green algae have evolved in two major lineages (Friedl, 1997; Karol et al., 2001; Lewis and McCourt, 2004). One lineage, the chlorophyte lineage (Chlorophyta *sensu* Bremer, 1985; Sluiman, 1985) comprises the majority of green algal diversity (i.e., the green algae in a more narrow sense or the majority of what have been traditionally called green algae) with a bewildering array of morphologies attributable to at least five different morphological organizations (Pröschold and Leliaert, 2007). Examples of microscopic green algae reported from freshwaters with high calcification levels are rather small coccoid forms (e.g., *Chlorella*-like, Figure 1a; *Desmochloris*, Figure 1d; *Pseudendocloniopsis*, Figure 1f), coccoids that form coenobia (e.g., *Scenedesmus*, Figure 1b) or are of filamentous organization which may easily disintegrate into unicells (e.g., *Stichococcus*, Figure 1c) or form small thalli of branched filaments (e.g., *Pseudendoclonium*, Figure 1e). Also some monadoid (flagellated unicells) green algae and the siphonocladous *Cladophora* are well known from highly calcifying freshwater habitats. Several marine green algae of coenocytic organization precipitate carbonate and belong to the

Cross-references

Anaerobic Transformation Processes, Microbiology
 Archaea
 Bacteria
 Carbon (Organic, Degradation)
 Critical Intervals in Earth History

ALGAE (EUKARYOTIC)

Thomas Friedl¹, Nicole Brinkmann¹, Kathrin I. Mohr^{1,2}

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Infection Research, Braunschweig, Germany

Algae (eukaryotic)

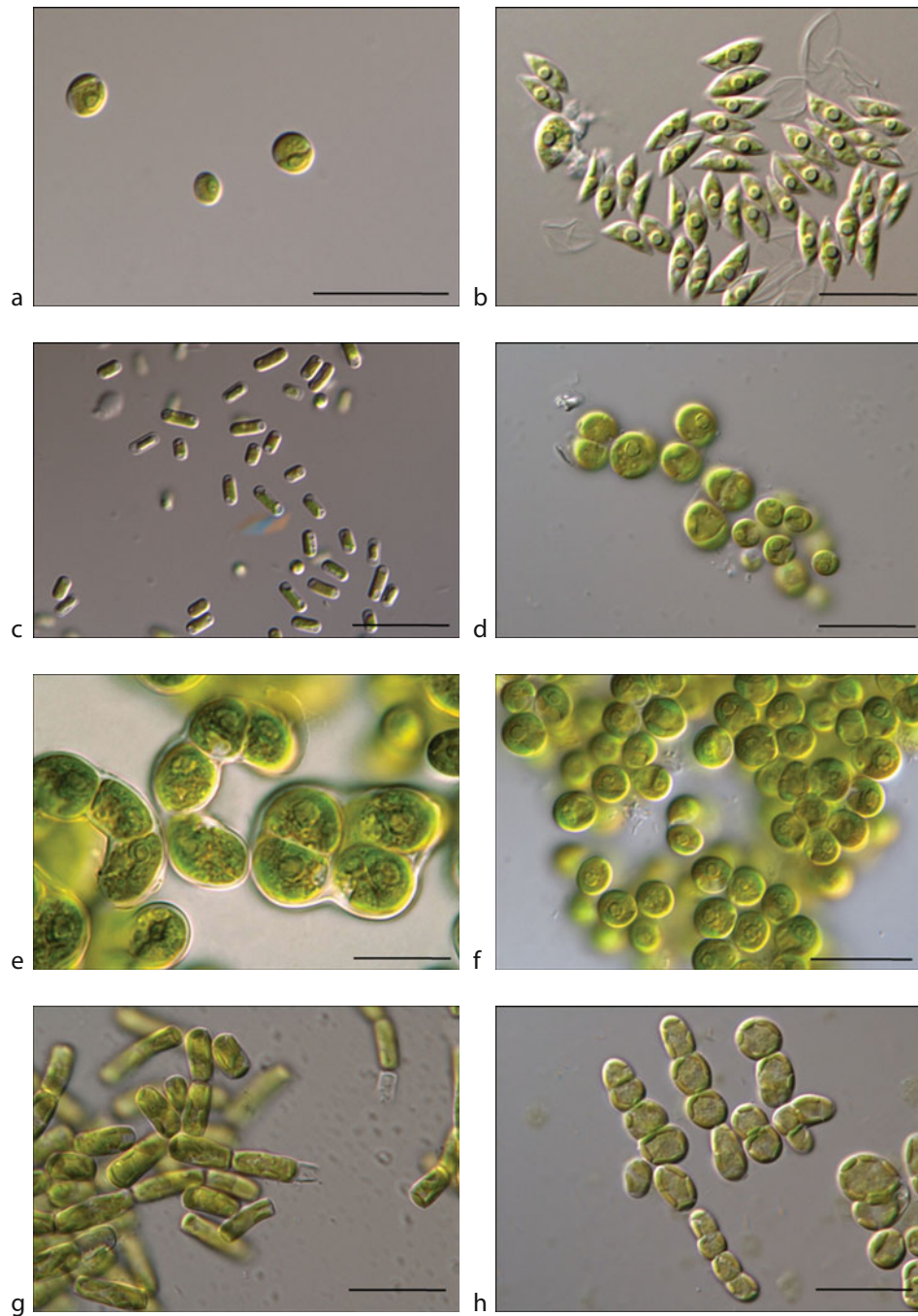
Eukaryotic algae are a collection of extremely diverse, nonrelated organisms that perform photosynthesis in plastids, permanent organelles of green, brown, or bluish colors derived from endosymbiosis. In contrast to plants, algae do not form embryos.

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids (Keeling, 2004). Many authors even include the prokaryotic cyanobacteria into the algae, because they exhibit a life-style rather similar to their eukaryotic counterparts and often share the same habitat with eukaryotic algae. Cyanobacteria form the origin of plastids (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Plastids are the organelles of plants and eukaryotic algae that harbor photosynthesis and synthesize many chemical compounds also important for other biochemical pathways (e.g., aromatic amino acids, heme, isoprenoids, and fatty acids); nonphotosynthetic plastids are known from plants and many algal groups (Williams and Keeling, 2003). Recently, from strategies of synthesizing various information, that is, mainly DNA sequence analyses of concatenated genes as well as the incorporation of discrete characters such as insertions, deletions, and gene fusion events, and consideration of morphology and biochemistry, a phylogeny of eukaryotes has emerged which consists of five “supergroups” (Keeling, 2004). Algae are scattered among four of the five major eukaryotic groups. The “Plantae” comprises exclusively eukaryotes with plastids derived from primary endosymbiosis, that is, they are bound by two membranes, derived from the inner and outer membranes of a cyanobacterium which was transformed into an organelle through uptake and retention by the host cell followed by the loss of much of its genome (Keeling, 2004). The Plantae are formed by three distinct but related lineages, the viridiplantae (which comprises all green algae and land plants or embryophytes), the rhodophytes (red algae), and the glaucophytes. The “Chromalveolates” comprises large and abundant algal groups such as the heterokont algae (e.g., diatoms and brown algae), the haptophytes,

the alveolates (which include the dinoflagellates and apicomplexans), and the cryptomonads. Only a single lineage of each of the “Excavates” and “Rhizaria” includes algae, the euglenoids and chlorarachniophytes. Except for the Plantae, all other eukaryotic algal lineages acquired their plastids through secondary endosymbiosis (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Secondary plastids are surrounded by four or three membranes which resulted from phagocytosis of a primary alga, which was either a red alga or a green alga. In secondary plastids of both red and green origin, the primary algal nucleus is highly reduced (in the cryptomonads and chlorarachniophytes) or more commonly lost altogether (e.g., in the heterokonts and haptophytes; Keeling, 2004).

Green algae (Plantae supergroup)

The green algae are photosynthetic eukaryotes with double membrane-bound plastids (derived from primary endosymbiosis), stacked thylakoids, and the chlorophylls *a* and *b*. Their other accessory pigments are the carotenoids, beta-carotene, and xanthophylls. The main reserve polysaccharide is starch which is deposited inside the plastid (Lewis and McCourt, 2004; Pröschold and Leliaert, 2007). A few genera (e.g., *Prototheca*) contain non-photosynthetic plastids which lost their pigments secondarily. Apart from plastid-associated features, a unique stellate structure linking nine pairs of microtubules in the flagellar base of those green algae that are motile or produce motile stages is characteristic, as well as cell walls composed of cellulose which is present in most green algae (Graham et al., 2009). Most of these characters are shared with embryophytes (land plants) and, therefore, the green algae are difficult to define to the exclusion of the latter. The green algae have evolved in two major lineages (Friedl, 1997; Karol et al., 2001; Lewis and McCourt, 2004). One lineage, the chlorophyte lineage (*Chlorophyta sensu* Bremer, 1985; Sluiman, 1985) comprises the majority of green algal diversity (i.e., the green algae in a more narrow sense or the majority of what have been traditionally called green algae) with a bewildering array of morphologies attributable to at least five different morphological organizations (Pröschold and Leliaert, 2007). Examples of microscopic green algae reported from freshwaters with high calcification levels are rather small coccoid forms (e.g., *Chlorella*-like, Figure 1a; *Desmochloris*, Figure 1d; *Pseudendocloniopsis*, Figure 1f), coccoids that form coenobia (e.g., *Scenedesmus*, Figure 1b) or are of filamentous organization which may easily disintegrate into unicells (e.g., *Stichococcus*, Figure 1c) or form small thalli of branched filaments (e.g., *Pseudendoclonium*, Figure 1e). Also some monadoid (flagellated unicells) green algae and the siphonocladous *Cladophora* are well known from highly calcifying freshwater habitats. Several marine green algae of coenocytic organization precipitate carbonate and belong to the



Algae (Eukaryotic), Figure 1 Eukaryotic algae recovered from tufa stromatolite biofilms of hardwater creeks: (a) *Chlorella*-like coccoid green alga (Trebouxiophyceae, pure culture), (b) coenobia of coccoid green alga *Scenedesmus* sp. (Chlorophyceae, pure culture), (c) barrel-shaped unicells of green alga *Stichococcus* sp. (Trebouxiophyceae, pure culture), (d) coccoid green alga *Desmochloris* sp. (Ulvophyceae, pure culture), (e) branched filaments of green alga *Pseudendoclonium* sp. (Ulvophyceae, pure culture), (f) coccoid green alga *Pseudendocloniopsis* sp. (Ulvophyceae, pure culture), (g) filamentous unbranched stramenopiles *Xanthonema* sp. (Xanthophyceae, pure culture), (h) filamentous branched *Heterococcus* sp. (Xanthophyceae, pure culture), scale bar 20 μm .

calcareous algae, for example, the orders Dasycladales (Kingsley et al., 2003) and Caulerpales (Stanley et al., 2010). Whether the aforementioned freshwater green algal taxa are involved in carbonate precipitation is still

unknown, but microscopic freshwater green algal genera with calcite deposits on their surfaces are several members of Zygnematales (e.g., *Oocardium*) and *Gongrosira* (Rott et al., 2009; Freytet and Verrecchia, 1998). The other major

green algal lineage, the charophyte clade (*sensu* Lewis and McCourt, 2004), contains a smaller number of widespread, mostly freshwater green algae. Examples from the charophyte clade which are associated with calcification are the orders Zygnematales and Charales, for examples, the genus *Chara* is known for its extracellular carbonate production (McConnaughey, 1991). The charophyte green algae represent the closer relatives of land plants (embryophytes) with the Charales being most closely related to embryophytes (Karol et al., 2001; Lewis and McCourt, 2004). In the three lineages of green algae where calcite accumulation is known, that is, the orders Caulerpales, Dasycladales and Zygnematales (Bilan and Usov, 2001), aragonite deposits are outside of the cell wall but inside a specialized sheath. The crystal bundles are organized in layers, which are parallel to the filaments of this porous multilayer sheath and have also calcium oxalate inclusions. The chemical composition of the sheath and its role in the calcification process are unknown for the majority of species. It may simply be a barrier preventing the diffusion into the surrounding medium, although its direct participation in the initiation of the crystal formation cannot be excluded (Bilan and Usov, 2001). A third group of green algal taxa, called prasinophytes, consists of “primitive”-appearing unicells of uncertain affinity. Most known prasinophyte green algae form several early diverging clades within the phylogeny of the Chlorophyta (Marin and Melkonian, 2010). The green flagellate genus *Mesostigma* may represent a very basal lineage of the charophyte clade (Charophyta), but some prasinophytes are likely representatives of even other early diverging clades (Fawley et al., 2000). With respect to the systematics of green algae, there is now consensus that the majority of known green algal species of the Chlorophyta belongs to three major lineages, the chlorophytes, trebouxiophytes, and ulvophytes, which are assigned to class rank (classes Chlorophyceae, Trebouxiophyceae, and Ulvophyceae; Pröschold and Leliaert, 2007). Green algae have an affinity to form symbioses with hosts of various phylogenetic positions. Well-known examples are green algae forming the photoautotrophic partners (photobionts) of fungal symbioses, that is, lichens, or representing the algal hosts (“Zoochlorellae”) of ciliates (Alveolates), heliozoa, sponges, and sea anemones (e.g., Summerer et al., 2008; Letsch et al., 2009).

Rhodophytes – red algae (Plantae supergroup)

Red algae are a very large and diverse group of microscopic algae and macroalgae. They are best known for their economic and ecological importance. Though they are present in freshwater with several genera, the majority of red algal genera occur on tropical and temperate marine shores, where they play important ecological roles (Graham et al., 2009). Certain calcified red algae, known as corallines, form hard, flat sheets

that consolidate and stabilize coral reef crests, that is, coralline red algae protect reefs from wave damage and, thus, they are regarded as important keystone organisms. Of well-known economic importance are the species of *Porphyra* and other red algal species which are grown in mariculture operations for use as human food. Several other marine genera are cultivated or harvested for the extraction of gelling polysaccharides such as agarose and carrageen (Graham et al., 2009).

Corallines are known from fossil records 500 million years old (Lower Ordovician; Riding et al., 1998). The oldest fossil record of a (non-coralline) red alga may represent the fossil taxon *Bangiomorpha pubescens* which was described from the 1,200-million-year-old Hunting Formation in the Canadian Arctic (Butterfield, 2000). It is most notable that *B. pubescens* even represents the earliest putative record for sex and taxonomically resolvable complex multicellularity among eukaryotes (Saunders and Hammersand, 2004). For a review of fossil records of the various taxonomic groups of red algae, see Saunders and Hammersand (2004).

The plastids of red algae lack chlorophyll *b* and *c*, but contain phycobilins (allophycocyanin, phycocyanin, and the red pigment phycoerythrin) as accessory pigments which are located in phycobilisomes on the outer surface of the thylakoids. The red algal plastids are bounded by two membranes and derived from a cyanobacterial primary endosymbiosis (Keeling, 2004). They produce floridean starch which is deposited in the cytoplasm. The rhodophytes lack flagella and centrioles in all stages of their life history (Graham et al., 2009).

The red algae (Rhodophyta) are a distinct eukaryotic lineage, whose members are united in the phylogenetic analyses of a nuclear, plastid, and mitochondrial genes (for review see Yoon et al., 2006). They belong to the Plantae supergroup of eukaryotes (Keeling, 2004) and are believed to form a sister group with the green line (Martin et al., 2002). Their divergence has been estimated ca. 1,500 million years ago (Yoon et al., 2004). Based on molecular evidence, the red algae are partitioned into seven classes, six of which have a rather simple body structure and reproduction (Yoon et al., 2006). The florideophytes (class Florideophyceae) are the monophyletic group of red algae that exhibit more complex bodies and reproduction (Saunders and Hammersand, 2004). The ecologically significant corallines, the economically important genus *Chondrus* (used as food and for the extraction of carrageen; Graham et al., 2009), and the vast majority of other modern red algal species are florideophytes; they are divided into several monophyletic groups (subclasses; Saunders and Hammersand, 2004; Le Gall and Saunders, 2007).

Coralline red algae represent not only a large carbon reservoir, but they also consolidate sediment and build landforms, and they occupy harder substrate in the

world's oceans than any other group of photosynthetic organisms. Unlike most other calcified algae that deposit carbonate as aragonite, the coralline red algae form calcite and it is believed that each family of coralline red algae is defined by only one crystalline modification of calcium carbonate (Borowitzka, 1977). The red algae show evidence of a surprising variety of calcification mechanisms, but the location of deposition is even more diverse. Mineralization may occur throughout the walls of nearly all vegetative cells, it may occur within the walls of only specialized cells, or it may be located in an intercellular organic matrix between cells, but not within the cell walls (Pueschel et al., 1992). Calcification in corallines is poorly understood, however, it seems clear that corallines deposit high magnesium calcite in a definitive pattern within their cell walls via the membrane pumps that control the flux of calcium and carbonate with the control of crystal orientation by organic wall components (Zankl, 2007). Coralline red algae can be the deepest algae (−50 to −110 m, Dullo et al., 1990) and many species occur in great abundance into the Arctic and subarctic. Coralline red algae in coral reefs are ubiquitous and dominant (Glynn et al., 1996; Keats et al., 1997), and their abundance in cryptic and shaded environments can be greatly underestimated (Littler, 1972). In temperate systems, the accumulation of unattached living or dead coralline algae forms large maerl beds (Martin et al., 2006). They also provide food for herbivores with hardened mouthparts (Steneck and Dethier, 1994) and surfaces for the settlement of invertebrate larvae (Adey, 1998). Coralline algal abundance, size, shape, and species composition in maerl beds vary considerably depending on their location, and several environmental factors influence coralline algal distribution. Maerl beds are highly sensitive to desiccation and are found from the low intertidal zone to depths of 150 m (Foster, 2001). Furthermore, coralline species are sensitive to seasonal variations in temperature inducing changes in their physiology, for example, *Lithothamnion* spp. growth rate increases with water temperature (Potin et al., 1990). Despite their ecological importance in temperate systems, primary production and calcification of coralline algae has been mainly investigated in tropical areas (Chisholm, 2003; Payri et al., 2001). A number of different associations between sponges and several species of red macroalgae have been described in literature (Trautman et al., 2000, 2002). The symbiotic association between the haplosclerid sponge *Haliclona cymiformis* and the red macroalga *Ceratodictyon spongiosum* is common on shallow coral reefs of the Indo-West Pacific, where it has been described as being one of the most conspicuous organisms found in these areas (van Soest, 1990). This symbiosis seems to be an obligatory association as neither sponge nor alga has ever been identified growing independently or in association with other species (Morrissey, 1980).

Glaucophytes (Plantae supergroup)

Glaucophytes (or glaucocystophytes) form a small group of microscopic algae exclusively found in freshwater environments; only about 13 species of glaucophytes have been described so far. They represent the third lineage of the plant supergroup, which is defined by plastids bound by a double membrane, that is, which is derived from primary cyanobacterial endosymbiosis (Keeling, 2004). Glaucophyte plastids are unique among plastids in that they are retaining the prokaryotic peptidoglycan layer between their two membranes. Therefore, they may represent an intermediate in the transition from a cyanobacterial endosymbiont to plastid; molecular phylogenetic analyses show the glaucophytes as the earliest divergence within the plant supergroup (Keeling, 2004; Martin et al., 2002). Glaucophyte plastids share the accessory photosynthetic pigments, phycobilins, which are organized in phycobilisomes (small particles on the outer surface of thylakoid membranes) with cyanobacteria and rhodophytes. For a review on glaucophytes, see Bhattacharya and Schmidt (1997) and Steiner and Löffelhardt (2002).

Photosynthetic stramenopiles (chromalveolates)

Stramenopiles form a monophyletic group of photoautotrophic as well as heterotrophic organisms which are characterized by swimming cells possessing at least one flagellum with distinct tripartite tubular hairs (Graham et al., 2009; Andersen, 2004). Most stramenopiles have two distinctly different flagella and, therefore, are also known as heterokonts. They have a long flagellum with tripartite hairs (tinsel or immature flagellum) to pull the cells through the water and a shorter smooth one (whiplash or mature flagellum) that lacks tripartite hairs, often with a light-sensing flagellar swelling at its base (Graham et al., 2009; Andersen, 2004). Photosynthetic stramenopiles or heterokont algae usually appear brown or golden brown (and are thus sometimes referred to as chromophytic algae) due to the presence of characteristic accessory pigments, fucoxanthin or vaucheriaxanthin, and at least one form of chlorophyll *c* (except in eustigmatophytes). The plastids are derived from a secondary endosymbiosis event with a red alga involved and surrounded by four membranes (McFadden, 2001; Keeling, 2004; Palmer, 2003). In most photosynthetic stramenopiles, a chloroplast endoplasmic reticulum is present, that is, plastid and nucleus are structurally connected. The photosynthetic stramenopiles comprise a tremendous morphological diversity: a large variety of unicellular or colonial algae, including the diatoms with silica frustules as walls, multicellular simple filaments (e.g., in the xanthophytes) as well as the macroscopic complex brown algae forming a brown canopy at sea shores. Based on detailed analyses of pigment composition, ultrastructure and molecular phylogenies more than a dozen of different classes of photosynthetic stramenopiles have been described (Graham

et al., 2009; Andersen, 2004). Some photosynthetic stramenopiles do not form mineralized cysts or scales and, therefore, they have no fossil record. Examples are the brown seaweeds (Phaeophyceae), the yellow green algae (Xanthophyceae), and the inconspicuous eustigmatophytes. In the context of geobiology also xanthophytes and eustigmatophytes may be important, as they have been reported from freshwaters with high calcite precipitation (see Figure 1g, e; Arp et al., 2010). However, their role in the calcification processes is still unclear. Therefore, in this entry, only those classes of photosynthetic stramenopiles will be discussed that produce mineralized cell coverings which are important in fossil deposits and, therefore, may be of particular interest in geobiology. Siliceous frustules define the diatoms (Bacillariophyta; see entry “*Diatoms*”), silica skeletons are characteristic for silicoflagellates (class Dictyochophyceae) and silica scales and cysts are commonly formed in synurophytes and chrysophytes.

Silicoflagellates are unicellular, flagellated, and photoautotrophic stramenopiles which are characterized in at least one life cycle stage by the formation of distinctive, one-piece external silica skeleton that is highly perforate. They form the order Dictyochaetales, one of three orders that have been described for the class Dictyochophyceae (Daugbjerg and Henriksen, 2001; Graham et al., 2009). Siliceous skeletons from silicoflagellates are common in fossil deposits dating back to the middle Cretaceous (ca. 120 million years ago). The skeletal remains in fossil material also indicate that species diversity reached a peak in the Miocene (ca. 23–25 million years ago), with more than 100 described species, but the diversity of silicoflagellates has decreased thereafter, leaving behind only two genera (Daugbjerg and Henriksen, 2001; Tappan, 1980). Biostratigraphic zonations have been developed for silicoflagellates in all oceans throughout the Cenozoic, though the zones typically range over longer intervals of time than foraminiferal or diatom zones (McCartney and Wise, 1990; McCartney and Harwood, 1992). Silicoflagellate skeletons may have considerable potential as ecophenotypic indicators (McCartney and Wise, 1990). Modern silicoflagellates are widespread in the oceans with a tendency to be more abundant in colder waters where they can even grow up to form blooms (Graham et al., 2009). Dictyochophytes occur in both marine and freshwater habitats (Moestrup, 1995; Moestrup and O’Kelly, 2000). *Synurophytes* and *chrysophytes* (often summarized as “Chrysophyte Algae”) produced an exceptional fossil record owing to the high preservation potential of their siliceous stomatocysts and scales (Smol, 1995; Andersen, 2004; Graham et al., 2009). In addition, they are highly sensitive to an array of limnological parameters. Synurophytes and Chrysophytes are close relatives in molecular phylogenies (Andersen, 2004); both share the capability to form stomatocysts, silica-walled resting stages, which arise sexually or asexually under unfavorable environmental conditions. The walls of stomatocysts

are so heavily silicified that they resist silica dissolution processes and thus accumulate in the sediments of lakes (Graham et al., 2009). Fossil records of stomatocysts extend back to the Lower Cretaceous (at least 150 million years ago). Synurophytes and chrysophytes are able to switch between autotrophy, heterotrophy, and even phagotrophy, which puts them at a distinct advantage in aquatic ecosystems that are low in nutrient concentrations and have reduced light penetration (Betts-Piper et al., 2004). From patterns of stomatocyst assemblages, past environmental conditions can be inferred making stomatocysts very important for paleolimnology (Duff et al., 1995; Siver, 1995; Wilkinson et al., 2001; Zeeb and Smol, 2001).

Synurophytes are unicellular or colonial silicated flagellates and despite they are photoautotrophic, many species are also capable of phagotrophy. Synurophycean cell surfaces are covered by overlapping silica scales, sometimes with spiny bristles, and are perforated (Graham et al., 2009). The scales’ perforation patterns are used as taxonomic features to delimitate species of synurophytes. A number of adhesive polysaccharides are involved in the scale attachment. The scales are produced in certain vesicles located on the surface of one of the plastids. In contrast to diatoms, synurophytes can continue to divide and function also in the absence of an external silica covering. In cultures of naked cells most cells will recover a complete cell covering when silicate is resupplied to silica-depleted cells (Leadbeater and Barker, 1995). Fossil scales similar to those of modern synurophytes have been reported from deposits of the middle Eocene (about 47 million years ago; Siver and Wolfe, 2005). Synurophyte scales can be used like stomatocysts for tracking environmental change using lake sediments (Zeeb and Smol, 2001; Wolfe and Perren, 2001). Synurophytes are abundant in neutral to slightly acidic freshwaters. Some species are regarded as indicators of low levels of pollution, but others are characteristic of eutrophic lakes (Graham et al., 2009).

Chrysophytes are golden-brown microalgae that are mostly unicellular or colonial which occur as flagellates or nonmotile cells; they include many unique and interesting morphologies (Graham et al., 2009). In contrast to synurophytes, scales covering the cell surface are absent in chrysophytes. Chrysophytes typically favor slightly acidic freshwaters of moderate to low productivity; their abundance and species richness increase with lake eutrophic status (Elloranta, 1995). Chrysophytes may have strong ecological impacts because many are mixotrophs, able to take up and metabolize dissolved organic compounds and particulate food as well as photosynthesize (Graham et al., 2009). Several chrysophytes are associated with the formation of undesirable blooms because living cells of certain species can produce toxic fatty acids that affect fish or excrete aldehydes and ketones into the water which can give it an unpleasant taste and odor.

Coccolithophorids and haptophyte algae (chromalveolates)

Haptophyte algae are a monophyletic group that includes all photosynthetic organisms with a haptonema. However, haptophytes also include some nonphotosynthetic relatives, and some that have secondarily lost the haptonema (Andersen, 2004). The haptonema, from which the group derives its name, is a microtubule-supported appendage that lies between two approximately equal flagella (for a review, see Inouye and Kawachi, 1994). Most haptophytes are marine, occurring in significant abundances even at greater water depth (up to 200 m). Haptophyte algae share many features, for example, pigment composition, presence of a chloroplast endoplasmatic reticulum, and plastids derived from red algal secondary endosymbiosis with the photosynthetic stramenopiles (Andersen, 2004). Based on structural and molecular evidences the haptophytes form a monophyletic lineage, treated as phylum Haptophyta which is divided into two classes (Edvardsen et al., 2000; Andersen, 2004). The larger group, class Coccolithophyceae, comprises the coccolithophorids which are haptophytes with a cell coat of mineralized scales (coccoliths) and two equal flagella or lack flagella altogether. Coccoliths are largely composed of calcium carbonate crystals in the form of calcite. There are two types of coccoliths, holococcoliths and heterococcoliths. In case of holococcoliths, an organic scale consisting largely of cellulose and produced by the Golgi body is secreted to the cell surface and then calcite crystals which are held together by organic material are deposited on the scale. In contrast, heterococcoliths develop on organic scales before being secreted from the cell, that is, calcite crystal deposition is already within the cell. The calcite crystals grow and interlock gradually forming the complex shape of the mature coccolith (deVrind-deJong et al., 1994; Probert et al., 2007; Graham et al., 2009). Coccoliths do not degrade at normal ocean pH and thus accumulate on the ocean floor. Therefore, coccolithophorids have produced huge amounts of sedimentary carbonates.

Coccolithophorids remove large quantities of atmospheric CO₂ through their photosynthesis and calcification and, therefore, are an important component of the global carbon cycle (McConnaughey, 1994), accounting for a substantial part of the ocean floor limestone sediments. Coccolithophorids contribute at least 25% of the total annual vertical transport of inorganic carbon to the deep ocean (Rost and Riebesell, 2004). Coccolithophorids have an excellent fossil record with fossil coccoliths well over 200 million years old. The abundance of coccolith fossils peaked during the late Cretaceous (63–95 million years ago) when extensive chalk deposits were laid down. Coccoliths are widely used as stratigraphic indicators and fossil coccoliths are widely used as bioindicators in the oil industry and as indexes of past climate and ocean chemistry conditions (Young et al., 1994).

Haptophytes are ecologically significant in terms of both biotic interactions and biogeochemistry. Haptophyte algae are considered as high-quality foods for zooplankton; several species contain nutritionally important polyunsaturated fatty acids which make them also commercially valuable for the production of fish in aquaculture systems. Other coccolithophytes may produce toxins that destroy cell membranes or produce copious amounts of organic slime and foam (Graham et al., 2009). Many coccolithophorids form blooms in ocean waters and produce large amounts of a volatile sulfur-containing molecule, dimethyl sulfide (DMS) that enhances cloud formation and increases acid rain (Malin and Steinke, 2004). Another cooling effect on the climate comes from the coccoliths which readily reflect light, thereby increasing reflectance of the ocean's surface.

Dinoflagellates (chromalveolates)

The dinoflagellates are an important group of phytoplankton in marine and freshwaters. Their adaptation to a wide variety of environments is reflected by a tremendous diversity in form and nutrition and an extensive fossil record dating back several hundred million years (Graham et al., 2009). Dinoflagellate cells have two structurally distinct flagella whose motion causes the cells to rotate as they swim, but also nonflagellate unicells and filamentous forms of dinophytes are known. Two different cell types can be distinguished on the basis of the cell-wall covering or theca. The “naked” or unarmored forms have an outer plasmalemma surrounding a single layer of flattened vesicles (membrane sacs or alveoli). Armored dinoflagellates have cellulose or other polysaccharides within each vesicle, giving the cells a more rigid, inflexible wall. These cellulose plates are arranged in distinct patterns which are extensively used as taxonomic “fingerprints” (Hackett et al., 2004). Cells of armored dinoflagellates display conspicuous anterior–posterior and dorsal–ventral differentiation. Their cells consist of two parts, separated by a groove that encircles the cell and contains the transverse flagellum which is flattened, ribbon-shaped, and with a single row of hairs. A smaller groove is mostly extending into the posterior cell part and contains the longitudinal flagellum which has two rows of hairs; it is directed posterior and emerges from the cell (Graham et al., 2009; Hackett et al., 2004).

Success of dinoflagellates as phytoplankton may be due in part to unique behavior patterns, including diel vertical migration and their capability to live phagotrophic (feeding on particles such as the cells of other organism) in addition to photoautotrophy (mixotrophic life-style). Some of the armored or thecate heterotrophic dinoflagellates have developed a remarkable pseudopod-like structure that is extruded from the cell and flows around the prey, enveloping it so the contents can then be digested (Hackett et al., 2004). Some dinoflagellates produce toxins that are dangerous to man, marine mammals, fish,

seabirds, and other components of the marine food chain (Van Dolah, 2000). Others are bioluminescent and emit light; some function as parasites or symbionts that rely on host organisms for part of their nutrition. Many photosynthetic dinoflagellates live as endosymbionts with reef-building corals. Stimulated by chemical signals from their animal hosts, dinoflagellate endosymbionts provide the corals with essential organic food.

Therefore, the dinoflagellate symbionts are critical components of the coral reef ecosystems whose loss during stress-related “bleaching” events can lead to mass mortality of coral hosts and associated collapse of reef ecosystems (Baker, 2003). Also, the high rates of calcification and production that characterize typically oligotrophic coral reef ecosystems are largely credited to the mutualistic symbiosis between the scleractinian corals and photosynthetic dinoflagellates belonging to the genus *Symbiodinium* (Apprill and Gates, 2007). Corals with a dinoflagellate symbiont calcify much faster than those without – an effect linked to photosynthetic fixation of CO₂ by the dinoflagellates (Marshall, 1996). A significant amount of photosynthetic product is excreted by the symbiotic dinoflagellates, primarily as glycerol. Up to 50% of the fixed carbon may be transferred to the host (Paracer and Ahmadjian, 2000), in which it is converted mainly to lipids and proteins. On the dinoflagellate side, many of these symbioses occur in oligotrophic waters in which nutrients are scarce in the water column (Hackett et al., 2004). In other symbiotic dinoflagellate associations, the hosts include foraminifera, radiolarians, flatworms, anemones, jellyfish, and even bivalve mollusks (Hackett et al., 2004).

Undisputed structural fossils of dinoflagellates occur some 200 million years or so ago (Graham et al., 2009). These fossils, known as hystrichospheres, resemble resting stages of some modern dinoflagellates. Some modern dinoflagellates produce resting cysts or zygotes with an organic compound resistant to decay and thus aid the survival of dinoflagellate fossils, of which a considerable diversity has been described (Fensome et al., 2003). Other chemical compounds thought to be specific to dinoflagellates have already been found in Proterozoic (Precambrian) rocks and such chemical fossils suggest that dinoflagellate-like organisms might have existed already more than 600 million years ago (Graham et al., 2009).

Many dinoflagellates are photosynthetic and, through endosymbiosis, have acquired a wide diversity of plastids from distant evolutionary lineages. The most common plastid in dinoflagellates is golden brown with a unique accessory pigment, peridinin; they also contain chlorophyll *c*. Such peridinin plastids are bounded by three membranes and are derived from red algal secondary endosymbiosis (Delwiche, 2007; Hackett et al., 2004). A number of dinoflagellates have plastids from haptophytes, diatoms, green algae, or cryptomonads which may have replaced preexisting peridinin plastids (tertiary endosymbiosis; McFadden, 2001; Keeling, 2004; Palmer, 2003).

A small group of dinoflagellates with peridinin plastids produce calcareous structures, formed during the life cycle or found in vegetative stages (Gottschling et al., 2005). The potential to produce calcareous structures has been considered as apomorphic within alveolates (Kohring et al., 2005), arguing for the monophyly of the family Calciodinellaceae which comprises approximately 30 (recognized) extant species that are distributed in cold through tropical seas of the world. Calcareous cysts are deposited in both sediments coastal (Montresor et al., 1998; Persson et al., 2000) and oceanic (Vink, 2004; Zonneveld et al., 1999). According to the fossil record, calcareous dinoflagellates originate in the Upper Triassic (Janofske, 1992) and are highly diverse during the Cretaceous and throughout the Tertiary (Keupp, 1991; Kohring, 1993; Willems, 1994), thus many fossil species (namely their cysts) have been described (Gottschling et al., 2005). Based on morphological and molecular data, calcareous dinoflagellates (Thoracosphaeraceae, Peridinales) are a monophyletic group comprising three major clades (Gottschling et al., 2008).

Other chromalveolates

Cryptomonads. Recent molecular phylogenetic analyses indicate that the cryptomonads and the haptophyte algae together form a monophyletic group (Hackett et al., 2007) and, therefore, also belong to the Chromalveolates supergroup. Cryptomonads form an abundant group of marine and freshwater unicellular flagellates that contain a plastid derived from red algal secondary endosymbiosis with chlorophyll *a* and *c* surrounded by four membranes. Cryptomonad plastids have received some attention because they are one of only two groups in which the primary algal nucleus has not been completely lost; they retain a small relict nucleus called a nucleomorph (McFadden, 2001; Keeling, 2004; Palmer, 2003).

Apicomplexa are a large group composed entirely of obligate intracellular parasites, including several that cause significant diseases such as malaria. As intracellular parasites, the discovery of a relict plastid (or apicoplast) in apicomplexa bounded by four membranes has drawn a great deal of attention as an evolutionary novelty and possible drug target (Keeling, 2004). There is current evidence indicating that this plastid is derived from a red alga and that the apicomplexa share a monophyletic origin with all other groups with plastids from red algal secondary endosymbiosis. The apicomplexa may be most closely related with the dinoflagellates (Moore, 2008). For a review of apicomplexa relating to their plastids, see Foth and McFadden (2003).

Algal groups with plastids derived from green algal secondary endosymbiosis

Chlorarachniophytes (Rhizaria supergroup). Chlorarachniophytes are a relatively rare group of marine amoeboid flagellates and flagellates that contain a green algal plastid (chlorophyll *a* and *b*) bounded by four membranes. They

contain a relict nucleus, nucleomorph, which has been retained from the green algal symbiont (McFadden, 2001; Keeling, 2004; Palmer, 2003). Chlorarachniophytes form a monophyletic group within the Rhizaria supergroup of eukaryotes (Ishida et al., 1999).

Euglenids (Excavates supergroup). Euglenids are a diverse group of common marine and freshwater flagellates, about half of which contain a plastid derived from green algal secondary endosymbiosis which is bounded by three membranes and contains chlorophyll *b* as accessory pigment. The remainder of the group are osmotrophs or heterotrophs that feed on bacteria or other eukaryotes. Molecular phylogenies show that photosynthetic euglenids may have acquired their plastids from a green alga relatively late in evolution, despite the Euglenozoa may be a rather old group. Euglenids are closely related to the parasitic trypanosomes (Kinetoplastids), together with diplomonids, making up the monophyletic Euglenozoa. Distinguishing features of euglenids are that they produce a storage carbohydrate which is a β -1,3-linked glucan (paramylon) in their cytoplasm and that they display a unique surface structure composed of parallel ribbon-like proteinaceous strips. For reviews of euglenids, see Leedale and Vickerman (2000), Milanowski et al. (2006) or Ciugulea and Triemer (2010).

Summary

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids. In addition, the photoautotrophic cyanobacteria are regarded as algae by some authors. In a recent phylogeny of the eukaryotes, the algae are scattered among four of the five recognized supergroups. A single primary endosymbiosis with a cyanobacterium may have been the start of all eukaryotic algae which first diverged into the green algae, red algae, and glaucophytes (Plantae supergroup). The plastids of all other algal lineages derived from secondary endosymbiosis involving either a red algal cell (algae of the Chromalveolates supergroup) or green algal cells (euglenids and chlorarchniophytes, Excavates and Rhizaria supergroups). Many algae form symbioses with ciliates and some metazoa. Algae are very important primary producers in almost every aquatic or terrestrial habitats and are important key players within the carbon and silica cycles for the major part of Earth history. Many lineages have produced significant fossil records due to their ability to precipitate calcium carbonate on their cell surfaces (red algae, some dinoflagellates) or form calcified (haptophytes) or silicified body scales, silica endoskeletons, resting stages, or cell walls (photoautotrophic stramenopiles, including dictyochophytes, synurophytes, chrysophytes, and diatoms). Due to their high sensitivity to certain environmental parameters and their high preservation potential, these algae also play important roles as sedimentary bioindicators in tracking environmental changes.

Bibliography

- Adey, W. H., 1998. Coral reefs: algal structured and mediated ecosystems in shallow, turbulent, alkaline waters. *Journal of Phycology*, **34**, 393–406.
- Andersen, R. A., 2004. Biology and systematics of heterokont and haptophyte algae. *American Journal of Botany*, **91**, 1508–1522.
- Apprill, A. M., and Gates, R. D., 2007. Recognizing diversity in coral symbiotic dinoflagellate communities. *Molecular Ecology*, **16**, 1127–1134.
- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., De Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. Tufa-forming biofilms of German karstwater streams: microorganisms, exopolymers, hydrochemistry and calcification. *Geological Society, London, Special Publications 2010*, **336**, 83–118.
- Baker, A. C., 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of symbiodinium. *Annual Review of Ecology, Evolution and Systematics*, **34**, 661–689.
- Betts-Piper, A. M., Zeeb, B. A., and Smol, J. P., 2004. Distribution and autecology of chrysophyte cysts from high Arctic Svalbard lakes: preliminary evidence of recent environmental change. *Journal of Paleolimnology*, **31**, 467–481.
- Bhattacharya, D., and Schmidt, H. A., 1997. Division glaucocystophyta. In Bhattacharya, D. (ed.), *Origin of Algae and Their Plastids*, Wien: Springer-Verlag, pp. 139–148.
- Bilan, M. I., and Usov, A. I., 2001. Polysaccharides of calcareous algae and their effect on the calcification process. *Russian Journal of Bioorganic Chemistry*, **27**, 2–16.
- Borowitzka, M. A., 1977. Algal calcification. *Oceanography and Marine Biology: An Annual Review*, **15**, 189–223.
- Bremer, K., 1985. Summary of green plant phylogeny and classification. *Cladistics*, **1**, 369–385.
- Butterfield, N. J., 2000. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology*, **26**, 386–404.
- Chisholm, J. R. M., 2003. Primary productivity of reef-building crustose coralline algae. *Limnology and Oceanography*, **48**, 1376–1387.
- Ciugulea, I., and Triemer, R. E., 2010. *A color atlas of photosynthetic Euglenoids*. East Lansing: Michigan State University Press.
- Daugbjerg, N., and Henriksen, P., 2001. Pigment composition and *rbcL* sequence data from the silicoflagellate *Dictyocha speculum*: a heterokont alga with pigments similar to some haptophytes. *Journal of Phycology*, **37**, 1110–1120.
- Delwiche, C. F., 2007. The origin and evolution of dinoflagellates. In Falkowski, P. G., and Knoll, A. H., (eds.), *Evolution of Primary Producers of the Sea*, New York: Academic, pp. 191–205.
- DeVrind-deJong, E. W., van Emburg, P. R., and deVrind, J. P. M., 1994. Mechanisms of calcification: *Emiliania huxleyi* as a model system. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*, Oxford: Clarendon, pp. 149–166.
- Duff, K. E., Zeeb, B. A., and Smol, J. P., 1995. *Atlas of Chrysophyte Cysts*. Dordrecht: Kluwer Academic, 189 pp.
- Dullo, W. C., Moussavian, E., and Brachert, T., 1990. The foralgal crust facies of the deeper fore reefs in the Red Sea. *Geobios*, **23**, 261–281.
- Edwardsen, B., Eikkrem, W., Green, J. C., Andersen, R. A., van der Staay, S. Y. M., and Medlin, L. K., 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. *Phycologia*, **39**, 19–35.
- Elloranta, P., 1995. Biogeography of chrysophytes in Finnish lakes. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae. Ecology, Phylogeny and Development*, Cambridge: Cambridge University Press, pp. 214–231.

- Fawley, M. W., Yun, Y., and Qin, M., 2000. Phylogenetic analyses of 18S rDNA sequences reveal a new coccoid lineage of the Prasinophyceae (Chlorophyta). *Journal of Phycology*, **36**, 387–393.
- Fensome, R. A., MacRae, R. A., Moldovan, J. M., Taylor, F. J. R., and Williams, G. L., 2003. The early Mesozoic radiation of dinoflagellates. *Paleobiology*, **22**, 329–338.
- Foster, M. S., 2001. Rhodoliths: between rocks and soft places. *Journal of Phycology*, **37**, 659–667.
- Foth, B. J., and McFadden, G. I., 2003. The apicoplast: a plastid in *Plasmodium falciparum* and other apicomplexan parasites. *International Review of Cytology*, **224**, 57–110.
- Freytet, P., and Verrecchia, E. P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*, **45**, 535–563.
- Friedl, T., 1997. The evolution of the green algae. *Plant Systematics and Evolution*, **11**, 87–101.
- Glynn, P. W., Veron, J. E. N., and Wellington, G. M., 1996. Clipperton Atoll (eastern Pacific): oceanography, geomorphology, reef-building coral ecology and biogeography. *Coral Reefs*, **15**, 71–99.
- Gottschling, M., Keupp, H., Plötnner, J., Knop, R., Willems, H., and Kirsch, M., 2005. Phylogeny of calcareous dinoflagellates as inferred from ITS and ribosomal sequence data. *Molecular Phylogenetics and Evolution*, **36**, 444–455.
- Gottschling, M., Renner, S. S., Meier, K. J. S., Willems, H., and Keupp, H., 2008. Timing deep divergence events in calcareous dinoflagellates. *Journal of Phycology*, **44**, 429–438.
- Graham, L. E., Graham, J. M., and Wilcox, L. W., 2009. *Algae*, 2nd edn. San Francisco: Pearson Benjamin Cummings.
- Hackett, J. D., Anderson, D. M., Erdner, D. L., and Bhattacharya, D., 2004. Dinoflagellates: a remarkable evolutionary experiment. *American Journal of Botany*, **91**, 1523–1534.
- Hackett, J. D., Yoon, H. S., Li, S., Reyes-Prieto, A., Rümmele, S. E., and Bhattacharya, D., 2007. Phylogenetic analysis supports the monophyly of cryptophytes and haptophytes and the association of Rhizaria with chromalveolates. *Molecular Biology and Evolution*, **24**, 1702–1713.
- Inouye, I., and Kawachi, M., 1994. The haptonema. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*. Oxford: Clarendon, pp. 73–89.
- Ishida, K., Green, B. R., and Cavalier-Smith, T., 1999. Diversification of a chimaeric algal group, the chlorarachniophytes: Phylogeny of nuclear and nucleomorph small-subunit rRNA genes. *Molecular Biology and Evolution*, **16**, 321–331.
- Janofske, D., 1992. Kalkiges Nannoplankton, insbesondere kalkige Dinoflagellaten-Zysten der alpinen Ober-Trias: Taxonomie, Biostratigraphie und Bedeutung für die Phylogenie der Peridinales. *Berliner Geowiss. Abh. (E)*, **4**, 1–53.
- Karol, K. G., McCourt, R. M., Cimino, M. T., and Delwiche, C. F., 2001. The closest living relatives of land plants. *Science*, **294**, 2351–2353.
- Keats, D. W., Knight, M., and Pueschel, C., 1997. Antifouling effects of epithelial shedding in three crustose coralline algae (Rhodophyta, Corallinales) on a coral reef. *Journal of Experimental Marine Biology and Ecology*, **213**, 281–293.
- Keeling, P. J., 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany*, **91**, 1481–1493.
- Keupp, H., 1991. Fossil calcareous dinoflagellate cysts. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 267–286.
- Kingsley, R. J., Van Gilder, R., LeGeros, R. Z., and Watabe, N., 2003. Multimineral calcareous deposits in the marine alga *Acetabularia acetabulum* (Chlorophyta; Dasycladaceae). *Journal of Phycology*, **39**, 937–947.
- Kohring, R., 1993. Kalkdinoflagellaten aus dem Mittel- und Obereozän von Jütland (Dänemark) und dem Pariser Becken (Frankreich) im Vergleich mit anderen Tertiär-Vorkommen. *Berliner Geowiss. Abh. (E)*, **6**, 1–164.
- Kohring, R., Gottschling, M., and Keupp, H., 2005. Examples for character traits and palaeoecological significance of calcareous dinoflagellates. *Paläontologische Zeitschrift*, **79**, 79–91.
- Le Gall, L., and Saunders, G. W., 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the Corallinophycidae subclassis nov. *Molecular Phylogenetics and Evolution*, **43**, 1118–1130.
- Leadbeater, B. S. C., and Barker, D. A. N., 1995. Biomineralization and scale production in the Chrysophyta. In Sandgren, C. D. J., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae: Ecology, Phylogeny and Development*. Cambridge, UK: Cambridge University Press, pp. 141–164.
- Leedale, G. F., and Vickerman, K., 2000. Phylum Euglenozoa. In Lee, J. J., Leedale, G. F., and Bradbury P. (eds.), *An Illustrated Guide to the Protozoa*, 2nd edn. Lawrence: Society of Protozoologists, pp. 1135–1185.
- Letsch, M. R., Muller-Parker, G., Friedl, T., and Lewis, L. A., 2009. *Elliptochloris marina* sp. nov. (Trebouxiophyceae, Chlorophyta), symbiotic green alga of the temperate pacific sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Anthozoa, Cnidaria). *Journal of Phycology*, **47**, 1127–1135.
- Lewis, L. A., and McCourt, R. M., 2004. Green algae and the origin of land plants. *American Journal of Botany*, **91**, 1535–1556.
- Littler, M. M., 1972. The crustose Corallinales. *Oceanography and Marine Biology Annual Review*, **10**, 311–347.
- Malin, G., and Steinke, M., 2004. Dimethyl sulfide production: What is the contribution of the coccolithophores? In Thierstein, H. R., and Young, J. R. (eds.), *Coccolithophores – From Molecular Process to Global Impact*. New York: Springer, pp. 127–164.
- Marshall, A. T., 1996. Calcification in hermatypic and ahermatypic corals. *Science*, **271**, 637–639.
- Marin, B., and Melkonian, M., 2010. Molecular phylogeny and classification of the mamiellophyceae class. nov. (Chlorophyta) based on sequence comparisons of the nuclear- and plastid-encoded rRNA operons. *Protist*, **161**, 304–336.
- Martin, S., Castets, M. D., and Clavier, J., 2006. Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*. *Aquatic Botany*, **85**, 121–128.
- Martin, W., Rujan, T., Richly, E., Hansen, A., Ornelsen, S., Lins, T., Lesiter, D., Stoebe, B., Hasegawa, M., and Penny, D., 2002. Evolutionary analysis of Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proceedings of the National Academy of Sciences, USA*, **99**, 12246–12251.
- McCartney, K., and Wise, S. W. Jr., 1990. Cenozoic silicoflagellates and ebridians from ODP Leg 113: biostratigraphy and notes on morphologic variability. In Barker, P. F., and Kennett, J. P., et al. (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results. College Station, TX: (Ocean Drilling Program)*, Vol. 113, pp. 729–760.
- McCartney, K., and Harwood, D. M., 1992. Silicoflagellates from Leg 120 on the Kerguelen Plateau, southeast Indian Ocean. In Wise, S. W. Jr., Schlich, R., et al. (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results, College Station, TX: Ocean Drilling Program*, Vol. 120, pp. 811–831.
- McConnaughey, T., 1991. Calcification in Chara corallina: CO₂ hydroxylation generates protons for bicarbonate assimilation. *Limnology and Oceanography*, **36**, 619–628.
- McConnaughey, T. A., 1994. Calcification, photosynthesis, global carbon cycles. In Doumenge, E., and Allemand Toulemon, A. (eds.), *Past and Present Biomineralization Processes Consideration about the Carbonate IUCNCOE Workshop Monaco*, Nov 1993. *Bulletin Oceanographique* **13**, 137–156.
- McFadden, G. I., 2001. Primary and secondary endosymbiosis and the origin of plastids. *Journal of Phycology*, **37**, 951–959.

- Milanowski, R., Kosmala, S., Zakrys, B., and Kwiatowski, J., 2006. Phylogeny of photosynthetic euglenophytes based on combined chloroplast and cytoplasmic SSU rDNA sequence analysis. *Journal of Phycology*, **42**, 721–730.
- Moestrup, Ø., 1995. Current status of chrysophyte splinter groups: synurophytes, pedinellids, silicoflagellates. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae*. Cambridge: Cambridge University Press, pp. 75–91.
- Moestrup, Ø., and O'Kelly, C. J., 2002. Class Silicoflagellata Lemmermann, 1901. In Lee, J. J., Leedale, G. F., and Bradbury, P. (eds.), *An Illustrated Guide to the Protozoa*, 2nd edn. Lawrence, Kansas, USA: Society of Protozoologists, vol. 2, pp. 775–776.
- Montresor, M., Zingone, A., and Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. *Journal of Plankton Research*, **20**, 2291–2312.
- Moore, R. B., 2008. A photosynthetic alveolate closely related to apicomplexan parasites. *Nature*, **451**, 959–963.
- Morrissey, J., 1980. Community structure and zonation of macroalgae and hermatypic corals on a fringing reef flat of Magnetic Island (Queensland, Australia). *Aquatic Botany*, **8**, 91–139.
- Nicholas, J. B., Knoll, A. H., and Swett, K., 1990. A bangiophyte red alga from the proterozoic of Arctic Canada. *Science*, **250**, 104–107.
- Palmer, J., 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology*, **39**, 4–12.
- Paracer, S., and Ahmadjian, V., 2000. *Symbiosis: An Introduction to Biological Associations*. New York: Oxford University Press.
- Payri, C. E., Maritorea, S., Bizeau, M., and Rodiere, M., 2001. Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinacea) from a French Polynesian reef. *Journal of Phycology*, **37**, 223–234.
- Persson, A., Godhe, A., and Karlson, B., 2000. Dinoflagellate cysts in recent sediments from the west coast of Sweden. *Botanica Marina*, **43**, 69–79.
- Potin, P., Floch, J. Y., Augris, C., and Cabioch, J., 1990. Annual growth rate of the calcareous red algae *Lithothamnion corallioides* (Corallinales, Rhodophyta) in the Bay of Brest, France. *Hydrobiologia*, **204/205**, 263–267.
- Probert, I., Fresnel, J., Billard, C., Geisen, M., and Young, J. R., 2007. Light and electron microscope observations of *Algirosphaera robusta* (Prymnesiophyceae). *Journal of Phycology*, **43**, 319–332.
- Pröschold, T., and Leliaert, F., 2007. Systematics of the green algae: conflict of classic and modern approaches. In Brodie, J., and Lewis, J. (eds.), *Unravelling the Algae: the Past, Present, and Future of the Algae Systematics*. London: Taylor and Francis, pp. 123–153.
- Pueschel, C. M., Eichelberger, H. H., and Trick, H. N., 1992. Specialized calciferous cells in the marine alga *Rhodogorgon caribbowensis* and their implications for models of red algal calcification. *Protoplasma*, **166**, 89–98.
- Riding, R., Cope, J. C. W., and Taylor, P. D., 1998. A coralline-like red alga from the lower Ordovician of Wales. *Palaeontology*, **41**, 1069–1076.
- Rost, B., and Riebesell, U., 2004. Coccolithophore calcification and the biological pump: Response to environmental changes. In Thierstein, H. R., and Young, J. R. (eds.), *Coccolithophores – From Molecular Processes to Global Impact*. New York: Springer, pp. 99–126.
- Rott, E., Holzinger, A., Gesierich, D., Kofler, D., and Sanders, D., 2009. Cell morphology, ultrastructure, and calcification pattern of *Oocardium stratum*, a peculiar lotic desmid. *Protoplasma*, 10.1007/s00709-009-0050-y.
- Saunders, G. W., and Hammersand, M. H., 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany*, **91**, 1494–1507.
- Siver, P. A., and Wolfe, A. P., 2005. Eocene scaled chrysophytes with pronounced modern affinities. *International Journal of Plant Sciences*, **166**, 533–536.
- Siver, P. A., 1995. The distribution of chrysophytes along environmental gradients: their use as bioindicators. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae*. Cambridge: Cambridge University Press, pp. 232–268.
- Sluiman, H. J., 1985. A cladistic evaluation of the lower and higher green plants (Viridiplantae). *Plant Systematics and Evolution*, **149**, 217–232.
- Smol, J. P., 1995. Applications of chrysophytes to problems in paleoecology. In Sangren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae: Ecology, Phylogeny and Development*. Cambridge: Cambridge University Press, pp. 303–329.
- Stanley, S. M., Ries, J. B., and Hardie, L. A., 2010. Increased production of calcite and slower growth for the major sediment-producing alga *Halimeda* as the Mg/Ca ratio of seawater is lowered to a “Calcite Sea” level. *Journal of Sedimentary Research*, **80**, 6–16.
- Steiner, J. M., and Löffelhardt, W., 2002. Protein import into cyanelles. *Trends in Plant Sciences*, **7**, 72–77.
- Steneck, R. S., and Dethier, M. N., 1994. A functional group approach to the structure of algal-dominated communities. *Oikos*, **69**, 476–498.
- Summerer, M., Sonntag, B., and Sommaruga, R., 2008. Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxiphyceae, Chlorophyta). *Journal of Phycology*, **44**, 7–84.
- Tappan, H., 1980. *The Paleobiology of Plant Protists*. San Francisco: Freeman, p. 1029.
- Trautman, D. A., Hinde, R., and Borowitzka, M. A., 2000. Population dynamics of an association between a coral reef sponge and a red macroalga. *Journal of Experimental Marine Biology and Ecology*, **244**, 87–105.
- Trautman, D. A., Hinde, R., and Borowitzka, M. A., 2002. The role of habitat in determining the distribution of a sponge-red alga symbiosis on a coral reef. *Journal of Experimental Marine Biology and Ecology*, **283**, 1–20.
- van Dolah, F. M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, **108**, 133–141.
- van Soest, R. W. M., 1990. Shallow-water reef sponges of eastern Indonesia. In Rützler, K. (ed.), *New Perspectives in Sponge Biology*. Washington, DC: Smithsonian Institution Press, pp. 302–308.
- Vink, A., 2004. Calcareous Dinoflagellate cysts in South and equatorial Atlantic surface sediments: diversity, distribution, ecology and potential for palaeoenvironmental reconstruction. *Marine Micropaleontology*, **50**, 43–88.
- Wilkinson, A. N., Zeeb, B. A., and Smol, J. P., 2001. *Atlas of Chrysophyte Cysts*. Dordrecht: Kluwer, 169 pp.
- Willems, H., 1994. New calcareous dinoflagellates from the upper cretaceous white chalk of northern Germany. *Review Palaeobotanic Palynology*, **84**, 57–72.
- Williams, B. A. P., and Keeling, P. J., 2003. Cryptic organelles in parasitic protists and fungi. *Advances in Parasitology*, **54**, 9–67.
- Wolfe, A. P., and Perren, B. P., 2001. Chrysophyte microfossils record marked responses to recent environmental changes in high- and mid-arctic lakes. *Canadian Journal of Botany*, **79**, 747–752.
- Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., and Bhattacharya, D., 2004. A molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution*, **21**, 809–818.
- Yoon, H. S., Müller, K. M., Sheath, R. G., and Bhattacharya, D., 2006. Defining the major lineages of red algae (Rhodophyta). *Journal of Phycology*, **42**, 482–492.
- Young, J. R., Brown, P. R., and Burnett, J. A., 1994. Paleontological perspectives. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*. Oxford, UK: Clarendon Press, pp. 379–392.

- Zankl, H., 2007. The origin of High-Mg-Calcite microbialites in cryptic habitats of Caribbean coral reefs – their dependence on light and turbulence. *Facies*, **29**, 55–60.
- Zeeb, B. A., and Smol, J. P., 2001. Chrysophyte scales and cysts. In John, P., Smol, H., Birks, J. B., and Last, W. M. (eds.), *Tracking Environmental Change Using Lake Sediments. Vol 3. Terrestrial, Algal, and Siliceous Indicators*. Dordrecht: Kluwer, pp. 203–223.
- Zonneveld, K. A. F., Höll, C., Janofske, D., Karwath, B., Kerntopf, B., Rühlemann, C., Willems, H., 1999. Calcareous dinoflagellate cysts as paleo-environmental tools. In Fischer, G., and Wefer, G. (eds.), *Use of Proxies in Paleoceanography*. Berlin: Springer, pp. 145–164.

Cross-references

Cyanobacteria
Diatoms
Early Precambrian Eukaryotes
Fungi and Lichens
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Photosynthesis
Protozoa (Heterotroph, Eukaryotic)
Symbiosis

ALKALINITY

Andreas Reimer, Gernot Arp
University of Göttingen, Göttingen, Germany

The term “Alkalinity,” commonly denoted by TA (also A_T , ALK, and others) and then called titration alkalinity or total alkalinity, refers to a very important chemical concept in aquatic chemistry. Total alkalinity is one of the few measurable quantities in natural waters that allows, together with other properties, to calculate concentrations of single species of the carbonate system such as CO_2 , HCO_3^- , CO_3^{2-} , H^+ , and OH^- (Wolf-Gladrow et al., 2007), and further on, the saturation state of the waters with respect to certain carbonate minerals. Therefore, alkalinity represents a hydrogeochemical key parameter for the understanding of carbonate precipitation, either biologically mediated or not, and its variation through the geological history.

Definition

From its determination by titrimetric techniques, alkalinity has historically been defined as the number of equivalents of a strong acid required to neutralize 1 L of water at 20°C. The obtained end-point, corresponding to the second equivalence point of the carbonate system, was operationally defined and results were then commonly given as eq L^{-1} . The recent definition of alkalinity is based on the implementation of the Brønsted–Lowry concept of acids and bases by Rakestrow (1949) who defined alkalinity as the excess of bases (proton acceptors) over acids (proton donors). Considering seawater, alkalinity is mostly determined by the ions bicarbonate and carbonate, also by the borate ion, and to a smaller extent by OH^- and H^+ from

dissociation of water. Alkalinity then refers to the proton condition with reference to a zero level of protons defined by the species H_2CO_3 , $\text{B}(\text{OH})_3$, and H_2O , where the proton acceptors are HCO_3^- , CO_3^{2-} , $\text{B}(\text{OH})_4^-$, and OH^- and the proton donor is H^+ . That is, alkalinity is the equivalent sum of bases that have one or two protons less than the reference species minus H^+ (or H_3O^+) that has one proton more than H_2O (Dickson, 1981; Stumm and Morgan, 1996; Zeebe and Wolf-Gladrow, 2001). The expression for the total alkalinity is thus presented as

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] - [\text{H}^+],$$

where conceptually the right hand term can be divided up in carbonate alkalinity ($\text{CA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]$) as the most dominant contributor, borate alkalinity, and water alkalinity.

Natural waters may contain various acid–base systems that can accept and donate protons other than or in addition to carbonic acid or boric acid. These complications have been taken into account currently in the most precise definition of total alkalinity of Dickson (1981) (compare also Dickson, 1992; DOE, 1994; and recent extension by Wolf-Gladrow et al., 2007). The definition is unequivocally based on a chemical model of occurring acid–base processes and to be independent of temperature and pressure expressed in gravimetric units (mol kg^{-1}). In Dickson’s concept, the total alkalinity (TA) is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors over proton donors with respect to the proton condition defined by the value $\text{p}K = 4.5$ in 1 kg of sample. Bases formed from weak acids with a dissociation constant $K \leq 10^{-4.5}$ are considered proton acceptors, acids with $K > 10^{-4.5}$ are proton donors, and the chemical species defining the zero level of protons of the various acid–base systems are H_2CO_3 , $\text{B}(\text{OH})_3$, H_2O , H_2PO_4^- , H_4SiO_4 , NH_4^+ , H_2S , SO_4^{2-} , F^- , and NO_2^- . From this definition the expression for the total alkalinity is presented as

$$\begin{aligned} \text{TA} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] \\ & + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_3\text{SiO}_4^-] \\ & + [\text{NH}_3] + [\text{HS}^-] + \dots - [\text{H}^+] - [\text{HSO}_4^-] \\ & - [\text{HF}] - [\text{H}_3\text{PO}_4] - [\text{HNO}_2] \dots \end{aligned}$$

where the ellipses stand for additional acid–base species (Wolf-Gladrow et al., 2007).

An alternative definition for alkalinity derives when considering the charge balance of natural waters (Stumm and Morgan, 1996). From the principle of electroneutrality total alkalinity can be expressed in terms of the charge imbalance between the equivalent sum of conservative cations (base cations of strong bases) and the sum of conservative anions (conjugate bases of strong acids). For the total alkalinity of sea water dominated by

- Zankl, H., 2007. The origin of High-Mg-Calcite microbialites in cryptic habitats of Caribbean coral reefs – their dependence on light and turbulence. *Facies*, **29**, 55–60.
- Zeeb, B. A., and Smol, J. P., 2001. Chrysophyte scales and cysts. In John, P., Smol, H., Birks, J. B., and Last, W. M. (eds.), *Tracking Environmental Change Using Lake Sediments. Vol 3. Terrestrial, Algal, and Siliceous Indicators*. Dordrecht: Kluwer, pp. 203–223.
- Zonneveld, K. A. F., Höll, C., Janofske, D., Karwath, B., Kerntopf, B., Rühlemann, C., Willems, H., 1999. Calcareous dinoflagellate cysts as paleo-environmental tools. In Fischer, G., and Wefer, G. (eds.), *Use of Proxies in Paleoceanography*. Berlin: Springer, pp. 145–164.

Cross-references

Cyanobacteria
Diatoms
Early Precambrian Eukaryotes
Fungi and Lichens
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Photosynthesis
Protozoa (Heterotroph, Eukaryotic)
Symbiosis

ALKALINITY

Andreas Reimer, Gernot Arp
University of Göttingen, Göttingen, Germany

The term “Alkalinity,” commonly denoted by TA (also A_T , ALK, and others) and then called titration alkalinity or total alkalinity, refers to a very important chemical concept in aquatic chemistry. Total alkalinity is one of the few measurable quantities in natural waters that allows, together with other properties, to calculate concentrations of single species of the carbonate system such as CO_2 , HCO_3^- , CO_3^{2-} , H^+ , and OH^- (Wolf-Gladrow et al., 2007), and further on, the saturation state of the waters with respect to certain carbonate minerals. Therefore, alkalinity represents a hydrogeochemical key parameter for the understanding of carbonate precipitation, either biologically mediated or not, and its variation through the geological history.

Definition

From its determination by titrimetric techniques, alkalinity has historically been defined as the number of equivalents of a strong acid required to neutralize 1 L of water at 20°C. The obtained end-point, corresponding to the second equivalence point of the carbonate system, was operationally defined and results were then commonly given as eq L^{-1} . The recent definition of alkalinity is based on the implementation of the Brønsted–Lowry concept of acids and bases by Rakestrow (1949) who defined alkalinity as the excess of bases (proton acceptors) over acids (proton donors). Considering seawater, alkalinity is mostly determined by the ions bicarbonate and carbonate, also by the borate ion, and to a smaller extent by OH^- and H^+ from

dissociation of water. Alkalinity then refers to the proton condition with reference to a zero level of protons defined by the species H_2CO_3 , $\text{B}(\text{OH})_3$, and H_2O , where the proton acceptors are HCO_3^- , CO_3^{2-} , $\text{B}(\text{OH})_4^-$, and OH^- and the proton donor is H^+ . That is, alkalinity is the equivalent sum of bases that have one or two protons less than the reference species minus H^+ (or H_3O^+) that has one proton more than H_2O (Dickson, 1981; Stumm and Morgan, 1996; Zeebe and Wolf-Gladrow, 2001). The expression for the total alkalinity is thus presented as

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] - [\text{H}^+],$$

where conceptually the right hand term can be divided up in carbonate alkalinity ($\text{CA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]$) as the most dominant contributor, borate alkalinity, and water alkalinity.

Natural waters may contain various acid–base systems that can accept and donate protons other than or in addition to carbonic acid or boric acid. These complications have been taken into account currently in the most precise definition of total alkalinity of Dickson (1981) (compare also Dickson, 1992; DOE, 1994; and recent extension by Wolf-Gladrow et al., 2007). The definition is unequivocally based on a chemical model of occurring acid–base processes and to be independent of temperature and pressure expressed in gravimetric units (mol kg^{-1}). In Dickson’s concept, the total alkalinity (TA) is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors over proton donors with respect to the proton condition defined by the value $\text{pK} = 4.5$ in 1 kg of sample. Bases formed from weak acids with a dissociation constant $K \leq 10^{-4.5}$ are considered proton acceptors, acids with $K > 10^{-4.5}$ are proton donors, and the chemical species defining the zero level of protons of the various acid–base systems are H_2CO_3 , $\text{B}(\text{OH})_3$, H_2O , H_2PO_4^- , H_4SiO_4 , NH_4^+ , H_2S , SO_4^{2-} , F^- , and NO_2^- . From this definition the expression for the total alkalinity is presented as

$$\begin{aligned} \text{TA} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] \\ & + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_3\text{SiO}_4^-] \\ & + [\text{NH}_3] + [\text{HS}^-] + \dots - [\text{H}^+] - [\text{HSO}_4^-] \\ & - [\text{HF}] - [\text{H}_3\text{PO}_4] - [\text{HNO}_2] \dots \end{aligned}$$

where the ellipses stand for additional acid–base species (Wolf-Gladrow et al., 2007).

An alternative definition for alkalinity derives when considering the charge balance of natural waters (Stumm and Morgan, 1996). From the principle of electroneutrality total alkalinity can be expressed in terms of the charge imbalance between the equivalent sum of conservative cations (base cations of strong bases) and the sum of conservative anions (conjugate bases of strong acids). For the total alkalinity of sea water dominated by

HCO_3^- , CO_3^{2-} , and B(OH)_4^- , the expression may be presented by

$$\text{TA} = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] \\ - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - 2[\text{SO}_4^{2-}],$$

which is equivalent to the above definition in terms of proton acceptors and donors

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] \\ + [\text{OH}^-] - [\text{H}^+].$$

In the charge balance expression quantities are defined to be conservative with respect to mixing and changes in temperature (T) and pressure (P) provided that concentrations are on the gravimetric unit scale (mol kg^{-1}). Thus, from the TA expression entirely in terms of conservative ions TA is a conservative quantity too. In contrast, the concentrations of individual species of, e.g., the carbonate system may change with temperature and pressure because of the T - P dependency of the equilibrium constants.

Following the charge balance approach, Zeebe and Wolf-Gladrow (2001) and Wolf-Gladrow et al. (2007) have derived an expression in terms of total concentrations of conservative ions and conservative total concentrations of the various acid-base species under concern in the definition of Dickson (1981) in terms of an acid-base balance. This derived expression for TA is denoted as the explicit conservative alkalinity expression (TA_{ec}).

$$\text{TA}_{\text{ec}} = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] + \dots \\ - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - \dots \\ - 2\text{TSO}_4 - \text{THF} - \text{TPO}_4 - \text{THNO}_2 + \text{TNH}_3,$$

where total concentrations terms represent concentrations sums of the species of the acid-base systems $\text{TSO}_4 = [\text{SO}_4^{2-}] + [\text{HSO}_4^-]$, $\text{THF} = [\text{F}^-] + [\text{HF}]$, $\text{TPO}_4 = [\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}]$, $\text{THNO}_2 = [\text{NO}_2^-] + [\text{HNO}_2]$, $\text{TNH}_3 = [\text{NH}_3] + [\text{NH}_4^+]$, and ellipses stand for additional ions with minor concentrations.

The definition of TA_{ec} has the advantage that each term in the expression is conservative with respect to T - P changes, even though single species in the total concentration terms may be not. In particular, the approach is very convenient to derive changes of TA due to certain physicochemical and biogeochemical processes such as nutrient uptake in biological production and release during remineralization.

Sources, changes, and ranges

The primary source of alkalinity in surficial (and subsurface) waters is carbonic acid-controlled chemical weathering of carbonates, aluminum-silicate minerals like feldspars or Mg-Fe-silicates. The overall concentration and subsequent changes in alkalinity of water are therefore determined by the geology of the watershed, the

predominant weathering reactions and ion exchange in the soils, and concomitant biogeochemical processes.

TA as a conservative quantity will first alter with changes in salinity as a result of precipitation, evaporation, and mixing. Contrariwise to its increase from dissolution of limestone carbonates TA decreases when biogenic or non-biogenic CaCO_3 or other carbonate minerals precipitate. Further important changes are due to various biogeochemical processes. It has to be noted that total alkalinity does not change as a result of CO_2 exchange with the atmosphere. Invasion or degassing of CO_2 affects the adjustment of the carbonate species (ratio of $[\text{HCO}_3^-]$ to $[\text{CO}_3^{2-}]$) and the pH but not TA. Therefore, it is also not the consumption of CO_2 during photosynthesis or release during aerobic respiration that brings about a change in TA, but the utilization or remineralization of nitrate and phosphate involved in the processes. Nitrogen assimilation in form of ammonia decreases TA and no change of TA results from molecular nitrogen uptake (Stumm and Morgan, 1996; Wolf-Gladrow et al., 2007). Several microbially mediated processes lead to a change in alkalinity where deviation in the charge balance is compensated by yield or consumption of H^+ or OH^- . When oxygen is consumed in processes like nitrification, oxygenation of soluble ferrous iron to ferric oxide or sulfide oxidation from HS^- or pyrite total alkalinity decreases. Under anoxic conditions, remineralization of organic matter by denitrification, reduction of iron and manganese oxides and sulfate reduction frequently result in large increases of total alkalinity (Lerman and Stumm, 1989; Stumm and Morgan, 1996).

Depending on the rock mineralogy of the catchment area and prevalent vegetation total alkalinity in streams and inland lakes commonly ranges below 3 mmol kg^{-1} . Very low alkalinity is bound to tributaries and lakes in crystalline rock areas or to acidic bog and peatland waters. Here, H^+ released by biomass exceeding H^+ consumption by soil weathering results in an excess of protons that is referred to as “mineral acidity” or in turn “negative alkalinity” (Stumm and Morgan, 1996). On the other hand, in hard-water creeks that gain their alkalinity preferably from the dissolution of limestone rocks TA may reach values of 4 mmol kg^{-1} to 6 mmol kg^{-1} close to their spring sites.

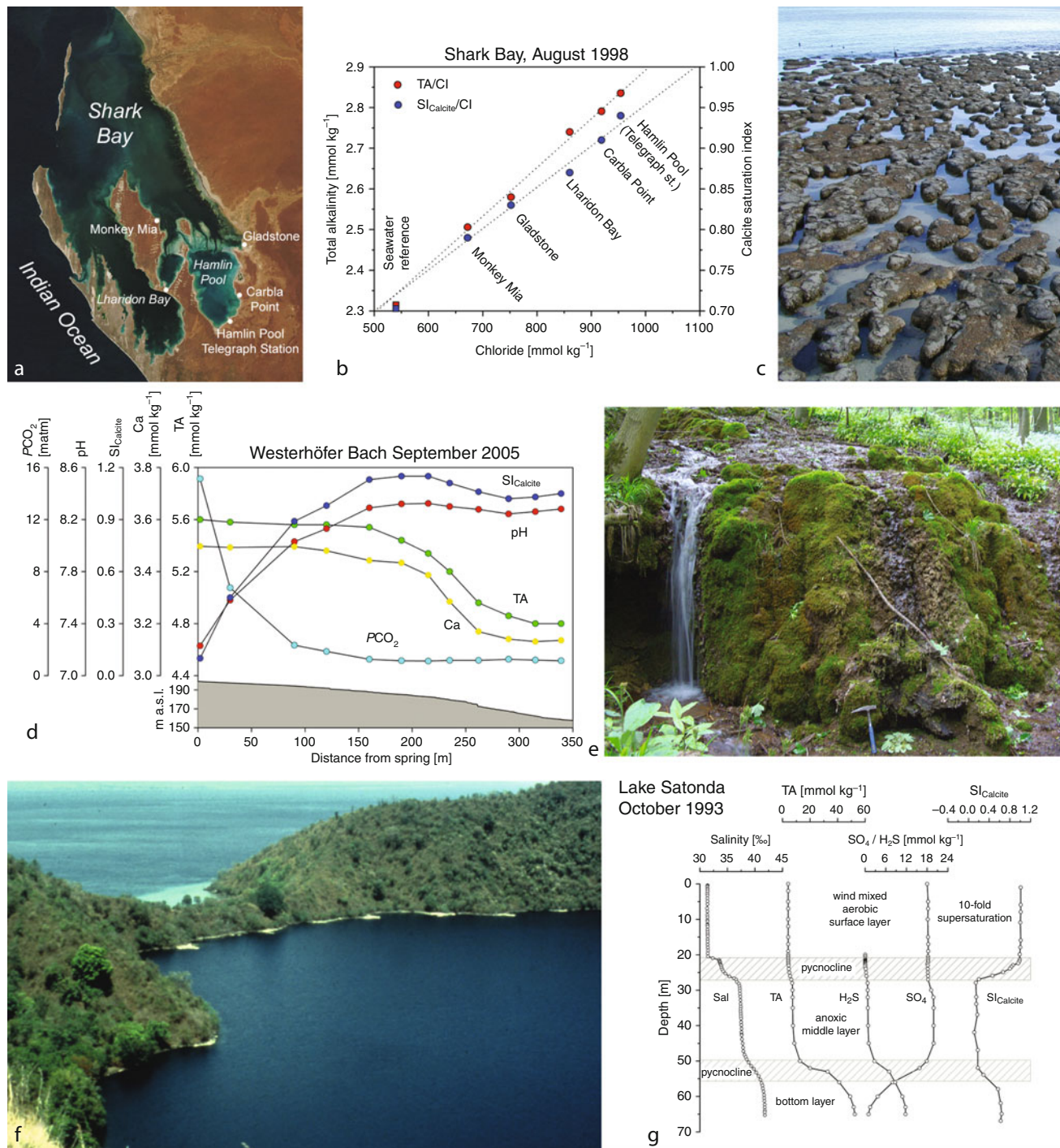
In the oceans, TA typically ranges from 2.2 mmol kg^{-1} to 2.5 mmol kg^{-1} where changes with depth are mainly associated with the production and dissolution of calcareous organisms (Broecker and Peng, 1982). Higher TA is found in today's only anaerobic ocean basin, the Black Sea, where alkalinity increases to about 4.5 mmol kg^{-1} in the anoxic bottom waters in the course of oxidation of organic material by sulfate reduction (Kempe, 1990; Hiscock and Millero, 2006). Also due to enhanced sulfate reduction and frequently complementing methane oxidation, TA in the order of $10^{-2} \text{ mol kg}^{-1}$ can accumulate in pore waters of marine sedimentary successions (Schulz and Zabel, 2000).

Even higher total alkalinity is typical for highly alkaline salt lakes, so-called soda lakes, which receive an equivalent load of HCO_3^- and CO_3^{2-} in excess to Mg^{2+} and Ca^{2+} from their riverine inflows. Here, TA can rise to

more than $10^{-1} \text{ mol kg}^{-1}$ and pH increase above 10 in the course of evaporation and subsequent Ca-carbonate precipitation while alkali cations, in particular sodium and potassium, increasingly maintain the charge balance of bicarbonate and carbonate ions (e.g., Lerman and Stumm, 1989; Kempe and Kazmierczak, 1994; Reimer et al., 2009).

Alkalinity and carbonate precipitation

Alkalinity, together with any of the three other measurable properties of the carbonate system – pH, dissolved inorganic carbon (DIC or $C_T = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$), or PCO_2 (partial pressure of CO_2) – can be used to compute the remaining quantities including concentrations of



Alkalinity, Figure 1 (Continued)

single species such as HCO_3^- and CO_3^{2-} . From CO_3^{2-} and Ca^{2+} concentrations (or more precise activities) saturation with respect to carbonate minerals is determined, where saturation Ω is given by the product of the actual ion activities ($\text{IAP} = \{\text{Ca}^{2+}\} \times \{\text{CO}_3^{2-}\}$) divided by the solubility product of the carbonate (K_{mineral}) at ambient temperature and pressure ($\Omega_{\text{mineral}} = \text{IAP}/K_{\text{mineral}}$). A commonly used alternative notation is the saturation index (SI) which is defined as $\log \Omega$ ($\text{SI}_{\text{mineral}} = \log (\text{IAP}/K_{\text{mineral}})$).

The oceanic CaCO_3 saturation state is primarily determined by variation of the CO_3^{2-} ion concentration, because the Ca^{2+} concentration closely depends on salinity and does not vary substantially. In the upper ocean, CO_2 removal by primary production increases CO_3^{2-} concentrations resulting in an about fivefold supersaturation ($\text{SI} = 0.7$) with respect to calcite. On the other hand, biologically controlled calcification of calcareous plankton or reefal organisms is responsible for the lower alkalinity of about $2.2\text{--}2.3 \text{ mmol kg}^{-1}$ in surface seawater. In turn, supersaturation decreases with depth when CO_2 is released by remineralization of sinking organic matter when temperature and pressure changes. In the deeper ocean, total alkalinity then increases to about $2.4\text{--}2.5 \text{ mmol kg}^{-1}$ reflecting the dissolution of sinking calcium carbonate particles in response to the lower saturation state (e.g., Broecker and Peng, 1982; Morse and Mackenzie, 1990).

As one of the major factors in the oceanic carbonate system, alkalinity distribution and behavior in the oceans has thoroughly been investigated during the last decades. Dissolution-preservation patterns of biogenic carbonates in surface to deep oceans and the changes brought about by a rising atmospheric CO_2 level (Feely et al., 2004) have been assessed within global ocean research programs, as well as general feedbacks between the oceanic carbonate and the global climate system. Contrary to this rising knowledge on the modern marine environment, estimates

of ocean alkalinity throughout geological history are rare and, in the lack of direct analytical proxies, mainly rely on model assumptions. Ocean alkalinity comparably high as modern values is suggested for the Cenozoic and late Mesozoic (Tyrrell and Zeebe, 2004; Ridgwell, 2005). During periods of the Mesozoic and Paleozoic, alkalinity about two to three times higher than recent can be assumed according to the evolution of the Phanerozoic atmospheric CO_2 (Ridgwell, 2005), fluctuations in the rate of continental weathering (Locklair and Lerman, 2005), or the more widespread occurrence of anoxic basins (Kempe and Kazmierczak, 1994). Elevated alkalinity concentrations of some tenths of mmol kg^{-1} are suggested for the Earth's primordial ocean in favor of a slightly acidic to neutral Na-Cl-dominated chemistry under an early CO_2 -rich atmosphere (Grotzinger and Kasting, 1993; Morse and Mackenzie, 1998). Alternatively, even higher alkalinity has been postulated for a Precambrian alkaline "Soda Ocean" in analogy to modern Na-carbonate-dominated soda lakes (Kempe and Degens, 1985).

In principle, precipitation of CaCO_3 is kinetically unfavorable even at the fivefold supersaturation of the modern ocean (e.g., Morse and He, 1993). Mechanisms to overcome this kinetic inhibition evolved at the Precambrian-Cambrian boundary with the advent of controlled biomineralization that became a major regulator of carbonate precipitation since the Mesozoic proliferation of the calcareous plankton. Prior to the Cambrian, carbonate deposition was characterized by sea-floor encrustations, cement beds, and the widespread occurrence of microbialites (Grotzinger, 1990; Riding, 2000), which are attributed to calcification of biofilms and microbial mats. In the lack of direct metabolic control on carbonate production, these Precambrian deposits are thought to have been formed under the pressure of a higher supersaturated environment, possibly still accounting for the dominance

Alkalinity, Figure 1 Recent environments that sustain microbialite growth illustrated in terms of alkalinity behavior and carbonate mineral saturation. (a–c) Shark Bay in Western Australia is one of the few recent marine examples that sustains calcifying biofilms involved in the formation of agglutinated stromatolites. Here, Indian Ocean water entering the embayment and running over the Faure sill into the Hamlin Pool to the SE is concentrated upon evaporation (a, courtesy to NASA). According to the twofold increase in salinity, alkalinity, and carbonate supersaturation rises continuously (b). The famous Hamlin Pool stromatolites (c) occur in the southernmost bay where alkalinity has increased to more than 2.8 mmol kg^{-1} and waters are nearly ten times supersaturated with respect to calcite. (d and e) Today, microbialite growth is also sustained in environments such as hardwater creeks such as the Westerhöfer Bach (Shiraishi et al., 2008), which are commonly characterized by calcium-bicarbonate type waters. From take-up of soil CO_2 and subsequent subsurface dissolution of limestone rocks, these creeks have gained already high total alkalinities ranging from 4 to 6 mmol kg^{-1} . Otherwise, at their spring sites initial pH (around 7) and carbonate saturation ($\text{SI}_{\text{cc}} = 0$) are low due to partial CO_2 pressures much higher than the atmospheric level ($\text{PCO}_2 = 10^{-2} \text{ atm}$). Continuous physicochemical CO_2 -degassing along the flowpath increases pH and CO_3^{2-} and therefore enforces supersaturation (d). Precipitation of CaCO_3 and formation of calcareous microbialites, so-called tufa stromatolites (e), commences when supersaturation reaches a certain maximum threshold level of 10- to 15-fold ($\text{SI} = 1.0\text{--}1.2$) (Arp et al., 2001), and results in the subsequent decline of alkalinity and Ca^{2+} concentrations in the creeks. (f and g) The crater lake of Satonda Island (f), Indonesia, filled with water of nearly marine composition, represents the case of a subrecent microbialite formation obviously induced by an enormous increase of alkalinity in its anoxic deep waters (Kempe and Kazmierczak, 1990). The multiple salinity stratification of the lake is a residue of former evaporitic phases. Especially in the bottom layer, sulfate has nearly been consumed due to bacterial reduction resulting in an exceptionally high total alkalinity of more than 50 mmol kg^{-1} , sulfide concentrations of up to 12 mmol kg^{-1} , and extremely high PCO_2 (more than 10^{-1} atm) (g). Alkalinity and carbonate supersaturation in the photic surface layer is determined by the seasonal mixing transfer of alkalinity from the monimolimnion top layer to the mixolimnion, degassing, rain water dilution, and evaporation (Arp et al., 2003). In the surface layer alkalinity reaches twice the value of open marine waters (4.1 mmol kg^{-1}) and the tenfold supersaturation may constitute the prerequisite for the present-day, though minor, calcification of biofilms.

of microbial carbonates in the early to middle Paleozoic (Riding and Liang, 2005). Today, the occurrence of carbonate deposits comparable to ancient microbialites is confined to particular environments such as restricted evaporational marine settings, freshwater habitats like tufa stromatolite forming hardwater creeks, or alkaline salt lakes (Figure 1). Although at first glance very different, these environments in common exhibit a significantly higher alkalinity than recent ocean values and a 10–15 fold supersaturation (i.e., $SI = 1\text{--}1.2$) with respect to calcite. Maintaining elevated carbonate supersaturation as found in these recent environments and as suggested for certain periods in Earth's history calls for variation in alkalinity in paleo-ocean model calculations (Locklair and Lerman, 2005). Therefore, changes in alkalinity may be considered as an underappreciated hydrogeochemical key mechanism with regard to carbonate supersaturation and carbonate deposition in its various forms through geologic times.

Bibliography

- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Broecker, W. S., and Peng, T.-H., 1982. *Tracers in the Sea*. New York: Lamont-Doherty Geological Observatory Publishers, 690 pp.
- Dickson, A. G., 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep-Sea Research*, **28A**, 609–623.
- Dickson, A. G., 1992. The development of the alkalinity concept in marine chemistry. *Marine Chemistry*, **40**, 49–63.
- DOE, 1994. In Dickson, A. G., and Goyet, C. (eds.), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water. Version 2*. ORNL/CDIAC-74.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J., 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Hiscock, W. T., and Millero, F. J., 2006. Alkalinity of the anoxic waters in the Western Black Sea. *Deep Sea Research Part II*, **53**, 1787–1801.
- Kempe, S., 1990. Alkalinity: the link between anaerobic basins and shallow water carbonates? *Naturwissenschaften*, **77**, 426–427.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Chemistry and stromatolites of the sea-linked Satonda Crater Lake, Indonesia: a recent model for the Precambrian sea? *Chemical Geology*, **81**, 299–310.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Monaco: Bulletin de l'Institut Océanographique, no. spec. 13, pp. 61–117.
- Lerman, A., and Stumm, W., 1989. CO_2 storage and alkalinity trends in lakes. *Water Research*, **23**, 139–146.
- Locklair, R. E., and Lerman, A., 2005. A model of Phanerozoic cycles of carbon and calcium in the global ocean: evaluation and constraints on the ocean chemistry and input fluxes. *Chemical Geology*, **217**, 113–126.
- Morse, J. W., and He, S., 1993. Influences of T , S , and P_{CO_2} on the pseudo-homogeneous precipitation of $CaCO_3$ from seawater: implications for whiting formation. *Marine Chemistry*, **41**, 291–297.
- Morse, J. W., and Mackenzie, F. T., 1990. Geochemistry of sedimentary carbonates. *Developments in Sedimentology*. Amsterdam: Elsevier, vol. 48, 707 pp.
- Morse, J. W., and Mackenzie, F. T., 1998. Hadean ocean carbonate geochemistry. *Aquatic Geochemistry*, **4**, 301–319.
- Rakestrow, N. W., 1949. The conception of alkalinity or excess base of seawater. *Journal of Marine Research*, **8**, 14–20.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.
- Ridgwell, A., 2005. A mid Mesozoic revolution in the regulation of ocean chemistry. *Marine Geology*, **217**, 339–357.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Schulz, H. D., and Zabel, M., 2000. *Marine Geochemistry*. Berlin, Heidelberg: Springer, 455 pp.
- Shiraishi, F., Reimer, A., Bissett, A., de Beer, D., and Arp, G., 2008. Microbial effects on biofilm calcification, ambient water chemistry and stable isotope records (Westerhöfer Bach, Germany). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **262**, 91–106.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd edn. New York: Wiley, 1022 pp.
- Tyrrell, T., and Zeebe, R. E., 2004. History of carbonate ion concentration over the last 100 million years. *Geochimica et Cosmochimica Acta*, **68**, 3521–3530.
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G., 2007. Total alkalinity: the explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, **106**, 287–300.
- Zeebe, R. E., and Wolf-Gladrow, D. A., 2001. *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Oceanography Series, Amsterdam: Elsevier, Vol 65, 346 pp.

Cross-references

[Calcite Precipitation, Microbially Induced Carbonate Environments](#)
[Carbonates](#)
[Saline Lakes](#)
[Salinity History of the Earth's Ocean](#)
[Soda Ocean Hypothesis](#)

AMBER

Eugenio Ragazzi¹, Alexander R. Schmidt²

¹University of Padova, Padova, Italy

²University of Göttingen, Göttingen, Germany

Synonyms

Fossil resin; Resinite

of microbial carbonates in the early to middle Paleozoic (Riding and Liang, 2005). Today, the occurrence of carbonate deposits comparable to ancient microbialites is confined to particular environments such as restricted evaporational marine settings, freshwater habitats like tufa stromatolite forming hardwater creeks, or alkaline salt lakes (Figure 1). Although at first glance very different, these environments in common exhibit a significantly higher alkalinity than recent ocean values and a 10–15 fold supersaturation (i.e., $SI = 1\text{--}1.2$) with respect to calcite. Maintaining elevated carbonate supersaturation as found in these recent environments and as suggested for certain periods in Earth's history calls for variation in alkalinity in paleo-ocean model calculations (Locklair and Lerman, 2005). Therefore, changes in alkalinity may be considered as an underappreciated hydrogeochemical key mechanism with regard to carbonate supersaturation and carbonate deposition in its various forms through geologic times.

Bibliography

- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Broecker, W. S., and Peng, T.-H., 1982. *Tracers in the Sea*. New York: Lamont-Doherty Geological Observatory Publishers, 690 pp.
- Dickson, A. G., 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep-Sea Research*, **28A**, 609–623.
- Dickson, A. G., 1992. The development of the alkalinity concept in marine chemistry. *Marine Chemistry*, **40**, 49–63.
- DOE, 1994. In Dickson, A. G., and Goyet, C. (eds.), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water. Version 2*. ORNL/CDIAC-74.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J., 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Hiscock, W. T., and Millero, F. J., 2006. Alkalinity of the anoxic waters in the Western Black Sea. *Deep Sea Research Part II*, **53**, 1787–1801.
- Kempe, S., 1990. Alkalinity: the link between anaerobic basins and shallow water carbonates? *Naturwissenschaften*, **77**, 426–427.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Chemistry and stromatolites of the sea-linked Satonda Crater Lake, Indonesia: a recent model for the Precambrian sea? *Chemical Geology*, **81**, 299–310.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Monaco: Bulletin de l'Institut Océanographique, no. spec. 13, pp. 61–117.
- Lerman, A., and Stumm, W., 1989. CO_2 storage and alkalinity trends in lakes. *Water Research*, **23**, 139–146.
- Locklair, R. E., and Lerman, A., 2005. A model of Phanerozoic cycles of carbon and calcium in the global ocean: evaluation and constraints on the ocean chemistry and input fluxes. *Chemical Geology*, **217**, 113–126.
- Morse, J. W., and He, S., 1993. Influences of T , S , and P_{CO_2} on the pseudo-homogeneous precipitation of $CaCO_3$ from seawater: implications for whiting formation. *Marine Chemistry*, **41**, 291–297.
- Morse, J. W., and Mackenzie, F. T., 1990. Geochemistry of sedimentary carbonates. *Developments in Sedimentology*. Amsterdam: Elsevier, vol. 48, 707 pp.
- Morse, J. W., and Mackenzie, F. T., 1998. Hadean ocean carbonate geochemistry. *Aquatic Geochemistry*, **4**, 301–319.
- Rakestrow, N. W., 1949. The conception of alkalinity or excess base of seawater. *Journal of Marine Research*, **8**, 14–20.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.
- Ridgwell, A., 2005. A mid Mesozoic revolution in the regulation of ocean chemistry. *Marine Geology*, **217**, 339–357.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Schulz, H. D., and Zabel, M., 2000. *Marine Geochemistry*. Berlin, Heidelberg: Springer, 455 pp.
- Shiraishi, F., Reimer, A., Bissett, A., de Beer, D., and Arp, G., 2008. Microbial effects on biofilm calcification, ambient water chemistry and stable isotope records (Westerhöfer Bach, Germany). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **262**, 91–106.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd edn. New York: Wiley, 1022 pp.
- Tyrrell, T., and Zeebe, R. E., 2004. History of carbonate ion concentration over the last 100 million years. *Geochimica et Cosmochimica Acta*, **68**, 3521–3530.
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G., 2007. Total alkalinity: the explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, **106**, 287–300.
- Zeebe, R. E., and Wolf-Gladrow, D. A., 2001. *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Oceanography Series, Amsterdam: Elsevier, Vol 65, 346 pp.

Cross-references

[Calcite Precipitation, Microbially Induced Carbonate Environments](#)
[Carbonates](#)
[Saline Lakes](#)
[Salinity History of the Earth's Ocean](#)
[Soda Ocean Hypothesis](#)

AMBER

Eugenio Ragazzi¹, Alexander R. Schmidt²

¹University of Padova, Padova, Italy

²University of Göttingen, Göttingen, Germany

Synonyms

Fossil resin; Resinite

Definition

Fossilized plant resin from various botanical sources.

Introduction

Amber is a fossilized plant resin from various botanical sources and of different geological ages, ranging from the Carboniferous to the Pliocene, although it is particularly frequent in the Cretaceous and in the Eocene to Miocene. The term “amber” is used most commonly as a synonym for fossil resin of every provenance, geological, and palaeobotanical source. A more reductive meaning of amber has been considered, referring only to *succinite*, the mineralogical species occurring in the Baltic coast deposits and neighboring localities containing significant amounts of succinic acid (up to 8%). However, most commonly, *amber* and *fossil resin* are the terms used interchangeably for indicating macroscopic material (Anderson and Crelling, 1995; Langenheim, 2003). Another term used by coal geologists for fossil resins is *resinite*, which is a microscopic material often found in coal maceral. Amber, fossil resin, and resinite, according to Anderson and Crelling (1995) should therefore be considered “solid, discrete organic material found in coals and other sediments as macroscopic or microscopic particles, which are derived from the resins of higher plants.”

The term amber derives from the Arabic word *anbar*, which was first used to indicate *ambergris* (a waxy substance secreted by the sperm whale, used in perfumery as a fixative) and then also fossil resin, both natural products with the common feature of being carried ashore by sea waves. The Romans called amber *succinum* (from *succus*, literally “juice,” “sap”). The ancient Greek name for amber is *elektron*, that means “shining thing,” a term which was related to the Sun God *Elektor* (the awakener), and the Moon, called *Elektris* (Stoppani, 1886). The modern term *electron* was coined in 1891 by the Irish physicist George Johnstone Stoney, using the Greek word for amber because static electricity could be generated on the surface of rubbed amber. The German word *Bernstein* (or *Börnstein*), the Dutch *Barnsteen*, and the Swedish *bärnsten* mean “burning stone” because amber may burn when exposed to flame.

Geological names are often attributed to fossil resins on the basis of the locality of discovery or of the discoverer, and these names are not based on specific knowledge of the composition; however, they are often used and also quoted in the scientific literature as common names. For example, *Simetite* is for amber collected in Sicily (Italy) near the Simeto river, *Burmite*, for amber found in Burma (Myanmar), and *Rumanite* for that found in Romania; *Beckerite* and *Stantienite* were named for Becker and Stantien, two developers of amber dredging and mining operations in the Samland region in the 1800s. Over a hundred of different fossil resins have been described, so far.

Fossil resins are considered the archetype of a “chemical fossil” (chemical evidence of life processes preserved in the fossil record), and besides their property of

preserving embedded organisms, they can also maintain particular details of their original composition better than any other sedimentary material.

Amber has been known by humans since prehistorical times and most bizarre hypotheses about its origin and composition have been formulated. In the antiquity amber has been considered even as produced by the rays of the Sun (Pliny the Elder, Book 37: *Nicias solis radiorum succum intellegi voluit hoc*) and that was not such a wrong idea, since amber, as a resin of ancient trees, is a metabolite of plants, originated from products of photosynthesis, that is, from the energy of the Sun.

Amber composition and formation

Several different parameters can play relevant roles in amber composition and consequently, they all should be considered in the study and comparison of different amber samples. These are for example:

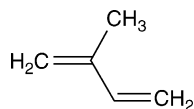
1. *Original environment*: temperature, insolation, and climate influence the resin composition before it is embedded in the sediment (biostratonomic processes);
2. *Age*: complex chemical arrangements occur with time; in general, low molecular weight components disappear with increasing age;
3. *Plant source*: resin produced by different plants exhibit different chemical composition;
4. *Diagenetic history*: following the embedding in the sediment, resin maturation is affected by temperature, pressure and permeating fluids which can modify the chemical composition and the physicochemical properties of fossil resin;
5. *Alteration processes after amber mining* (of relevance especially in archaeological context): direct sunlight and exposition to air and heat may change the color and physical properties (and, consequently, the chemical composition) of the sample.

Resins consist of secondary components of plants, that is, chemicals occurring only in some groups of plants, without an apparent role in primary physiological or metabolic pathways. Most natural resins are derived from *terpenes*, a large and varied class of hydrocarbons, which are chemicals composed by structures based on the isoprene unit (C_5H_8) (Figure 1). The basic molecular formula of terpenes can be expressed as multiples of isoprene, $(C_5H_8)_n$, where n is the number of linked isoprene units. The isoprene units can link together “head to tail” to form linear chains, or alternatively they can arrange to form rings. The overall terpene structure undergoes oxidation or rearrangement of the carbon skeleton, producing compounds, which are generally indicated as *terpenoids* or *isoprenoids*. Mono-, sesqui-, di-, and tri-terpenoids are polymers formed by two, three, four, and six isoprene units, respectively.

The biosynthesis of resins starts from simple molecules, namely mevalonic acid, which can be considered the precursor of isoprene units. Photosynthetically

produced carbohydrates are broken down to give pyruvate, which represents the basis for the synthesis of mevalonic acid. Mevalonic acid is converted into the activated form of isopentenyl pyrophosphate, which gives origin to geranyl pyrophosphate (C₁₀), the basis for the monoterpene synthesis, or can further undergo condensation into farnesyl pyrophosphate (C₁₅) for the production of sesquiterpenes, or geranyl-geranyl pyrophosphate (C₂₀), which yields diterpenes and tetraterpenes, or squalene (C₃₀), which is the basis of triterpenes (Figure 2).

Fluid resins, after their exudation from plant, are characterized by a large volatile fraction, up to 50%, mainly constituted by monoterpenes and sesquiterpenes, while the nonvolatile fraction is composed of di- and tri-terpene acids (carboxylic acids with double bonds and functional groups), with alcohols, aldehydes, and esters. The volatile components of the resins are mainly involved in the defensive role of the resin against pathogens and insects. Evaporation of volatile components, following polymerization of the remaining nonvolatile constituents, mainly presenting dienic functions, causes the hardening of resin, which, if allowed to be embedded in appropriate sediment, poses the basis for transformation into a fossil resin.



Amber, Figure 1 The basic isoprene unit (C₅H₈).

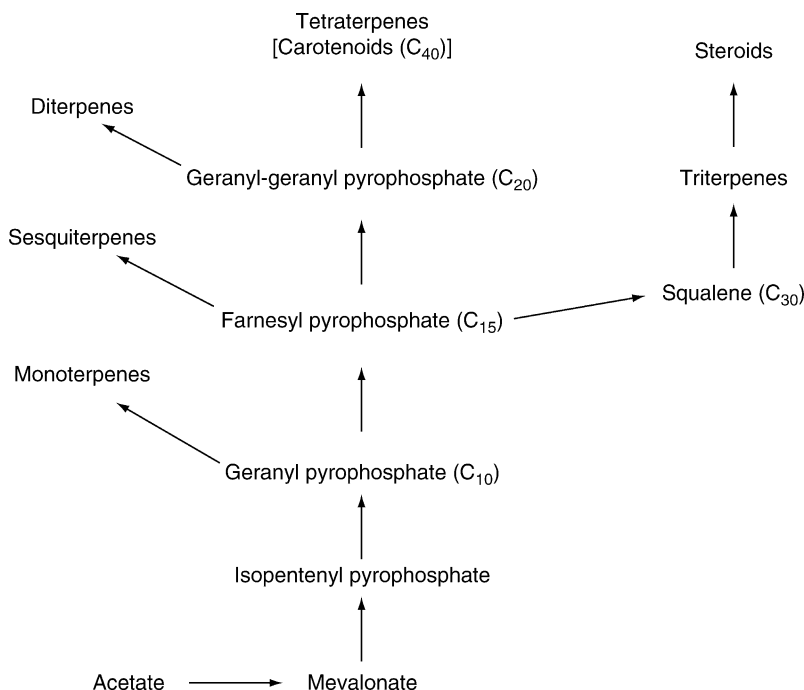
Diterpenoid resins, mainly composed of diterpene acids (C₂₀), are produced by gymnosperms (conifers) and angiosperms (mostly in the Leguminosae family), and they are particularly prone to polymerization and thus being preserved during fossilization into amber.

Gymnosperm diterpenes are mainly represented by diterpene acids of the abietane, pimarane, and labdane types (Figure 3). Abietane- and pimarane-type diterpene acids, such as abietic and pimaric acids, abundant in the Pinaceae family, tend to remain unpolymerized; on the contrary, labdane-type acids (e.g., agathic and communic acids, common in the Cupressaceae family), easily polymerize thanks to conjugated diene compounds (Langenheim, 2003). Resins of the Araucariaceae family may contain all three diterpene groups.

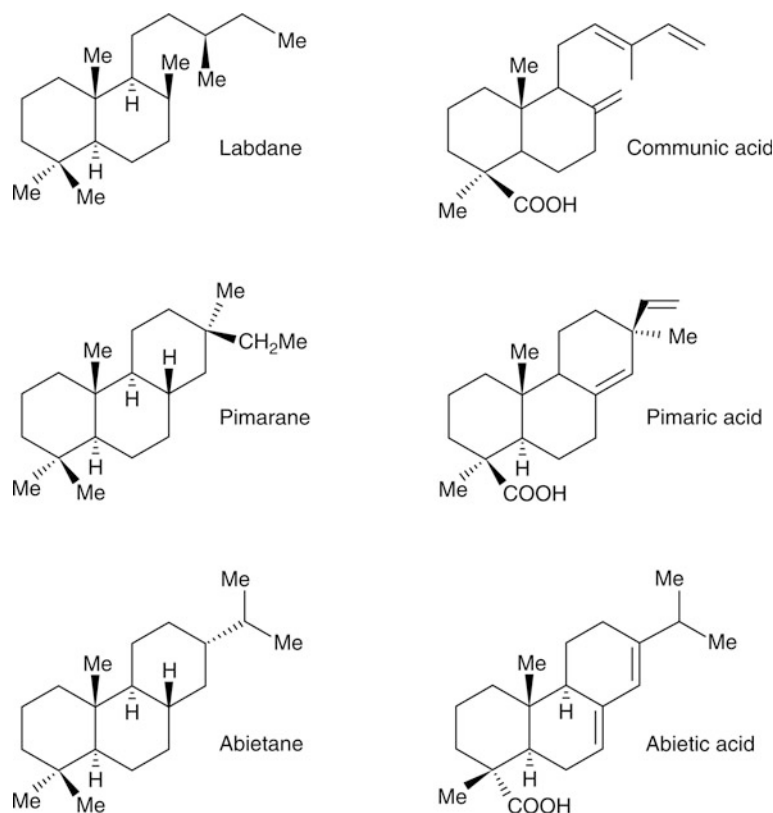
Also the nonvolatile fraction of angiosperm resins, for example, from the genus *Hymenaea* of the Leguminosae (Fabaceae) family, which yields copal, is constituted by diterpenes of the labdadiene group. Resins from other angiosperms, such as Dipterocarpaceae, contain mainly triterpenes.

Therefore, resins containing particular diterpenes, such as abietic acid, cannot form amber *sensu stricto* because the constituent does not contain functional groups that could lead to a cross-linked polymer (Beck, 1999). Also triterpenoid resins (C₃₀), which are mainly produced by broad-leaved trees, generally do not polymerize.

The process of polymerization and resin hardening starts just after resin exudation, and involves free radical mechanisms, which are initiated by exposure to sunlight and air (Langenheim, 2003). Initial polymerization can



Amber, Figure 2 Biosynthesis of terpenoid resins from mevalonate (adapted from Langenheim, 1969).



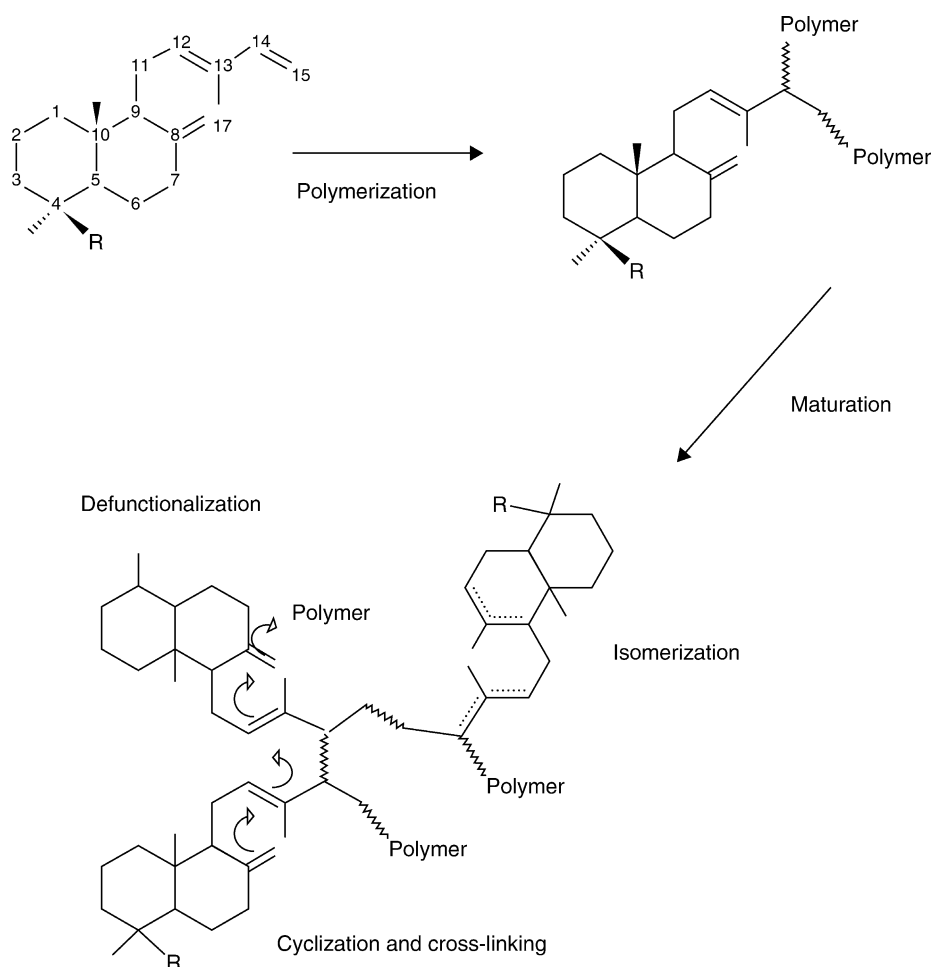
Amber, Figure 3 Chemical structure of major conifer resin components. Left column presents the general skeleton, and right column, an example of specific compounds (for further details, see Langenheim, 2003).

occur involving the terminal groups of a side chain in the molecule of terpenoids, mainly diterpenes with a labdanoid structure (unsaturated labdatriene acids and alcohols, and biformene), leading to the formation of a general polymeric structure (Anderson et al., 1992; Clifford and Hatcher, 1995; Clifford et al., 1997) (Figure 4). As a complex mixture, amber contains also nonpolymerizing compounds such as succinic acid in succinite, or monoterpenes, which are trapped (cross-linked) into the polymer network (Clifford and Hatcher, 1995; Clifford et al., 1997) and function as plasticizers.

Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and burial into the sediment, due to diagenetic and catagenetic processes. The chemical reactions in maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization (Clifford and Hatcher, 1995; Clifford et al., 1997). One typical reaction in polylabdanoid fossil resins is the depletion of exocyclic methylenes (Figure 4) in the C8–C17 double bond of the molecule, and the formation of intramolecular cyclization. The modification of amber structure during its maturation leads to a progressive loss of unsaturated bonds, a decrease in functionalized groups and an increased proportion of aromatized groups (Grimalt et al., 1988).

Although the degree of resin maturation is dependent on the age of the resin, the rate at which this process occurs depends on several geological conditions, including the thermal history of the sample (Anderson et al., 1992) and on the structure and resin composition (Langenheim, 2003). Although a certain direct correlation between resin maturation and the age has also been demonstrated, by means of thermal analysis (Ragazzi et al., 2003), maturity and age cannot always be directly linked, since chemical transformation of resin increases at higher temperatures, and therefore the geothermal variable should be considered. Other physicochemical properties of amber, such as hardness and solvent solubility, may suggest the degree of maturation (Poinar, 1992; Rodgers and Currie, 1999; Langenheim, 2003; Ragazzi et al., 2003).

An alternative approach to define the age of a resin was proposed by Anderson (1997) on the basis of carbon-14 content. Resins that are older than the detection limit of carbon-14, that is, at about 40,000 years, have been considered *fossil resins*, and their age should be determined based on the stratigraphical data of the embedding sediment. Resins of age between 40,000 and 5,000 years are considered *subfossil*, while resins between 5,000 and 250 years are *ancient resins*, and those younger than 250 years are considered as *modern* or *recent*. This



Amber, Figure 4 Schematic representation of the polymeric structure in polylabdanoid fossil resins and of the maturation process (adapted from Clifford and Hatcher, 1995; Clifford et al., 1997).

terminology should be preferred to that referring to “young amber” or “copal,” which is often misleading with respect to the age of the sample.

Physicochemical characteristics of amber

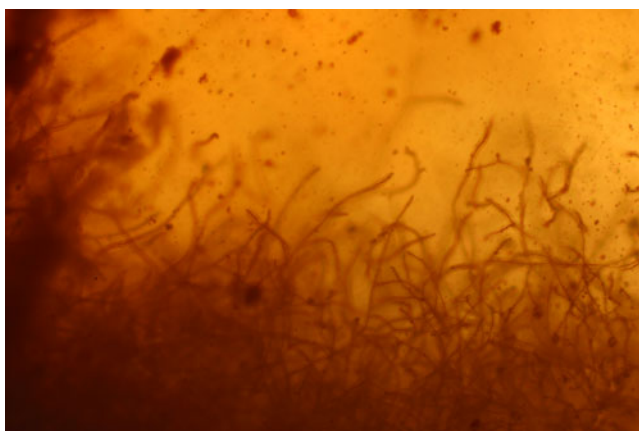
Most amber pieces are minute ranging from millimeter to centimeter size. Resin pieces may be found preserved in their original shape as solidified in the amber forest (e.g., drops, elongate stalactites, lumps with a flattened side which was attached to the bark or to other surfaces). Fossil resin is sometimes still attached to fossil wood, or even *in situ* in wood or twigs which can help to identify the amber-bearing trees of a particular deposit. Amber pieces are more or less rounded after being transported and redeposited.

Amber has been extensively investigated under the physicochemical aspect. The relative hardness according to Mohs’ scale is 2–2.5 (corresponding to 199–290 MPa) for most types of amber, indicating that it is slightly harder than gypsum and that it is possible to scratch the surface with calcite or copper. The specific

gravity, that is, the ratio of the density of a substance to the density of water, ranges from about 0.96–1.12. For this reason most amber is nearly floating in sea water (specific gravity of the Baltic sea water is very low, about 1.005) and it sinks to the bottom in quiet water, but it can easily be carried along by disturbed water and washed ashore. Because of its low density, amber floats on a salt solution (about 15 g of NaCl/100 ml), while several types of plastic, often used as amber imitation, sink due to their higher density (e.g., the phenolic plastic bakelite, which has a specific gravity of about 1.4). Amber has resinous luster, and might appear translucent or opaque. Its refractive index ranges between 1.539 and 1.545. The color of translucent amber varies from a honey-like yellow and yellow-orange to light and dark red; the latter color is often caused by weathering. Rare specimens of blue (e.g., some Dominican amber) and green (e.g., some Mexican amber) samples have been found. Resins may be opaque because of microscopic bubbles or tiny detritus particles. Sometimes, densely arranged filaments of bacteria and fungi

that grew into the resin in its liquid stage cause opacity of the outer parts of resin pieces (Figure 5). If exposed to ultraviolet light, amber often produces fluorescence of blue, yellow, or green color. When amber is heated, it becomes soft at 150–180°C and decomposes around 280–300°C, emitting fumes with aromatic odor. After touching amber with a hot needle (hot point test), it produces smoke and burnt-resinous odor; this method is empirically used to distinguish between amber and plastic or copal. Exposed to a flame, amber burns easily. Amber is insoluble in water, and partly soluble in organic solvents,

such as diethyl ether, acetone, and dichloromethane, as well as in mixtures of turpentine oil and acetone. After applying a drop of solvent to the surface, recent resins (copal) or plastic are readily dissolved and the surface becomes sticky, while amber (polymerized fossil resin) is unaffected. Although resin is macroscopically amorphous, some of its constituents may form microscopic drop- or bubble-like and elongated microstructures. Some of these “pseudoinclusions” may resemble enclosed microorganisms but can be distinguished by their variable size and shape and by the absence of diagnostic surface features (Figure 6).



Amber, Figure 5 Filamentous sheathed bacteria of ca 10 μm diameter preserved in French Cretaceous amber after growing into the liquid resin (photograph by Alexander R. Schmidt).

Chemical analyses and classification of amber

Since early chemical investigations in the nineteenth century, amber has been identified as being composed of carbon, hydrogen, and oxygen. It is not possible to indicate a univocal composition formula of amber since it is an amorphous, noncrystalline material. The composition of different types of amber, as a result of elemental analysis, has been indicated approximately in the following range: carbon 75–87%, hydrogen 8.5–11%, oxygen 5–15%, with traces of sulfur (up to 1.7%) and other elements (Kosmowska-Ceranowicz, 1984; Ragazzi et al., 2003).

Since amber is mostly insoluble in organic solvents, a complete characterization of its chemical composition has been obtained using the total solid sample, by means of specific analytical techniques that have become available, such as infrared spectroscopy (IRS), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and nuclear magnetic resonance (NMR) spectroscopy.



Amber, Figure 6 Pseudoinclusions in Cenomanian amber from Fourtou in the French Pyreneans (photograph by Alexander R. Schmidt).

Infrared spectroscopy of powdered samples, since the first application in the study of fossil resins (Beck et al., 1964), has become one of the main techniques for obtaining structural information and identification of amber from different origins (Kosmowska-Ceranowicz, 1999). Infrared spectra allow to define the fingerprinting of any fossil resin, and in particular it has been successful as diagnostic for Baltic amber, observing the presence of the characteristic Baltic shoulder in the 1,150–1,260 cm^{-1} region of the spectrum, attributed to the absorption of ester groups, in particular esterified succinic acid. It is noteworthy that the free succinic acid levels in Baltic amber range between 50 and 400 ppm (Tonidandel et al., 2009), despite the high total succinic acid content of 1–8%.

Anderson et al. (1992; also Anderson and Crelling, 1995), on the basis of various physicochemical methods, mainly Py-GC/MS, proposed a classification of fossil resins into five main classes:

Class I	The macromolecular structures are derived from polymers of primarily labdanoid diterpenes, which include labdatriene carboxylic acids, alcohols, and hydrocarbons
Ia	Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, including communic acid, communol, and succinic acid; example: succinite (Baltic amber)
Ib	Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, often including communic acid, communol, biformene, but not succinic acid; example: New Zealand's fossil kauri resin
Ic	Derived from/based on polymers and copolymers of labdanoid diterpenes with enantio [1S, 4aS, 5R, 8aS] configuration, including ozic acid, ozol, and enantio biformenes; examples: Mexican and Dominican amber, African resinates
Class II	Derived from/based on polymers of bicyclic sesquiterpenoid hydrocarbons, especially cadinene, and related isomers; examples: Utah and Indonesian amber
Class III	Natural (fossil) polystyrene; example: some New Jersey amber, Siegburgite from Germany
Class IV	Nonpolymeric, incorporating sesquiterpenoids based on the cedrane carbon skeleton; example: Moravian amber from the Czech Republic
Class V	Nonpolymeric diterpenoid carboxylic acid, especially based on abietane, pimarane, and isopimarane carbon skeletons; example: Highgate copalite and other retinites in European brown coals

The high-resolution NMR spectra of the carbon nuclei in powdered samples provided detailed information on the types of carbon functionality in the fossil resin, over the entire range of resonances, indicating the presence of

saturated carbons (resonances 0–100 ppm), unsaturated carbons such as alkenic and aromatic (resonances 100–160 ppm), and carbonyl groups (resonances above 160 ppm) (Lambert and Frye, 1982). Using NMR, comparative analysis of samples from different origin permitted to distinguish four major groupings (Group A through D) of fossil resins (Lambert and Poinar, 2002), in good agreement with Anderson's et al. classification based on Py-GC/MS.

Age, global occurrence, and botanical origin of amber

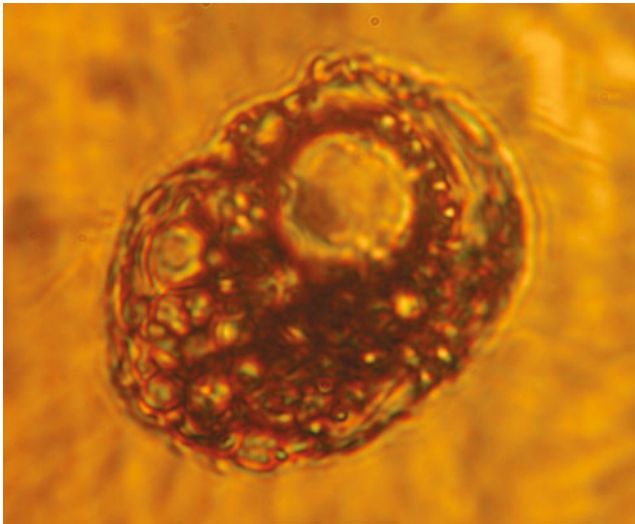
Amber might be found on every continent but most amber deposits have been discovered in the northern hemisphere, so far. A few ambers are known from South America, Africa, Australia, and New Zealand. Many new Mesozoic and Cenozoic amber localities have been described in the last decade (see Poinar, 1992; Grimaldi, 1996; Krumbiegel and Krumbiegel, 2005, for age and global occurrence of ambers).

A few resins were reported from the Carboniferous of Europe and the USA (Bray and Anderson, 2009). The first conifers which produced significant amounts of resin appeared in the Carnian stage of the late Triassic (about 220 million years ago). Remarkable amounts of small Triassic amber pieces have been found in the petrified forests of Arizona (Litwin and Ash, 1991), in the Molteno Formation of Lesotho (Ansorge, 2007) and the Heiligkreuz-Santa Croce Formation of the Italian Dolomites (Roghi et al., 2006, Figure 7). The latter is the oldest fossiliferous amber containing various microorganisms (Schmidt et al., 2006, see Figure 8). The Jurassic is particularly poor in amber and just few samples have been reported from the late Jurassic age of Portugal, Denmark (Bornholm), Germany, and Thailand.

The oldest Cretaceous ambers come from the Wealden Formation of southern England (Nicholas et al., 1993) and from the Kirkwood Formation of South Africa (Gomez et al., 2002), both being Valanginian in age. The most important fossiliferous Cretaceous ambers are (1) the *Middle East ambers* from Lebanon, Israel, and Jordan, ranging from the Hauterivian to the Aptian, (2) the Aptian–Albian *Álava amber* from Peñacerrada in the Basque county of northern Spain, (3) the *Siberian amber* from the Albian to Santonian of the Taimyr Peninsula, (4) the Albian–Cenomanian *ambers from Charente and Charente-Maritime* in southwestern France (see Perrichot et al., 2007, for these newly discovered ambers), (5) the *Burmese amber* from Myanmar which is Albian in age (Grimaldi et al., 2002; Cruickshank and Ko, 2003), (6) the *Atlantic coastal plain ambers* of the USA which comprise several localities of various Upper Cretaceous ages from Maryland and New Jersey to South Carolina, and (7) the Upper Cretaceous *Canadian amber* (Cedar Lake in Manitoba and other localities). Further remarkable Cretaceous resins come from Alaska, from several Albian to Coniacian localities in Japan, and from



Amber, Figure 7 Triassic amber drop from the Italian Dolomites (photograph by Guido Roghi, *Nature*, 444, 835, 2006). The piece is about 3 mm in length.



Amber, Figure 8 Shell of a testate amoeba enclosed in Triassic amber from Italy. This shell is 35 μm in size and morphologically identical to the modern species *Centropyxis hirsuta* (photograph by Alexander R. Schmidt, *Nature*, 444, 835, 2006).

the Aptian age of Golling in Austria, but these ambers yield few fossils.

Cenozoic ambers are largely Eocene to Miocene in age, and some deposits already produced tons of resin. The most well-known fossil resin is the *Baltic amber* which

can be found in the Baltic sea area (especially along the coast of Russia and Poland) in Eocene sediments, washed ashore and also as glacial erratic. The sediments bearing the Baltic amber are considered to be 38–47, some up to 55 million years old (Ritzkowski, 1999). Other fossiliferous Eocene ambers come from the *Paris Basin* in France (50–55 million years old; Nel et al., 1999), from Vastan (Gujarat) in western India (52 million years old; Alimohammadian et al., 2003) and from Fu Shun in the Chinese Liaoning province. The Bitterfeld amber from a coal mine near the city of Bitterfeld in central Germany is uppermost Oligocene in age (24 million years old, see Wimmer et al., 2006) and also produced a plethora of inclusions, mainly arthropods (Knuth et al., 2002). The most fossiliferous Cenozoic New World ambers come from the Dominican Republic and from Chiapas in Mexico (see Poinar, 1992). Recent studies suggest a Miocene age (15–20 million years old) for these Caribbean ambers (Iturralde-Vinent and MacPhee, 1996; Solórzano Kraemer, 2007). Further fossiliferous Miocene ambers were found in western Amazonia in Peru, and in Sicily and Borneo.

Representatives of various gymnosperm and angiosperm families have produced resin in the Earth's history (see Langenheim, 2003; Krumbiegel and Krumbiegel, 2005, for overview). The Italian Triassic amber was possibly produced by members of the Cheirolepidiaceae, an extinct gymnosperm family. Among others, various representatives of the Araucariaceae (Figures 9 and 10) and Cupressaceae are considered to be resin-bearing trees in the Cretaceous. Angiosperm resins are recorded since the Eocene, for example, *Glessite* stems from Burseraceae and *Siegburgite* were produced by Hamamelidaceae. The tropical genus *Hymenaea* (Leguminosae) produced the Dominican and Mexican ambers. The main resin-bearing trees of the Baltic amber forest probably belonged to conifers of the family Sciadopityaceae, as recently suggested by Wolfe et al. (2009).

Embedding and preservation of organisms in resin

Resins are produced by plants as defensive reaction against mechanical damage (injury to the bark, fire, herbivores) and potential invasion by pathogens, such as bacteria and fungi. Also, climatic reasons and ecological disturbance have been discussed to be the reasons for extensive resin production (Henwood, 1993).

The exceptional preservation of organisms included in amber is due to the dehydration environment made possible by the resin and by natural embalming properties due to antiseptic activity of the resin constituents. Components that do not participate in polymerization of the resin, such as some diterpene resin acids (e.g., copalic acid), can remain available for the interactions with biota embedded in resin (Henwood, 1993) and contribute to the preservation even of soft-bodied inclusions. Even cell organelles such as chloroplasts of algae are sometimes well-preserved in amber (Figure 11).

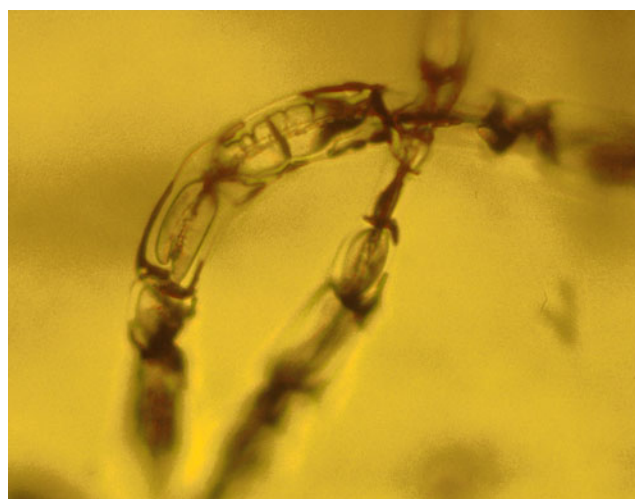


Amber, Figure 9 Resinous forest of *Araucaria columnaris* growing at the coast of Maré, New Caledonia (photograph by Alexander R. Schmidt).



Amber, Figure 10 Extensive resin flows of ~30 cm length induced by fire at the bark of *Araucaria columnaris* in New Caledonia (photograph by Alexander R. Schmidt).

Extraction of ancient DNA from amber has been of particular interest in working with fossils embedded in amber, and in the early 1990s first attempts to establish methods for extraction, amplification, and sequencing of fossil



Amber, Figure 11 Preservation of chloroplasts in the conjugatophyte *Palaeozygnema spiralis* from the Cenomanian resin of Schliersee in the Bavarian Alps. The cells are ~15 μm in diameter (photograph by Alexander R. Schmidt).

DNA from amber were carried out (e.g., Cano et al., 1994; Cano and Borucki, 1995). However, critical reinvestigations suggest that the DNA obtained in these studies was modern contamination and because even the amplification of DNA from copal failed, Austin et al. (1997) concluded that resin is not predestined for the preservation of complex molecules, although morphological structures are recorded very well.

Attached to fresh sticky resin (Figure 10), organisms or parts of them are entirely covered by further resin flows. Amber inclusions are therefore usually found at the surfaces of successive resin flows in amber. Invertebrates of appropriate size, especially arthropods (e.g., diptera, hymenoptera, see Figure 12), and small plant remnants fallen onto the resin are therefore predestined for getting stuck and embedded. Arthropods, plant remnants (flowers, leaves, stellate hairs, wood, pollen, spores) and microorganisms are typical amber inclusions and sometimes, also small vertebrates (frogs, lizards) and parts of larger animals (reptile skin, feathers, hairs) are preserved. Some groups of organisms are only recorded as amber fossils, since few other fossilization processes occur in terrestrial environments which may preserve morphological structures even of soft-bodied organisms very well.

Many life forms and representatives of all trophic levels of a biocoenosis may be found in a single piece of resin: bacteria, algae as producers, protozoans, micrometazoans, molluscs, arthropods as consumers, and fungi as decomposers (Perrichot and Girard, 2009). Although many members of a biocoenosis can potentially become embedded in resin, selective embedding has been observed (Schmidt and Dilcher, 2007). Because of their motility, moving or flying arthropods have a higher probability of encountering and becoming attached to the resin. Therefore, the amount of amber inclusions of a species is not correlated with its abundance in the ancient ecosystem.

Most amber inclusions are fossils of terrestrial organisms which lived at or close to the resin-bearing trees. Much resin solidified at the forest floor, not on the bark (Henwood, 1993) and the term *litter amber* has been proposed by Perrichot (2004) for amber pieces which largely contain soil and litter-dwelling arthropods (Figure 13).



Amber, Figure 12 Hymenoptera (Scelionidae), fossilized in Lower Cenomanian amber of Fouras, Charente-Maritime, southwestern France. The inclusion is 1.8 mm in length (courtesy of Vincent Perrichot).

To find aquatic organisms in tree resin may seem to be highly unlikely but the fossil record provides amber-preserved limnetic arthropods (e.g., water beetles, water striders, crustaceans, larvae of caddisflies and mayflies) and microorganisms (e.g., algae, ciliates, testate amoebae, rotifers). Limnetic organisms may get stuck or enclosed when resin comes into contact with water or even flows into water (Schmidt and Dilcher, 2007).

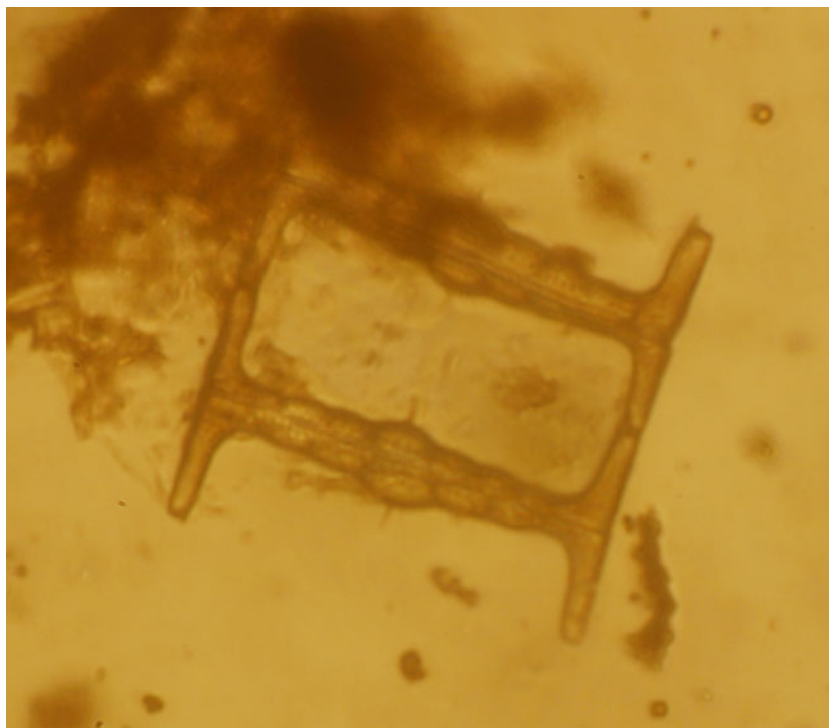
Few amber inclusions of marine organisms (crustaceans, diatoms) have been reported from amber forests which grew directly at the coast (Vonk and Schram, 2007; Girard et al., 2008; see Figure 14). These organisms were probably introduced by wind or spray from beach and sea onto the resin flows in the nearby woods.

Although resin has fungicidal properties, some fungi and bacteria are able to grow into liquid resin and can therefore be found in almost every fossil resin. Usually, these filaments grow in random orientation as long as the resin is liquid. Growth stops when the resin solidifies and the filiform inclusions become well-preserved in the resin. Resinicolous fungi such as ascomycetes of the Mycocaliciales are adapted to their special substrates and are also able to grow on fresh resin. Therefore, they may be found in places where herbivore insects induce long-term resin flows (Rikkinen, 1999; Rikkinen and Poinar, 2000).

Resin which dried at the bark is exposed to the air, and processes of weathering and degradation start rapidly. Long-time preservation of resins and its inclusions and



Amber, Figure 13 *Marchandia magnifica*, a mole cricket from Albian amber from southwestern France. It is 3.8 mm in length (photograph by Vincent Perrichot, courtesy of Elsevier/ Academic Press, *Cretaceous Research*, 23, 307–314, 2002).



Amber, Figure 14 Two frustules of a marine diatom of the genus *Hemiaulus* enclosed in a piece of amber from a coastal Cretaceous forest from Archingeay in south-western France. The cells are ca 40 μm in size (photograph by Alexander R. Schmidt).

formation of amber therefore generally needs redeposition into marine sediments or at least a cover by sediment layers to stop processes of oxidation.

Summary

Amber is fossilized plant resin, which is largely found in the Cretaceous and in the Eocene to Miocene when various representatives of gymnosperms and angiosperms produced considerable amounts of resin. After exudation from plants, the volatile components evaporate and the remaining nonvolatile constituents polymerize which leads to the hardening of the resin. Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and embedding into the sediment. The chemical reactions in the maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization. Several different parameters can play relevant roles in amber composition, such as original environment, age, plant source, diagenetic history, and also alteration processes after amber mining. Inclusions of many life forms may be found in amber, although selective embedding has been observed. The taphocoenosis in a piece of amber may potentially contain organisms of different habitats such as tree bark, soil and litter, freshwater ponds, and littoral.

Bibliography

- Alimohammadian, H., Sahni, A., Patnaik, R., Rana, R. S., and Singh, H., 2003. First record of an exceptionally diverse and well preserved amber-embedded biota from Lower Eocene (~52 Ma) lignites, Vastan, Gujarat. *Current Science*, **89**, 1328–1330.
- Anderson, K. B., 1997. The nature and fate of natural resins in the geosphere – VII. A Radiocarbon (^{14}C) age scale for description of immature natural resins: an invitation to scientific debate. *Organic Geochemistry*, **25**, 251–253.
- Anderson, K. B., and Crelling, J. C., 1995. *Amber, Resinite, and Fossil Resins*. Washington: American Chemical Society.
- Anderson, K. B., Winans, R. E., and Botto, R. E., 1992. The nature and fate of natural resins in the geosphere – II. Identification, classification and nomenclature of resinates. *Organic Geochemistry*, **18**, 829–841.
- Ansorge, J., 2007. Upper Triassic insects and amber from Lesotho (South Africa). In *FossilsX3: Insects, Arthropods, Amber*. Vitoria-Gasteiz, Spain, May 4–9, 2007, Abstracts: p. 52.
- Austin, J. J., Ross, A. J., Smith, A. B., Fortey, R. A., and Thomas, R. H., 1997. Problems of reproducibility – does geologically ancient DNA survive in amber-preserved insects? *Proceedings of the Royal Society of London, Series B*, **264**, 467–474.
- Beck, C. W., 1999. The chemistry of amber. *Estudios del Museo de Ciencias Naturales de Alava*, **14**(Numero especial 2), 33–48.
- Beck, C. W., Wilbur, E., and Meret, S., 1964. Infrared spectra and the origin of amber. *Nature*, **201**, 256–257.
- Bray, P. S., and Anderson, K. B., 2009. Identification of Carboniferous (320 Million Years Old) Class Ic amber. *Science*, **326**, 132–134.

- Cano, R. J., and Borucki, M. K., 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. *Science*, **268**, 1060–1064.
- Cano, R. J., Borucki, M. K., Higby-Schweitzer, M., Poinar, H. N., Poinar, G. O., Jr., and Pollard, K. J., 1994. *Bacillus*-DNA in fossil bees: an ancient symbiosis? *Applied and Environmental Microbiology*, **60**, 2164–2167.
- Clifford, D. J., and Hatcher, P. G., 1995. Structural transformations of polylabdanoid resins during maturation. *Organic Geochemistry*, **23**, 407–418.
- Clifford, D. J., Hatcher, P. G., Botto, R. E., Muntean, J. V., Michels, B., and Anderson, K. B., 1997. The nature and fate of natural resins in the geosphere – VIII. NMR and Py-GC-MS characterization of soluble Labdanoid polymers, isolated from Holocene class I resins. *Organic Geochemistry*, **27**, 449–464.
- Cruickshank, R. D., and Ko, K., 2003. Geology of an amber locality in the Hukawng valley, Northern Myanmar. *Journal of Asian Earth Sciences*, **21**, 441–445.
- Girard, V., Schmidt, A. R., Saint Martin, S., Struwe, S., Perrichot, V., Saint Martin, J.-P., Grosheny, D., Breton, G., and Néraudeau, D., 2008. Evidence for marine microfossils from amber. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17426–17429.
- Gomez, B., Martinez-Delclos, X., Bamford, M., and Philippe, M., 2002. Taphonomy and palaeoecology of plant remains from the oldest African Early Cretaceous amber locality. *Lethaia*, **35**, 300–308.
- Grimaldi, D. A., 1996. *Amber: Window to the Past*. New York: Harry N. Abrams Inc. in Association with the American Museum of Natural History.
- Grimaldi, D. A., Engel, M. S., and Nascimbene, P. C., 2002. Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates*, **3361**, 1–72.
- Grimalt, J. O., Simoneit, B. R. T., Hatcher, P. G., and Nissenbaum, A., 1988. The molecular composition of ambers. *Organic Geochemistry*, **13**, 677–690.
- Henwood, A., 1993. Recent plant resins and the taphonomy of organisms in amber: a review. *Modern Geology*, **19**, 35–59.
- Iturralde-Vinent, M. A., and MacPhee, R. D. E., 1996. Age and paleogeographical origin of Dominican amber. *Science*, **273**, 1850–1852.
- Knuth, G., Koch, T. h., Rappsilber, I., and Volland, L., 2002. Concerning amber in the Bitterfeld Region – geologic and genetic aspects. *Hallesches Jahrbuch für Geowissenschaften*, **24**, 35–46.
- Kosmowska-Ceranowicz, B., 1984. Amber and its origins. In Kosmowska-Ceranowicz, B. (ed.), *Amber in Nature*. Warsaw: PAN Muzeum Ziemi, pp. 17–18.
- Kosmowska-Ceranowicz, B., 1999. Succinite and some other fossil resins in Poland and Europe (deposits, finds, features and differences in IRS). *Estudios del Museo de Ciencias Naturales de Alava*, **14**(Número especial 2), 73–117.
- Krumbiegel, G., and Krumbiegel, B., 2005. *Bernstein. Fossile Harze aus aller Welt*. Wiebelsheim, Germany: Quelle & Meyer.
- Lambert, J. B., and Frye, J. S., 1982. Carbon functionalities in amber. *Science*, **217**, 55–57.
- Lambert, J. B., and Poinar, G. O., Jr., 2002. Amber: the organic gemstone. *Accounts of Chemical Research*, **35**, 628–636.
- Langenheim, J. H., 1969. Amber: a botanical inquiry. *Science*, **163**, 1157–1169.
- Langenheim, J. H., 2003. *Plant Resins. Chemistry, Evolution, Ecology, Ethnobotany*. Portland: Timber Press.
- Litwin, R. J., and Ash, S. R., 1991. First early Mesozoic amber in the western hemisphere. *Geology*, **19**, 273–276.
- Nel, A., De Plöeg, G., Dejaj, J., Dutheil, D., de Franceschi, D., Gheerbrant, E., Godinot, M., Hervet, S., Menier, J. J., Augé, M., Bignot, G., Cavagnetto, C., Duffaud, S., Gaudant, J., Hua, S., Jossang, A., De Lapparent de Broin, F., Pozzi, J. P., Paicheler, J. C., Bouchet, F., and Rage, J. C., 1999. Un gisement sparnacien exceptionnel à plantes, arthropodes et vertébrés (Éocène basal, MP7): Le Quesnoy (Oise, France). *Comptes Rendus de l'Académie des Sciences, Sciences de la Terre et des Planètes*, **329**, 65–72.
- Nicholas, C. J., Henwood, A. A., and Simpson, M., 1993. A new discovery of Early Cretaceous (Wealden) amber from the Isle of Wight. *Geological Magazine*, **130**, 847–850.
- Perrichot, V., 2004. Early Cretaceous amber from south-western France: insight into the Mesozoic litter fauna. *Geologica Acta*, **2**, 9–22.
- Perrichot, V., and Girard, V., 2009. A unique piece of amber and the complexity of ancient forest ecosystems. *Palaïos*, **24**, 137–139.
- Perrichot, V., Néraudeau, D., Nel, A., and De Ploëg, G., 2007. A reassessment of the Cretaceous amber deposits from France and their palaeontological significance. *African Invertebrates*, **48**, 213–227.
- Poinar, G. O., Jr., 1992. *Life in Amber*. Palo Alto: Stanford University Press.
- Ragazzi, E., Roghi, G., Giarretta, A., and Gianolla, P., 2003. Classification of amber based on thermal analysis. *Thermochimica Acta*, **404**, 43–54.
- Rikkinen, J., 1999. Two new species of resinicolous *Chaenothecopsis* (Mycocaliciaceae) from western North America. *The Bryologist*, **102**, 366–369.
- Rikkinen, J., and Poinar, G. O., Jr., 2000. A new species of resinicolous *Chaenothecopsis* (Mycocaliciaceae, Ascomycota) from 20 million year old Bitterfeld amber, with remarks on the biology of resinicolous fungi. *Mycological Research*, **104**, 7–15.
- Ritzkowski, S., 1999. Das geologische Alter der bernsteinführenden Sedimente in Sambia (Bezirk Kaliningrad), bei Bitterfeld (Sachsen-Anhalt) und bei Helmstedt (SE-Niedersachsen). In: *Investigations into Amber. Proceedings of the International Interdisciplinary Symposium: Baltic Amber and Other Fossil Resins*, September 2–6, 1997, Gdansk. The Archaeological Museum in Gdansk, Museum of the Earth, Polish Academy of Sciences, Gdansk, pp. 33–40.
- Rodgers, K. A., and Currie, S., 1999. A thermal analytical study of some modern and fossil resins from New Zealand. *Thermochimica Acta*, **326**, 143–149.
- Roghi, G., Ragazzi, E., and Gianolla, P., 2006. Triassic amber of the southern Alps (Italy). *Palaïos*, **21**, 143–154.
- Schmidt, A. R., and Dilcher, D. L., 2007. Aquatic organisms as amber inclusions and examples from a modern swamp forest. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16581–16585.
- Schmidt, A. R., Ragazzi, E., Coppellotti, O., and Roghi, G., 2006. A microworld in Triassic amber. *Nature*, **444**, 835.
- Solórzano Kraemer, M. M., 2007. Systematic, palaeoecology, and palaeobiogeography of the insect fauna from the Mexican amber. *Palaeontographica Abteilung A*, **282**, 1–133.
- Stoppani, A., 1886. *L'ambra nella storia e nella geologia, con speciale riguardo agli antichi popoli d'Italia nei loro rapporti colle origini e collo svolgimento della civiltà in Europa*. Milan, Italy: Dumolard.
- Tonidandel, L., Ragazzi, E., and Traldi, P., 2009. Mass spectrometry in the characterization of ambers. II. Free succinic acid in fossil resins of different origins. *Rapid Communication in Mass Spectrometry*, **23**, 403–408.
- Vonk, R., and Schram, F. R., 2007. Three new tanaid species (Crustacea, Peracarida, Tanaidacea) from the Lower Cretaceous Álava amber in Northern Spain. *Journal of Paleontology*, **81**, 1502–1509.

- Wimmer, R., Pester, L., and Eissmann, L., 2006. Das bernsteinführende Tertiär zwischen Leipzig und Bitterfeld. *Mauritiana (Altenburg)*, **19**, 373–421.
- Wolfe, A. P., Tappert, R., Muehlenbachs, K., Boudreau, M., McKellar, R. C., Basinger, J. F., and Garrett, A., 2009. A new proposal concerning the botanical origin of Baltic amber. *Proceedings of the Royal Society B*, **276**, 3403–3412.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Molecular Fossils)
 Diatoms
 Fungi and Lichens
 Geomycology
 Protozoa (Heterotroph, Eukaryotic)

ANAEROBIC OXIDATION OF METHANE WITH SULFATE

Katrin Knittel, Antje Boetius
 Max Planck Institute for Marine Microbiology, Bremen,
 Germany

Definition

Anaerobic oxidation of methane (AOM): microbially mediated oxidation of methane to CO₂ by electron acceptors other than oxygen.

Introduction

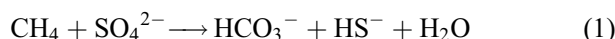
Methane is the most abundant hydrocarbon in the atmosphere, and an important greenhouse gas (see *Methane, Origin*). A great deal of research has focused on the cause and climatic consequences of the variation in fluxes of methane to the atmosphere, throughout the Earth's history. Three key functional groups of microbial organisms play a central role in regulating the fluxes of methane on the Earth, namely the methanogens, the aerobic methanotrophic bacteria, and the more recently discovered anaerobic methanotrophic archaea (ANME). It is estimated that AOM is a major sink for methane on the Earth, and of similar relevance as its photooxidation in the atmosphere (Hinrichs and Boetius, 2002; Reeburgh, 2007).

Today, most methane is produced by methanogenesis, i.e., the final step in the fermentation of organic matter taking place in soils, wetlands, landfills, rice fields, freshwater and marine sediments, as well as in the guts of animals. Almost all of the methane produced in ocean sediments is consumed by AOM within the sulfate penetrated seafloor zones. Hence, the ocean does not contribute significantly to the atmospheric methane budget (<2%; Reeburgh, 2007). The first evidence for the removal of methane within anoxic sediments and seawaters came from

geochemical observations in the 1970s and 1980s (Reeburgh, 1969, 1976; Barnes and Goldberg, 1976; Martens and Berner, 1977). The involvement of sulfate as terminal electron acceptor was demonstrated by radiotracer measurements of methane oxidation and sulfate reduction in parallel incubations of undisturbed sediment samples (Iversen and Jørgensen, 1985). Field observations and experiments led to the hypothesis of a coupled mechanism, in which both methanotrophic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (Hoehler et al., 1994). Finally, consortia of such microorganisms were detected in sediments from methane seeps, and the substantial depletion of their specific biomarkers in the stable carbon isotope ¹³C suggested that methane or a methane-derived compound is used as carbon source by these organisms (Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001b). Till recently, all known anaerobic methanotrophs were clades related to the methanogenic Euryarchaeota termed ANME. In 2008, bacteria were found capable of oxidizing methane anaerobically with nitrate (Ettwig et al., 2008; 2010). In the past decade, much has been learned about the distribution, activity, and physiology of the ANME, however, not a single member of these groups has been obtained in culture and the biochemical functioning of AOM remains unknown.

The process of AOM

The AOM with sulfate as the final electron acceptor according to



is the major sink of methane in the oceans and hence a significant process in the global carbon cycle and methane budget (Reeburgh, 2007). Aggregated methanotrophic archaea (ANME) and SRB are assumed to act syntrophically (Hoehler et al., 1994; Hansen et al., 1998; Hinrichs et al., 1999; Boetius et al., 2000) where the archaeal partner activates and metabolizes methane, leading to an intermediate that is scavenged as electron donor by the sulfate-reducing partner. So far, there is no evidence for the involvement of extracellular intermediates such as hydrogen, formate, or acetate (Nauhaus et al., 2002; Boetius et al., 2008). The process of AOM with sulfate has been maintained in vitro in the laboratory and investigated with respect to substrate-product stoichiometry and dependence on pressure, temperature, and a variety of other environmental factors (Nauhaus et al., 2002, 2005, 2007; Girguis et al., 2003, 2005). The ANME doubling time was reported to be approximately 7 months, and the molar growth yield of AOM was 0.6 g cell dry weight (mol CH₄ oxidized)⁻¹. Corresponding to this estimate, only 1% of the consumed methane is channeled into the ANME biomass

- Wimmer, R., Pester, L., and Eissmann, L., 2006. Das bernsteinführende Tertiär zwischen Leipzig und Bitterfeld. *Mauritiana (Altenburg)*, **19**, 373–421.
- Wolfe, A. P., Tappert, R., Muehlenbachs, K., Boudreau, M., McKellar, R. C., Basinger, J. F., and Garrett, A., 2009. A new proposal concerning the botanical origin of Baltic amber. *Proceedings of the Royal Society B*, **276**, 3403–3412.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Molecular Fossils)
 Diatoms
 Fungi and Lichens
 Geomycology
 Protozoa (Heterotroph, Eukaryotic)

ANAEROBIC OXIDATION OF METHANE WITH SULFATE

Katrin Knittel, Antje Boetius
 Max Planck Institute for Marine Microbiology, Bremen,
 Germany

Definition

Anaerobic oxidation of methane (AOM): microbially mediated oxidation of methane to CO₂ by electron acceptors other than oxygen.

Introduction

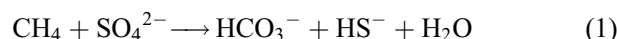
Methane is the most abundant hydrocarbon in the atmosphere, and an important greenhouse gas (see *Methane, Origin*). A great deal of research has focused on the cause and climatic consequences of the variation in fluxes of methane to the atmosphere, throughout the Earth's history. Three key functional groups of microbial organisms play a central role in regulating the fluxes of methane on the Earth, namely the methanogens, the aerobic methanotrophic bacteria, and the more recently discovered anaerobic methanotrophic archaea (ANME). It is estimated that AOM is a major sink for methane on the Earth, and of similar relevance as its photooxidation in the atmosphere (Hinrichs and Boetius, 2002; Reeburgh, 2007).

Today, most methane is produced by methanogenesis, i.e., the final step in the fermentation of organic matter taking place in soils, wetlands, landfills, rice fields, freshwater and marine sediments, as well as in the guts of animals. Almost all of the methane produced in ocean sediments is consumed by AOM within the sulfate penetrated seafloor zones. Hence, the ocean does not contribute significantly to the atmospheric methane budget (<2%; Reeburgh, 2007). The first evidence for the removal of methane within anoxic sediments and seawaters came from

geochemical observations in the 1970s and 1980s (Reeburgh, 1969, 1976; Barnes and Goldberg, 1976; Martens and Berner, 1977). The involvement of sulfate as terminal electron acceptor was demonstrated by radiotracer measurements of methane oxidation and sulfate reduction in parallel incubations of undisturbed sediment samples (Iversen and Jørgensen, 1985). Field observations and experiments led to the hypothesis of a coupled mechanism, in which both methanotrophic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (Hoehler et al., 1994). Finally, consortia of such microorganisms were detected in sediments from methane seeps, and the substantial depletion of their specific biomarkers in the stable carbon isotope ¹³C suggested that methane or a methane-derived compound is used as carbon source by these organisms (Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001b). Till recently, all known anaerobic methanotrophs were clades related to the methanogenic Euryarchaeota termed ANME. In 2008, bacteria were found capable of oxidizing methane anaerobically with nitrate (Ettwig et al., 2008; 2010). In the past decade, much has been learned about the distribution, activity, and physiology of the ANME, however, not a single member of these groups has been obtained in culture and the biochemical functioning of AOM remains unknown.

The process of AOM

The AOM with sulfate as the final electron acceptor according to



is the major sink of methane in the oceans and hence a significant process in the global carbon cycle and methane budget (Reeburgh, 2007). Aggregated methanotrophic archaea (ANME) and SRB are assumed to act syntrophically (Hoehler et al., 1994; Hansen et al., 1998; Hinrichs et al., 1999; Boetius et al., 2000) where the archaeal partner activates and metabolizes methane, leading to an intermediate that is scavenged as electron donor by the sulfate-reducing partner. So far, there is no evidence for the involvement of extracellular intermediates such as hydrogen, formate, or acetate (Nauhaus et al., 2002; Boetius et al., 2008). The process of AOM with sulfate has been maintained in vitro in the laboratory and investigated with respect to substrate-product stoichiometry and dependence on pressure, temperature, and a variety of other environmental factors (Nauhaus et al., 2002, 2005, 2007; Girguis et al., 2003, 2005). The ANME doubling time was reported to be approximately 7 months, and the molar growth yield of AOM was 0.6 g cell dry weight (mol CH₄ oxidized)⁻¹. Corresponding to this estimate, only 1% of the consumed methane is channeled into the ANME biomass

(Nauhaus et al., 2007). Further steps toward an understanding of the metabolic pathway were the purification of a candidate enzyme (Ni-protein I) that may catalyze methane activation in a reverse terminal methyl-coenzyme M reductase reaction (Krüger et al., 2003) and genome-based observations of nearly all genes involved in methane production present in one specific ANME type (Hallam et al., 2004; Meyerdierks et al., 2009) both supporting the hypothesis that ANME carry out AOM by reverse methanogenesis.

Anaerobic methanotrophs

Discovery of ANME

Hinrichs et al. (1999) discovered a new archaeal group in sediments from a methane seep in the Eel River basin off California, together with characteristic archaeal lipid biomarkers strongly depleted in ^{13}C , indicating that methane must be the carbon source (Hinrichs et al., 1999). The dominant archaeal group was defined as ANME-1 and a second major group associated with was identified later as ANME-2 (Orphan et al., 2001a). Boetius et al. (2000) provided the first microscopic evidence for a syntrophic association of ANME archaea and SRB (Boetius et al., 2000). In both studies specific archaeal lipid biomarkers, i.e., archaeol, hydroxyarchaeol, crocetane, and pentamethylcosane (PMI), and bacterial lipid biomarkers common for SRB, i.e., *iso*- and *anteiso*- C_{15} fatty acids, were strongly depleted in ^{13}C . The first direct link between the identity and metabolic activity of ANME was provided by using a combination of ion microprobe mass spectrometry (SIMS) and fluorescence in situ hybridization (FISH) (Orphan et al., 2001b, 2002). These experiments confirmed the extreme ^{13}C depletion in the aggregate biomass, as predicted from the biomarker extractions. In 2006, a third archaeal ANME group, ANME-3, was discovered at the submarine mud volcano Haakon Mosby in the Barents Sea (Niemann et al., 2006b). Recently, a new clade of ANME-associated archaea (AAA) was identified in a bioreactor inoculated with sediments from a Dutch canal, but their relevance in freshwater and marine sediments remains unknown (Raghoebarsing et al., 2006; Knittel and Boetius 2009).

Diversity and morphology of ANME organisms

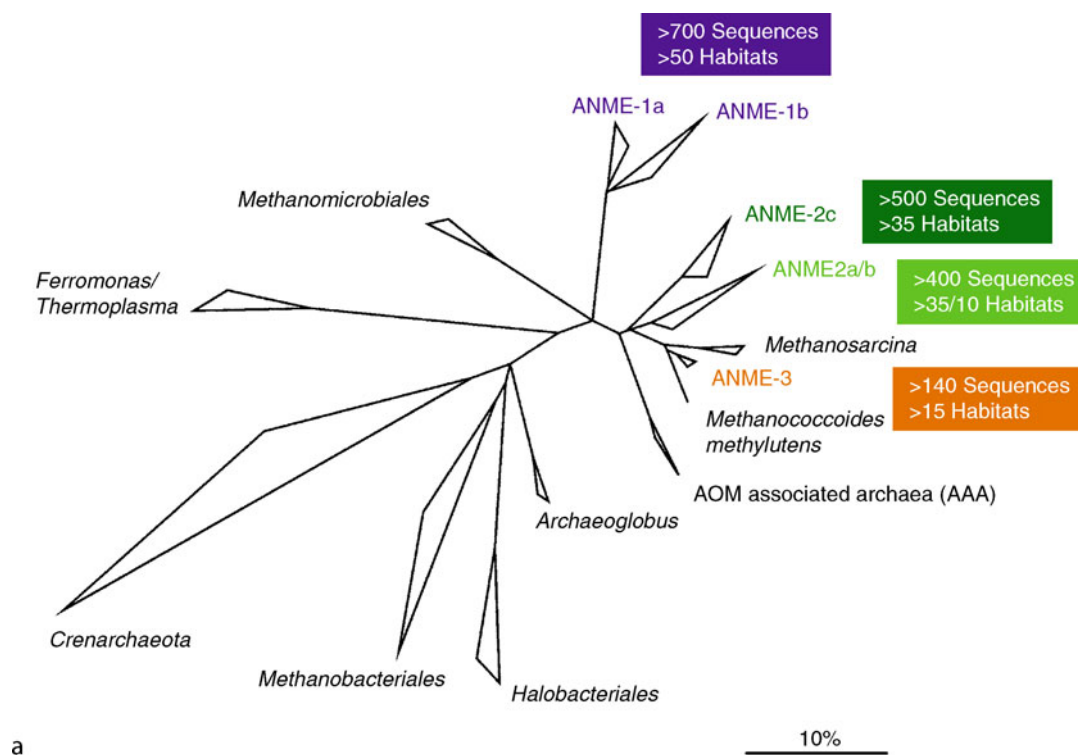
All yet known ANMEs are Euryarchaeota, and they form clusters distantly or closely related to the orders *Methanosarcinales* and *Methanomicrobiales*, which comprise a major part of the cultivated methanogens (Figure 1a).

The phylogenetic distance between the three ANME groups is large with 75–92% sequence similarity. Thus, members of ANME-1 and ANME-2 certainly belong to different orders or families, which have apparently similar physiological properties, i.e., the capability to oxidize methane anaerobically.

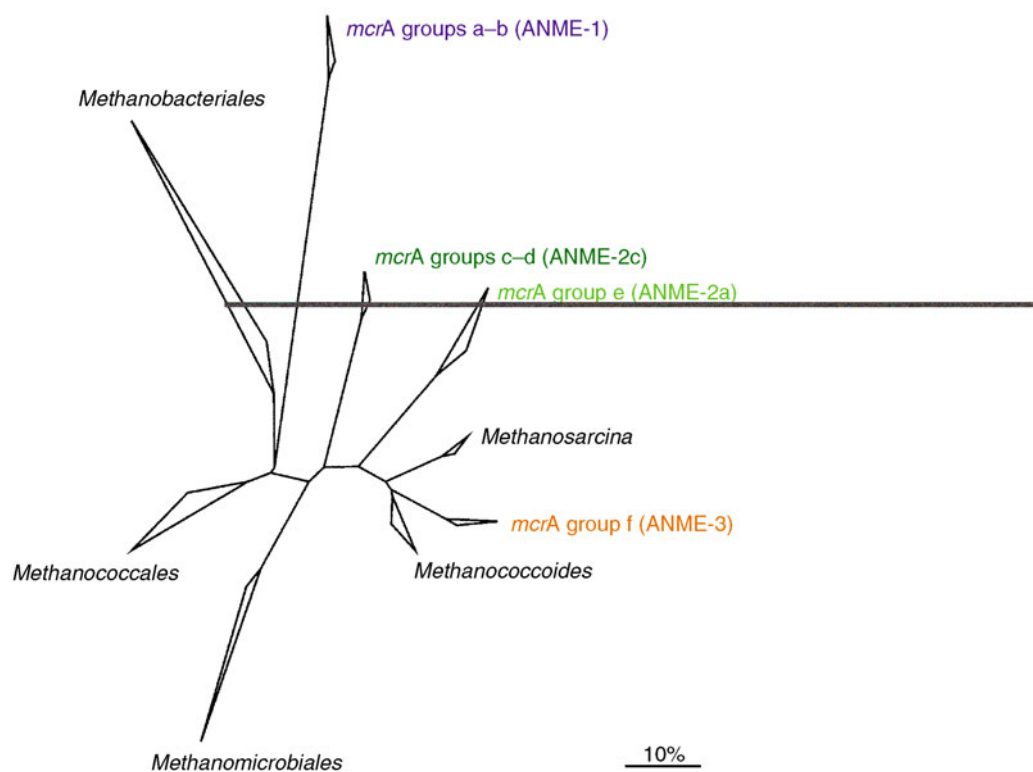
ANME-1 cells have a typical rectangular morphology and are autofluorescent under UV light, a feature characteristic for methanogenic archaea containing coenzyme F_{420} . ANME-1 archaea most often occur as single cells (Figure 2a) or in chains of two to four cells. In the Black Sea, “mat-type” associations of ANME-1 archaea and sulfate-reducing partners (Figure 2b) or ANME-1 and ANME-2 archaea (Figure 2c) form microbial mats (Michaelis et al., 2002; Knittel et al., 2005; Treude et al., 2007).

ANME-2 archaea belong to the order *Methanosarcinales*, but sequence similarity to cultivated representatives is below 90%. Similarity between subgroups, ANME-2a to ANME-2c, varies with an inter- and intra-group similarity of 75–90% and 90–100%, respectively. Diverse forms of associations between ANME-2 and their partner bacteria have been found (Figure 2d–k). Single ANME-2 cells were also reported (Figure 2d, l; Treude et al., 2007) as aggregated ANME-2 without any partner (Figure 2m, n; Orphan et al., 2002; Treude et al., 2007). In addition to the differences in phylogenetic origin, the two subpopulations ANME-2a and -2c could be distinguished from each other by their aggregate morphology. In general, ANME-2a/*Desulfosarcina* (DSS) aggregates represent the “mixed-type.” Here, archaea and SRB are completely mixed and the aggregates are not always spherical (Figure 2f–k). Typical ANME-2c/DSS aggregates represent the common “shell-type” with an inner core of ANME-2 which is partially or fully surrounded by an outer shell of SRB (Figure 2o–x). Sometimes SRB grow into the inner ANME-2c core, but they do not seem to mix completely (Figure 2u–w; Knittel et al., 2005). Microscopical analysis of a growing ANME-2 population showed that population growth occurred both as an increase in the number as well as in size of the shell-type consortia (Nauhaus et al., 2007). Starting from a few archaea and SRB cells, consortia seem to develop from smaller sized consortia to big aggregates of up to 100,000 cells (Holler and Knittel, unpublished data). Larger consortia separate evenly into two or unevenly into more aggregates when SRB grow into the archaeal colonies. The average diameter of consortia is 3–5 μm in situ with the smallest detected aggregate consisting of one ANME and one SRB cell and the largest 20 μm (Boetius et al., 2000; Knittel et al., 2005; Lösekann et al., 2007). Even much larger aggregates have been observed in enrichment cultures with >40 μm in diameter (Nauhaus et al., 2007; Holler, unpublished data). After reaching a specific size they appear to burst, releasing single cells into the environment (Figure 2ab).

The ANME-3 clade also belongs to the order *Methanosarcinales* and is closely related to cultivated species of the genus *Methanococcoides* with 95% 16S rRNA sequence similarity (Figure 1a). They form shell-type aggregates with a *Desulfobulbus* population as sulfate-reducing partner, however, only very few bacterial cells are associated (Figure 2y–z, aa).



a



b

Anaerobic Oxidation of Methane with Sulfate, Figure 1 (Continued)

Identification of ANME by methyl coenzyme M reductase gene phylogeny

The first genomic and proteomic analyses of sediments and microbial mats naturally enriched in the ANME biomass revealed a nickel protein similar to methyl coenzyme M reductase (MCR) as well as genomic fragments coding for nearly all genes typically associated with methane production. Based on these observations, earlier hypotheses on the role of reverse methanogenesis in archaea (Zehnder and Brock, 1979, 1980; Hoehler et al., 1994) appeared in a new light. Subsequently, molecular studies were conducted targeting the enzyme MCR, which catalyzes the terminal step in biogenic methane production (Shima and Thauer, 2005). Investigations have revealed a remarkably high phylogenetic diversity within *mcrA* genes among ANME archaea (Hallam et al., 2003; Inagaki et al., 2004, 2006a; Dhillon et al., 2005; Kelley et al., 2005; Alain et al., 2006; Lloyd et al., 2006; Nunoura et al., 2006; Lösekann et al., 2007) which have been classified into four subgroups (Hallam et al., 2003; Lösekann et al., 2007), group a–b (ANME-1), group c–d (ANME-2c), group e (ANME-2a), and group f (ANME-3) (Figure 1b). These groups are all distinct from those formed by methanogens. ANME *mcrA* gene phylogeny appears to be partially phylogenetically congruent to the 16S rRNA gene (Figure 3; Hallam et al., 2003; Nunoura et al., 2006; Lösekann et al., 2007). Quantification of specific ANME groups based on their *mcrA* gene abundance is now an alternative method to 16S rRNA based FISH to study distributional patterns of anaerobic methanotrophs in AOM zones (Nunoura et al., 2006).

Identification of ANME by their specific lipid biomarkers

Another molecular method, which has been crucial in the investigation of the natural distribution of ANME populations, is the identification of specific lipid biomarkers (see *Biomarkers*) and their stable carbon isotope signatures. This method can integrate phylogenetic information with function (^{13}C signatures indicating methane assimilation) and can be used for comparative analyses of community biomass. All three known ANME groups and their partner bacteria incorporate light (^{13}C -depleted) methane-derived carbon into their membrane lipids (Orphan et al., 2002; Niemann et al., 2006b). Using either naturally ^{13}C depleted methane (Nauhaus et al., 2007) or ^{13}C labeled methane as substrate (Blumenberg et al., 2004) for active populations of ANME in environmental samples have helped to identify typical membrane lipid

profiles. Crocetane is the only biomarker lipid specific for ANME, i.e., not found in methanogen cultures. Glycerol dibiphytanyl glycerol tetraethers (GDGT) were also used as indicator of ANME distribution (Schouten et al., 2003), but the ANME GDGTs appear to show some overlap with those of benthic Crenarchaeota, which often share the same niche in the seabed (Knittel et al., 2005). Recently, the biomarker approach was extended to include intact polar lipids (IPLs) which are of higher taxonomic specificity (level of families to orders) and property to select for living biomass (Sturt et al., 2004; Biddle et al., 2006; Rossel et al., 2008). Characteristic IPL molecular fingerprints have been reported for each specific ANME type: the lipids of ANME-1 archaea were dominated by diglycosidic GDGT derivatives, while IPLs of ANME-2 and ANME-3 archaea are dominated by phosphate-based polar derivatives of archaeol and hydroxyarchaeol (Rossel et al., 2008).

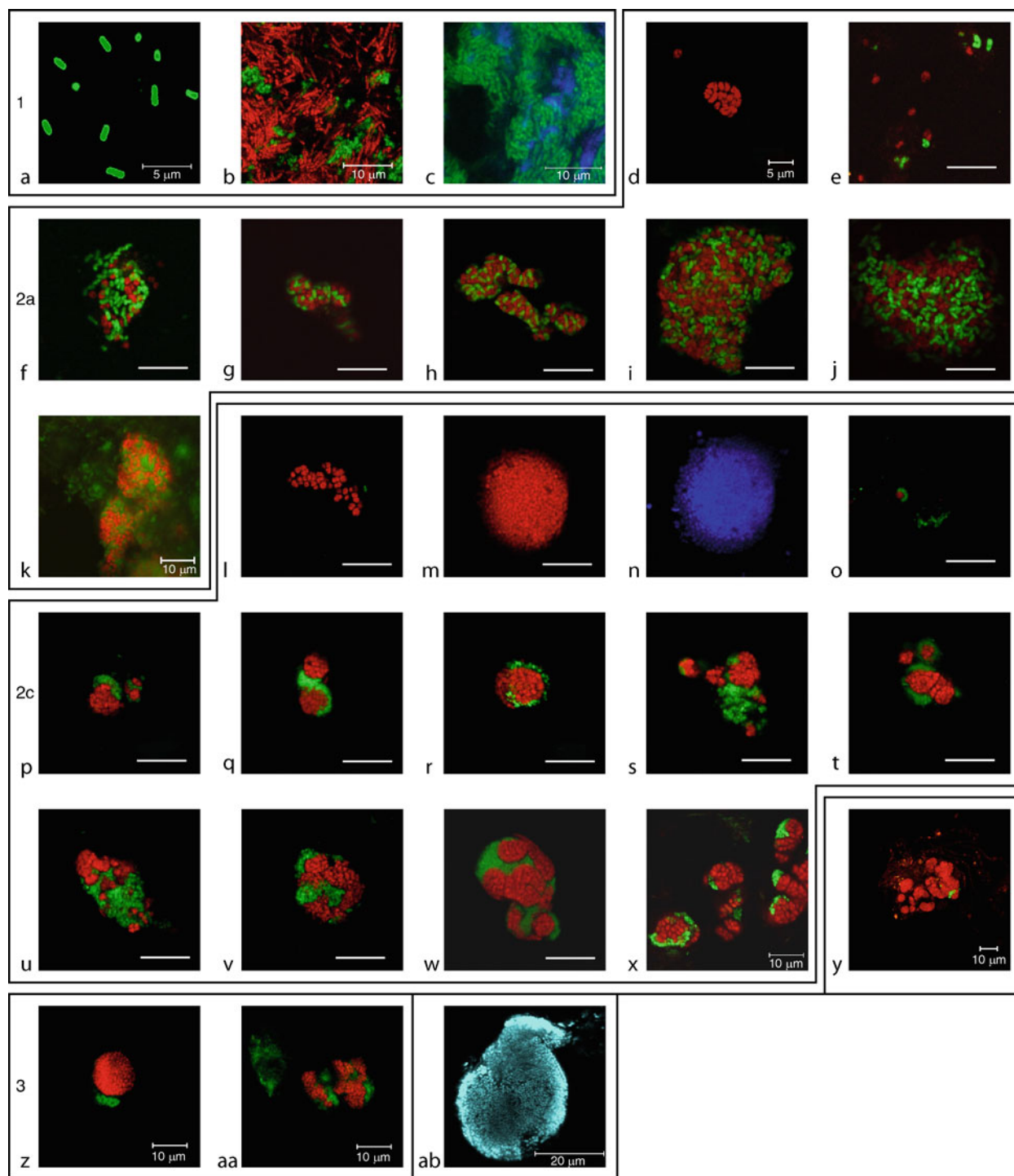
Bacterial partners of ANME

ANME-1 and ANME-2 archaea are usually associated with SRB of the SEEP-SRB I branch, subgroup a, of the *Desulfosarcina/Desulfococcus* (DSS) group (Figure 2; Schreiber et al., 2010).

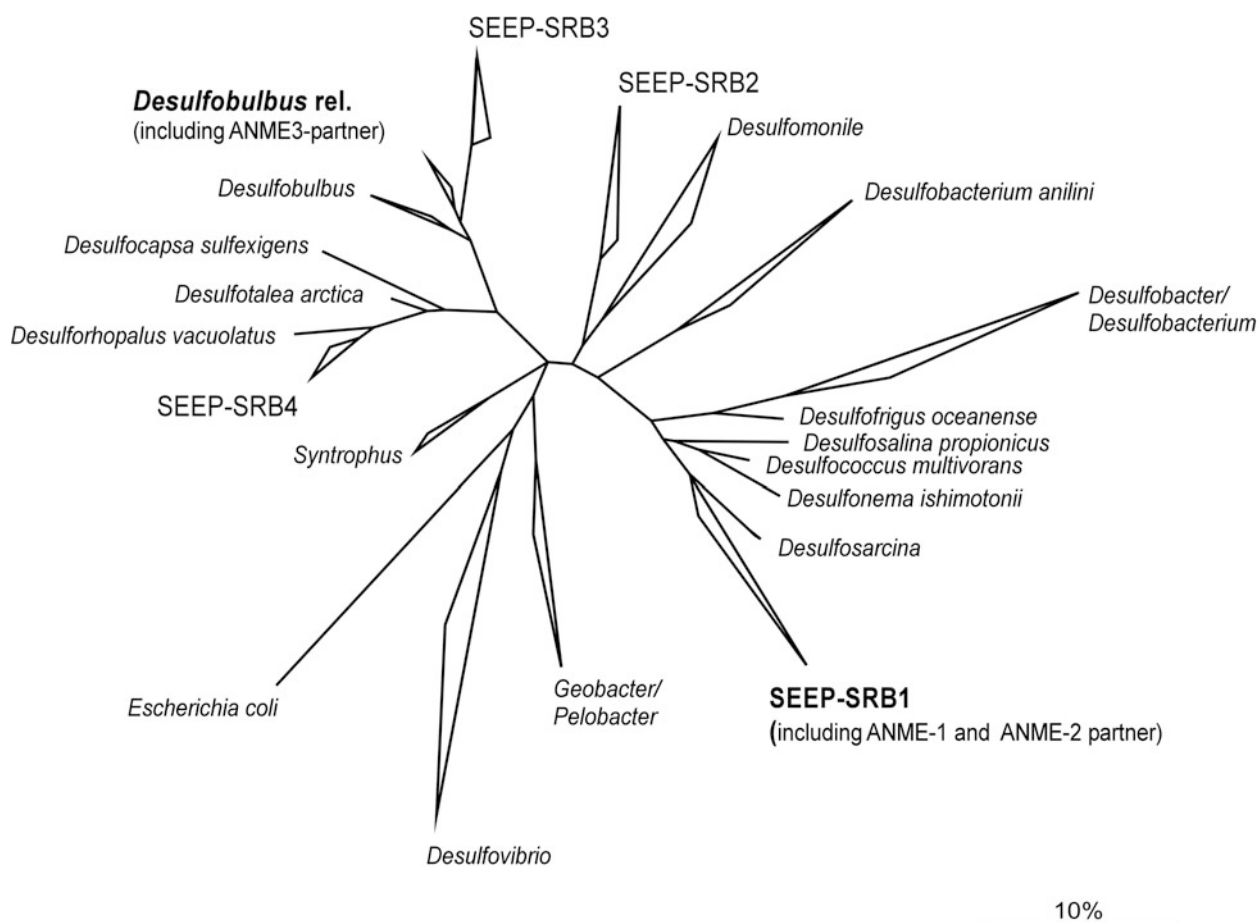
These SRB are physically attached to the ANME-2 archaea, whereas ANME-1 is most often found without close physical association to other cells. The diversity within the SEEP-SRB1 cluster is high, and it is still unknown if the partners of the ANME-2a, -2b, and -2c subgroups belong to the same species. The morphology of the DSS cells varies from cocci (mostly associated with ANME-2c cells, Figure 2p–x) to rods (mostly associated with ANME-2a cells, Figure 2f–j). Other SRB have not yet been identified to be associated with ANME-1 and -2. ANME-3 archaea are most often associated with SRB of the *Desulfobulbus* branch (Figure 2), but have also been detected together with SRB of the *Desulfosarcina/Desulfococcus* group in shallow subsurface sediments of cold seeps from the Hydrate Ridge (Lösekann et al., unpublished data). Single cells of ANME-1, -2, and -3 have also been found in situ as well as in vitro enrichments, indicating that the physical association may be not obligate, but certainly the typical life mode of the anaerobic methanotrophs in most habitats.

There is also some evidence that the diversity of bacteria associated with ANME may be larger than anticipated. The analysis of ANME-2c consortia captured by whole-cell magneto-FISH showed a diversity of bacterial partners of ANME-2 far beyond the Deltaproteobacteria

Anaerobic Oxidation of Methane with Sulfate, Figure 1 Phylogenetic trees showing the affiliations of (a) anaerobic methanotrophic archaea (ANME) 16S rRNA gene sequences to selected reference sequences of the domain archaea. The tree was calculated with nearly full-length sequences (>1,300 bp) by maximum-likelihood analysis in combination with filters excluding highly variable positions. Data in colored boxes give information about the distribution and abundance of sequence retrieval and (b) ANME gene sequences coding for the alpha subunit of methyl coenzyme M reductase (*mcrA*) to selected sequences of the domain archaea. The tree was generated from deduced amino acid sequences (>247 amino acids) by neighbor-joining analysis with a 30% amino acid frequency filter. *mcrA* tree by courtesy of Lösekann. Bar, 10% estimated sequence divergence.



Anaerobic Oxidation of Methane with Sulfate, Figure 2 Epifluorescence micrographs of different ANME single cells and aggregates visualized by fluorescence in situ hybridization (FISH) or CARD-FISH. (a) Single ANME-1 cells living in a microbial mat from the Black Sea; (b) mat-type consortium formed by ANME-1 (red) and DSS cells (green); (c) aggregated ANME-1 (green) and ANME-2 cells (blue) in a microbial mat from the Black Sea; (d) single ANME-2a cells in an enrichment culture from Hydrate Ridge sediments; (e–k) mixed-type consortia of ANME-2a (red) and DSS (green) cells observed in different seep sediments; (l) single ANME-2a cells; (m) monospecific ANME-2a aggregate; (n) corresponding DAPI staining in an enrichment culture from Hydrate Ridge sediments; (o–x) shell-type consortia of ANME-2c (red) and DSS (green) cells of different sizes and structure observed in different seep sediments; (y–aa): ANME-3; (y) monospecific aggregation of ANME-3 at Haakon Mosby mud volcano, (z, aa) ANME-3/*Desulfobulbus* consortia; and (ab): DAPI staining of a large ANME-2c aggregate. Unless otherwise indicated, scale bar 10 μm.



Anaerobic Oxidation of Methane with Sulfate, Figure 3 Phylogenetic tree showing the affiliations of ANME-sulfate-reducing bacteria partner 16S rRNA gene sequences to selected reference sequences of the *Deltaproteobacteria*. The tree was calculated with nearly full-length sequences (>1,450 bp) by maximum-likelihood analysis in combination with filters excluding highly variable positions. Groups known as ANME partners are printed in boldface. Scale bar gives 10% estimated sequence divergence.

(Pernthaler et al., 2008). Lösekann et al. found evidence for ANME-3 aggregates with unidentified partners showing a mixed-type morphology in sediments from the Arctic mud volcano Haakon Mosby (Lösekann et al., 2007).

Habitats of ANME

Since their discovery, the distribution of the ANME organisms has been studied intensively, mainly based on 16S rRNA gene phylogeny. More than 1800 16S rRNA gene sequences are available now from more than 50 different marine methane seeps (Hinrichs et al., 1999; Orphan et al., 2001a; Aloisi et al., 2002; Tourova et al., 2002; Mills et al., 2003, 2004, 2005; Inagaki et al., 2004; Heijs et al., 2005, 2007; Knittel et al., 2005; Lanoil et al., 2005; Stadnitskaia et al., 2005; Arakawa et al., 2006a, b; Fang et al., 2006; Lloyd et al., 2006; Martinez et al., 2006; Niemann et al., 2006a; Nunoura et al., 2006; Reed et al., 2006; Lösekann et al., 2007), vents (Takai and Horikoshi, 1999; Teske et al., 2002; Kelley et al., 2005; Brazelton et al., 2006; Inagaki et al., 2006a), and “sulfate–methane

transition zone” (SMTZ) (Thomsen et al., 2001; Niemann et al., 2005; Parkes et al., 2007) differing in e.g., temperature, methane flux, salinity, and pH. Furthermore, the presence of ANME organisms in the water column (Black Sea; Vetriani et al., 2003; Schubert et al., 2006a) and in a terrestrial mud volcano in the Carpathian mountains (Romania; Alain et al., 2006) has also been reported. A comparison of these reports shows that globally, the two most abundant and diverse phylogenetic groups of methane-oxidizing archaea are the ANME-1 and ANME-2 clades. The distribution of ANME-2a and ANME-2c is as wide as for ANME-1, but only few sequences have been reported belonging to subgroup ANME-2b. ANME-2b, originally defined on the basis of few sequences as a separate group (Orphan et al., 2001a), now seem to be rather a subgroup of ANME-2a than a monophyletic group (Figure 1a).

Today it is known that ANME are present wherever methane and sulfate co-occur, at a wide range of environmental conditions (Knittel and Boetius, 2009). However,

usually only one specific ANME group dominates a habitat and seems to be responsible for most of the measured AOM (Knittel et al., 2005). High rates of methane oxidation, sulfate reduction, and/or sulfide production can be used as an indicator for high densities of ANME populations (Boetius et al., 2008). Continuous high fluxes of both AOM substrates methane and sulfate at mM concentrations sustain high cell densities of $>10^{10}$ ANME cells cm^{-3} in the environment and can even form some of the densest and largest cell accumulations known to exist in nature (Michaelis et al., 2002).

Seep ecosystems

Cold seeps form where tectonic or gravitational forces advect free gas, methane-rich porewater and/or mud upward into the sulfate-penetrated surface sediments and sometimes into the hydrosphere (Judd and Hovland, 2007; see *Cold Seeps*). The increased availability of dissolved methane at higher hydrostatic pressures increases the energy yield of AOM and leads to a natural enrichment of ANME populations. The products of AOM, sulfide and carbonate accumulate in the seabed, forming the typical features of cold seep ecosystems such as carbonate precipitates and high sulfide fluxes. The sulfide is oxidized chemically and microbially with oxygen, nitrate, or iron close to the seafloor surface. One of the most striking visual features of submarine cold seep ecosystems are mats of thiotrophic bacteria covering the seafloor. These mats are usually associated with high AOM rates and dense ANME communities inhabiting the underlying sediments (Sahling et al., 2002; Treude et al., 2003; Joye et al., 2004; Niemann et al., 2006b). Also, a variety of thiotrophic microbe–animal symbioses profit from AOM, such as siboglinid tubeworms and mytilid as well as vesicomyid bivalves (Sibuet and Olu, 1998). Thiotrophic mats and invertebrate symbioses may enhance the growth of ANME populations by rapidly removing the toxic endproduct sulfide, and by replenishing sulfate in the sediments (Treude et al., 2003; Cordes et al., 2005; Niemann et al., 2006b). In active cold seep sediments, AOM rates of $500\text{--}5,000\text{ nmol CH}_4\text{ cm}^{-3}\text{ day}^{-1}$ ($>2\text{ mol m}^{-2}\text{ year}^{-1}$) are reached, associated with very dense ANME populations of $>10^{10}$ cells cm^{-3} (Boetius et al., 2000; Knittel et al., 2005; Niemann et al., 2006b).

Cold seep ecosystems from Eel River Basin (Hinrichs et al., 1999; Orphan et al., 2001b), Hydrate Ridge (Boetius et al., 2000; Knittel et al., 2003, 2005), Black Sea (Thiel et al., 2001, 2003, 2007; Michaelis et al., 2002; Blumenberg et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007), Gulf of Mexico (Orcutt et al., 2005; Lloyd et al., 2006; Martinez et al., 2006), the Tommeliten and Gullfaks area in the North Sea (Wegener et al., 2008), and mud volcanoes from the Mediterranean Sea (Omorgie et al., 2008) and the Barents Sea (Niemann et al., 2006b; Lösekann et al., 2007) have been intensively studied. With the exception of cold seeps in the Black Sea (see next) most of the studies indicate a

dominance of ANME-2 or ANME-3. A prominent example is the gas hydrate bearing sediment from the Hydrate Ridge. Hot spots of ANME-2 are surface layers just above the gas hydrates with up to 10^8 aggregates cm^{-3} . Interestingly, ANME-2 subgroups revealed preferences for either *Beggiatoa* (ANME-2a) or *Calyptogenia* (ANME-2c) fields (Knittel et al., 2005) indicating that different environmental conditions select for different ANME groups.

Two seep systems are known yet where other ANMEs dominate: (1) sediments overlaying a brine pool methane seep in the Gulf of Mexico are dominated by ANME-1b archaea. Here, the ANME-1b community was found in the sulfate-methane interface, where low methane concentrations of ca. 100–250 μM are present and coexist with sulfate concentrations of ca. 10 mM; and (2) microbial mats from Black Sea cold seeps. These seeps are unique ecosystems and AOM is important both in sediments and in the water column (Pimenov et al., 1997; Wakeham et al., 2003). Tall reef-like structures composed of porous carbonates and microbial mats are found on the seafloor. These mats have been shown to mediate AOM and consist mainly of densely aggregated ANME-1 cells and SRB (Michaelis et al., 2002; Blumenberg et al., 2004, 2005; Knittel et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007). Treude et al. (2007) combined radiotracer incubations, beta-microimaging, SIMS, and CARD-FISH to locate hot spots of methanotrophy. Incorporation of ^{14}C from radiolabeled CH_4 indicated a hot spot for methanotrophy close to the mat surface associated with a dominance of ANME-1 archaea (Treude et al., 2007). The mats, however, are very heterogeneous and also provide niches for ANME-2. Black nodules from the top of the reef seem to be dominated by ANME-2 as shown by specific ^{13}C depleted lipids and FISH (Blumenberg et al., 2004). Reitner et al. found intracellular precipitation of iron sulfide (greigite) by bacteria growing in close association with ANME-2 and suggested iron cycling as an additional pathway involved in AOM (Reitner et al., 2005b).

ANME-3 archaea are commonly found at submarine mud volcanoes (Niemann et al., 2006b; Heijs et al., 2007; Lösekann et al., 2007; Omorgie et al., 2008) but also at other cold seeps (Orphan et al., 2001a; Inagaki et al., 2004; Knittel et al., 2005), hydrothermal vents (Brazelton et al., 2006), and recently in subsurface sediments (Parkes et al., 2007).

Sulfate–methane transition zones (SMTZ)

The most common ANME habitat on the Earth is the so-called SMTZ in the seabed (Thomsen et al., 2001; Ishii et al., 2004; Niemann et al., 2005, 2006a; Treude et al., 2005b; Parkes et al., 2007). SMTZ are found in all seabed horizons where the diffusive transport of methane from below and sulfate from above leads to a zone of AOM. Methane is completely consumed in the SMTZ, which may be found at decimeters to tens of meters below the seafloor (D'Hondt et al., 2004), depending on the depth

of the methane production zone, on the transport velocity of methane and sulfate and on their consumption rates. In diffusive seabed systems, the distribution of ANME is restricted to SMTZ. Population densities of ANME are low with $<10^6$ cells cm^{-3} (Niemann et al., 2005) and associated AOM rates are below $10 \text{ nmol cm}^{-3} \text{ day}^{-1}$ ($<0.04 \text{ mol m}^{-2} \text{ year}^{-1}$). The 16S rRNA gene libraries of ANME populations associated with SMTZ zones are very similar to those of cold seeps. The in situ dominance of ANME groups varies between the habitats. In SMTZ, sediments of Eckernförde Bay (German Baltic) ANME-2 archaea dominated (Treude et al., 2005b) as in the Skagerrak off the Danish coast (Parkes et al., 2007) or in a tidal sand flat of the German Wadden Sea (Ishii et al., 2004) while in the SMTZ of the Tommeliten seep area or at Hydrate Ridge ANME-1 were dominant (Niemann et al., 2005; Orcutt et al., unpublished data). Most likely, the SMTZ ANME archaea represent the seed populations for cold seep communities. It remains unknown whether SMTZ ANME possess special physiological adaptations to their energetically less favorable habitat compared to those at cold seeps.

So far, no ANME-related sequences have been published from the deep subsurface (D'Hondt et al., 2004; Newberry et al., 2004; Biddle et al., 2006; Inagaki et al., 2006b; Sorensen and Teske, 2006; Webster et al., 2006). Even in an extensive study by Inagaki et al. sequencing of several thousands of clones obtained from methane hydrate sites from Peru and Cascadia Margin did not result in the retrieval of ANME sequences (Inagaki et al., 2006b). Instead, archaea of the Marine Benthic Group B (MBGB, synonym Deep Sea Archaeal Group, DSAG) and the Miscellaneous Crenarchaeotal Group (MCG) were consistently the dominant phylotypes. Sediments lacking methane hydrates showed no or few MBGB phylotypes. The study by Biddle et al. supported these findings and suggested, based on stable isotopic compositions and intact archaeal lipids that these archaea assimilate organic carbon other than methane (Biddle et al., 2006).

Hydrothermal vents

High methane fluxes are also found at hydrothermal vents of mid ocean ridges (see *Hydrothermal Environments, Marine*), but these ecosystems may not offer many niches for ANME populations. The seafloor of mid ocean ridges consists of basalts and lacks a sediment cover, hence, the habitats for ANME-related organisms are limited to small anoxic zones within vent chimneys (Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006). Furthermore, the fluids of most hydrothermal vents are sulfate free, and the seawater, which could provide sulfate, is oxygen rich – and hence toxic for ANME organisms. Temperatures range from $<40^\circ\text{C}$ to 90°C at pH 9 to 11. By contrast, sedimentary hydrothermal systems such as the Guaymas basin offer suitable habitats within the surface seafloor similar to the cold seep systems (Teske et al.,

2002; Schouten et al., 2003) although AOM accounts for $<5\%$ of sulfate reduction (Kallmeyer and Boetius, 2004). Another extreme environment in which ANME have been recently found is the CO_2 vented sediments of the Yonaguni Knoll hydrothermal field, southern Okinawa Trough, with an in situ pH <5 (Inagaki et al., 2006a).

Water column

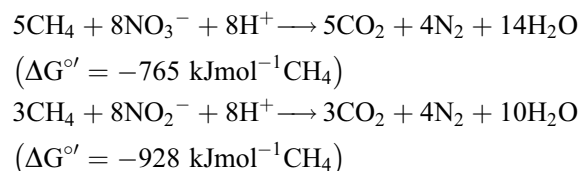
The efflux of methane from cold seeps on the continental margin and mud volcanoes on the abyssal plain make the Black Sea the world's largest surface water reservoir of dissolved methane (Schubert et al., 2006b). Methane concentrations of up to $12 \mu\text{M}$ and AOM rates in the range of nM day^{-1} have been measured (Reeburgh et al., 1991). ANME-1 and ANME-2 archaea are suggested to be responsible for pelagic AOM in the Black Sea (Vetriani et al., 2003; Schubert et al., 2006a, b) accounting each for 3–4% of total cells (Durisch-Kaiser et al., 2005). ANME-1 and ANME-2 appear as single cells in anoxic water, compared to the sediment, where they most often form cell aggregates. Similar to the findings for sediments, one ANME-group dominates, i.e., below approximately 600 m water depth ANME-1 archaea and above 600 m ANME-2 archaea are suggested to be responsible for AOM (Schubert et al., 2006a).

Terrestrial habitats

The presence of ANME has also been reported for terrestrial habitats. ANME-2a archaea are the organisms responsible for AOM activity in a mud volcano field located in the Carpathian Mountains (Romania). Here, thermal alteration of sedimentary organic compounds leads to the release of methane and higher hydrocarbons into the environment (Alain et al., 2006). Additionally, presence of ANME-1 and -2 archaea in a freshwater lake has been reported (Lake Plußsee; northern Germany (Eller et al., 2005)). Long anoxic periods during the summer together with a high organic load in Lake Plußsee favor anaerobic processes in the sediment. In a distinct layer in the anoxic water body, low numbers ($<1\%$) of single ANME archaea were found.

Methane oxidation coupled to denitrification

Methane oxidation could theoretically also be coupled to electron acceptors such as NO_3^- , Fe(III) , and Mn(IV) . Recently, Raghoebarsing et al. (2006) demonstrated AOM coupled to denitrification of nitrate and nitrite in an enrichment culture according to



Anoxic sediment from the Twente canal in the Netherlands was used as inoculum to grow these organisms. The canal contained nitrate concentrations of up to 1 mM and the sediment was saturated with methane, which is typical for freshwater habitats receiving agricultural runoff. The bioreactor biomass was dominated by bacteria of the NC10 clade, a deep branching diverse phylogenetic group (Rappe and Giovannoni, 2003; Raghoebarsing et al., 2006). Recently, the genome of the dominant bacteria *Candidatus Methyloirabilis oxyfera* has been sequenced and surprisingly this apparently anaerobic, denitrifying bacterium encoded, transcribed and expressed the well-established aerobic pathway for methane oxidation, whereas it lacked known genes for dinitrogen production. Isotopic labelling experiments indicated that 'M. oxyfera' bypassed the denitrification intermediate nitrous oxide by the conversion of two nitric oxide molecules to dinitrogen and oxygen, which was used to oxidize methane (Ettwig et al., 2010).

Summary

The AOM is a key biogeochemical process on the Earth, and represents a globally important sink for methane. ANME are present in all environments where methane and sulfate overlap. Unfortunately, no member of the ANME groups or of their sulfate-reducing partner bacteria has been cultivated yet, and the biochemical process is not understood. As long as pure biomass of the archaea is missing, culture-independent molecular tools are the most helpful techniques to investigate the functioning of AOM, including metagenomics and proteomics. Protein patterns of different enrichments help in elucidating the most abundant and hence the most relevant proteins for both partners. Questions to the genome of both partners include for example, whether the ANME themselves may possess the genes for sulfate reduction, and what carbon and energy sources the sulfate reducers could utilize. Answering such questions from metagenomic data sets, however, remains difficult due to the relatively high diversity in the environmental enrichments, and the high proportion of open reading frames in the metagenomes which cannot be assigned to a certain functional category. Here, single cell technologies may provide solutions in the future.

Bibliography

- Alain, K., Holler, T., Musat, F., Elvert, M., Treude, T., and Krüger, M., 2006. Microbiological investigation of methane- and hydrocarbon-discharging mud volcanoes in the Carpathian Mountains, Romania. *Environmental Microbiology*, **8**, 574–590.
- Aloisi, G., Bouloubassi, I., Heijs, S. K., Pancost, R. D., Pierre, C., Damsté, J. S. S., Gottschal, J. C., Forney, L. J., and Rouchy, J.M., 2002. CH₄-consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters*, **203**, 195–203.
- Arakawa, S., Sato, T., Sato, R., Zhang, J., Gamo, T., Tsunogai, U., Hirota, A., Yoshida, Y., Usami, R., Inagaki, F., and Kato, C., 2006a. Molecular phylogenetic and chemical analyses of the microbial mats in deep-sea cold seep sediments at the northeastern Japan Sea. *Extremophiles*, **10**, 311–319.
- Arakawa, S., Sato, T., Yoshida, Y., Usami, R., and Kato, C., 2006b. Comparison of microbial diversity in cold-seep sediments from different depths in the Nankai Trough. *Journal of General and Applied Microbiology*, **52**, 47–54.
- Barnes, R. O., and Goldberg, E. D., 1976. Methane production and consumption in anoxic marine sediments. *Geology*, **4**, 297–300.
- Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sorensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., Sogin, M. L., Brechley, J. E., Teske, A., House, C. H., and Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 3846–3851.
- Blumenberg, M., Seifert, R., Reitner, J., Pape, T., and Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 11111–11116.
- Blumenberg, M., Seifert, R., Nauhaus, K., Pape, T., and Michaelis, W., 2005. In vitro study of lipid biosynthesis in an anaerobically methane-oxidizing microbial mat. *Applied and Environmental Microbiology*, **71**, 4345–4351.
- Boetius, A., Ravensschlag, K., Schubert, C., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Boetius, A., Holler, T., Knittel, K., Felden, J., and Wenzhöfer, F., 2008. The seabed as natural laboratory: lessons from uncultivated methanotrophs. In Epstein, S. S. (ed.), *Uncultivated Microorganisms*. Heidelberg, Germany: Springer.
- Brazelton, W. J., Schrenk, M. O., Kelley, D. S., and Baross, J. A., 2006. Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Applied and Environmental Microbiology*, **72**, 6257–6270.
- Cordes, E. E., Arthur, M. A., Shea, K., Arvidson, R. S., and Fisher, C. R., 2005. Modeling the mutualistic interactions between tubeworms and microbial consortia. *PLoS Biology*, **3**, 1–10.
- Dhillon, A., Lever, M., Lloyd, K. G., Albert, D. B., Sogin, M. L., and Teske, A., 2005. Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes in hydrothermal sediments of the Guaymas Basin. *Applied and Environmental Microbiology*, **71**, 4592–4601.
- D'Hondt, S., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K.-U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegand, J., Padilla, C. N., and Acosta, J. L. S., 2004. Distributions of microbial activities in deep seafloor sediments. *Science*, **306**, 2216–2221.
- Durisch-Kaiser, E., Klauser, L., Wehrli, B., and Schubert, C., 2005. Evidence of intense archaeal and bacterial methanotrophic activity in the Black Sea water column. *Applied and Environmental Microbiology*, **71**, 8099–8106.
- Eller, G., Kanel, L. K., and Krüger, M., 2005. Cooccurrence of aerobic and anaerobic methane oxidation in the water column of Lake Plusssee. *Applied and Environmental Microbiology*, **71**, 8925–8928.
- Ettwig, K. F., Shima, S., van de Pas-Schoonen K. T., Kahnt, J., Medema, M. H., Op den Camp, H. J., Jetten, M. S., Strous, M., 2008. Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. *Environ. Microbiol.* **10**, 3164–3173.
- Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M., Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H. J. C. T., van Alen, T., Luesken, F., Wu, M. L., van de Pas-Schoonen, K. T., Op den

- Camp, H. J. M., Janssen-Megens, E. M., Francoijs, K.-J., Stunnenberg, H., Weissenbach, J., Jetten, M. S. M., Strous, M., 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*, **464**, 543–548.
- Fang, J. S., Shizuka, A., Kato, C., and Schouten, S., 2006. Microbial diversity of cold-seep sediments in Sagami Bay, Japan, as determined by 16S rRNA gene and lipid analyses. *FEMS Microbiology Ecology*, **57**, 429–441.
- Girguis, P. R., Orphan, V. J., Hallam, S. J., and DeLong, E. F., 2003. Growth and methane oxidation rates of anaerobic methanotrophic archaea in a continuous-flow bioreactor. *Applied and Environmental Microbiology*, **69**, 5472–5482.
- Girguis, P. R., Cozen, A. E., and DeLong, E. F., 2005. Growth and population dynamics of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria in a continuous-flow bioreactor. *Applied and Environmental Microbiology*, **71**, 3725–3733.
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F., 2003. Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing archaea. *Applied and Environmental Microbiology*, **69**, 5483–5491.
- Hallam, S. J., Putnam, N., Preston, C. M., Detter, J. C., Rokhsar, D., Richardson, P. M., and DeLong, E. F., 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science*, **305**, 1457–1462.
- Hansen, L. B., Finster, K., Fossing, H., and Iversen, N., 1998. Anaerobic methane oxidation in sulfate depleted sediments: effects of sulfate and molybdate additions. *Aquatic Microbial Ecology*, **14**, 195–204.
- Heijs, S. K., Sinninghe Damsté, J. S., and Forney, L. J., 2005. Characterization of a deep-sea microbial mat from an active cold seep at the Milano mud volcano in the Eastern Mediterranean Sea. *FEMS Microbiology Ecology*, **54**, 47–56.
- Heijs, S. K., Haese, R. R., van der Wielen, P., Forney, L. J., and van Elsas, J. D., 2007. Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microbial Ecology*, **53**, 384–398.
- Hinrichs, K.-U., and Boetius, A., 2002. The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In Wefer, G., Billett, D., Hebbeln, D., Jørgensen, B. B., Schlüter, M., and van Weering, T. (ed.), *Ocean Margin Systems*. Berlin, Heidelberg: Springer, pp. 457–477.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S., 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate reducer consortium. *Global Biogeochemical Cycles*, **8**, 451–463.
- Inagaki, F., Tsunogai, U., Suzuki, M., Kosaka, A., Machiyama, H., Takai, K., Nunoura, T., Nealson, K. H., and Horikoshi, K., 2004. Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, Southern Ryukyu Arc, by analyzing *pmoA*, *mmoX*, *mxrA*, *mcrA*, and 16S rRNA genes. *Applied and Environmental Microbiology*, **70**, 7445–7455.
- Inagaki, F., Kuypers, M. M. M., Tsunogai, U., Ishibashi, J., Nakamura, K., Treude, T., Ohkubo, S., Nakaseama, M., Gena, K., Chiba, H., Hirayama, H., Nunoura, T., Takai, K., Jørgensen, B. B., Horikoshi, K., and Boetius, A., 2006a. Microbial community in a sediment-hosted CO₂ lake of the southern Okinawa Trough hydrothermal system. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14164–14169.
- Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., Nealson, K. H., Horikoshi, K., D'Hondt, S., and Jørgensen, B. B., 2006b. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean margin. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 2815–2820.
- Ishii, K., Mußmann, M., MacGregor, B. J., and Amann, R., 2004. An improved fluorescence in situ hybridization protocol for the identification of bacteria and archaea in marine sediments. *FEMS Microbiology Ecology*, **50**, 203–212.
- Iversen, N., and Jørgensen, B. B., 1985. Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnology and Oceanography*, **30**, 944–955.
- Joye, S. B., Boetius, A., Orcutt, B. N., Montoya, J. P., Schulz, H. N., Erickson, M. J., and Lugo, S. K., 2004. The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. *Chemical Geology*, **205**, 219–238.
- Judd, A., and Hovland, M. (eds.), 2007. *Seabed Fluid Flow*. Cambridge: Cambridge University Press, p. 475.
- Kallmeyer, J., and Boetius, A., 2004. Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Applied and Environmental Microbiology*, **70**, 1231–1233.
- Kelley, D. S., Karson, J. A., Fruh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- Knittel, K., and Boetius, A., 2009. The anaerobic oxidation of methane - progress with an unknown process. *Annual Reviews of Microbiology*, **63**, 311–334.
- Knittel, K., Boetius, A., Lemke, A., Eilers, H., Lochte, K., Pfannkuche, O., Linke, P., and Amann, R., 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, OR). *Geomicrobiology Journal*, **20**, 269–294.
- Knittel, K., Lösekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea (ANME) at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Krüger, M., Meyerderks, A., Glöckner, F. O., Amann, R., Widdel, F., Kube, M., Reinhardt, R., Kahnt, J., Böcher, R., Thauer, R. K., and Shima, S., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature*, **426**, 878–881.
- Lanoil, B. D., La Duc, M. T., Wright, M., Kastner, M., Nealson, K. H., and Bartlett, D., 2005. Archaeal diversity in ODP legacy borehole 892b and associated seawater and sediments of the Cascadia Margin. *FEMS Microbiology Ecology*, **54**, 167–177.
- Lloyd, K. G., Lapham, L., and Teske, A., 2006. An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Applied and Environmental Microbiology*, **72**, 7218–7230.
- Lösekann, T., Knittel, K., Nadalig, T., Fuchs, B., Niemann, H., Boetius, A., and Amann, R., 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby mud volcano, Barents Sea. *Applied and Environmental Microbiology*, **73**, 3348–3362.
- Martens, C. S., and Berner, R. A., 1977. Interstitial water chemistry of Long Island sound sediments, I, dissolved gases. *Limnology and Oceanography*, **22**, 10–25.
- Martinez, R. J., Mills, H. J., Story, S., and Sobecky, P. A., 2006. Prokaryotic diversity and metabolically active microbial populations in sediments from an active mud volcano in the Gulf of Mexico. *Environmental Microbiology*, **8**, 1783–1796.

- Meyerdierks, A., Kube, M., Kostadinov, I., Teeling, H., Glöckner, F. O., Reinhardt, R., and Amann, R., 2009. Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. *Environmental Microbiology*, **12**, 422–439.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., and Gulin, M. B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Mills, H. J., Hodges, C., Wilson, K., MacDonald, I. R., and Sobecky, P. A., 2003. Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. *FEMS Microbiology Ecology*, **46**, 39–52.
- Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A., 2004. Identification of members of the metabolically active microbial populations associated with Beggiatoa species mat communities from Gulf of Mexico cold-seep sediments. *Applied and Environmental Microbiology*, **70**, 5447–5458.
- Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A., 2005. Characterization of microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA-derived clone libraries. *Applied and Environmental Microbiology*, **71**, 3225–3247.
- Nauhaus, K., Boetius, A., Krüger, M., and Widdel, F., 2002. In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. *Environmental Microbiology*, **4**, 296–305.
- Nauhaus, K., Treude, T., Boetius, A., and Krüger, M., 2005. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I- and ANME-II-communities. *Environmental Microbiology*, **7**, 98–106.
- Nauhaus, K., Albrecht, M., Elvert, M., Boetius, A., and Widdel, F., 2007. In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. *Environmental Microbiology*, **9**, 187–196.
- Newberry, C. J., Webster, G., Cragg, B. A., Parkes, R. J., Weightman, A. J., and Fry, J. C., 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, ocean drilling program leg 190. *Environmental Microbiology*, **6**, 274–287.
- Niemann, H., Elvert, M., Hovland, M., Orcutt, B., Judd, A., Suck, I., Gutt, J., Joye, S., Damm, E., Finster, K., and Boetius, A., 2005. Methane emission and consumption at a North Sea gas seep (Tommeliten Area). *Biogeosciences*, **2**, 335–351.
- Niemann, H., Duarte, J., Hensen, C., Omorgie, E., Magalhaes, V. H., Elvert, M., Pinheiro, L. M., Kopf, A., and Boetius, A., 2006a. Microbial methane turnover at mud volcanoes of the Gulf of Cadiz. *Geochimica et Cosmochimica Acta*, **70**, 5336–5355.
- Niemann, H., Lösekann, T., DeBeer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E. J., Schlüter, M., Klages, M., Foucher, J. P., and Boetius, A., 2006b. Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature*, **443**, 854–858.
- Nunoura, T., Oida, H., Toki, T., Ashi, J., Takai, K., and Horikoshi, K., 2006. Quantification of *mcrA* by quantitative fluorescent PCR in sediments from methane seep of the Nankai Trough. *FEMS Microbiology Ecology*, **57**, 149–157.
- Omorgie, E. O., Mastalerz, V., de Lange, G., Straub, K. L., Kappler, A., et al. 2008. Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Chefred Mud volcano (Nile Deep Sea Fan, Eastern Mediterranean). *Applied and Environmental Microbiology*, **74**, 3198–3215.
- Orcutt, B., Boetius, A., Elvert, M., Samarkin, V., and Joye, S. B., 2005. Molecular biogeochemistry of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. *Geochimica et Cosmochimica Acta*, **69**, 4267, 5633–5633.
- Orphan, V. J., Hinrichs, K.-U., Ussler, W. III, Paull, C. K., Taylor, L. T., Sylva, S. P., Hayes, J. M., and DeLong, E. F., 2001a. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Applied and Environmental Microbiology*, **67**, 1922–1934.
- Orphan, V. J., House, C. H., Hinrichs, K.-U., McKeegan, K. D., and DeLong, E. F., 2001b. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., House, C. H., Hinrichs, K.-U., McKeegan, K. D., and DeLong, E. F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7663–7668.
- Parkes, R. J., Cragg, B. A., Banning, N., Brock, F., Webster, G., Fry, J. C., Hornibrook, E., Pancost, R. D., Kelly, S., Knab, N., Jørgensen, B. B., Rinna, J., and Weightman, A. J., 2007. Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environmental Microbiology*, **9**, 1146–1161.
- Pernthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., and Orphan, V. J., 2008. Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 7052–7057.
- Pimenov, N. V., Rusanov, I. I., Poglavzova, M. N., Mityushina, L. L., Sorokin, D. Y., Khmelenina, V. N., and Trotsenko, Y. A., 1997. Bacterial mats on coral-like structures at methane seeps in the Black Sea. *Microbiology (translated from Mikrobiologiya)*, **66**, 354–360.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig, K. F., Rijpstra, W. I. C., Schouten, S., Damsté, J. S. S., Op den Camp, H. J. M., Jetten, M. S. M., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 918–921.
- Rappe, M. S., and Giovannoni, S. J., 2003. The uncultured microbial majority. *Annual Review of Microbiology*, **57**, 369–394.
- Reeburgh, W. S., 1969. Observations of gases in Chesapeake Bay sediments. *Limnology and Oceanography*, **14**, 368–375.
- Reeburgh, W. S., 1976. Methane consumption in Cariaco trench waters and sediments. *Earth and Planetary Science Letters*, **28**, 337–344.
- Reeburgh, W. S., 2007. Oceanic methane biogeochemistry. *Chemical Reviews*, **107**, 486–513.
- Reeburgh, W. S., Ward, B. B., Whalen, S. C., Sandbeck, K. A., Kilpatrick, K. A., and Kerkhof, L. J., 1991. Black Sea methane geochemistry. *Deep Sea Research*, **38** (Supplement 2), S1189–S1210.
- Reed, A. J., Lutz, R. A., and Vetriani, C., 2006. Vertical distribution and diversity of bacteria and archaea in sulfide and methane-rich cold seep sediments located at the base of the Florida escarpment. *Extremophiles*, **10**, 199–211.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005a. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005b. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean Shelf (Black Sea). *Facies*, **51**, 66–79.
- Rossel, P., Fredricks, H., Lipp, J., Arnds, J., Boetius, A., Elvert, M., and Hinrichs, K.-U., 2008. Intact polar lipids of anaerobic methanotrophic archaea and associated bacteria. *Organic Geochemistry*, **39**, 992–999.

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schouten, S., Wakeham, S. G., Hopmans, E. C., and Sinninghe Damsté, J. S., 2003. Biogeochemical evidence that thermophilic archaea mediate the anaerobic oxidation of methane. *Applied and Environmental Microbiology*, **69**, 1680–1686.
- Schreiber, L., Holler, T., Knittel, K., Meyerdierks, A., Amann, R. (in press). Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2 clade. *Environmental Microbiology*.
- Schrenk, M. O., Kelley, D. S., Bolton, S. A., and Baross, J. A., 2004. Low archaeal diversity linked to seafloor geochemical processes at the Lost City hydrothermal field, Mid-Atlantic Ridge. *Environmental Microbiology*, **6**, 1086–1095.
- Schubert, C. J., Coolen, M. J. L., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E. C., Sinninghe Damsté, J. S., Wakeham, S., and Kuypers, M. M. M., 2006a. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**, 1844–1856.
- Schubert, C. J., Durisch-Kaiser, E., Holzner, C. P., Klauser, L., Wehrli, B., Schmale, O., Greinert, J., McGinnis, D. F., DeBastis, M., and Kipfer, R., 2006b. Methanotrophic microbial communities associated with bubble plumes above gas seeps in the Black Sea. *Geochemistry, Geophysics, Geosystems*, **7**, Q04002, doi:10.1029/2005GC001049.
- Shima, S., and Thauer, R. K., 2005. Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic archaea. *Current Opinion in Microbiology*, **8**, 643–648.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep-Sea Research II*, **45**, 517–567.
- Sorensen, K. B., and Teske, A., 2006. Stratified communities of active archaea in deep marine subsurface sediments. *Applied and Environmental Microbiology*, **72**, 4596–4603.
- Stadnitskaia, A., Muyzer, G., Abbas, B., Coolen, M. J. L., Hopmans, E. C., Baas, M., van Weering, T. C. E., Ivanov, M. K., Poludetkina, E., and Sinninghe Damsté, J. S., 2005. Biomarker and 16S rDNA evidence for anaerobic oxidation of methane and related carbonate precipitation in deep-sea mud volcanoes of the Sorokin Trough, Black Sea. *Marine Geology*, **217**, 67–96.
- Sturt, H. F., Summons, R. E., Smith, K., Elvert, M., and Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. *Rapid Communications in Mass Spectrometry*, **18**, 617–628.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Hinrichs, K.-U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S. P., Sogin, M. L., and Jannasch, H. W., 2002. Microbial diversity of hydrothermal sediments in the Guaymas basin: evidence for anaerobic methanotrophic communities. *Applied and Environmental Microbiology*, **68**, 1994–2007.
- Thiel, V., Peckmann, J., Richnow, H. H., Luth, U., Reitner, J., and Michaelis, W., 2001. Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat. *Marine Chemistry*, **73**, 97–112.
- Thiel, V., Blumenberg, M., Pape, T., Seifert, R., and Michaelis, W., 2003. Unexpected occurrence of hopanoids at gas seeps in the Black Sea. *Organic Geochemistry*, **34**, 81–87.
- Thiel, V., Heim, C., Arp, G., Hahmann, U., Sjövall, P., and Lausmaa, J., 2007. Biomarkers at the microscopic range: ToF-SIMS molecular imaging of archaea-derived lipids in a microbial mat. *Geobiology*, **5**, 413–421.
- Thomsen, T. R., Finster, K., and Ramsing, N. B., 2001. Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. *Applied and Environmental Microbiology*, **67**, 1646–1656.
- Tourova, T. P., Kolganov, T. V., Kuznetsov, B. B., and Pimenov, N. V., 2002. Phylogenetic diversity of the archaeal component in microbial mats on coral-like structures associated with methane seeps in the Black Sea. *Microbiology (translated from Mikrobiologiya)*, **71**, 230–236.
- Treude, T., Boetius, A., Knittel, K., Wallmann, K., and Jørgensen, B. B., 2003. Anaerobic oxidation of methane above gas hydrates at Hydrate Ridge, NE Pacific Ocean. *Marine Ecology Progress Series*, **264**, 1–14.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005a. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**, 6375–6378.
- Treude, T., Krüger, M., Boetius, A., and Jørgensen, B. B., 2005b. Environmental control on anaerobic oxidation of methane in the gassy sediments of Eckernförde Bay (German Baltic). *Limnology and Oceanography*, **50**, 1771–1786.
- Treude, T., Orphan, V., Knittel, K., Gieseke, A., House, C., and Boetius, A., 2007. Consumption of methane and CO₂ by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. *Applied and Environmental Microbiology*, **73**, 2271–2283.
- Vettriani, C., Tran, H. V., and Kerkhof, L. J., 2003. Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea. *Applied and Environmental Microbiology*, **69**, 6481–6488.
- Wakeham, S. G., Lewis, C. M., Hopmans, E. C., Schouten, S., and Sinninghe Damsté, J. S., 2003. Archaea mediate anaerobic oxidation of methane in deep euxinic waters of the Black Sea. *Geochimica et Cosmochimica Acta*, **67**, 1359–1374.
- Webster, G., Parkes, R. J., Cragg, B. A., Newberry, C. J., Weightman, A. J., and Fry, J. C., 2006. Prokaryotic community composition and biogeochemical processes in deep subsurface sediments from the Peru Margin. *FEMS Microbiology Ecology*, **58**, 65–85.
- Wegener, G., Shovviti, M., Knittel, K., Niemann, H., Hovland, M., and Boetius, A., 2008. Gullfaks and Tommeliten: microbiological and geochemical description of two of the most active seepage sites of the North Sea. *Biogeosciences*, **5**, 1127–1144.
- Zehnder, A. J. B., and Brock, T. D., 1979. Methane formation and methane oxidation by methanogenic bacteria. *Journal of Bacteriology*, **137**, 420–432.
- Zehnder, A. J. B., and Brock, T. D., 1980. Anaerobic methane oxidation: occurrence and ecology. *Applied and Environmental Microbiology*, **39**, 194–204.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Biosignatures in Rocks](#)
[Carbon \(Organic, Cycling\)](#)
[Cold Seeps](#)
[Fluorescence in Situ Hybridisation \(FISH\)](#)
[Isotopes and Geobiology](#)
[Methane Oxidation \(Aerobic\)](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Nickel, Biology](#)
[Sulfate-Reducing Bacteria](#)

- Yochelson, E. L., and Kisselev, G. N., 2003. Early Cambrian *Salterella* and *Volborthella* (Phylum Agmata) re-evaluated. *Lethaia*, **36**, 8–20.
- Zhuravlev, A. Yu., and Wood, R. A., 2008. Eve of biomineralization: Controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Animal Biocalcification, Evolution
 Breakup of Rodinia
 Ediacaran Biota
 Gondwanaland, Formation
 Origins of the Metazoa
 Trace Fossils: Neoproterozoic

ARCHAEA

Volker Thiel
 University of Göttingen, Göttingen, Germany

Synonyms

Archaeobacteria (term abandoned)

Definition

The Archaea are single-celled or filamentous prokaryotes that constitute the third phylogenetic domain of life, besides the Bacteria and the Eukarya. The word “Archaea” (singular archaeum, archaeon) is derived from the Greek word for “the old ones”.

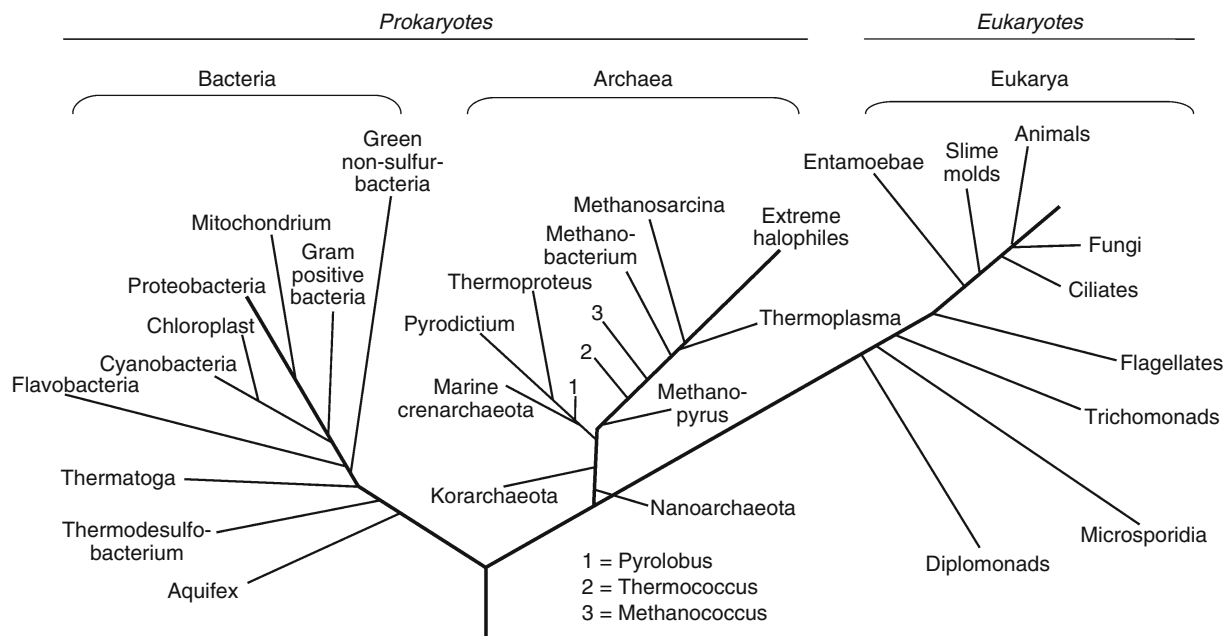
History

The discovery of the Archaea dates back to 1976 when Carl Woese, at his laboratory at Illinois University, compared prokaryotic small subunit ribosomal RNA sequences using oligonucleotide catalogs (Woese, 2007). Woese recognized *Methanobacterium thermoautotrophicum* as the first member of a fundamentally distinct group of prokaryotes that clustered away from all other bacteria. Consequently, Woese and Fox (1977) established the concept of two separate prokaryotic “urkingdoms,” Eubacteria and Archaeobacteria. Later, the term “Archaeobacteria” was changed to “Archaea” to emphasize the fundamental differences between both groups. Based on these discoveries, Woese and his coworkers proposed the now-accepted division of life into the three domains of Archaea, Bacteria, and Eukarya (Figure 1; Woese et al., 1990).

Specific traits

Like the Bacteria, most Archaea show a cell size between 0.5 and 2 μm and lack a cell nucleus, internal membranes, and organelles. However, a number of traits distinguish the Archaea from members of the other domains of life:

1. The genetic machinery of Archaea is different from Bacteria and Eukarya. The distinctive archaeal “signature” contains more than 1,000 archaeal protein-encoding genes. These signature genes account for a significant portion (9–15%) of archaeal genomic DNA (Graham et al., 2000). The archaeal RNA polymerase, an enzyme producing RNA chains from DNA gene templates, consists of eight (methanogens



Archaea, Figure 1 The phylogenetic tree of life. (Redrawn after Madigan and Martinko, 2006.)

- and halophiles) or ten (hyperthermophiles) individual polypeptides. These polymerases are quite different from those of the Bacteria (four polypeptides), but somewhat similar to those of the Eukarya (10–12 polypeptides; Madigan and Martinko, 2006).
2. Unlike bacteria, the cell walls of the Archaea contain no peptidoglycan (a polymer consisting of sugars and amino acids, also known as murein). Rather, archaeal cell walls exhibit a variety of compositions (Kandler and König, 1998). They may consist of the murein analog pseudomurein, polysaccharides, proteins, or glycoproteins (Madigan and Martinko, 2006).
 3. Archaea possess glycerol-based phospholipids as building blocks of their cell membranes, as do bacteria and eukaryotes. However, some features of the archaeal membrane lipids set them apart, namely (a) the stereochemistry of the glycerol moiety (L instead of R), (b) the linkage between the alkyl chains and the glycerol molecules (ether instead of ester bonds), and (c) the design of the alkyl chains (isoprene-based instead of acyl-based; DeRosa and Gambacorta, 1988). In many archaea, these chains are long enough (C₄₀) to span the whole membrane, forming a monolayer instead of the lipid bilayer typical for other organisms (Koga et al., 1993). These isoprenoid lipids, or degradation products thereof, are excellent tracers for archaea-derived organic matter inputs and biogeochemical processes in modern and ancient sediments (Hopmans et al., 2000; Kuypers et al., 2001; Peckmann and Thiel, 2004; see entry *Biomarkers*).

Phylogeny

Most Archaea, and all cultured species, belong to two major, well-established subdivisions, the Euryarchaeota, which include methanogens and extreme halophiles, and the Crenarchaeota, which encompass predominantly hyperthermophiles and anaerobic respirers (Figure 1). Further lineages, namely Korarchaeota (Barns et al., 1996), Nanoarchaeota (Huber and Rachel, 2007), and the Ancient Archaeal Group (Takai and Horikoshi, 1999), have been proposed based on 16S rDNA amplification from environmental samples and/or co-cultivation in mixed laboratory enrichments (Gribaldo and Brochier-Armanet, 2006; Schleper, 2007). However, most of these newly proposed groups still await detailed characterization.

It is as yet unclear at which time in Earth history the domain Archaea arose (Gribaldo and Brochier-Armanet, 2006). Isotopic signatures indicate the presence of methane-cycling archaea 2.8–2.6 billion years ago. Other molecular estimates placed the divergence between Euryarchaeota and Crenarchaeota even 4.1 billion years ago, but this was inferred by using the plant/animal divergence as a calibration point (Battistuzzi et al., 2004). Unequivocal archaeal biosignatures in Precambrian rocks are therefore urgently needed to provide reliable calibration

points for the molecular dating of Archaea and prokaryotes in general (Gribaldo and Brochier-Armanet, 2006).

Ecology

Until recently, it had been thought that archaea are largely restricted to particular ecological niches. Indeed, many archaea are extremophiles (see entry *Extreme Environments*), with growth optima at the most extreme pH values, ranging from pH 1 (*Sulfolobus acidocaldarius*) to pH 12 (*Natronococcus*). Moreover, numerous species are hyperthermophiles, with growth optima above 80°C. The current record for growth temperatures (122°C) is held by a methanogen, *Methanopyrus kandleri* (Takai et al., 2008). Likewise, many halophilic archaea can tolerate extremely high salt concentrations, even at the saturation limit for NaCl (5.5 M; Barns and Nierzwicki-Bauer, 1997; see entry *Halobacteria – Halophiles*). However, ongoing research employing culture-independent molecular techniques and biomarker studies revealed that archaea are by no means restricted to extreme environments. Rather, they are globally distributed and occur in virtually all “normal” settings as well, including soils and freshwater environments (Schleper, 2007), marine planktonic communities (>20% of the total rRNA, >14% of the total cell counts in sea water; Giovannoni and Rappé, 2000), and marine sediments (Lipp et al., 2008). Archaea are also found as partners in symbioses, e.g., with other archaea (Huber and Rachel, 2007), bacteria (Boetius et al., 2000; Stams and Plugge, 2009), ciliates (van Hoek et al., 2000), sponges (Margot et al., 2002), ruminants (Wright et al., 2006), and even in human intestines (Eckburg et al., 2005; see entry *Symbiosis*).

Metabolism

Some examples for biochemical pathways used by the Archaea are given in Table 1.

The Archaea exhibit a wide range of metabolic capacities, however, all members are chemotrophic. Phototrophy, in a strict sense, does not occur among the Archaea, although some halophilic archaea produce pigments, bacteriorhodopsin-like, light driven proton, or ion pumps that allow them to utilize sunlight for biosynthesis, but in a way very different from phototrophic organisms (Madigan and Martinko, 2006). *Chemoheterotrophic* archaea utilize organic substances, particularly metabolic products of other microbes formed during organic matter degradation (Barns and Nierzwicki-Bauer, 1997). These substrates usually consist of simple C₁ and C₂ compounds, such as methanol or acetic acid. Methane and CO₂ are typical metabolic products of archaeal chemoheterotrophy. *Chemolithotrophic* archaea fix inorganic carbon, with hydrogen gas derived from geochemical processes or microbial metabolism being widely used as an energy source (Pedersen, 1997; Berg et al., 2010).

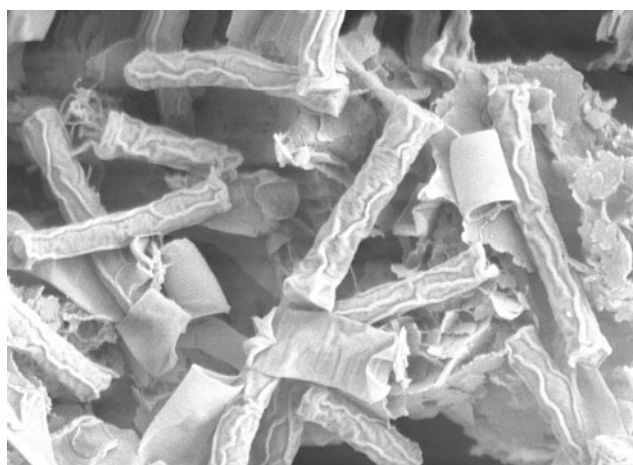
Some archaea perform iron reduction using Fe(III) as an electron acceptor and hydrogen gas or organic compounds as an electron donor (Bond and Lovley, 2002;

Archaea, Table 1 Some examples for biochemical pathways used by archaea

Reaction	
$2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$	Hydrogen oxidation
$\text{H}_2 + \text{S}^0 \rightarrow \text{H}_2\text{S}$	Sulfur reduction
$4\text{H}_2\text{S} + 2\text{O}_2 \rightarrow 4\text{H}_2\text{SO}_4$	Sulfide oxidation
$2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4$	Sulfur oxidation
$\text{H}_2 + \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	Nitrate reduction
$\text{H}_2 + 2\text{Fe}^{3+} \rightarrow 2\text{Fe}^{2+} + 2\text{H}^+$	Iron reduction
$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	Methanogenesis (autotrophic)
$4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O}$	Methanogenesis (heterotrophic)
$4\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow 3\text{CH}_4 + \text{HCO}_3^-$	Methanogenesis ("acetoclastic," heterotrophic)
Organic compound + $\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{CO}_2$	Aerobic respiration

Kashefi et al., 2002; see entry *Fe(III)-Reducing Prokaryotes*). Other members of the Archaea drive pathways of the nitrogen cycle, such as ammonia oxidation, nitrate respiration and denitrification, and assimilatory pathways like N_2 fixation and nitrate assimilation (Cabello et al., 2004; see entry *Nitrogen*). In addition, sulfur is a central component for reactions performed by numerous thermophilic archaea. Elemental sulfur (S^0), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), sulfite (SO_3^-), or sulfate (SO_4^{2-}) may be used as electron acceptors, and elemental sulfur (S^0) or H_2S as electron donors, depending on species and environmental conditions (Barns and Nierzwicki-Bauer, 1997, see entry *Sulfur Cycle*).

Methanogenic archaea (methanogens) are anaerobic euryarchaeotes that produce methane as a metabolic end product of various reactions (Table 1, see entry *Methane, Origin* for details). For several reasons, these archaea are of particularly interest from a geobiological perspective. (a) Chemoorganotrophic methanogens thriving on simple metabolic products of other (micro)organisms such as methanol or acetate (acetoclastic methanogenesis, Table 1) are key players in the degradation of organic matter in sediments (see entry *Carbon (Organic, Degradation)*). (b) Chemolithotrophic methanogens, utilizing only hydrogen gas and CO_2 may thrive in dark and extremely organic-lean environments. (c) Many methanogens are thermophiles (growth temperatures $>60^\circ\text{C}$) or hyperthermophiles (growth temperatures $>80^\circ\text{C}$) with some species being even capable of withstanding temperatures above 100°C (Huber et al., 1990; see entry *Extreme Environments*). Archaea are therefore predestined to pioneering life in extreme settings such as the Deep Biosphere (Hallbeck and Pedersen, 2008), Hot Springs, and Hydrothermal Environments (see these entries). Third, the product of archaeal methanogenesis, "biogenic" methane, may accumulate in sedimentary reservoirs either as free natural gas or, under certain low-temperature/high-pressure conditions, frozen as solid gas hydrates (see entry *Cold Seeps*). Sedimentary methane reservoirs play a crucial role as an energy resource for man, but when released into the atmosphere, methane may act as a strong driving force of global change (Mascarelli, 2009). In as much as methane is an effective



Archaea, Figure 2 Cylindrical cells of archaea belonging to the ANME-1 group of methanotrophic archaea. Individual cells are about $0.5\ \mu\text{m}$ in diameter. (Image courtesy of J. Reitner.)

greenhouse gas being more than 20 times as potent as CO_2 per molecule, it presently contributes more than 20% to the current global greenhouse effect (IPCC, 2007). Several dramatic events in Earth history, such as the Paleocene/Eocene thermal maximum, have been attributed to sudden increases of atmospheric methane concentrations, possibly induced by rapid methane release due to gas hydrate dissolution (Zachos et al., 2008).

Methanotrophic archaea (ANME, *anaerobic methane oxidizers*, Figure 2), organisms that reversed the methanogenic metabolism, have recently been specified as members of sedimentary microbial communities in anoxic sediments. Living together in a syntrophic consortium with sulfate reducing bacteria, ANME oxidize methane by sulfate or nitrate (Raghoebarsing et al., 2006; Knittel and Boetius, 2009; see entry *Anaerobic Oxidation of Methane with Sulfate*).

Biom mineralization

In methane-rich environments, ANME are key players in the formation of calcium carbonate (CaCO_3) deposits, the so-called seep carbonates or methane-derived



Archaea, Figure 3 A Miocene (~16–20 million years old) carbonate deposit that formed as a result of archaeal methanotrophy (Terzi et al., 1994). Croce della Moggiona, Lower Vicchio Formation, Tuscan Apennines, Italy. (Image courtesy of J. Peckmann.)

carbonates (Figure 3; Peckmann and Thiel, 2004; Reitner et al., 2005; see entry *Cold Seeps*). This biologically induced mineralization process is driven by an increase in *Alkalinity* resulting from the anaerobic oxidation of methane. The anaerobic oxidation of methane may also be involved in dolomite ($\text{Ca, Mg}(\text{CO}_3)_2$) precipitation in marine sediments (Moore et al., 2004). Likewise, methanogens have been linked to dolomite formation in shallow groundwater aquifers (Roberts et al., 2004). Recent laboratory experiments identified acetoclastic and autotrophic methanogenesis associated with dolomite formation (Kenward et al., 2009; see entry *Dolomite, Microbial*).

Summary

The Archaea are a group of unicellular prokaryotes representing the third domain of life, in addition to Bacteria and Eukarya. The Archaea differ from the latter by aspects of their genomic machinery, cell wall composition, and membrane lipid structure. Although the timing of their emergence has not been determined, it is believed that the Archaea have been key players within the carbon, nitrogen, and sulfur cycles for the major part of Earth history. Regarding their ecophysiology, individual archaeal groups display unique capacities, such as methanogenesis,

extreme salt tolerance, and survival at temperatures even higher than 100°C. However, environmental surveys revealed the Archaea to be abundant and metabolically versatile not only in extreme environments, but also in “normal” habitats such as soil, oceans, and freshwater. Archaea involved in methane cycling have also been identified as drivers of biologically induced mineralization resulting in (carbonate) rock formation.

Bibliography

- Barns, S. M., Delwiche, C. F., Palmer, J. D., and Pace, N. R., 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 9188–9193.
- Barns, S. M., and Nierzwicki-Bauer, S. A., 1997. Microbial diversity in ocean, surface and subsurface environments. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals. Reviews in Mineralogy and Geochemistry*. Washington, DC: Mineralogical Society of America and Geochemical Society, Vol. 35, pp. 35–79.
- Battistuzzi, F. U., Feijao, A., and Hedges, S. B., 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC Evolutionary Biology*, **4**, 44–58.
- Berg, I. A., Kockelkorn, D., Ramos-Vera, W. H., Say, R. F., Zarzycki, J., Hügler, M., Alber, B. E., and Fuchs, G., 2010. Autotrophic carbon fixation in Archaea. *Nature Reviews Microbiology*, **8**, 447–460.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Bond, D. R., and Lovley, D. R., 2002. Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinines. *Environmental Microbiology*, **4**(2), 115–124.
- Cabello, P., Roldán, M. D., and Moreno-Vivián, C., 2004. Nitrate reduction and the nitrogen cycle in archaea. *Microbiology*, **150**, 3527–3546.
- DeRosa, M., and Gambacorta, A., 1988. The lipids of Archaeobacteria. *Progress in Lipid Research*, **27**, 153–175.
- Eckburg, P. B., Bik, E. M., Bernstein, C. N., et al., 2005. Diversity of the human intestinal microbial flora. *Science*, **308**, 1635–1638.
- Giovannoni, S., and Rappé, M., 2000. Evolution, diversity and molecular ecology of marine prokaryotes. In Kirchman, D. L. (ed.), *Microbial Ecology of the Oceans*. New York: Wiley, pp. 47–84.
- Graham, D. E., Overbeek, R., Olsen, G. J., and Woese, C. R., 2000. An archaeal genomic signature. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 3304–3308.
- Gribaldo, S., and Brochier-Armanet, C., 2006. The origin and evolution of Archaea: a state of the art. *Philosophical Transactions of the Royal Society B*, **361**, 1007–1022.
- Hallbeck, L., and Pedersen, K., 2008. Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. *Applied Geochemistry*, **23**, 1796–1819.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S., 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate

- reducer consortium. *Global Biogeochemical Cycles*, **8**, 451–463.
- Hopmans, E. C., Schouten, S., Pancost, R. D., van der Meer, M. T. J., and Sinninghe Damsté, J. S., 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, **14**, 585–589.
- Huber, H., and Rachel, R., 2007. Nanoarchaeota. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 51–59.
- Huber, R., Stoffers, P., Cheminee, J. L., Richnow, H. H., and Stetter, K. O., 1990. Hyperthermophilic archaeobacteria within the crater and open-sea plume of erupting Macdonald Seamount. *Nature*, **345**, 179–181.
- Intergovernmental Panel on Climate Change (IPCC), Fourth Assessment Report 2007 (AR4); Working Group I Report “The physical science basis”, Ch. 2.
- Kandler, O., and König, H., 1998. Cell wall polymers in Archaea (Archaeobacteria). *Cellular and Molecular Life Sciences*, **54**, 305–308.
- Kashefi, K., Tor, J. M., Holmes, D. E., Gaw Van Praagh, C. V., Reysenbach, A. L., and Lovley, D. R., 2002. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *International Journal of Systematic and Evolutionary Microbiology*, **52**, 719–728.
- Kenward, P. A., Goldstein, R. G., Gonzalez, L. A., and Roberts, J. A., 2009. Precipitation of low-temperature dolomite from an anaerobic microbial consortium: the role of methanogenic Archaea. *Geobiology*, **7**, 556–565.
- Knittel, K., and Boetius, A., 2009. Anaerobic oxidation of methane: progress with an unknown process. *Annual Review of Microbiology*, **63**, 311–334.
- Koga, Y., Nishihara, M., Morii, H., and Akagawa-Matsushita, M., 1993. Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosyntheses. *Microbiological Reviews*, **57**, 164–182.
- Kuypers, M. M. M., Blokker, P., Erbacher, J., Kinkel, H., Pancost, R. D., Schouten, S., and Sinninghe Damsté, J. S., 2001. Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. *Science*, **293**, 92–94.
- Lipp, J. S., Morono, Y., Inagaki, F., and Hinrichs, K. U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature*, **454**, 991–994.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock Biology of Microorganisms*, 11th edn. Englewood Cliffs NJ: Prentice Hall.
- Margot, H., Acebal, C., Toril, E., Amils, R., and Fernandez Puentes, J. L., 2002. Consistent association of crenarchaeal Archaea with sponges of the genus *Axinella*. *Marine Biology*, **140**, 739–745.
- Mascarelli, A. L., 2009. A sleeping giant? *Nature Reports, Climate Change*, **3**, 46–49.
- Moore, T. S., Murray, R. W., Kurtz, A. C., and Schrag, D. P., 2004. Anaerobic methane oxidation and the formation of dolomite. *Earth and Planetary Science Letters*, **229**, 141–154.
- Nicol, G. W., and Schleper, C., 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology*, **14**, 207–212.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Pedersen, K., 1997. Microbial life in deep granitic rock. *FEMS (Federation of European Microbiological Societies) Microbiology Reviews*, **20**, 399–414.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J., Ettwig, K. F., Rijpstra, W. I., Schouten, S., Damsté, J. S., Op den Camp, H. J., Jetten, M. S., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 878–879.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 66–79.
- Roberts, J. A., Bennett, P. C., Gonzalez, L. A., Macpherson, G. L., and Milliken, K. L., 2004. Microbial precipitation of dolomite in methanogenic groundwater. *Geology*, **32**, 277–280.
- Schleper, C., 2007. Diversity of uncultivated Archaea: perspectives from microbial ecology and metagenomics. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 39–53.
- Stams, A. J. M., and Plugge, C. M., 2009. Electron transfer in syntrophic communities of anaerobic bacteria and Archaea. *Nature Reviews Microbiology*, **7**, 568–577.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., and Horikoshi, K., 2008. Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10949–10954.
- Terzi, C., Aharon, P., Ricci Lucchi, F., and Vai, G. B., 1994. Petrography and stable isotope aspects of cold-vent activity imprinted on Miocene-age “calcarei a Lucina” from Tuscan and Romagna Apennines, Italy. *Geo-Marine Letters*, **14**, 177–184.
- van Hoek, A. H. A. M., van Alen, T. A., Sprakel, V. S. I., Leunissen, J. A. M., Brigge, T., Vogels, G. D., and Hackstein, J. H. P., 2000. Multiple acquisition of methanogenic archaeal symbionts by anaerobic ciliates. *Molecular Biology and Evolution*, **17**, 251–258.
- Woese, C. R., 2007. The birth of the Archaea: a personal retrospective. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 1–15.
- Woese, C. R., and Fox, G. E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 5088–5090.
- Woese, C. R., Kandler, O., and Wheelis, M., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4576–4579.
- Wright, A. D. G., Toovey, A. F., and Pimm, C. L., 2006. Molecular identification of methanogenic archaea from sheep in Queensland, Australia reveal more uncultured novel archaea. *Anaerobe*, **12**, 134–139.
- Zachos, J. C., Dickens, G. R., and Zeebe, R. E., 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature*, **451**, 279–283.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Anaerobic Transformation Processes, Microbiology
 Bacteria
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Carbon (Organic, Degradation)
 Chemolithotrophy

[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Dolomite, Microbial](#)
[Extreme Environments](#)
[Halobacteria – Halophiles](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Methane, Origin](#)
[Microbial Biomineralization](#)
[Nitrogen](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

ARSENIC

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Arsenic, element 33, is a metalloid in row VB of the periodic table. The main oxidation states are arsine (−3) elemental As(0), arsenite (+3), and arsenate (+5). Arsenate, As(V), and arsenite, As(III), are most common, with HAsO_4^{2-} and $\text{H}_2\text{AsO}_4^{1-}$ predominating under oxic conditions and H_3AsO^0 and $\text{H}_2\text{AsO}_3^{2-}$ prevalent in anoxic environments. Arsenic is found in minerals such as arsenosulfides (e.g., arsenopyrite, realgar), iron hydroxides and aluminosilicates (e.g., clays), fossil fuels, geothermal sources, and weathered volcanic and evaporitic deposits. Anthropogenic inputs may also be considerable as organoarsenicals such as roxarsone (3-nitro-4-hydroxy benzene arsonic acid) and monosodium and disodium methyl arsonate continue to see wide spread use. The toxicology of arsenic is well known; As(V) is an analog of phosphate and can disrupt oxidative phosphorylation, while As(III) binds to sulfhydryl groups and can inhibit respiratory enzymes. Nevertheless, arsenic is readily metabolized by microorganisms, undergoing redox reactions involved in resistance or energy generation. Organarsenicals are used for energy generation or as a carbon source. Environments with elevated concentrations can support a diverse arsenic metabolizing microbial community and a robust biogeochemical cycle (Oremland and Stolz, 2003).

As(III)-oxidizing prokaryotes are found among the alpha-, beta-, and gamma-proteobacteria, Deinocci (i.e., *Thermus*), and Crenarchaeota. Physiologically diverse, they include heterotrophic and chemolithoautotrophic species. The oxidation of arsenite involves the arsenite oxidase (AoxAB or AroBA), a member of the molybdenum family of enzymes. The gene sequence of the catalytic subunit (*aoxB* or *aroA*) is highly conserved, and oligonucleotide primers have been used to investigate the occurrence and distribution of As(III)-oxidizing

species. The dissimilatory reduction of As(V) has been demonstrated in Crenarchaeota, thermophilic bacteria, gamma-, delta-, and epsilon-proteobacteria, and low and high mole %GC gram positive bacteria. The enzyme, ArrAB, is also a molybdoenzyme, but differs significantly from AoxBA. The *arrA* gene has also been used as an environmental probe. Arsenic resistance involves a completely different type of arsenate reductase (ArsC, ACR2). Three different but comparable resistance systems (e.g., *E. coli*, *Staphylococcus*, *Saccharomyces*) have been described. ArsC catalyzes the reduction of arsenate to arsenite, while an arsenite-specific efflux pump (ArsB, ACR3) expels As(III) from the cell. Additional components include an ATPase (ArsA) and regulatory elements (ArsR, ArsD). Far less is known about arsenic methylation and demethylation, however, a SAM-dependent methylase has recently been described (Stolz et al., 2006).

Different microbially mediated processes can result in arsenic contaminated aquifers including (1) the oxidation of arsenic-containing pyrites, (2) the release of As(V) from reduction of iron oxides by autochthonous organic matter, (3) the reduction of iron oxides by allochthonous organic matter from dissolved organics in recharging waters, (4) the exchange of adsorbed As(V) with fertilizer phosphates, and (5) recharge with water contaminated with inorganic arsenic derived from the application or production of wood preservatives, pesticides (e.g., calcium arsenate), and organoarsenicals (e.g., roxarsone). Thus organisms that metabolize arsenic can impact the speciation, mobility, and toxicity of arsenic in the environment (Oremland and Stolz, 2005).

Bibliography

- Oremland, R. S., and Stolz, J. F., 2003. The ecology of arsenic. *Science*, **300**, 939–944.
 Oremland, R. S., and Stolz, J. F., 2005. Arsenic, microbes, and contaminated aquifers. *Trends in Microbiology*, **13**, 45–49.
 Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

Cross-references

[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Geomycology](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Shewanella](#)
[Sulfide Mineral Oxidation](#)

ASTEROID AND COMET IMPACTS

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The impact of an extraterrestrial object (either an asteroid or comet) with the Earth's surface.

[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Dolomite, Microbial](#)
[Extreme Environments](#)
[Halobacteria – Halophiles](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Methane, Origin](#)
[Microbial Biomineralization](#)
[Nitrogen](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

ARSENIC

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Arsenic, element 33, is a metalloid in row VB of the periodic table. The main oxidation states are arsine (−3) elemental As(0), arsenite (+3), and arsenate (+5). Arsenate, As(V), and arsenite, As(III), are most common, with HAsO_4^{2-} and $\text{H}_2\text{AsO}_4^{1-}$ predominating under oxic conditions and H_3AsO^0 and $\text{H}_2\text{AsO}_3^{2-}$ prevalent in anoxic environments. Arsenic is found in minerals such as arsenosulfides (e.g., arsenopyrite, realgar), iron hydroxides and aluminosilicates (e.g., clays), fossil fuels, geothermal sources, and weathered volcanic and evaporitic deposits. Anthropogenic inputs may also be considerable as organoarsenicals such as roxarsone (3-nitro-4-hydroxy benzene arsonic acid) and monosodium and disodium methyl arsonate continue to see wide spread use. The toxicology of arsenic is well known; As(V) is an analog of phosphate and can disrupt oxidative phosphorylation, while As(III) binds to sulfhydryl groups and can inhibit respiratory enzymes. Nevertheless, arsenic is readily metabolized by microorganisms, undergoing redox reactions involved in resistance or energy generation. Organarsenicals are used for energy generation or as a carbon source. Environments with elevated concentrations can support a diverse arsenic metabolizing microbial community and a robust biogeochemical cycle (Oremland and Stolz, 2003).

As(III)-oxidizing prokaryotes are found among the alpha-, beta-, and gamma-proteobacteria, Deinocci (i.e., *Thermus*), and Crenarchaeota. Physiologically diverse, they include heterotrophic and chemolithoautotrophic species. The oxidation of arsenite involves the arsenite oxidase (AoxAB or AroBA), a member of the molybdenum family of enzymes. The gene sequence of the catalytic subunit (*aoxB* or *aroA*) is highly conserved, and oligonucleotide primers have been used to investigate the occurrence and distribution of As(III)-oxidizing

species. The dissimilatory reduction of As(V) has been demonstrated in Crenarchaeota, thermophilic bacteria, gamma-, delta-, and epsilon-proteobacteria, and low and high mole %GC gram positive bacteria. The enzyme, ArrAB, is also a molybdoenzyme, but differs significantly from AoxBA. The *arrA* gene has also been used as an environmental probe. Arsenic resistance involves a completely different type of arsenate reductase (ArsC, ACR2). Three different but comparable resistance systems (e.g., *E. coli*, *Staphylococcus*, *Saccharomyces*) have been described. ArsC catalyzes the reduction of arsenate to arsenite, while an arsenite-specific efflux pump (ArsB, ACR3) expels As(III) from the cell. Additional components include an ATPase (ArsA) and regulatory elements (ArsR, ArsD). Far less is known about arsenic methylation and demethylation, however, a SAM-dependent methylase has recently been described (Stolz et al., 2006).

Different microbially mediated processes can result in arsenic contaminated aquifers including (1) the oxidation of arsenic-containing pyrites, (2) the release of As(V) from reduction of iron oxides by autochthonous organic matter, (3) the reduction of iron oxides by allochthonous organic matter from dissolved organics in recharging waters, (4) the exchange of adsorbed As(V) with fertilizer phosphates, and (5) recharge with water contaminated with inorganic arsenic derived from the application or production of wood preservatives, pesticides (e.g., calcium arsenate), and organoarsenicals (e.g., roxarsone). Thus organisms that metabolize arsenic can impact the speciation, mobility, and toxicity of arsenic in the environment (Oremland and Stolz, 2005).

Bibliography

- Oremland, R. S., and Stolz, J. F., 2003. The ecology of arsenic. *Science*, **300**, 939–944.
 Oremland, R. S., and Stolz, J. F., 2005. Arsenic, microbes, and contaminated aquifers. *Trends in Microbiology*, **13**, 45–49.
 Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

Cross-references

[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Geomycology](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Shewanella](#)
[Sulfide Mineral Oxidation](#)

ASTEROID AND COMET IMPACTS

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The impact of an extraterrestrial object (either an asteroid or comet) with the Earth's surface.

A

ACETOGENS

Kirsten Küsel¹, Harold L. Drake²

¹Friedrich Schiller University Jena, Jena, Germany

²University of Bayreuth, Bayreuth, Germany

Synonyms

Homoacetogens

Definition

Acetogens are defined as anaerobic prokaryotes that use the acetyl-CoA pathway for the (a) reductive synthesis of the acetyl moiety of acetyl-CoA from CO₂, (b) conservation of energy, and (c) assimilation of CO₂ into biomass.

Introduction

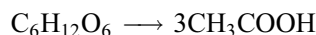
Acetogens utilize the acetyl-CoA “Wood–Ljungdahl” pathway as a terminal electron-accepting, energy-conserving, CO₂-fixing process. The reductive synthesis of acetate from CO₂ differentiates acetogens from organisms that synthesize acetate by other metabolic processes. Although the production of acetate as a sole reduced end product is the classic hallmark of acetogens, the production of acetate is not a part of the definition, because the acetogen might not form acetate in situ or when cultured in the laboratory.

The first acetogen, *Clostridium aceticum*, was isolated from soil by the Dutch microbiologist K. T. Wieringa in 1936. This spore-forming, mesophilic bacterium was shown to grow at the expense of H₂–CO₂ and synthesizes acetate according to the following stoichiometry (Wieringa, 1939–1940):



In 1936, this reaction constituted a unique mechanism for the fixation of CO₂. Unfortunately, the culture of

C. aceticum was lost and no further work was done until it was re-isolated in the early 1980s (Braun et al., 1981). In 1942, F. E. Fontaine and coworkers isolated the second acetogen, *Clostridium thermoaceticum* (later reclassified as *Moorella thermoacetica*), a spore-forming, thermophilic bacterium that catalyzed the near stoichiometric conversion of glucose to acetate (Fontaine et al., 1942):

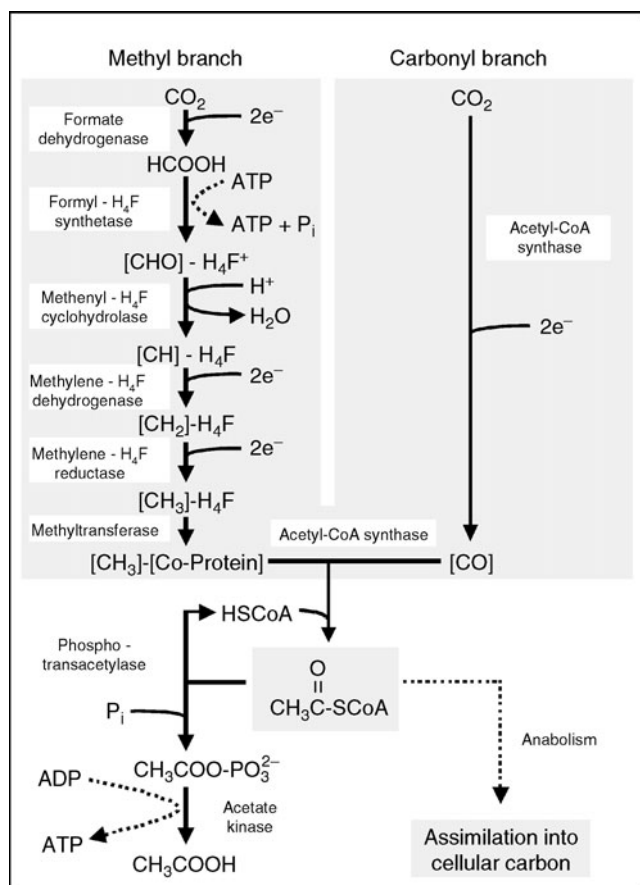


C. thermoaceticum became the most extensively studied acetogen and was used to resolve the enzymology of the acetyl-CoA pathway in the laboratories of two biochemists, H. G. Wood and L. G. Ljungdahl (Drake and Daniel, 2004).

The demonstration that ¹⁴CO₂ was incorporated equally into both carbons of acetate by *C. thermoaceticum* was the first ¹⁴C-study in biology (Barker and Kamen, 1945). The synthesis of acetate from two molecules of CO₂ was later confirmed by Wood using ¹³CO₂ and mass spectrometry (Wood, 1952). Both studies demonstrated that acetogens featured a new autotrophic mechanism for the fixation of CO₂. The acetyl-CoA pathway, also referred to as the Wood–Ljungdahl pathway, is now recognized as a fundamental component of the global carbon cycle, important to primary production in subsurface-ecosystems, and essential to the metabolic potentials of not only acetogens but also methanogens, sulfate-reducers, and anammox bacteria (Wood and Ljungdahl, 1991; Drake, 1994; Schouten et al., 2004).

The acetyl-CoA Wood–Ljungdahl pathway

The acetyl-CoA pathway is a reductive, linear, “one-carbon” process (Figure 1) in contrast to cyclic CO₂-fixing processes (Calvin cycle, reductive tricarboxylic acid cycle, and hydroxypropionate cycle). The two branches of the acetyl-CoA pathway merge at the synthesis of acetyl-CoA that is converted to either acetate or



Acetogens, Figure 1 The acetyl-CoA “Wood-Ljungdahl” pathway. H_4F , tetrahydrofolate; HSCoA , coenzyme A; P_i , inorganic phosphate; e^- , electron; co-protein, corrinoid enzyme; ATP, adenosine-5'-triphosphate. The pathway is from Müller et al. (2004) and used with kind permission of Horizon Bioscience.

assimilated into biomass (Drake, 1994; Drake et al., 2006). Acetyl-CoA synthase (ACS) not only catalyzes the reduction of CO_2 to CO and the synthesis of acetyl-CoA but also oxidizes CO to CO_2 . Thus, the acetyl-CoA synthase is also termed CO dehydrogenase (CODH). Energy conservation occurs during the reductive synthesis of acetate by substrate-level phosphorylation and chemiosmotic processes. For each glucose that is converted to three acetates, four molecules of ATP are produced by substrate-level phosphorylation (ATP_{SLP}). However, when growth occurs under autotrophic conditions, there is no net gain in ATP_{SLP} and growth is dependent upon translocation of protons or sodium ions.

The relatively simple features of the linear acetyl-CoA pathway and the fact that metals and metal sulfides do the biochemical work of CO_2 fixation in the key enzymes of the pathway (i.e., CODH and ACS) suggest that the acetyl-CoA pathway might have been the first autotrophic and early respiratory process on earth and, thus, important in the evolution of life (Wood, 1991; Miyakawa et al.,

2002; Russel and Martin, 2004). It has been proposed that biochemistry started when marine CO_2 from volcanoes and hydrothermal H_2 met at a hydrothermal vent rich in metal sulfides, where an analogue of the exergonic acetyl-CoA pathway catalyzed the synthesis of organic precursors to fuel primordial biochemical reactions.

Phylogenetic diversity of acetogens

Over 100 acetogenic bacterial species have been isolated to date from very diverse habitats, including marine and freshwater sediments; salt lake soda deposits; tundra, forest, and agricultural soils; the subsurface; fecal material; and marine and salt marsh plants (Drake et al., 2006, 2008). Acetogens display extreme genetic diversity, having genomic G + C contents that vary between 22 mol% (*Clostridium ljungdahli*) to 62 mol% (*Holophaga foetida*). Acetogens have been assigned to 22 different bacterial genera including *Acetivibrio*, *Acetobacterium*, *Acetohalobium*, *Acetonebium*, *Bryantella*, “*Butyrivibrio*,” *Caloramator*, *Clostridium*, *Eubacterium*, *Holophaga*, *Moorella*, *Natroninella*, *Natronincola*, *Oxobacter*, *Ruminococcus*, *Sporomusa*, *Syntrophococcus*, *Tindallia*, *Thermoacetogenium*, *Thermoanaerobacter*, and *Treponema* (the name in quotation marks has not been validated). The diverse habitat range of acetogens demonstrates that they are adapted to a broad range of in situ conditions. Many acetogens are sporeformers (e.g., Byrner et al., 2000), a feature that aids in surviving unfavorable in situ conditions, and some acetogens have connecting filaments (Küsel et al., 2001) that might facilitate communication. The usage of the acetyl-CoA pathway for autotrophic assimilation of carbon and acetate utilization by methanogens suggests that Archaea also can grow via acetogenesis. Indeed, the methanogen *Methanosarcina acetivorans* C2A uses the acetyl-CoA pathway to convert carbon monoxide to both acetate and methane, that is, methane is not the sole reduced end product of this methanogen (Rother and Metcalf, 2004; Lessner et al., 2006). Similarly, the archaea *Archaeoglobus fulgidus* VC16 can grow via CO -dependent acetogenesis (Henstra et al., 2008).

Only some acetogenic genera, like *Moorella* and *Sporomusa*, are monophyletic, but many acetogens are phylogenetically dispersed within genera that contain non-acetogenic species. Thus, the phylogenetic position of 16S rRNA gene sequences is usually inadequate for resolving the functional identity of a potential acetogen. To date, only some highly specific 16S rRNA-based probes and primers have been designed to target subsets of acetogenic taxa (Küsel et al., 1999). Molecular approaches based on the analysis of functional genes central to the acetyl-CoA pathway (e.g., a gene for formyltetrahydrofolate synthetase) are still limited due to problems of probe specificity (Leapheart et al., 2003).

Functional diversity of acetogens

Acetogens utilize a wide variety of electron donors and electron acceptors and can engage alternative terminal

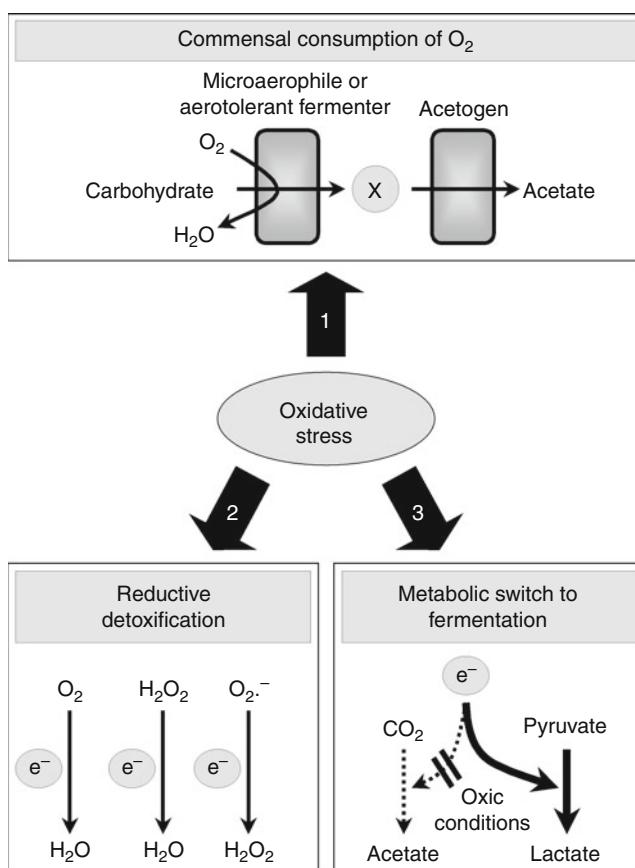
electron-accepting processes when challenged with O_2 (Drake et al., 2006). Many acetogens can utilize one or more terminal electron-accepting processes in addition to acetogenesis. For example, nitrate is a preferred electron acceptor for *M. thermoacetica* and is dissimilated to nitrite and ammonium (Seifritz et al., 1993), and perchlorate is a preferred terminal electron acceptor for *Moorella perchloratireducens* (Balk et al., 2008). The engagement of diverse redox couples enables acetogens to form junction points within and between biological cycles at the ecosystem level (Drake and Küsel, 2005). Acetogens are known to use the following alternative terminal electron acceptors that yield diverse end products listed below:

Fumarate → succinate
 Methoxylated phenylacrylates → methoxylated phenyl propionates
 Nitrate → nitrite
 Nitrite → ammonium
 Thiosulfate → sulfide
 Dimethylsulfoxide → dimethylsulfide
 Perchlorate → chloride
 Chlorate → chloride
 Pyruvate → lactate
 Acetaldehyde → ethanol
 Protons → hydrogen gas

CO , H_2 , carbohydrates, alcohols, carboxylic acids, dicarboxylic acids, aldehydes, substituent groups of various aromatic compounds, and certain halogenated substrates are examples of substrates that acetogens can oxidize. Although hexoses or pentoses are utilized by most of the acetogens isolated to date, none of these isolates appear to be able to degrade high molecular weight polymers, such as cellulose and lignin. Most acetogens have the capacity to produce more than acetate as their sole reduced end product, and this capacity is dependent on the availability of both reductant and terminal electron acceptors, including CO_2 . Thus, referring to acetogens as homoacetogens is usually a misnomer.

In anoxic habitats, acetogens compete with primary fermentors for monomeric compounds that are derived from the initial breakdown of cellulose and lignin and with secondary fermentors for fermentation products. Thermodynamic considerations suggest that acetogenesis should not be a highly competitive microbial process. Nonetheless, acetogens can outcompete methanogens for H_2 in freshwater sediments with low pH and low temperature and in the hindgut of certain termites (Conrad et al., 1989; Breznak and Kane, 1990; Drake et al., 2006). Microbially produced acetate can provide up to 100% of the energy requirement of wood-feeding termites. The attachment of H_2 -consuming acetogenic spirochetes to H_2 -producing protozoa provides H_2 concentrations above the known H_2 -threshold values for acetogens.

Another ecological advantage might be the low oxygen sensitivity of certain acetogens (Figure 2). Acetogens have been classically referred to as obligate, if not strict, anaerobes, and many enzymes central to acetogenesis are



Acetogens, Figure 2 Mechanisms by which acetogens cope with oxidative stress. X, products (e.g., H_2 , formate, lactate) that are derived from the partial oxidation of carbohydrates (in some cases, short-chain polymers [e.g., stachyose] that are not substrates for the acetogen); e^- , electron. (Modified from Müller et al., 2004, and used with kind permission from Horizon Bioscience.)

extremely sensitive to O_2 . However, acetogens have been isolated from oxic habitats, like soils and the rhizosphere of macrophytes that release O_2 through their roots, indicating that such acetogenic species must cope with periods of oxidative stress. Acetogens contain numerous enzymes that can reductively remove O_2 and its toxic by-products (e.g., superoxide and peroxide), when the concentration of O_2 is relatively low (Drake et al., 2006). These enzymes include peroxidase, NADH-oxidase, rubredoxin oxidoreductase (a superoxide reductase), rubrerythrin (a peroxidase), superoxide dismutase, catalase, and cytochrome *bd* oxidase (Das et al., 2001, 2005; Küsel et al., 2001). Acetogens can shift the flow of reductant away from the acetyl-CoA pathway to alternative terminal electron-accepting processes that are less sensitive to O_2 and operate at higher redox potentials than the very low standard redox potential of the CO_2 /acetate half-cell reaction (-290 mV). For example, *Clostridium glycolicum* RD-1 (isolated from seagrass roots) is an aerotolerant acetogen that switches from acetogenesis to

classic fermentation in response to O₂ (Küsel et al., 2001). Acetogens can also form symbiotic relationships with O₂-consuming microaerophiles and aerotolerant fermenters (Göbner et al., 1999). The microaerophile can be a fermentative non-acetogen that has the capacity to consume O₂ and forms fermentation products (e.g., lactate, formate, and H₂) that can be used by the acetogen for acetogenesis. Such interactions can protect acetogens from oxidative stress and form trophic linkages in habitats with fluctuating redox conditions.

Conclusions

It has been estimated that approximately 10¹² kg of acetate is synthesized per year via H₂-driven acetogenesis in sediments and the hindgut of termites (Breznak and Kane, 1990), a number that is fivefold greater than the annual amount of methane produced via the methanogenic reduction of CO₂. Such estimates accentuate the potential importance of acetogens and acetogenesis to the global carbon cycle. However, in situ acetate turnover measurements are complicated and acetogens catalyze a large number of redox reactions, suggesting that their in situ activities are not restricted to acetogenesis. Thus, although acetate forms an important trophic link in a wide variety of ecosystems, in situ information on acetogens and their activities are often theoretical.

Bibliography

- Andreesen, J. R., and Ljungdahl, L. G., 1973. Formate dehydrogenase of *Clostridium thermoaceticum*: incorporation of selenium-75, and the effects of selenite, molybdate, and tungstate on the enzyme. *The Journal of Bacteriology*, **116**, 867–873.
- Balk, M., van Gelder, T., Weelink, S. A., and Stams, A. J. M., 2008. (Per)chlorate reduction by the thermophilic bacterium *Moorella perchloratireducens* sp. nov., isolated from underground gas storage. *Applied and Environmental Microbiology*, **74**, 403–409.
- Barker, H. A., and Kamen, M. D., 1945. Carbon dioxide utilization in the synthesis of acetic acid by *Clostridium thermoaceticum*. *Proceedings of the National Academy of Sciences of the United States of America*, **31**, 219–225.
- Braun, M., Mayer, F., and Gottschalk, G., 1981. *Clostridium acetium* (Wieringa), a microorganism producing acetic acid from molecular hydrogen and carbon dioxide. *Archives of Microbiology*, **128**, 288–293.
- Breznak, J. A., and Kane, M. D., 1990. Microbial H₂/CO₂ acetogenesis in animal guts: nature and nutritional significance. *FEMS Microbiology Reviews*, **7**, 309–313.
- Byer, D. E., Rainey, F. A., and Wiegel, J., 2000. Novel strains of *Moorella thermoacetica* form unusually heat-resistant spores. *Archives of Microbiology*, **174**, 334–339.
- Conrad, R., Bak, F., Seitz, H. J., Thebrath, B., Mayer, H. P., and Schütz, H., 1989. Hydrogen turnover by psychrotrophic homoacetogenic and mesophilic methanogenic bacteria in anoxic paddy soil and lake sediment. *FEMS Microbiology Ecology*, **62**, 285–294.
- Das, A., Coulter, E. D., Kurtz, D. M., and Ljungdahl, L. G., 2001. Five-gene cluster in *Clostridium thermoaceticum* consisting of two divergent operons encoding rubredoxin oxidoreductase-rubredoxin and rubrerythrin-type A flavoprotein-high-molecular-weight rubredoxin. *The Journal of Bacteriology*, **183**, 1560–1567.
- Das, A., Silaghi-Dumitrescu, R., Ljungdahl, L. G., and Kurtz, D. M., Jr., 2005. Cytochrome *bd* oxidase, oxidative stress, and dioxygen tolerance of the strictly anaerobic bacterium *Moorella thermoacetica*. *The Journal of Bacteriology*, **187**, 2020–2029.
- Drake, H. L., 1994. Acetogenesis, acetogenic bacteria, and the acetyl-CoA “Wood/Ljungdahl” pathway: past and current perspectives. In Drake, H. L. (ed.), *Acetogenesis*. New York: Chapman & Hall, pp. 3–60.
- Drake, H. L., and Daniel, S. L., 2004. Physiology of the thermophilic acetogen *Moorella thermoacetica*. *Research in Microbiology*, **155**, 869–883.
- Drake, H. L., and Küsel, K., 2005. Acetogenic clostridia. In Dürre, P. (ed.), *Handbook on Clostridia*. Boca Raton, FL: CRC Press, pp. 719–746.
- Drake, H. L., Küsel, K., and Matthies, C., 2006. Acetogenic prokaryotes. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes*, Vol. 2. New York: Springer-Verlag, pp. 354–420.
- Drake, H. L., Göbner, A. S., and Daniel, S. L., 2008. Old acetogens, new light. *Annals New York Academy of Sciences*, **1125**, 100–128.
- Fontaine, F. E., Peterson, W. H., McCoy, E., and Johnson, M. J., 1942. A new type of glucose fermentation by *Clostridium thermoaceticum* n. sp. *The Journal of Bacteriology*, **43**, 701–715.
- Göbner, A. S., Devereux, R., Ohnemüller, N., Acker, G., Stackebrandt, E., and Drake, H. L., 1999. *Thermicanus aegyptius* gen. nov., sp. nov., isolated from oxic soil, a fermentative microaerophile that grows commensally with the thermophilic acetogen *Moorella thermoacetica*. *Applied and Environmental Microbiology*, **65**, 5124–5133.
- Henstra, A. M., Dijkema, C., and Stams, A. J. M., 2008. *Archaeoglobus fulgidus* couples CO oxidation to sulfate reduction and acetogenesis with transient formate accumulation. *Environmental Microbiology*, **9**, 1836–1841.
- Küsel, K., Pinkart, H. C., Drake, H. L., and Devereux, R., 1999. Acetogenic and sulfate-reducing bacteria inhabiting the rhizosphere and deeper cortex cells of the sea grass *Halodule wrightii*. *Applied and Environmental Microbiology*, **65**, 5117–5123.
- Küsel, K., Karnholz, A., Trinkwalter, T., Devereux, R., Acker, G., and Drake, H. L., 2001. Physiological ecology of *Clostridium glycolicum* RD-1, an aerotolerant acetogen isolated from sea grass roots. *Applied and Environmental Microbiology*, **67**, 4734–4741.
- Leaphart, A. B., Friez, M. J., and Lovell, C. R., 2003. Formyltetrahydrofolate synthetase sequences from salt marsh plant roots reveal a diversity of acetogenic bacteria and other bacterial functional groups. *Applied and Environmental Microbiology*, **69**, 693–696.
- Lessner, D. J., Li, L., Li, Q., Rejtar, T., Andreev, V. P., Reichlen, M., Hill, K., Moran, J. J., Karger, B. L., and Ferry, J. G., 2006. An unconventional pathway for reduction of CO₂ to methane in CO-grown *Methanosarcina acetivorans* revealed by proteomics. *Proceedings of the National Academy of Science of the United States of America*, **103**, 17921–17926.
- Ljungdahl, L. G., 1986. The autotrophic pathway of acetate synthesis in acetogenic bacteria. *Annual Reviews of Microbiology*, **40**, 415–450.
- Miyakawa, S., Yamanashi, H., Kobayashi, K., Cleaves, H. J., and Miller, S. L., 2002. Prebiotic synthesis from CO atmospheres: implications for the origins of life. *Proceedings of the National Academy of Science of the United States of America*, **99**, 14628–14631.
- Müller, V., Inkamp, F., Rauwolf, A., Küsel, K., and Drake, H. L., 2004. Molecular and cellular biology of acetogenic bacteria. In Nakano, M., and Zuber, P. (eds.), *Strict and Facultative Anaerobes: Medical and Environmental Aspects*. Norfolk, UK: Horizon Scientific Press, pp. 251–281.

- Rother, M., and Metcalf, W. W., 2004. Anaerobic growth of *Methanosarcina acetivorans* C2A on carbon monoxide: an unusual way of life for a methanogenic archaeon. *Proceedings of the National Academy of Science of the United States of America*, **101**, 16929–16934.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Schouten, S., Strous, M., Kuypers, M. M. M., Irene, W., Rijpstra, C., Baas, M., Schubert, C. J., Jetten, M. S. M., and Damste, J. S. S., 2004. Stable carbon isotopic fractionations associated with inorganic carbon fixation by anaerobic ammonium-oxidizing bacteria. *Applied and Environmental Microbiology*, **70**, 3785–3788.
- Seifritz, C., Daniel, S. L., Gößner, A., and Drake, H. L., 1993. Nitrate as a preferred electron sink for the acetogen *Clostridium thermoaceticum*. *The Journal of Bacteriology*, **175**, 8008–8013.
- Wieringa, K. T., 1939–1940. The Formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. *Antonie Van Leeuwenhoek Journal of Microbiology and Serology*, **6**, 251–262.
- Wood, H. G. 1952. A study of carbon dioxide fixation by mass determination on the types of C^{13} -acetate. *Journal of Biological Chemistry*, **194**, 905–931.
- Wood, H. G. 1991. Life with CO or CO_2 and H_2 as a source of carbon and energy. *Journal of the Federation of American Societies for Experimental Biology*, **5**, 156–163.
- Wood, H. G., and Ljungdahl, L. G., 1991. Autotrophic character of acetogenic bacteria. In Shively, J. M., and Barton, L. L. (eds.), *Variations in Autotrophic Life*. San Diego, CA: Academic, pp. 201–250.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Bacteria](#)
[Carbon \(Organic, Cycling\)](#)
[Fermentation](#)
[Hydrogen](#)
[Methanogens](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)

ACID ROCK DRAINAGE

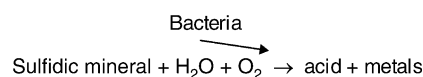
Lesley A. Warren
 McMaster University, Hamilton, ON, Canada

Synonyms

Acid mine drainage; AMD; ARD

Definition

Acid mine and acid rock drainage (AMD/ARD) refer to the extremely acidic ($pH < 3$), metal-rich waters that are derived from the weathering of sulfidic minerals when exposed to air, water, and microorganisms ([Figure 1](#); Nordstrom and Alpers, [1999](#); Bond et al., [2000](#)):



Acid Rock Drainage, Figure 1 An AMD stream at a nickel-copper mine in northern Ontario, Canada. The orange and red colours in the stream are Fe-oxyhydroxide and Fe-sulphate mineral precipitates.

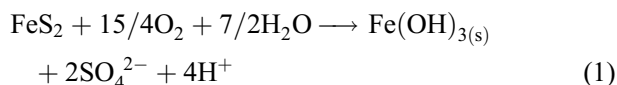
Environmental significance

AMD/ARD is considered to be the most important and widespread global-mining-industry-related pollution problem (Rowe et al., [2007](#)). The main characteristics of ARD/AMD are (1) low pH, (2) high concentrations of dissolved heavy metals, and (3) high concentrations of sulfate (SO_4^{2-} , Tsukanoto et al., [2004](#)). Total dissolved metal concentrations as high as 200,000 mg/L and dissolved SO_4^{2-} as high as 760,000 mg/L have been reported to be associated with AMD (Nordstrom et al., [2000](#)).

AMD/ARD generation

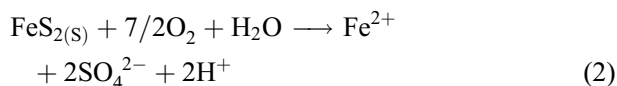
AMD is generated through a combination of chemical and biological (microorganism) processes by which sulfidic minerals are converted to sulfates and iron oxyhydroxides

when exposed to water and oxygen (see [Sulfide Mineral Oxidation](#)). The overall oxidative dissolution of pyrite (FeS_2), a widespread common mine constituent, and associated with AMD generation is given by [Equation 1](#):

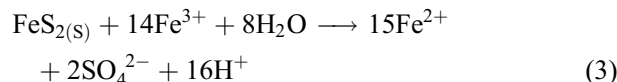


This process progressively increases water acidity, resulting in the mobilization of metals from mine wastes. AMD generation is typically amplified when the reactions involved in the overall process, described in [Equation 1](#), are catalyzed by aerobic Fe- and S-oxidizing bacteria, such as *Acidithiobacilli* (formerly *Thiobacilli*, e.g., *Acidithiobacillus ferrooxidans*; see [Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#); [Sulfide Mineral Oxidation](#)). However, factors such as levels of microorganism activity, pH, sulfide mineral area, crystallography, type of sulfide mineral, temperature, and oxygen concentration all interactively influence the rate of AMD generation (Berghorn and Hunzeker, 2001).

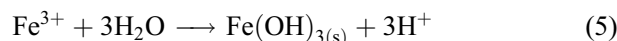
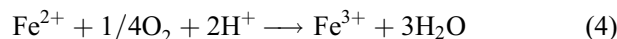
[Figure 2](#) provides a schematic illustrating the overall AMD generation process. Initiation at neutral pH occurs through the release of ferrous iron (Fe^{2+}) and acid (H^+) into solution ([Equation 2](#)):



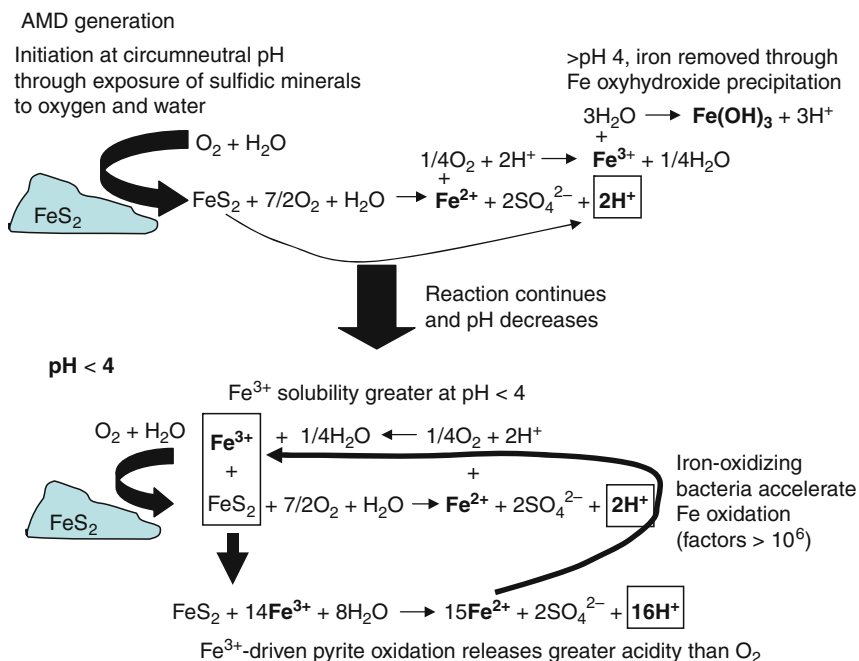
At pH values <4 , increased solubility of ferric iron promotes Fe^{3+} pyrite oxidation ([Equation 3](#)) at far greater rates than those associated with O_2 oxidation:



At pH values greater than 4, O_2 -driven oxidation of ferrous iron to ferric iron ([Equation 4](#)) results in Fe oxyhydroxide formation ([Equation 5](#)) and iron removal through precipitation:



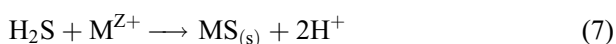
In addition, as the pH decreases below 4, iron-oxidizing bacteria, many of which are adapted to pH values of 2–4, substantively increase rates of Fe oxidation ([Equation 4](#)) by factors greater than 10^6 . For these reasons, the oxidation of Fe^{2+} to Fe^{3+} is often referred to as the rate-determining step in the acid generation process. Thus, once the system reaches pH values less than 4, a self-perpetuating cycle of Fe^{3+} -driven oxidation of pyrite and regeneration of Fe^{3+} through Fe oxidizers is initiated, producing more acidity and Fe^{2+} until either ferric iron or the pyrite waste is depleted ([Figure 2](#)).



Acid Rock Drainage, Figure 2 A schematic of AMD generation based on FeS_2 , indicating the relative importance of O_2 vs Fe^{3+} -driven oxidation rates and the role of microorganisms as a function of pH. At low pH, Fe^{3+} -driven pyrite oxidation releases greater acidity than O_2 -driven pyrite oxidation, which is more favourable at circumneutral pH values.

Treatment of acid mine drainage

AMD waters must be treated to remove metals and raise the pH before they are discharged to a receiving environment (Neculita et al., 2007). Numerous approaches, often characterized as active or passive, exist to treat AMD. Active processes refer to operations that require a relatively high degree of management and continuous input of consumables, while passive processes theoretically require minimal management and other costs once they are established (Johnson, 2006). The most widespread method is active treatment involving the addition of a chemical neutralizing agent or base (i.e., lime or Ca oxide, CaCO_3 , Na/Mg carbonates, Na/Mg hydroxides) to increase water pH and precipitate metals as hydroxides and carbonates. Over the last 20 years, there have been considerable new developments for AMD treatment including constructed wetlands (Johnson and Hallberg, 2002), bioreactors, and permeable reactive barriers (Blowes et al., 2000), which take advantage of the increasing knowledge of microbially driven reactions. Much of the biotechnology developments rely on sulfate-reducing bacteria (SRB) to treat AMD. Fundamentally, bioreactors use SRB to drive sulfide precipitation, removing metals from the AMD solution (Equations 6 and 7):



where M^{Z+} is a cationic metal such as Cu or Ni.

In addition, SRB will drive alkalinity generation (Equation 6) (Koschorreck and Tittel, 2007). However, all emerging treatment approaches are challenged with regard to long-term efficiency. In large part, this reflects the challenges in identifying the complex and dynamic reactions involved in the overall AMD process. The exact mechanisms and controls involved in reactions as well as an understanding and identification of the microbial flora required to efficiently drive desired reactions for bioreactors over the long-term have not yet been elucidated (Edwards et al., 2000; Bernier and Warren, 2005; Bernier and Warren, 2007). In particular, growing evidence of the importance of consortia or mixed microorganism communities in driving AMD-associated processes underscores the need to more fully investigate the interactive roles of bacteria, system pH, $[\text{Fe}]$, $[\text{O}_2]$, and other [metals] involved in the process to narrow the gap in determining effective long-term strategies to mitigate AMD.

Summary

AMD/ARD refer to metal-rich acidic waters that are generated through the exposure of sulfidic minerals in mine wastes to water, oxygen, and microorganisms. These discharges are a significant global pollution issue associated with mining activities. Both sulfur- and iron-oxidizing bacteria are involved with Fe-oxidizing bacteria playing a key role in the increased rates of AMD generation compared to abiotic (nonbiologically stimulated oxidation) sulfidic wastes. AMD must be treated for the high levels of acidity and metals before it can be released to the

environment. New treatment strategies are emerging that include some combination of chemical and biological approaches; however, all are challenged with regard to long-term efficacy. New research evaluating the roles of environmentally mixed communities of microorganisms demonstrates the opportunities to harness environmentally occurring microorganisms to combat AMD.

Bibliography

- Berghom, G. H., and Hunzeker, G. R., 2001. *Passive Treatment Alternatives for Remediating Abandoned-Mine Drainage*. New York: Wiley, pp. 111–127.
- Bernier, L., and Warren, L. A., 2005. Acidity generation in a mine tailings lake. *Geobiology*, **3**, 115–133.
- Bernier, L., and Warren, L. A., 2007. Geochemical diversity in S processes mediated by culture-adapted and environmental enrichments of *Acidithiobacillus* spp. *Geochimica et Cosmochimica Acta*, **71**, 5684–5697.
- Blowes, D. W., Ptacek, C. J., Benner, S. G., McRae, C. W. T., Bennett, T. A., and Puls, R. W., 2000. Treatment of inorganic contaminants using permeable reactive barriers. *Journal of Contaminant Hydrogeology*, **45**, 123–137.
- Bond, P. L., Druschel, G. K., and Banfield, J. K., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Edwards, K. J., Bond, P. L., Druschel, G. K., McGuire, M. M., Hamers, R. J., and Banfield, J. F., 2000. Geochemical and biological aspects of sulfide mineral dissolution: lessons from iron mountain, California. *Chemical Geology*, **169**, 383–397.
- Johnson, D. B., 2006. Biohydrometallurgy and the environment: intimate and important interplay. *Hydrometallurgy*, **83**, 153–166.
- Johnson, D. B., and Hallberg, K. B., 2002. Pitfalls of passive mine water treatment. *Reviews in Environmental Sciences and Biotechnology*, **1**, 335–343.
- Koschorreck, M., and Tittel, J., 2007. Natural alkalinity generation in neutral lakes affected by acid mine drainage. *Journal of Environmental Quality*, **36**, 1163–1171.
- Neculita, C.-M., Zagury, G. J., and Bussiere, B., 2007. Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. *Journal of Environmental Quality*, **36**, 1–16.
- Nordstrom, D. K., and Alpers, C. N., 1999. Geochemistry of acid mine waters. In Plumlee G. S., and Logsdon, M. J. (eds.), *The Environmental Geochemistry of Mineral Deposits. Part A: Processes, Techniques, and Health Issues*. Littleton, CO: The Society of Economic Geologists, pp. 133–1160.
- Nordstrom, D. K., and Southam, G., 1997. The geomicrobiology of acid mine drainage. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals*. Washington, DC: Reviews in Mineralogy Mineralogical Society of America, pp. 361–390.
- Nordstrom, D. K., Alpers, C. N., Ptacek, C. J., and Blowes, D. W., 2000. Negative pH and extremely acidic mine waters from Iron Mountain, California. *Environmental Science and Technology*, **34**, 254–258.
- Rowe, O. F., Sanchez-Espana, J., Hallberg, K. B., and Johnson, D. B., 2007. Microbial communities and geochemical dynamics in an extremely acidic, metal-rich stream at an abandoned sulfide mine (Huelva, Spain) underpinned by two functional primary production systems. *Environmental Microbiology*, **9**, 1761–1771.
- Tsukamoto, T. K., Killion, H. A., and Miller, G. C., 2004. Column experiments for microbiological treatment of acid mine drainage: low temperature, low-pH and matrix investigation. *Water Research*, **38**, 1405–1418.

Cross-references

Biomining (Mineral Bioleaching, Mineral Biooxidation)
Copper
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Iron Sulfide Formation
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation

ACIDOPHILES

“Acidophiles” are organisms thriving in environments below pH 5. For details, see entries “*Extreme Environments*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Hot Springs and Geysers*,” *Hydrothermal Environments (Marine)* and “*Acid Rock Drainage*.”

ACRITARCHS

Acritarchs are organic-walled acid-resistant microfossils known from the Proterozoic and throughout the Phanerozoic. The position of these microfossils is still uncertain. A number of acritarch genera have been assigned to Green Algae; others are considered to bear some resemblance to the cysts of dinoflagellates. For more information, please refer to entries “Algae, Eukaryotic” and “Protozoa.”

AEROBIC METABOLISM

Heribert Cypionka
University of Oldenburg, Oldenburg, Germany

Synonyms

Aerobic respiration; Oxygen metabolism

Definition

Aerobic metabolism comprises the reduction of molecular oxygen as electron acceptor of aerobic respiration, its use as cosubstrate in the degradation of certain compounds, and reactions leading to the detoxification of partially reduced oxygen species.

Introduction

Life has evolved in the absence of molecular oxygen (see Chapter *Early Earth*). Therefore, it is not surprising that basic metabolic pathways involved in growth and cell division (like DNA replication or protein synthesis) do not depend on the presence of molecular O₂. The evolution of the oxygen-producing (oxygenic) phototrophs dramatically changed the situation of living organisms. Molecular oxygen raised the redox potential of the environment and enabled microorganisms to respire with a much higher energy yield than before. Although oxygen is involved in

a limited number of reactions only, its presence or absence has fundamental impact on biogeochemical processes.

Aerobic degradation of organic matter

Aerobic respiration consumes a major part of the photosynthetically produced O₂ and about 90% of the organic matter (see Chapter *Carbon Cycle*). Aerobic (oxygen-metabolizing) organisms normally oxidize their substrates completely to CO₂. In most cases, O₂ is not directly involved in this process. Substrate oxidation generates reduced electron carriers by means of the respiratory chain. Oxygen is reduced as the last step of the respiration process.

Respiratory chain

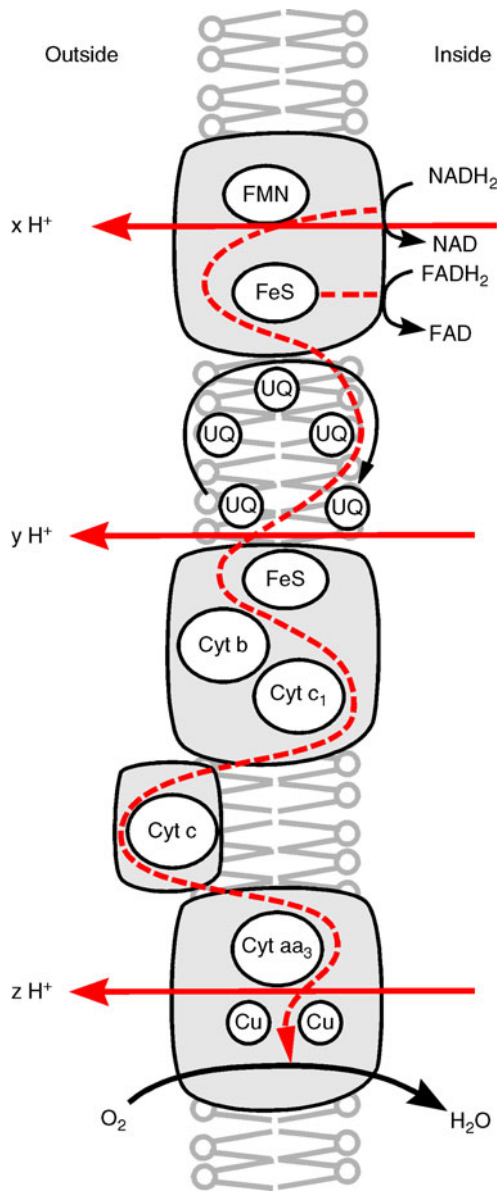
Respiration is a membrane-bound process that generates a chemiosmotic proton gradient across the membrane (Mitchell, 1966). Respiratory chains are composed of series of enzymes, which are organized in multienzyme complexes and use cofactors that allow the stepwise transport of reducing equivalents to oxygen (Figure 1). In eukaryotes (plants, animals, fungi) it takes place within the mitochondria, which are assumed to have evolved from endosymbiotic bacteria. In prokaryotes (Bacteria, Archaea), the respiratory chain is located in the cytoplasmic membrane. The respiratory chain of the bacterium *Paracoccus denitrificans* appears to have many similarities to the mitochondrial type (Harold, 1986). However, many bacteria have variations compared to the mitochondrial type presented in Figure 1.

Energetics and ATP conservation

Most reducing equivalents are fed into the respiration process via NADH₂, which has a standard redox potential (E_0') of -0.32 V. The terminal electron-accepting redox pair O₂/H₂O has redox potential of $+0.82$ V. Thus, the usable redox potential is about 1.14 V. From this redox potential one can calculate a standard $\Delta G_0'$ of -440 kJ per mol of O₂. Assuming that ATP conservation requires about -75 kJ per mol, the conservation of 5 to 6 ATP per O₂ reduced is possible. As a consequence of the high ATP yield, aerobic organisms can form more biomass from the same amount of substrate than anaerobic organisms.

Use of oxygen as cosubstrate

Molecular oxygen is used as a cosubstrate in some catabolic and anabolic reactions (Lengeler et al., 1999). The enzymes involved are called mono- or di-oxygenases, depending on the number of oxygen atoms transferred to their substrate. Oxygenases are used by aerobes to activate certain substrates that do not have functional groups that allow a simple degradation. They catalyze the initial hydroxylation of hydrocarbons to yield alcohols that can easily be metabolized further. They are also involved in the ring cleavage of aromatic compounds and in the first oxidation step of ammonia oxydation. Anaerobic organisms are either unable to oxidize the substrates activated



Aerobic Metabolism, Figure 1 Principle of respiratory electron transport and vectorial proton translocation. The first proton translocating complex (with FMN and FeS as prosthetic groups) channels electrons to the quinones (UQ), which perform a cycle, again coupled to proton translocation. Electrons move on to the cytochrome bc_1 -complex, via cytochrome c to the copper-containing terminal cytochrome oxidase (Cyt aa_3). O_2 is reduced in the last step only. The ATP synthesis coupled to reentry of the translocated protons is independent of the electron transport process. (From Cypionka, 2006.)

by means of oxygenases or possess different degradation pathways.

Anabolic reactions making use of molecular oxygen are the biosynthesis pathways of tetrapyrroles, ubiquinones, pyrimidines, sterols, and unsaturated fatty acids. Again,

either the products are absent in strict anaerobes (e.g., sterols), or anaerobes have found alternative pathways for their biosynthesis.

Toxic forms of oxygen and detoxification

Aerobic metabolism inevitably generates small amounts of partially reduced toxic oxygen species (Lengeler et al., 1999). These are the superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($\cdot OH$). These species can be formed via nonenzymatic one-electron reduction steps (e.g., with Fe^{2+} , quinols, or H_2S) or by nonspecific reactions with reduced flavoproteins. Aerobic bacteria can remove toxic oxygen species by nonenzymatic reactions (e.g., with glutathione) or by means of enzymes: Superoxide dismutase (SOD) converts superoxide to H_2O_2 , and catalyze dismutates H_2O_2 to water and O_2 . These enzymes often lack in strict anaerobes, which might explain their sensitivity toward oxygen.

Distribution of the aerobic lifestyle

Many microorganisms can thrive in oxic (oxygen-containing) as well as in anoxic (free of oxygen) environments. Those are classified as facultative aerobes (or facultative anaerobes). This means that they can rely on aerobic respiration as well as on respiration with other electron acceptors or on fermentative energy conservation. Groups of strict anaerobes comprise methanogens, sulfate reducers, and Clostridia. Even in strict anoxic (oxygen-free) environments facultative aerobes can be found (Süß et al., 2006).

Summary

Aerobic respiration is the most effective way of energy conservation. It is used to oxidize the major part of the organic matter on Earth. During aerobic respiration oxygen is reduced to water. In some cases, O_2 is used by oxygenases for the hydroxylation of inert substrates. Aerobic organisms have enzymatic and nonenzymatic mechanisms to detoxify partially reduced oxygen species such as superoxide or hydrogen peroxide.

Bibliography

- Cypionka, H., 2006. *Grundlagen der Mikrobiologie*. Heidelberg: Springer.
- Harold, F. M., 1986. *The Vital Force: A Study of Bioenergetics*. New York: Freeman and Company.
- Lengeler, J. W., Drews, G., and Schlegel, H. G., 1999. *Biology of the Prokaryotes*. Stuttgart: Thieme.
- Mitchell, P., 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biological Reviews of the Cambridge Philosophical Society*, **41**, 445–502.
- Süß, J., Schubert, K., Sass, H., Cypionka, H., Overmann, J., and Engelen, B., 2006. Widespread distribution and high abundance of *Rhizobium radiobacter* within Mediterranean subsurface sediments. *Environmental Microbiology*, **8**, 1753–1763.

Cross-references

Anaerobic Transformation Processes, Microbiology
 Archaea
 Bacteria
 Carbon (Organic, Degradation)
 Critical Intervals in Earth History

ALGAE (EUKARYOTIC)

Thomas Friedl¹, Nicole Brinkmann¹, Kathrin I. Mohr^{1,2}

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Infection Research, Braunschweig, Germany

Algae (eukaryotic)

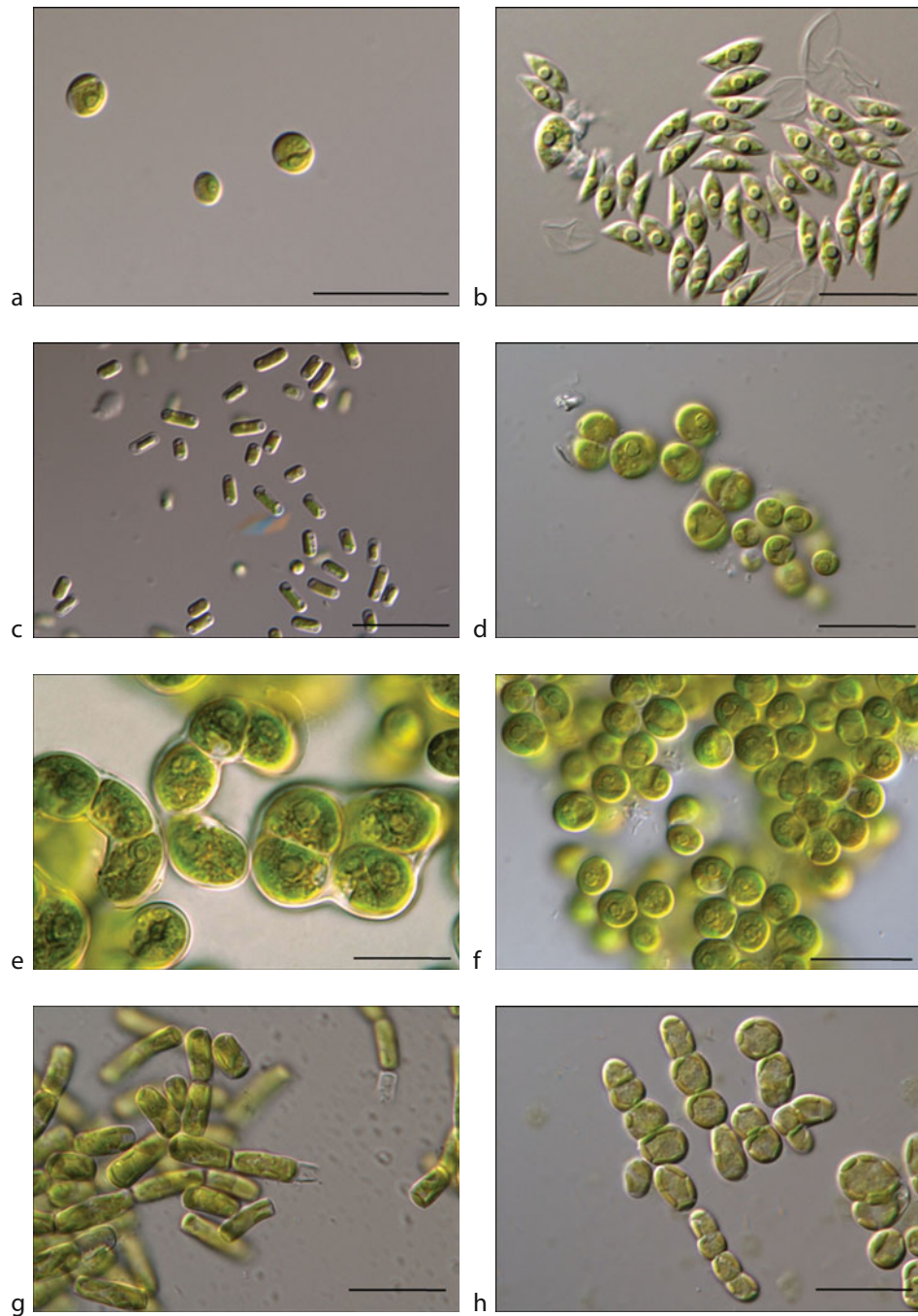
Eukaryotic algae are a collection of extremely diverse, nonrelated organisms that perform photosynthesis in plastids, permanent organelles of green, brown, or bluish colors derived from endosymbiosis. In contrast to plants, algae do not form embryos.

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids (Keeling, 2004). Many authors even include the prokaryotic cyanobacteria into the algae, because they exhibit a life-style rather similar to their eukaryotic counterparts and often share the same habitat with eukaryotic algae. Cyanobacteria form the origin of plastids (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Plastids are the organelles of plants and eukaryotic algae that harbor photosynthesis and synthesize many chemical compounds also important for other biochemical pathways (e.g., aromatic amino acids, heme, isoprenoids, and fatty acids); nonphotosynthetic plastids are known from plants and many algal groups (Williams and Keeling, 2003). Recently, from strategies of synthesizing various information, that is, mainly DNA sequence analyses of concatenated genes as well as the incorporation of discrete characters such as insertions, deletions, and gene fusion events, and consideration of morphology and biochemistry, a phylogeny of eukaryotes has emerged which consists of five “supergroups” (Keeling, 2004). Algae are scattered among four of the five major eukaryotic groups. The “Plantae” comprises exclusively eukaryotes with plastids derived from primary endosymbiosis, that is, they are bound by two membranes, derived from the inner and outer membranes of a cyanobacterium which was transformed into an organelle through uptake and retention by the host cell followed by the loss of much of its genome (Keeling, 2004). The Plantae are formed by three distinct but related lineages, the viridiplantae (which comprises all green algae and land plants or embryophytes), the rhodophytes (red algae), and the glaucophytes. The “Chromalveolates” comprises large and abundant algal groups such as the heterokont algae (e.g., diatoms and brown algae), the haptophytes,

the alveolates (which include the dinoflagellates and apicomplexans), and the cryptomonads. Only a single lineage of each of the “Excavates” and “Rhizaria” includes algae, the euglenoids and chlorarachniophytes. Except for the Plantae, all other eukaryotic algal lineages acquired their plastids through secondary endosymbiosis (for reviews see McFadden, 2001; Keeling, 2004; Palmer, 2003). Secondary plastids are surrounded by four or three membranes which resulted from phagocytosis of a primary alga, which was either a red alga or a green alga. In secondary plastids of both red and green origin, the primary algal nucleus is highly reduced (in the cryptomonads and chlorarachniophytes) or more commonly lost altogether (e.g., in the heterokonts and haptophytes; Keeling, 2004).

Green algae (Plantae supergroup)

The green algae are photosynthetic eukaryotes with double membrane-bound plastids (derived from primary endosymbiosis), stacked thylakoids, and the chlorophylls *a* and *b*. Their other accessory pigments are the carotenoids, beta-carotene, and xanthophylls. The main reserve polysaccharide is starch which is deposited inside the plastid (Lewis and McCourt, 2004; Pröschold and Leliaert, 2007). A few genera (e.g., *Prototheca*) contain non-photosynthetic plastids which lost their pigments secondarily. Apart from plastid-associated features, a unique stellate structure linking nine pairs of microtubules in the flagellar base of those green algae that are motile or produce motile stages is characteristic, as well as cell walls composed of cellulose which is present in most green algae (Graham et al., 2009). Most of these characters are shared with embryophytes (land plants) and, therefore, the green algae are difficult to define to the exclusion of the latter. The green algae have evolved in two major lineages (Friedl, 1997; Karol et al., 2001; Lewis and McCourt, 2004). One lineage, the chlorophyte lineage (Chlorophyta *sensu* Bremer, 1985; Sluiman, 1985) comprises the majority of green algal diversity (i.e., the green algae in a more narrow sense or the majority of what have been traditionally called green algae) with a bewildering array of morphologies attributable to at least five different morphological organizations (Pröschold and Leliaert, 2007). Examples of microscopic green algae reported from freshwaters with high calcification levels are rather small coccoid forms (e.g., *Chlorella*-like, Figure 1a; *Desmochloris*, Figure 1d; *Pseudendocloniopsis*, Figure 1f), coccoids that form coenobia (e.g., *Scenedesmus*, Figure 1b) or are of filamentous organization which may easily disintegrate into unicells (e.g., *Stichococcus*, Figure 1c) or form small thalli of branched filaments (e.g., *Pseudendoclonium*, Figure 1e). Also some monadoid (flagellated unicells) green algae and the siphonocladous *Cladophora* are well known from highly calcifying freshwater habitats. Several marine green algae of coenocytic organization precipitate carbonate and belong to the



Algae (Eukaryotic), Figure 1 Eukaryotic algae recovered from tufa stromatolite biofilms of hardwater creeks: (a) *Chlorella*-like coccoid green alga (Trebouxiophyceae, pure culture), (b) coenobia of coccoid green alga *Scenedesmus* sp. (Chlorophyceae, pure culture), (c) barrel-shaped unicells of green alga *Stichococcus* sp. (Trebouxiophyceae, pure culture), (d) coccoid green alga *Desmochloris* sp. (Ulvophyceae, pure culture), (e) branched filaments of green alga *Pseudendoclonium* sp. (Ulvophyceae, pure culture), (f) coccoid green alga *Pseudendocloniopsis* sp. (Ulvophyceae, pure culture), (g) filamentous unbranched stramenopiles *Xanthonema* sp. (Xanthophyceae, pure culture), (h) filamentous branched *Heterococcus* sp. (Xanthophyceae, pure culture), scale bar 20 μm .

calcareous algae, for example, the orders Dasycladales (Kingsley et al., 2003) and Caulerpales (Stanley et al., 2010). Whether the aforementioned freshwater green algal taxa are involved in carbonate precipitation is still

unknown, but microscopic freshwater green algal genera with calcite deposits on their surfaces are several members of Zygnematales (e.g., *Oocardium*) and *Gongrosira* (Rott et al., 2009; Freytet and Verrecchia, 1998). The other major

green algal lineage, the charophyte clade (*sensu* Lewis and McCourt, 2004), contains a smaller number of widespread, mostly freshwater green algae. Examples from the charophyte clade which are associated with calcification are the orders Zygnematales and Charales, for examples, the genus *Chara* is known for its extracellular carbonate production (McConnaughey, 1991). The charophyte green algae represent the closer relatives of land plants (embryophytes) with the Charales being most closely related to embryophytes (Karol et al., 2001; Lewis and McCourt, 2004). In the three lineages of green algae where calcite accumulation is known, that is, the orders Caulerpales, Dasycladales and Zygnematales (Bilan and Usov, 2001), aragonite deposits are outside of the cell wall but inside a specialized sheath. The crystal bundles are organized in layers, which are parallel to the filaments of this porous multilayer sheath and have also calcium oxalate inclusions. The chemical composition of the sheath and its role in the calcification process are unknown for the majority of species. It may simply be a barrier preventing the diffusion into the surrounding medium, although its direct participation in the initiation of the crystal formation cannot be excluded (Bilan and Usov, 2001). A third group of green algal taxa, called prasinophytes, consists of “primitive”-appearing unicells of uncertain affinity. Most known prasinophyte green algae form several early diverging clades within the phylogeny of the Chlorophyta (Marin and Melkonian, 2010). The green flagellate genus *Mesostigma* may represent a very basal lineage of the charophyte clade (Charophyta), but some prasinophytes are likely representatives of even other early diverging clades (Fawley et al., 2000). With respect to the systematics of green algae, there is now consensus that the majority of known green algal species of the Chlorophyta belongs to three major lineages, the chlorophytes, trebouxiophytes, and ulvophytes, which are assigned to class rank (classes Chlorophyceae, Trebouxiophyceae, and Ulvophyceae; Pröschold and Leliaert, 2007). Green algae have an affinity to form symbioses with hosts of various phylogenetic positions. Well-known examples are green algae forming the photoautotrophic partners (photobionts) of fungal symbioses, that is, lichens, or representing the algal hosts (“Zoochlorellae”) of ciliates (Alveolates), heliozoa, sponges, and sea anemones (e.g., Summerer et al., 2008; Letsch et al., 2009).

Rhodophytes – red algae (Plantae supergroup)

Red algae are a very large and diverse group of microscopic algae and macroalgae. They are best known for their economic and ecological importance. Though they are present in freshwater with several genera, the majority of red algal genera occur on tropical and temperate marine shores, where they play important ecological roles (Graham et al., 2009). Certain calcified red algae, known as corallines, form hard, flat sheets

that consolidate and stabilize coral reef crests, that is, coralline red algae protect reefs from wave damage and, thus, they are regarded as important keystone organisms. Of well-known economic importance are the species of *Porphyra* and other red algal species which are grown in mariculture operations for use as human food. Several other marine genera are cultivated or harvested for the extraction of gelling polysaccharides such as agarose and carrageen (Graham et al., 2009).

Corallines are known from fossil records 500 million years old (Lower Ordovician; Riding et al., 1998). The oldest fossil record of a (non-coralline) red alga may represent the fossil taxon *Bangiomorpha pubescens* which was described from the 1,200-million-year-old Hunting Formation in the Canadian Arctic (Butterfield, 2000). It is most notable that *B. pubescens* even represents the earliest putative record for sex and taxonomically resolvable complex multicellularity among eukaryotes (Saunders and Hammersand, 2004). For a review of fossil records of the various taxonomic groups of red algae, see Saunders and Hammersand (2004).

The plastids of red algae lack chlorophyll *b* and *c*, but contain phycobilins (allophycocyanin, phycocyanin, and the red pigment phycoerythrin) as accessory pigments which are located in phycobilisomes on the outer surface of the thylakoids. The red algal plastids are bounded by two membranes and derived from a cyanobacterial primary endosymbiosis (Keeling, 2004). They produce floridean starch which is deposited in the cytoplasm. The rhodophytes lack flagella and centrioles in all stages of their life history (Graham et al., 2009).

The red algae (Rhodophyta) are a distinct eukaryotic lineage, whose members are united in the phylogenetic analyses of a nuclear, plastid, and mitochondrial genes (for review see Yoon et al., 2006). They belong to the Plantae supergroup of eukaryotes (Keeling, 2004) and are believed to form a sister group with the green line (Martin et al., 2002). Their divergence has been estimated ca. 1,500 million years ago (Yoon et al., 2004). Based on molecular evidence, the red algae are partitioned into seven classes, six of which have a rather simple body structure and reproduction (Yoon et al., 2006). The florideophytes (class Florideophyceae) are the monophyletic group of red algae that exhibit more complex bodies and reproduction (Saunders and Hammersand, 2004). The ecologically significant corallines, the economically important genus *Chondrus* (used as food and for the extraction of carrageen; Graham et al., 2009), and the vast majority of other modern red algal species are florideophytes; they are divided into several monophyletic groups (subclasses; Saunders and Hammersand, 2004; Le Gall and Saunders, 2007).

Coralline red algae represent not only a large carbon reservoir, but they also consolidate sediment and build landforms, and they occupy harder substrate in the

world's oceans than any other group of photosynthetic organisms. Unlike most other calcified algae that deposit carbonate as aragonite, the coralline red algae form calcite and it is believed that each family of coralline red algae is defined by only one crystalline modification of calcium carbonate (Borowitzka, 1977). The red algae show evidence of a surprising variety of calcification mechanisms, but the location of deposition is even more diverse. Mineralization may occur throughout the walls of nearly all vegetative cells, it may occur within the walls of only specialized cells, or it may be located in an intercellular organic matrix between cells, but not within the cell walls (Pueschel et al., 1992). Calcification in corallines is poorly understood, however, it seems clear that corallines deposit high magnesium calcite in a definitive pattern within their cell walls via the membrane pumps that control the flux of calcium and carbonate with the control of crystal orientation by organic wall components (Zankl, 2007). Coralline red algae can be the deepest algae (–50 to –110 m, Dullo et al., 1990) and many species occur in great abundance into the Arctic and subarctic. Coralline red algae in coral reefs are ubiquitous and dominant (Glynn et al., 1996; Keats et al., 1997), and their abundance in cryptic and shaded environments can be greatly underestimated (Littler, 1972). In temperate systems, the accumulation of unattached living or dead coralline algae forms large maerl beds (Martin et al., 2006). They also provide food for herbivores with hardened mouthparts (Steneck and Dethier, 1994) and surfaces for the settlement of invertebrate larvae (Adey, 1998). Coralline algal abundance, size, shape, and species composition in maerl beds vary considerably depending on their location, and several environmental factors influence coralline algal distribution. Maerl beds are highly sensitive to desiccation and are found from the low intertidal zone to depths of 150 m (Foster, 2001). Furthermore, coralline species are sensitive to seasonal variations in temperature inducing changes in their physiology, for example, *Lithothamnion* spp. growth rate increases with water temperature (Potin et al., 1990). Despite their ecological importance in temperate systems, primary production and calcification of coralline algae has been mainly investigated in tropical areas (Chisholm, 2003; Payri et al., 2001). A number of different associations between sponges and several species of red macroalgae have been described in literature (Trautman et al., 2000, 2002). The symbiotic association between the haplosclerid sponge *Haliclona cymiformis* and the red macroalga *Ceratodictyon spongiosum* is common on shallow coral reefs of the Indo-West Pacific, where it has been described as being one of the most conspicuous organisms found in these areas (van Soest, 1990). This symbiosis seems to be an obligatory association as neither sponge nor alga has ever been identified growing independently or in association with other species (Morrissey, 1980).

Glaucophytes (Plantae supergroup)

Glaucophytes (or glaucocystophytes) form a small group of microscopic algae exclusively found in freshwater environments; only about 13 species of glaucophytes have been described so far. They represent the third lineage of the plant supergroup, which is defined by plastids bound by a double membrane, that is, which is derived from primary cyanobacterial endosymbiosis (Keeling, 2004). Glaucophyte plastids are unique among plastids in that they are retaining the prokaryotic peptidoglycan layer between their two membranes. Therefore, they may represent an intermediate in the transition from a cyanobacterial endosymbiont to plastid; molecular phylogenetic analyses show the glaucophytes as the earliest divergence within the plant supergroup (Keeling, 2004; Martin et al., 2002). Glaucophyte plastids share the accessory photosynthetic pigments, phycobilins, which are organized in phycobilisomes (small particles on the outer surface of thylakoid membranes) with cyanobacteria and rhodophytes. For a review on glaucophytes, see Bhattacharya and Schmidt (1997) and Steiner and Löffelhardt (2002).

Photosynthetic stramenopiles (chromalveolates)

Stramenopiles form a monophyletic group of photoautotrophic as well as heterotrophic organisms which are characterized by swimming cells possessing at least one flagellum with distinct tripartite tubular hairs (Graham et al., 2009; Andersen, 2004). Most stramenopiles have two distinctly different flagella and, therefore, are also known as heterokonts. They have a long flagellum with tripartite hairs (tinsel or immature flagellum) to pull the cells through the water and a shorter smooth one (whiplash or mature flagellum) that lacks tripartite hairs, often with a light-sensing flagellar swelling at its base (Graham et al., 2009; Andersen, 2004). Photosynthetic stramenopiles or heterokont algae usually appear brown or golden brown (and are thus sometimes referred to as chromophytic algae) due to the presence of characteristic accessory pigments, fucoxanthin or vaucheriaxanthin, and at least one form of chlorophyll *c* (except in eustigmatophytes). The plastids are derived from a secondary endosymbiosis event with a red alga involved and surrounded by four membranes (McFadden, 2001; Keeling, 2004; Palmer, 2003). In most photosynthetic stramenopiles, a chloroplast endoplasmic reticulum is present, that is, plastid and nucleus are structurally connected. The photosynthetic stramenopiles comprise a tremendous morphological diversity: a large variety of unicellular or colonial algae, including the diatoms with silica frustules as walls, multicellular simple filaments (e.g., in the xanthophytes) as well as the macroscopic complex brown algae forming a brown canopy at sea shores. Based on detailed analyses of pigment composition, ultrastructure and molecular phylogenies more than a dozen of different classes of photosynthetic stramenopiles have been described (Graham

et al., 2009; Andersen, 2004). Some photosynthetic stramenopiles do not form mineralized cysts or scales and, therefore, they have no fossil record. Examples are the brown seaweeds (Phaeophyceae), the yellow green algae (Xanthophyceae), and the inconspicuous eustigmatophytes. In the context of geobiology also xanthophytes and eustigmatophytes may be important, as they have been reported from freshwaters with high calcite precipitation (see Figure 1g, e; Arp et al., 2010). However, their role in the calcification processes is still unclear. Therefore, in this entry, only those classes of photosynthetic stramenopiles will be discussed that produce mineralized cell coverings which are important in fossil deposits and, therefore, may be of particular interest in geobiology. Siliceous frustules define the diatoms (Bacillariophyta; see entry “*Diatoms*”), silica skeletons are characteristic for silicoflagellates (class Dictyochophyceae) and silica scales and cysts are commonly formed in synurophytes and chrysophytes.

Silicoflagellates are unicellular, flagellated, and photoautotrophic stramenopiles which are characterized in at least one life cycle stage by the formation of distinctive, one-piece external silica skeleton that is highly perforate. They form the order Dictyochaetales, one of three orders that have been described for the class Dictyochophyceae (Daugbjerg and Henriksen, 2001; Graham et al., 2009). Siliceous skeletons from silicoflagellates are common in fossil deposits dating back to the middle Cretaceous (ca. 120 million years ago). The skeletal remains in fossil material also indicate that species diversity reached a peak in the Miocene (ca. 23–25 million years ago), with more than 100 described species, but the diversity of silicoflagellates has decreased thereafter, leaving behind only two genera (Daugbjerg and Henriksen, 2001; Tappan, 1980). Biostratigraphic zonations have been developed for silicoflagellates in all oceans throughout the Cenozoic, though the zones typically range over longer intervals of time than foraminiferal or diatom zones (McCartney and Wise, 1990; McCartney and Harwood, 1992). Silicoflagellate skeletons may have considerable potential as ecophenotypic indicators (McCartney and Wise, 1990). Modern silicoflagellates are widespread in the oceans with a tendency to be more abundant in colder waters where they can even grow up to form blooms (Graham et al., 2009). Dictyochophytes occur in both marine and freshwater habitats (Moestrup, 1995; Moestrup and O’Kelly, 2000). *Synurophytes* and *chrysophytes* (often summarized as “Chrysophyte Algae”) produced an exceptional fossil record owing to the high preservation potential of their siliceous stomatocysts and scales (Smol, 1995; Andersen, 2004; Graham et al., 2009). In addition, they are highly sensitive to an array of limnological parameters. Synurophytes and Chrysophytes are close relatives in molecular phylogenies (Andersen, 2004); both share the capability to form stomatocysts, silica-walled resting stages, which arise sexually or asexually under unfavorable environmental conditions. The walls of stomatocysts

are so heavily silicified that they resist silica dissolution processes and thus accumulate in the sediments of lakes (Graham et al., 2009). Fossil records of stomatocysts extend back to the Lower Cretaceous (at least 150 million years ago). Synurophytes and chrysophytes are able to switch between autotrophy, heterotrophy, and even phagotrophy, which puts them at a distinct advantage in aquatic ecosystems that are low in nutrient concentrations and have reduced light penetration (Betts-Piper et al., 2004). From patterns of stomatocyst assemblages, past environmental conditions can be inferred making stomatocysts very important for paleolimnology (Duff et al., 1995; Siver, 1995; Wilkinson et al., 2001; Zeeb and Smol, 2001).

Synurophytes are unicellular or colonial silicated flagellates and despite they are photoautotrophic, many species are also capable of phagotrophy. Synurophycean cell surfaces are covered by overlapping silica scales, sometimes with spiny bristles, and are perforated (Graham et al., 2009). The scales’ perforation patterns are used as taxonomic features to delimitate species of synurophytes. A number of adhesive polysaccharides are involved in the scale attachment. The scales are produced in certain vesicles located on the surface of one of the plastids. In contrast to diatoms, synurophytes can continue to divide and function also in the absence of an external silica covering. In cultures of naked cells most cells will recover a complete cell covering when silicate is resupplied to silica-depleted cells (Leadbeater and Barker, 1995). Fossil scales similar to those of modern synurophytes have been reported from deposits of the middle Eocene (about 47 million years ago; Siver and Wolfe, 2005). Synurophyte scales can be used like stomatocysts for tracking environmental change using lake sediments (Zeeb and Smol, 2001; Wolfe and Perren, 2001). Synurophytes are abundant in neutral to slightly acidic freshwaters. Some species are regarded as indicators of low levels of pollution, but others are characteristic of eutrophic lakes (Graham et al., 2009).

Chrysophytes are golden-brown microalgae that are mostly unicellular or colonial which occur as flagellates or nonmotile cells; they include many unique and interesting morphologies (Graham et al., 2009). In contrast to synurophytes, scales covering the cell surface are absent in chrysophytes. Chrysophytes typically favor slightly acidic freshwaters of moderate to low productivity; their abundance and species richness increase with lake eutrophic status (Elloranta, 1995). Chrysophytes may have strong ecological impacts because many are mixotrophs, able to take up and metabolize dissolved organic compounds and particulate food as well as photosynthesize (Graham et al., 2009). Several chrysophytes are associated with the formation of undesirable blooms because living cells of certain species can produce toxic fatty acids that affect fish or excrete aldehydes and ketones into the water which can give it an unpleasant taste and odor.

Coccolithophorids and haptophyte algae (chromalveolates)

Haptophyte algae are a monophyletic group that includes all photosynthetic organisms with a haptonema. However, haptophytes also include some nonphotosynthetic relatives, and some that have secondarily lost the haptonema (Andersen, 2004). The haptonema, from which the group derives its name, is a microtubule-supported appendage that lies between two approximately equal flagella (for a review, see Inouye and Kawachi, 1994). Most haptophytes are marine, occurring in significant abundances even at greater water depth (up to 200 m). Haptophyte algae share many features, for example, pigment composition, presence of a chloroplast endoplasmatic reticulum, and plastids derived from red algal secondary endosymbiosis with the photosynthetic stramenopiles (Andersen, 2004). Based on structural and molecular evidences the haptophytes form a monophyletic lineage, treated as phylum Haptophyta which is divided into two classes (Edwardsen et al., 2000; Andersen, 2004). The larger group, class Coccolithophyceae, comprises the coccolithophorids which are haptophytes with a cell coat of mineralized scales (coccoliths) and two equal flagella or lack flagella altogether. Coccoliths are largely composed of calcium carbonate crystals in the form of calcite. There are two types of coccoliths, holococcoliths and heterococcoliths. In case of holococcoliths, an organic scale consisting largely of cellulose and produced by the Golgi body is secreted to the cell surface and then calcite crystals which are held together by organic material are deposited on the scale. In contrast, heterococcoliths develop on organic scales before being secreted from the cell, that is, calcite crystal deposition is already within the cell. The calcite crystals grow and interlock gradually forming the complex shape of the mature coccolith (deVrind-deJong et al., 1994; Probert et al., 2007; Graham et al., 2009). Coccoliths do not degrade at normal ocean pH and thus accumulate on the ocean floor. Therefore, coccolithophorids have produced huge amounts of sedimentary carbonates.

Coccolithophorids remove large quantities of atmospheric CO₂ through their photosynthesis and calcification and, therefore, are an important component of the global carbon cycle (McConnaughey, 1994), accounting for a substantial part of the ocean floor limestone sediments. Coccolithophorids contribute at least 25% of the total annual vertical transport of inorganic carbon to the deep ocean (Rost and Riebesell, 2004). Coccolithophorids have an excellent fossil record with fossil coccoliths well over 200 million years old. The abundance of coccolith fossils peaked during the late Cretaceous (63–95 million years ago) when extensive chalk deposits were laid down. Coccoliths are widely used as stratigraphic indicators and fossil coccoliths are widely used as bioindicators in the oil industry and as indexes of past climate and ocean chemistry conditions (Young et al., 1994).

Haptophytes are ecologically significant in terms of both biotic interactions and biogeochemistry. Haptophyte algae are considered as high-quality foods for zooplankton; several species contain nutritionally important polyunsaturated fatty acids which make them also commercially valuable for the production of fish in aquaculture systems. Other coccolithophytes may produce toxins that destroy cell membranes or produce copious amounts of organic slime and foam (Graham et al., 2009). Many coccolithophorids form blooms in ocean waters and produce large amounts of a volatile sulfur-containing molecule, dimethyl sulfide (DMS) that enhances cloud formation and increases acid rain (Malin and Steinke, 2004). Another cooling effect on the climate comes from the coccoliths which readily reflect light, thereby increasing reflectance of the ocean's surface.

Dinoflagellates (chromalveolates)

The dinoflagellates are an important group of phytoplankton in marine and freshwaters. Their adaptation to a wide variety of environments is reflected by a tremendous diversity in form and nutrition and an extensive fossil record dating back several hundred million years (Graham et al., 2009). Dinoflagellate cells have two structurally distinct flagella whose motion causes the cells to rotate as they swim, but also nonflagellate unicells and filamentous forms of dinophytes are known. Two different cell types can be distinguished on the basis of the cell-wall covering or theca. The “naked” or unarmored forms have an outer plasmalemma surrounding a single layer of flattened vesicles (membrane sacs or alveoli). Armored dinoflagellates have cellulose or other polysaccharides within each vesicle, giving the cells a more rigid, inflexible wall. These cellulose plates are arranged in distinct patterns which are extensively used as taxonomic “fingerprints” (Hackett et al., 2004). Cells of armored dinoflagellates display conspicuous anterior–posterior and dorsal–ventral differentiation. Their cells consist of two parts, separated by a groove that encircles the cell and contains the transverse flagellum which is flattened, ribbon-shaped, and with a single row of hairs. A smaller groove is mostly extending into the posterior cell part and contains the longitudinal flagellum which has two rows of hairs; it is directed posterior and emerges from the cell (Graham et al., 2009; Hackett et al., 2004).

Success of dinoflagellates as phytoplankton may be due in part to unique behavior patterns, including diel vertical migration and their capability to live phagotrophic (feeding on particles such as the cells of other organism) in addition to photoautotrophy (mixotrophic life-style). Some of the armored or thecate heterotrophic dinoflagellates have developed a remarkable pseudopod-like structure that is extruded from the cell and flows around the prey, enveloping it so the contents can then be digested (Hackett et al., 2004). Some dinoflagellates produce toxins that are dangerous to man, marine mammals, fish,

seabirds, and other components of the marine food chain (Van Dolah, 2000). Others are bioluminescent and emit light; some function as parasites or symbionts that rely on host organisms for part of their nutrition. Many photosynthetic dinoflagellates live as endosymbionts with reef-building corals. Stimulated by chemical signals from their animal hosts, dinoflagellate endosymbionts provide the corals with essential organic food.

Therefore, the dinoflagellate symbionts are critical components of the coral reef ecosystems whose loss during stress-related “bleaching” events can lead to mass mortality of coral hosts and associated collapse of reef ecosystems (Baker, 2003). Also, the high rates of calcification and production that characterize typically oligotrophic coral reef ecosystems are largely credited to the mutualistic symbiosis between the scleractinian corals and photosynthetic dinoflagellates belonging to the genus *Symbiodinium* (Apprill and Gates, 2007). Corals with a dinoflagellate symbiont calcify much faster than those without – an effect linked to photosynthetic fixation of CO₂ by the dinoflagellates (Marshall, 1996). A significant amount of photosynthetic product is excreted by the symbiotic dinoflagellates, primarily as glycerol. Up to 50% of the fixed carbon may be transferred to the host (Paracer and Ahmadjian, 2000), in which it is converted mainly to lipids and proteins. On the dinoflagellate side, many of these symbioses occur in oligotrophic waters in which nutrients are scarce in the water column (Hackett et al., 2004). In other symbiotic dinoflagellate associations, the hosts include foraminifera, radiolarians, flatworms, anemones, jellyfish, and even bivalve mollusks (Hackett et al., 2004).

Undisputed structural fossils of dinoflagellates occur some 200 million years or so ago (Graham et al., 2009). These fossils, known as hystrichospheres, resemble resting stages of some modern dinoflagellates. Some modern dinoflagellates produce resting cysts or zygotes with an organic compound resistant to decay and thus aid the survival of dinoflagellate fossils, of which a considerable diversity has been described (Fensome et al., 2003). Other chemical compounds thought to be specific to dinoflagellates have already been found in Proterozoic (Precambrian) rocks and such chemical fossils suggest that dinoflagellate-like organisms might have existed already more than 600 million years ago (Graham et al., 2009).

Many dinoflagellates are photosynthetic and, through endosymbiosis, have acquired a wide diversity of plastids from distant evolutionary lineages. The most common plastid in dinoflagellates is golden brown with a unique accessory pigment, peridinin; they also contain chlorophyll *c*. Such peridinin plastids are bounded by three membranes and are derived from red algal secondary endosymbiosis (Delwiche, 2007; Hackett et al., 2004). A number of dinoflagellates have plastids from haptophytes, diatoms, green algae, or cryptomonads which may have replaced preexisting peridinin plastids (tertiary endosymbiosis; McFadden, 2001; Keeling, 2004; Palmer, 2003).

A small group of dinoflagellates with peridinin plastids produce calcareous structures, formed during the life cycle or found in vegetative stages (Gottschling et al., 2005). The potential to produce calcareous structures has been considered as apomorphic within alveolates (Kohring et al., 2005), arguing for the monophyly of the family Calciodinellaceae which comprises approximately 30 (recognized) extant species that are distributed in cold through tropical seas of the world. Calcareous cysts are deposited in both sediments coastal (Montresor et al., 1998; Persson et al., 2000) and oceanic (Vink, 2004; Zonneveld et al., 1999). According to the fossil record, calcareous dinoflagellates originate in the Upper Triassic (Janofske, 1992) and are highly diverse during the Cretaceous and throughout the Tertiary (Keupp, 1991; Kohring, 1993; Willems, 1994), thus many fossil species (namely their cysts) have been described (Gottschling et al., 2005). Based on morphological and molecular data, calcareous dinoflagellates (Thoracosphaeraceae, Peridinales) are a monophyletic group comprising three major clades (Gottschling et al., 2008).

Other chromalveolates

Cryptomonads. Recent molecular phylogenetic analyses indicate that the cryptomonads and the haptophyte algae together form a monophyletic group (Hackett et al., 2007) and, therefore, also belong to the Chromalveolates supergroup. Cryptomonads form an abundant group of marine and freshwater unicellular flagellates that contain a plastid derived from red algal secondary endosymbiosis with chlorophyll *a* and *c* surrounded by four membranes. Cryptomonad plastids have received some attention because they are one of only two groups in which the primary algal nucleus has not been completely lost; they retain a small relict nucleus called a nucleomorph (McFadden, 2001; Keeling, 2004; Palmer, 2003).

Apicomplexa are a large group composed entirely of obligate intracellular parasites, including several that cause significant diseases such as malaria. As intracellular parasites, the discovery of a relict plastid (or apicoplast) in apicomplexa bounded by four membranes has drawn a great deal of attention as an evolutionary novelty and possible drug target (Keeling, 2004). There is current evidence indicating that this plastid is derived from a red alga and that the apicomplexa share a monophyletic origin with all other groups with plastids from red algal secondary endosymbiosis. The apicomplexa may be most closely related with the dinoflagellates (Moore, 2008). For a review of apicomplexa relating to their plastids, see Foth and McFadden (2003).

Algal groups with plastids derived from green algal secondary endosymbiosis

Chlorarachniophytes (Rhizaria supergroup). Chlorarachniophytes are a relatively rare group of marine amoeboid flagellates and flagellates that contain a green algal plastid (chlorophyll *a* and *b*) bounded by four membranes. They

contain a relict nucleus, nucleomorph, which has been retained from the green algal symbiont (McFadden, 2001; Keeling, 2004; Palmer, 2003). Chlorarachniophytes form a monophyletic group within the Rhizaria supergroup of eukaryotes (Ishida et al., 1999).

Euglenids (Excavates supergroup). Euglenids are a diverse group of common marine and freshwater flagellates, about half of which contain a plastid derived from green algal secondary endosymbiosis which is bounded by three membranes and contains chlorophyll *b* as accessory pigment. The remainder of the group are osmotrophs or heterotrophs that feed on bacteria or other eukaryotes. Molecular phylogenies show that photosynthetic euglenids may have acquired their plastids from a green alga relatively late in evolution, despite the Euglenozoa may be a rather old group. Euglenids are closely related to the parasitic trypanosomes (Kinetoplastids), together with diplomonids, making up the monophyletic Euglenozoa. Distinguishing features of euglenids are that they produce a storage carbohydrate which is a β -1,3-linked glucan (paramylon) in their cytoplasm and that they display a unique surface structure composed of parallel ribbon-like proteinaceous strips. For reviews of euglenids, see Leedale and Vickerman (2000), Milanowski et al. (2006) or Ciugulea and Triemer (2010).

Summary

Algae is a term of convenience and refers to a collection of highly diverse organisms that undertake photosynthesis and/or possess plastids. In addition, the photoautotrophic cyanobacteria are regarded as algae by some authors. In a recent phylogeny of the eukaryotes, the algae are scattered among four of the five recognized supergroups. A single primary endosymbiosis with a cyanobacterium may have been the start of all eukaryotic algae which first diverged into the green algae, red algae, and glaucophytes (Plantae supergroup). The plastids of all other algal lineages derived from secondary endosymbiosis involving either a red algal cell (algae of the Chromalveolates supergroup) or green algal cells (euglenids and chlorarchniophytes, Excavates and Rhizaria supergroups). Many algae form symbioses with ciliates and some metazoa. Algae are very important primary producers in almost every aquatic or terrestrial habitats and are important key players within the carbon and silica cycles for the major part of Earth history. Many lineages have produced significant fossil records due to their ability to precipitate calcium carbonate on their cell surfaces (red algae, some dinoflagellates) or form calcified (haptophytes) or silicified body scales, silica endoskeletons, resting stages, or cell walls (photoautotrophic stramenopiles, including dictyochophytes, synurophytes, chrysophytes, and diatoms). Due to their high sensitivity to certain environmental parameters and their high preservation potential, these algae also play important roles as sedimentary bioindicators in tracking environmental changes.

Bibliography

- Adey, W. H., 1998. Coral reefs: algal structured and mediated ecosystems in shallow, turbulent, alkaline waters. *Journal of Phycology*, **34**, 393–406.
- Andersen, R. A., 2004. Biology and systematics of heterokont and haptophyte algae. *American Journal of Botany*, **91**, 1508–1522.
- Apprill, A. M., and Gates, R. D., 2007. Recognizing diversity in coral symbiotic dinoflagellate communities. *Molecular Ecology*, **16**, 1127–1134.
- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., De Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. Tufa-forming biofilms of German karstwater streams: microorganisms, exopolymers, hydrochemistry and calcification. *Geological Society, London, Special Publications 2010*, **336**, 83–118.
- Baker, A. C., 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of symbiodinium. *Annual Review of Ecology, Evolution and Systematics*, **34**, 661–689.
- Betts-Piper, A. M., Zeeb, B. A., and Smol, J. P., 2004. Distribution and autecology of chrysophyte cysts from high Arctic Svalbard lakes: preliminary evidence of recent environmental change. *Journal of Paleolimnology*, **31**, 467–481.
- Bhattacharya, D., and Schmidt, H. A., 1997. Division glaucocystophyta. In Bhattacharya, D. (ed.), *Origin of Algae and Their Plastids*, Wien: Springer-Verlag, pp. 139–148.
- Bilan, M. I., and Usov, A. I., 2001. Polysaccharides of calcareous algae and their effect on the calcification process. *Russian Journal of Bioorganic Chemistry*, **27**, 2–16.
- Borowitzka, M. A., 1977. Algal calcification. *Oceanography and Marine Biology: An Annual Review*, **15**, 189–223.
- Bremer, K., 1985. Summary of green plant phylogeny and classification. *Cladistics*, **1**, 369–385.
- Butterfield, N. J., 2000. *Bangiomorpha pubescens* n. gen., n. sp.: implications for the evolution of sex, multicellularity, and the Mesoproterozoic/Neoproterozoic radiation of eukaryotes. *Paleobiology*, **26**, 386–404.
- Chisholm, J. R. M., 2003. Primary productivity of reef-building crustose coralline algae. *Limnology and Oceanography*, **48**, 1376–1387.
- Ciugulea, I., and Triemer, R. E., 2010. *A color atlas of photosynthetic Euglenoids*. East Lansing: Michigan State University Press.
- Daugbjerg, N., and Henriksen, P., 2001. Pigment composition and *rbcL* sequence data from the silicoflagellate *Dictyocha speculum*: a heterokont alga with pigments similar to some haptophytes. *Journal of Phycology*, **37**, 1110–1120.
- Delwiche, C. F., 2007. The origin and evolution of dinoflagellates. In Falkowski, P. G., and Knoll, A. H., (eds.), *Evolution of Primary Producers of the Sea*, New York: Academic, pp. 191–205.
- DeVrind-deJong, E. W., van Emburg, P. R., and deVrind, J. P. M., 1994. Mechanisms of calcification: *Emiliania huxleyi* as a model system. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*, Oxford: Clarendon, pp. 149–166.
- Duff, K. E., Zeeb, B. A., and Smol, J. P., 1995. *Atlas of Chrysophyte Cysts*. Dordrecht: Kluwer Academic, 189 pp.
- Dullo, W. C., Moussavian, E., and Brachert, T., 1990. The foralgal crust facies of the deeper fore reefs in the Red Sea. *Geobios*, **23**, 261–281.
- Edwardsen, B., Eikkrem, W., Green, J. C., Andersen, R. A., van der Staay, S. Y. M., and Medlin, L. K., 2000. Phylogenetic reconstructions of the Haptophyta inferred from 18S ribosomal DNA sequences and available morphological data. *Phycologia*, **39**, 19–35.
- Elloranta, P., 1995. Biogeography of chrysophytes in Finnish lakes. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae. Ecology, Phylogeny and Development*, Cambridge: Cambridge University Press, pp. 214–231.

- Fawley, M. W., Yun, Y., and Qin, M., 2000. Phylogenetic analyses of 18S rDNA sequences reveal a new coccoid lineage of the Prasinophyceae (Chlorophyta). *Journal of Phycology*, **36**, 387–393.
- Fensome, R. A., MacRae, R. A., Moldovan, J. M., Taylor, F. J. R., and Williams, G. L., 2003. The early Mesozoic radiation of dinoflagellates. *Paleobiology*, **22**, 329–338.
- Foster, M. S., 2001. Rhodoliths: between rocks and soft places. *Journal of Phycology*, **37**, 659–667.
- Foth, B. J., and McFadden, G. I., 2003. The apicoplast: a plastid in *Plasmodium falciparum* and other apicomplexan parasites. *International Review of Cytology*, **224**, 57–110.
- Freytet, P., and Verrecchia, E. P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*, **45**, 535–563.
- Friedl, T., 1997. The evolution of the green algae. *Plant Systematics and Evolution*, **11**, 87–101.
- Glynn, P. W., Veron, J. E. N., and Wellington, G. M., 1996. Clipperton Atoll (eastern Pacific): oceanography, geomorphology, reef-building coral ecology and biogeography. *Coral Reefs*, **15**, 71–99.
- Gottschling, M., Keupp, H., Plötner, J., Knop, R., Willems, H., and Kirsch, M., 2005. Phylogeny of calcareous dinoflagellates as inferred from ITS and ribosomal sequence data. *Molecular Phylogenetics and Evolution*, **36**, 444–455.
- Gottschling, M., Renner, S. S., Meier, K. J. S., Willems, H., and Keupp, H., 2008. Timing deep divergence events in calcareous dinoflagellates. *Journal of Phycology*, **44**, 429–438.
- Graham, L. E., Graham, J. M., and Wilcox, L. W., 2009. *Algae*, 2nd edn. San Francisco: Pearson Benjamin Cummings.
- Hackett, J. D., Anderson, D. M., Erdner, D. L., and Bhattacharya, D., 2004. Dinoflagellates: a remarkable evolutionary experiment. *American Journal of Botany*, **91**, 1523–1534.
- Hackett, J. D., Yoon, H. S., Li, S., Reyes-Prieto, A., Rümmele, S. E., and Bhattacharya, D., 2007. Phylogenetic analysis supports the monophyly of cryptophytes and haptophytes and the association of Rhizaria with chromalveolates. *Molecular Biology and Evolution*, **24**, 1702–1713.
- Inouye, I., and Kawachi, M., 1994. The haptonema. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*. Oxford: Clarendon, pp. 73–89.
- Ishida, K., Green, B. R., and Cavalier-Smith, T., 1999. Diversification of a chimaeric algal group, the chlorarachniophytes: Phylogeny of nuclear and nucleomorph small-subunit rRNA genes. *Molecular Biology and Evolution*, **16**, 321–331.
- Janofske, D., 1992. Kalkiges Nannoplankton, insbesondere kalkige Dinoflagellaten-Zysten der alpinen Ober-Trias: Taxonomie, Biostratigraphie und Bedeutung für die Phylogenie der Peridinales. *Berliner Geowiss. Abh. (E)*, **4**, 1–53.
- Karol, K. G., McCourt, R. M., Cimino, M. T., and Delwiche, C. F., 2001. The closest living relatives of land plants. *Science*, **294**, 2351–2353.
- Keats, D. W., Knight, M., and Pueschel, C., 1997. Antifouling effects of epithelial shedding in three crustose coralline algae (Rhodophyta, Corallinales) on a coral reef. *Journal of Experimental Marine Biology and Ecology*, **213**, 281–293.
- Keeling, P. J., 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany*, **91**, 1481–1493.
- Keupp, H., 1991. Fossil calcareous dinoflagellate cysts. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 267–286.
- Kingsley, R. J., Van Gilder, R., LeGeros, R. Z., and Watabe, N., 2003. Multimineral calcareous deposits in the marine alga *Acetabularia acetabulum* (Chlorophyta; Dasycladaceae). *Journal of Phycology*, **39**, 937–947.
- Kohring, R., 1993. Kalkdinoflagellaten aus dem Mittel- und Obereozän von Jütland (Dänemark) und dem Pariser Becken (Frankreich) im Vergleich mit anderen Tertiär-Vorkommen. *Berliner Geowiss. Abh. (E)*, **6**, 1–164.
- Kohring, R., Gottschling, M., and Keupp, H., 2005. Examples for character traits and palaeoecological significance of calcareous dinoflagellates. *Paläontologische Zeitschrift*, **79**, 79–91.
- Le Gall, L., and Saunders, G. W., 2007. A nuclear phylogeny of the Florideophyceae (Rhodophyta) inferred from combined EF2, small subunit and large subunit ribosomal DNA: establishing the Corallinophycidae subclassis nov. *Molecular Phylogenetics and Evolution*, **43**, 1118–1130.
- Leadbeater, B. S. C., and Barker, D. A. N., 1995. Biomineralization and scale production in the Chrysophyta. In Sandgren, C. D. J., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae: Ecology, Phylogeny and Development*. Cambridge, UK: Cambridge University Press, pp. 141–164.
- Leedale, G. F., and Vickerman, K., 2000. Phylum Euglenozoa. In Lee, J. J., Leedale, G. F., and Bradbury P. (eds.), *An Illustrated Guide to the Protozoa*, 2nd edn. Lawrence: Society of Protozoologists, pp. 1135–1185.
- Letsch, M. R., Muller-Parker, G., Friedl, T., and Lewis, L. A., 2009. *Elliptochloris marina* sp. nov. (Trebouxiophyceae, Chlorophyta), symbiotic green alga of the temperate pacific sea anemones *Anthopleura xanthogrammica* and *A. elegantissima* (Anthozoa, Cnidaria). *Journal of Phycology*, **47**, 1127–1135.
- Lewis, L. A., and McCourt, R. M., 2004. Green algae and the origin of land plants. *American Journal of Botany*, **91**, 1535–1556.
- Littler, M. M., 1972. The crustose Corallinales. *Oceanography and Marine Biology Annual Review*, **10**, 311–347.
- Malin, G., and Steinke, M., 2004. Dimethyl sulfide production: What is the contribution of the coccolithophores? In Thierstein, H. R., and Young, J. R. (eds.), *Coccolithophores – From Molecular Process to Global Impact*. New York: Springer, pp. 127–164.
- Marshall, A. T., 1996. Calcification in hermatypic and ahermatypic corals. *Science*, **271**, 637–639.
- Marin, B., and Melkonian, M., 2010. Molecular phylogeny and classification of the mamiellophyceae class. nov. (Chlorophyta) based on sequence comparisons of the nuclear- and plastid-encoded rRNA operons. *Protist*, **161**, 304–336.
- Martin, S., Castets, M. D., and Clavier, J., 2006. Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*. *Aquatic Botany*, **85**, 121–128.
- Martin, W., Rujan, T., Richly, E., Hansen, A., Ornelsen, S., Lins, T., Lesiter, D., Stoebe, B., Hasegawa, M., and Penny, D., 2002. Evolutionary analysis of Arabidopsis, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proceedings of the National Academy of Sciences, USA*, **99**, 12246–12251.
- McCartney, K., and Wise, S. W. Jr., 1990. Cenozoic silicoflagellates and ebridians from ODP Leg 113: biostratigraphy and notes on morphologic variability. In Barker, P. F., and Kennett, J. P., et al. (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results. College Station, TX: (Ocean Drilling Program)*, Vol. 113, pp. 729–760.
- McCartney, K., and Harwood, D. M., 1992. Silicoflagellates from Leg 120 on the Kerguelen Plateau, southeast Indian Ocean. In Wise, S. W. Jr., Schlich, R., et al. (eds.), *Proceedings of the Ocean Drilling Program, Scientific Results, College Station, TX: Ocean Drilling Program*, Vol. 120, pp. 811–831.
- McConnaughey, T., 1991. Calcification in Chara corallina: CO₂ hydroxylation generates protons for bicarbonate assimilation. *Limnology and Oceanography*, **36**, 619–628.
- McConnaughey, T. A., 1994. Calcification, photosynthesis, global carbon cycles. In Doumenge, E., and Allemand Toulemon, A. (eds.), *Past and Present Biomineralization Processes Consideration about the Carbonate IUCNCOE Workshop Monaco*, Nov 1993. *Bulletin Oceanographique* **13**, 137–156.
- McFadden, G. I., 2001. Primary and secondary endosymbiosis and the origin of plastids. *Journal of Phycology*, **37**, 951–959.

- Milanowski, R., Kosmala, S., Zakrys, B., and Kwiatowski, J., 2006. Phylogeny of photosynthetic euglenophytes based on combined chloroplast and cytoplasmic SSU rDNA sequence analysis. *Journal of Phycology*, **42**, 721–730.
- Moestrup, Ø., 1995. Current status of chrysophyte splinter groups: synurophytes, pedinellids, silicoflagellates. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae*. Cambridge: Cambridge University Press, pp. 75–91.
- Moestrup, Ø., and O'Kelly, C. J., 2002. Class Silicoflagellata Lemmermann, 1901. In Lee, J. J., Leedale, G. F., and Bradbury, P. (eds.), *An Illustrated Guide to the Protozoa*, 2nd edn. Lawrence, Kansas, USA: Society of Protozoologists, vol. 2, pp. 775–776.
- Montresor, M., Zingone, A., and Sarno, D., 1998. Dinoflagellate cyst production at a coastal Mediterranean site. *Journal of Plankton Research*, **20**, 2291–2312.
- Moore, R. B., 2008. A photosynthetic alveolate closely related to apicomplexan parasites. *Nature*, **451**, 959–963.
- Morrissey, J., 1980. Community structure and zonation of macroalgae and hermatypic corals on a fringing reef flat of Magnetic Island (Queensland, Australia). *Aquatic Botany*, **8**, 91–139.
- Nicholas, J. B., Knoll, A. H., and Swett, K., 1990. A bangiophyte red alga from the proterozoic of Arctic Canada. *Science*, **250**, 104–107.
- Palmer, J., 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology*, **39**, 4–12.
- Paracer, S., and Ahmadjian, V., 2000. *Symbiosis: An Introduction to Biological Associations*. New York: Oxford University Press.
- Payri, C. E., Maritorea, S., Bizeau, M., and Rodiere, M., 2001. Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinacea) from a French Polynesian reef. *Journal of Phycology*, **37**, 223–234.
- Persson, A., Godhe, A., and Karlson, B., 2000. Dinoflagellate cysts in recent sediments from the west coast of Sweden. *Botanica Marina*, **43**, 69–79.
- Potin, P., Floch, J. Y., Augris, C., and Cabioch, J., 1990. Annual growth rate of the calcareous red algae *Lithothamnion corallioides* (Corallinales, Rhodophyta) in the Bay of Brest, France. *Hydrobiologia*, **204/205**, 263–267.
- Probert, I., Fresnel, J., Billard, C., Geisen, M., and Young, J. R., 2007. Light and electron microscope observations of *Algirosphaera robusta* (Prymnesiophyceae). *Journal of Phycology*, **43**, 319–332.
- Pröschold, T., and Leliaert, F., 2007. Systematics of the green algae: conflict of classic and modern approaches. In Brodie, J., and Lewis, J. (eds.), *Unravelling the Algae: the Past, Present, and Future of the Algae Systematics*. London: Taylor and Francis, pp. 123–153.
- Pueschel, C. M., Eichelberger, H. H., and Trick, H. N., 1992. Specialized calciferous cells in the marine alga *Rhodogorgon caribbowensis* and their implications for models of red algal calcification. *Protoplasma*, **166**, 89–98.
- Riding, R., Cope, J. C. W., and Taylor, P. D., 1998. A coralline-like red alga from the lower Ordovician of Wales. *Palaeontology*, **41**, 1069–1076.
- Rost, B., and Riebesell, U., 2004. Coccolithophore calcification and the biological pump: Response to environmental changes. In Thierstein, H. R., and Young, J. R. (eds.), *Coccolithophores – From Molecular Processes to Global Impact*. New York: Springer, pp. 99–126.
- Rott, E., Holzinger, A., Gesierich, D., Kofler, D., and Sanders, D., 2009. Cell morphology, ultrastructure, and calcification pattern of *Oocardium stratum*, a peculiar lotic desmid. *Protoplasma*, 10.1007/s00709-009-0050-y.
- Saunders, G. W., and Hammersand, M. H., 2004. Assessing red algal supraordinal diversity and taxonomy in the context of contemporary systematic data. *American Journal of Botany*, **91**, 1494–1507.
- Siver, P. A., and Wolfe, A. P., 2005. Eocene scaled chrysophytes with pronounced modern affinities. *International Journal of Plant Sciences*, **166**, 533–536.
- Siver, P. A., 1995. The distribution of chrysophytes along environmental gradients: their use as bioindicators. In Sandgren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae*. Cambridge: Cambridge University Press, pp. 232–268.
- Sluiman, H. J., 1985. A cladistic evaluation of the lower and higher green plants (Viridiplantae). *Plant Systematics and Evolution*, **149**, 217–232.
- Smol, J. P., 1995. Applications of chrysophytes to problems in paleoecology. In Sangren, C. D., Smol, J. P., and Kristiansen, J. (eds.), *Chrysophyte Algae: Ecology, Phylogeny and Development*. Cambridge: Cambridge University Press, pp. 303–329.
- Stanley, S. M., Ries, J. B., and Hardie, L. A., 2010. Increased production of calcite and slower growth for the major sediment-producing alga *Halimeda* as the Mg/Ca ratio of seawater is lowered to a “Calcite Sea” level. *Journal of Sedimentary Research*, **80**, 6–16.
- Steiner, J. M., and Löffelhardt, W., 2002. Protein import into cyanelles. *Trends in Plant Sciences*, **7**, 72–77.
- Steneck, R. S., and Dethier, M. N., 1994. A functional group approach to the structure of algal-dominated communities. *Oikos*, **69**, 476–498.
- Summerer, M., Sonntag, B., and Sommaruga, R., 2008. Ciliate-symbiont specificity of freshwater endosymbiotic *Chlorella* (Trebouxioiophyceae, Chlorophyta). *Journal of Phycology*, **44**, 7–84.
- Tappan, H., 1980. *The Paleobiology of Plant Protists*. San Francisco: Freeman, p. 1029.
- Trautman, D. A., Hinde, R., and Borowitzka, M. A., 2000. Population dynamics of an association between a coral reef sponge and a red macroalga. *Journal of Experimental Marine Biology and Ecology*, **244**, 87–105.
- Trautman, D. A., Hinde, R., and Borowitzka, M. A., 2002. The role of habitat in determining the distribution of a sponge-red alga symbiosis on a coral reef. *Journal of Experimental Marine Biology and Ecology*, **283**, 1–20.
- van Dolah, F. M., 2000. Marine algal toxins: origins, health effects, and their increased occurrence. *Environmental Health Perspectives*, **108**, 133–141.
- van Soest, R. W. M., 1990. Shallow-water reef sponges of eastern Indonesia. In Rützler, K. (ed.), *New Perspectives in Sponge Biology*. Washington, DC: Smithsonian Institution Press, pp. 302–308.
- Vink, A., 2004. Calcareous Dinoflagellate cysts in South and equatorial Atlantic surface sediments: diversity, distribution, ecology and potential for palaeoenvironmental reconstruction. *Marine Micropaleontology*, **50**, 43–88.
- Wilkinson, A. N., Zeeb, B. A., and Smol, J. P., 2001. *Atlas of Chrysophyte Cysts*. Dordrecht: Kluwer, 169 pp.
- Willems, H., 1994. New calcareous dinoflagellates from the upper cretaceous white chalk of northern Germany. *Review Palaeobotanic Palynology*, **84**, 57–72.
- Williams, B. A. P., and Keeling, P. J., 2003. Cryptic organelles in parasitic protists and fungi. *Advances in Parasitology*, **54**, 9–67.
- Wolfe, A. P., and Perren, B. P., 2001. Chrysophyte microfossils record marked responses to recent environmental changes in high- and mid-arctic lakes. *Canadian Journal of Botany*, **79**, 747–752.
- Yoon, H. S., Hackett, J. D., Ciniglia, C., Pinto, G., and Bhattacharya, D., 2004. A molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution*, **21**, 809–818.
- Yoon, H. S., Müller, K. M., Sheath, R. G., and Bhattacharya, D., 2006. Defining the major lineages of red algae (Rhodophyta). *Journal of Phycology*, **42**, 482–492.
- Young, J. R., Brown, P. R., and Burnett, J. A., 1994. Paleontological perspectives. In Green, J. C., and Leadbeater, B. S. C. (eds.), *The Haptophyte Algae*. Oxford, UK: Clarendon Press, pp. 379–392.

- Zankl, H., 2007. The origin of High-Mg-Calcite microbialites in cryptic habitats of Caribbean coral reefs – their dependence on light and turbulence. *Facies*, **29**, 55–60.
- Zeeb, B. A., and Smol, J. P., 2001. Chrysophyte scales and cysts. In John, P., Smol, H., Birks, J. B., and Last, W. M. (eds.), *Tracking Environmental Change Using Lake Sediments. Vol 3. Terrestrial, Algal, and Siliceous Indicators*. Dordrecht: Kluwer, pp. 203–223.
- Zonneveld, K. A. F., Höll, C., Janofske, D., Karwath, B., Kerntopf, B., Rühlemann, C., Willems, H., 1999. Calcareous dinoflagellate cysts as paleo-environmental tools. In Fischer, G., and Wefer, G. (eds.), *Use of Proxies in Paleoceanography*. Berlin: Springer, pp. 145–164.

Cross-references

Cyanobacteria
Diatoms
Early Precambrian Eukaryotes
Fungi and Lichens
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Photosynthesis
Protozoa (Heterotroph, Eukaryotic)
Symbiosis

ALKALINITY

Andreas Reimer, Gernot Arp
University of Göttingen, Göttingen, Germany

The term “Alkalinity,” commonly denoted by TA (also A_T , ALK, and others) and then called titration alkalinity or total alkalinity, refers to a very important chemical concept in aquatic chemistry. Total alkalinity is one of the few measurable quantities in natural waters that allows, together with other properties, to calculate concentrations of single species of the carbonate system such as CO_2 , HCO_3^- , CO_3^{2-} , H^+ , and OH^- (Wolf-Gladrow et al., 2007), and further on, the saturation state of the waters with respect to certain carbonate minerals. Therefore, alkalinity represents a hydrogeochemical key parameter for the understanding of carbonate precipitation, either biologically mediated or not, and its variation through the geological history.

Definition

From its determination by titrimetric techniques, alkalinity has historically been defined as the number of equivalents of a strong acid required to neutralize 1 L of water at 20°C. The obtained end-point, corresponding to the second equivalence point of the carbonate system, was operationally defined and results were then commonly given as eq L^{-1} . The recent definition of alkalinity is based on the implementation of the Brønsted–Lowry concept of acids and bases by Rakestrow (1949) who defined alkalinity as the excess of bases (proton acceptors) over acids (proton donors). Considering seawater, alkalinity is mostly determined by the ions bicarbonate and carbonate, also by the borate ion, and to a smaller extent by OH^- and H^+ from

dissociation of water. Alkalinity then refers to the proton condition with reference to a zero level of protons defined by the species H_2CO_3 , $\text{B}(\text{OH})_3$, and H_2O , where the proton acceptors are HCO_3^- , CO_3^{2-} , $\text{B}(\text{OH})_4^-$, and OH^- and the proton donor is H^+ . That is, alkalinity is the equivalent sum of bases that have one or two protons less than the reference species minus H^+ (or H_3O^+) that has one proton more than H_2O (Dickson, 1981; Stumm and Morgan, 1996; Zeebe and Wolf-Gladrow, 2001). The expression for the total alkalinity is thus presented as

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] - [\text{H}^+],$$

where conceptually the right hand term can be divided up in carbonate alkalinity ($\text{CA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}]$) as the most dominant contributor, borate alkalinity, and water alkalinity.

Natural waters may contain various acid–base systems that can accept and donate protons other than or in addition to carbonic acid or boric acid. These complications have been taken into account currently in the most precise definition of total alkalinity of Dickson (1981) (compare also Dickson, 1992; DOE, 1994; and recent extension by Wolf-Gladrow et al., 2007). The definition is unequivocally based on a chemical model of occurring acid–base processes and to be independent of temperature and pressure expressed in gravimetric units (mol kg^{-1}). In Dickson’s concept, the total alkalinity (TA) is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors over proton donors with respect to the proton condition defined by the value $\text{pK} = 4.5$ in 1 kg of sample. Bases formed from weak acids with a dissociation constant $K \leq 10^{-4.5}$ are considered proton acceptors, acids with $K > 10^{-4.5}$ are proton donors, and the chemical species defining the zero level of protons of the various acid–base systems are H_2CO_3 , $\text{B}(\text{OH})_3$, H_2O , H_2PO_4^- , H_4SiO_4 , NH_4^+ , H_2S , SO_4^{2-} , F^- , and NO_2^- . From this definition the expression for the total alkalinity is presented as

$$\begin{aligned} \text{TA} = & [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] \\ & + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{H}_3\text{SiO}_4^-] \\ & + [\text{NH}_3] + [\text{HS}^-] + \dots - [\text{H}^+] - [\text{HSO}_4^-] \\ & - [\text{HF}] - [\text{H}_3\text{PO}_4] - [\text{HNO}_2] \dots \end{aligned}$$

where the ellipses stand for additional acid–base species (Wolf-Gladrow et al., 2007).

An alternative definition for alkalinity derives when considering the charge balance of natural waters (Stumm and Morgan, 1996). From the principle of electroneutrality total alkalinity can be expressed in terms of the charge imbalance between the equivalent sum of conservative cations (base cations of strong bases) and the sum of conservative anions (conjugate bases of strong acids). For the total alkalinity of sea water dominated by

HCO_3^- , CO_3^{2-} , and B(OH)_4^- , the expression may be presented by

$$\text{TA} = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] \\ - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - 2[\text{SO}_4^{2-}],$$

which is equivalent to the above definition in terms of proton acceptors and donors

$$\text{TA} = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B(OH)}_4^-] \\ + [\text{OH}^-] - [\text{H}^+].$$

In the charge balance expression quantities are defined to be conservative with respect to mixing and changes in temperature (T) and pressure (P) provided that concentrations are on the gravimetric unit scale (mol kg^{-1}). Thus, from the TA expression entirely in terms of conservative ions TA is a conservative quantity too. In contrast, the concentrations of individual species of, e.g., the carbonate system may change with temperature and pressure because of the T - P dependency of the equilibrium constants.

Following the charge balance approach, Zeebe and Wolf-Gladrow (2001) and Wolf-Gladrow et al. (2007) have derived an expression in terms of total concentrations of conservative ions and conservative total concentrations of the various acid-base species under concern in the definition of Dickson (1981) in terms of an acid-base balance. This derived expression for TA is denoted as the explicit conservative alkalinity expression (TA_{ec}).

$$\text{TA}_{\text{ec}} = [\text{Na}^+] + [\text{K}^+] + 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}] + \dots \\ - [\text{Cl}^-] - [\text{Br}^-] - [\text{NO}_3^-] - \dots \\ - 2\text{TSO}_4 - \text{THF} - \text{TPO}_4 - \text{THNO}_2 + \text{TNH}_3,$$

where total concentrations terms represent concentrations sums of the species of the acid-base systems $\text{TSO}_4 = [\text{SO}_4^{2-}] + [\text{HSO}_4^-]$, $\text{THF} = [\text{F}^-] + [\text{HF}]$, $\text{TPO}_4 = [\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}]$, $\text{THNO}_2 = [\text{NO}_2^-] + [\text{HNO}_2]$, $\text{TNH}_3 = [\text{NH}_3] + [\text{NH}_4^+]$, and ellipses stand for additional ions with minor concentrations.

The definition of TA_{ec} has the advantage that each term in the expression is conservative with respect to T - P changes, even though single species in the total concentration terms may be not. In particular, the approach is very convenient to derive changes of TA due to certain physicochemical and biogeochemical processes such as nutrient uptake in biological production and release during remineralization.

Sources, changes, and ranges

The primary source of alkalinity in surficial (and subsurface) waters is carbonic acid-controlled chemical weathering of carbonates, aluminum-silicate minerals like feldspars or Mg-Fe-silicates. The overall concentration and subsequent changes in alkalinity of water are therefore determined by the geology of the watershed, the

predominant weathering reactions and ion exchange in the soils, and concomitant biogeochemical processes.

TA as a conservative quantity will first alter with changes in salinity as a result of precipitation, evaporation, and mixing. Contrariwise to its increase from dissolution of limestone carbonates TA decreases when biogenic or non-biogenic CaCO_3 or other carbonate minerals precipitate. Further important changes are due to various biogeochemical processes. It has to be noted that total alkalinity does not change as a result of CO_2 exchange with the atmosphere. Invasion or degassing of CO_2 affects the adjustment of the carbonate species (ratio of $[\text{HCO}_3^-]$ to $[\text{CO}_3^{2-}]$) and the pH but not TA. Therefore, it is also not the consumption of CO_2 during photosynthesis or release during aerobic respiration that brings about a change in TA, but the utilization or remineralization of nitrate and phosphate involved in the processes. Nitrogen assimilation in form of ammonia decreases TA and no change of TA results from molecular nitrogen uptake (Stumm and Morgan, 1996; Wolf-Gladrow et al., 2007). Several microbially mediated processes lead to a change in alkalinity where deviation in the charge balance is compensated by yield or consumption of H^+ or OH^- . When oxygen is consumed in processes like nitrification, oxygenation of soluble ferrous iron to ferric oxide or sulfide oxidation from HS^- or pyrite total alkalinity decreases. Under anoxic conditions, remineralization of organic matter by denitrification, reduction of iron and manganese oxides and sulfate reduction frequently result in large increases of total alkalinity (Lerman and Stumm, 1989; Stumm and Morgan, 1996).

Depending on the rock mineralogy of the catchment area and prevalent vegetation total alkalinity in streams and inland lakes commonly ranges below 3 mmol kg^{-1} . Very low alkalinity is bound to tributaries and lakes in crystalline rock areas or to acidic bog and peatland waters. Here, H^+ released by biomass exceeding H^+ consumption by soil weathering results in an excess of protons that is referred to as “mineral acidity” or in turn “negative alkalinity” (Stumm and Morgan, 1996). On the other hand, in hard-water creeks that gain their alkalinity preferably from the dissolution of limestone rocks TA may reach values of 4 mmol kg^{-1} to 6 mmol kg^{-1} close to their spring sites.

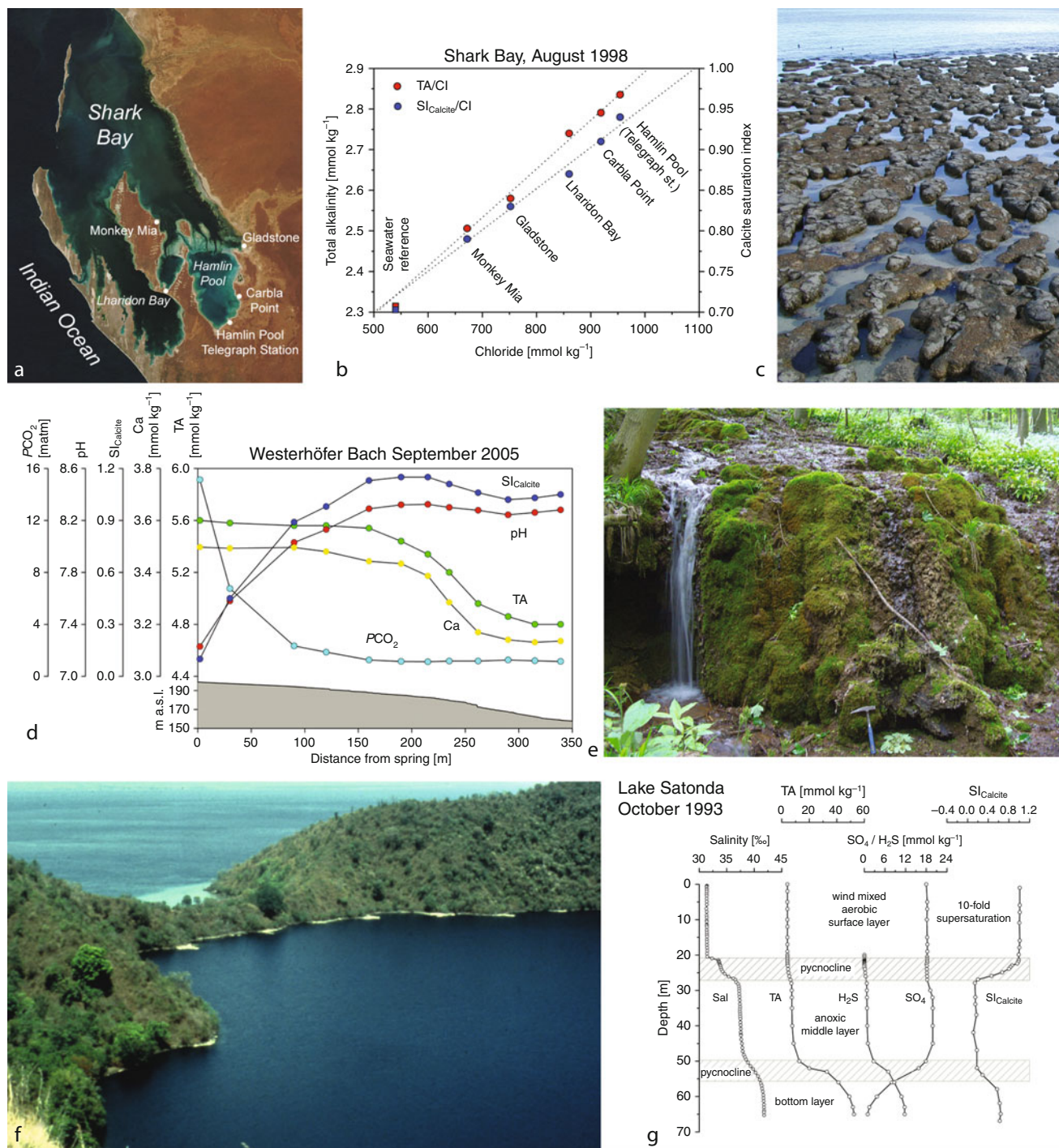
In the oceans, TA typically ranges from 2.2 mmol kg^{-1} to 2.5 mmol kg^{-1} where changes with depth are mainly associated with the production and dissolution of calcareous organisms (Broecker and Peng, 1982). Higher TA is found in today's only anaerobic ocean basin, the Black Sea, where alkalinity increases to about 4.5 mmol kg^{-1} in the anoxic bottom waters in the course of oxidation of organic material by sulfate reduction (Kempe, 1990; Hiscock and Millero, 2006). Also due to enhanced sulfate reduction and frequently complementing methane oxidation, TA in the order of $10^{-2} \text{ mol kg}^{-1}$ can accumulate in pore waters of marine sedimentary successions (Schulz and Zabel, 2000).

Even higher total alkalinity is typical for highly alkaline salt lakes, so-called soda lakes, which receive an equivalent load of HCO_3^- and CO_3^{2-} in excess to Mg^{2+} and Ca^{2+} from their riverine inflows. Here, TA can rise to

more than $10^{-1} \text{ mol kg}^{-1}$ and pH increase above 10 in the course of evaporation and subsequent Ca-carbonate precipitation while alkali cations, in particular sodium and potassium, increasingly maintain the charge balance of bicarbonate and carbonate ions (e.g., Lerman and Stumm, 1989; Kempe and Kazmierczak, 1994; Reimer et al., 2009).

Alkalinity and carbonate precipitation

Alkalinity, together with any of the three other measurable properties of the carbonate system – pH, dissolved inorganic carbon (DIC or $C_T = [\text{CO}_2] + [\text{HCO}_3^-] + [\text{CO}_3^{2-}]$), or PCO_2 (partial pressure of CO_2) – can be used to compute the remaining quantities including concentrations of



Alkalinity, Figure 1 (Continued)

single species such as HCO_3^- and CO_3^{2-} . From CO_3^{2-} and Ca^{2+} concentrations (or more precise activities) saturation with respect to carbonate minerals is determined, where saturation Ω is given by the product of the actual ion activities ($\text{IAP} = \{\text{Ca}^{2+}\} \times \{\text{CO}_3^{2-}\}$) divided by the solubility product of the carbonate (K_{mineral}) at ambient temperature and pressure ($\Omega_{\text{mineral}} = \text{IAP}/K_{\text{mineral}}$). A commonly used alternative notation is the saturation index (SI) which is defined as $\log \Omega$ ($\text{SI}_{\text{mineral}} = \log (\text{IAP}/K_{\text{mineral}})$).

The oceanic CaCO_3 saturation state is primarily determined by variation of the CO_3^{2-} ion concentration, because the Ca^{2+} concentration closely depends on salinity and does not vary substantially. In the upper ocean, CO_2 removal by primary production increases CO_3^{2-} concentrations resulting in an about fivefold supersaturation ($\text{SI} = 0.7$) with respect to calcite. On the other hand, biologically controlled calcification of calcareous plankton or reefal organisms is responsible for the lower alkalinity of about $2.2\text{--}2.3 \text{ mmol kg}^{-1}$ in surface seawater. In turn, supersaturation decreases with depth when CO_2 is released by remineralization of sinking organic matter when temperature and pressure changes. In the deeper ocean, total alkalinity then increases to about $2.4\text{--}2.5 \text{ mmol kg}^{-1}$ reflecting the dissolution of sinking calcium carbonate particles in response to the lower saturation state (e.g., Broecker and Peng, 1982; Morse and Mackenzie, 1990).

As one of the major factors in the oceanic carbonate system, alkalinity distribution and behavior in the oceans has thoroughly been investigated during the last decades. Dissolution-preservation patterns of biogenic carbonates in surface to deep oceans and the changes brought about by a rising atmospheric CO_2 level (Feely et al., 2004) have been assessed within global ocean research programs, as well as general feedbacks between the oceanic carbonate and the global climate system. Contrary to this rising knowledge on the modern marine environment, estimates

of ocean alkalinity throughout geological history are rare and, in the lack of direct analytical proxies, mainly rely on model assumptions. Ocean alkalinity comparably high as modern values is suggested for the Cenozoic and late Mesozoic (Tyrrell and Zeebe, 2004; Ridgwell, 2005). During periods of the Mesozoic and Paleozoic, alkalinity about two to three times higher than recent can be assumed according to the evolution of the Phanerozoic atmospheric CO_2 (Ridgwell, 2005), fluctuations in the rate of continental weathering (Locklair and Lerman, 2005), or the more widespread occurrence of anoxic basins (Kempe and Kazmierczak, 1994). Elevated alkalinity concentrations of some tenths of mmol kg^{-1} are suggested for the Earth's primordial ocean in favor of a slightly acidic to neutral Na-Cl-dominated chemistry under an early CO_2 -rich atmosphere (Grotzinger and Kasting, 1993; Morse and Mackenzie, 1998). Alternatively, even higher alkalinity has been postulated for a Precambrian alkaline "Soda Ocean" in analogy to modern Na-carbonate-dominated soda lakes (Kempe and Degens, 1985).

In principle, precipitation of CaCO_3 is kinetically unfavorable even at the fivefold supersaturation of the modern ocean (e.g., Morse and He, 1993). Mechanisms to overcome this kinetic inhibition evolved at the Precambrian-Cambrian boundary with the advent of controlled biomineralization that became a major regulator of carbonate precipitation since the Mesozoic proliferation of the calcareous plankton. Prior to the Cambrian, carbonate deposition was characterized by sea-floor encrustations, cement beds, and the widespread occurrence of microbialites (Grotzinger, 1990; Riding, 2000), which are attributed to calcification of biofilms and microbial mats. In the lack of direct metabolic control on carbonate production, these Precambrian deposits are thought to have been formed under the pressure of a higher supersaturated environment, possibly still accounting for the dominance

Alkalinity, Figure 1 Recent environments that sustain microbialite growth illustrated in terms of alkalinity behavior and carbonate mineral saturation. (a–c) Shark Bay in Western Australia is one of the few recent marine examples that sustains calcifying biofilms involved in the formation of agglutinated stromatolites. Here, Indian Ocean water entering the embayment and running over the Faure sill into the Hamlin Pool to the SE is concentrated upon evaporation (a, courtesy to NASA). According to the twofold increase in salinity, alkalinity, and carbonate supersaturation rises continuously (b). The famous Hamlin Pool stromatolites (c) occur in the southernmost bay where alkalinity has increased to more than 2.8 mmol kg^{-1} and waters are nearly ten times supersaturated with respect to calcite. (d and e) Today, microbialite growth is also sustained in environments such as hardwater creeks such as the Westerhöfer Bach (Shiraishi et al., 2008), which are commonly characterized by calcium-bicarbonate type waters. From take-up of soil CO_2 and subsequent subsurface dissolution of limestone rocks, these creeks have gained already high total alkalinities ranging from 4 to 6 mmol kg^{-1} . Otherwise, at their spring sites initial pH (around 7) and carbonate saturation ($\text{SI}_{\text{cc}} = 0$) are low due to partial CO_2 pressures much higher than the atmospheric level ($\text{PCO}_2 = 10^{-2} \text{ atm}$). Continuous physicochemical CO_2 -degassing along the flowpath increases pH and CO_3^{2-} and therefore enforces supersaturation (d). Precipitation of CaCO_3 and formation of calcareous microbialites, so-called tufa stromatolites (e), commences when supersaturation reaches a certain maximum threshold level of 10- to 15-fold ($\text{SI} = 1.0\text{--}1.2$) (Arp et al., 2001), and results in the subsequent decline of alkalinity and Ca^{2+} concentrations in the creeks. (f and g) The crater lake of Satonda Island (f), Indonesia, filled with water of nearly marine composition, represents the case of a subrecent microbialite formation obviously induced by an enormous increase of alkalinity in its anoxic deep waters (Kempe and Kazmierczak, 1990). The multiple salinity stratification of the lake is a residue of former evaporitic phases. Especially in the bottom layer, sulfate has nearly been consumed due to bacterial reduction resulting in an exceptionally high total alkalinity of more than 50 mmol kg^{-1} , sulfide concentrations of up to 12 mmol kg^{-1} , and extremely high PCO_2 (more than 10^{-1} atm) (g). Alkalinity and carbonate supersaturation in the photic surface layer is determined by the seasonal mixing transfer of alkalinity from the monimolimnion top layer to the mixolimnion, degassing, rain water dilution, and evaporation (Arp et al., 2003). In the surface layer alkalinity reaches twice the value of open marine waters (4.1 mmol kg^{-1}) and the tenfold supersaturation may constitute the prerequisite for the present-day, though minor, calcification of biofilms.

of microbial carbonates in the early to middle Paleozoic (Riding and Liang, 2005). Today, the occurrence of carbonate deposits comparable to ancient microbialites is confined to particular environments such as restricted evaporational marine settings, freshwater habitats like tufa stromatolite forming hardwater creeks, or alkaline salt lakes (Figure 1). Although at first glance very different, these environments in common exhibit a significantly higher alkalinity than recent ocean values and a 10–15 fold supersaturation (i.e., $SI = 1\text{--}1.2$) with respect to calcite. Maintaining elevated carbonate supersaturation as found in these recent environments and as suggested for certain periods in Earth's history calls for variation in alkalinity in paleo-ocean model calculations (Locklair and Lerman, 2005). Therefore, changes in alkalinity may be considered as an underappreciated hydrogeochemical key mechanism with regard to carbonate supersaturation and carbonate deposition in its various forms through geologic times.

Bibliography

- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Broecker, W. S., and Peng, T.-H., 1982. *Tracers in the Sea*. New York: Lamont-Doherty Geological Observatory Publishers, 690 pp.
- Dickson, A. G., 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep-Sea Research*, **28A**, 609–623.
- Dickson, A. G., 1992. The development of the alkalinity concept in marine chemistry. *Marine Chemistry*, **40**, 49–63.
- DOE, 1994. In Dickson, A. G., and Goyet, C. (eds.), *Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water: Version 2*. ORNL/CDIAC-74.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J., 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–366.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Hiscock, W. T., and Millero, F. J., 2006. Alkalinity of the anoxic waters in the Western Black Sea. *Deep Sea Research Part II*, **53**, 1787–1801.
- Kempe, S., 1990. Alkalinity: the link between anaerobic basins and shallow water carbonates? *Naturwissenschaften*, **77**, 426–427.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Chemistry and stromatolites of the sea-linked Satonda Crater Lake, Indonesia: a recent model for the Precambrian sea? *Chemical Geology*, **81**, 299–310.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Monaco: Bulletin de l'Institut Océanographique, no. spec. 13, pp. 61–117.
- Lerman, A., and Stumm, W., 1989. CO_2 storage and alkalinity trends in lakes. *Water Research*, **23**, 139–146.
- Locklair, R. E., and Lerman, A., 2005. A model of Phanerozoic cycles of carbon and calcium in the global ocean: evaluation and constraints on the ocean chemistry and input fluxes. *Chemical Geology*, **217**, 113–126.
- Morse, J. W., and He, S., 1993. Influences of T , S , and P_{CO_2} on the pseudo-homogeneous precipitation of $CaCO_3$ from seawater: implications for whiting formation. *Marine Chemistry*, **41**, 291–297.
- Morse, J. W., and Mackenzie, F. T., 1990. Geochemistry of sedimentary carbonates. *Developments in Sedimentology*. Amsterdam: Elsevier, vol. 48, 707 pp.
- Morse, J. W., and Mackenzie, F. T., 1998. Hadean ocean carbonate geochemistry. *Aquatic Geochemistry*, **4**, 301–319.
- Rakestrow, N. W., 1949. The conception of alkalinity or excess base of seawater. *Journal of Marine Research*, **8**, 14–20.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.
- Ridgwell, A., 2005. A mid Mesozoic revolution in the regulation of ocean chemistry. *Marine Geology*, **217**, 339–357.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Schulz, H. D., and Zabel, M., 2000. *Marine Geochemistry*. Berlin, Heidelberg: Springer, 455 pp.
- Shiraishi, F., Reimer, A., Bissett, A., de Beer, D., and Arp, G., 2008. Microbial effects on biofilm calcification, ambient water chemistry and stable isotope records (Westerhöfer Bach, Germany). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **262**, 91–106.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, 3rd edn. New York: Wiley, 1022 pp.
- Tyrrell, T., and Zeebe, R. E., 2004. History of carbonate ion concentration over the last 100 million years. *Geochimica et Cosmochimica Acta*, **68**, 3521–3530.
- Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G., 2007. Total alkalinity: the explicit conservative expression and its application to biogeochemical processes. *Marine Chemistry*, **106**, 287–300.
- Zeebe, R. E., and Wolf-Gladrow, D. A., 2001. *CO_2 in Seawater: Equilibrium, Kinetics, Isotopes*. Elsevier Oceanography Series, Amsterdam: Elsevier, Vol 65, 346 pp.

Cross-references

[Calcite Precipitation, Microbially Induced Carbonate Environments](#)
[Carbonates](#)
[Saline Lakes](#)
[Salinity History of the Earth's Ocean](#)
[Soda Ocean Hypothesis](#)

AMBER

Eugenio Ragazzi¹, Alexander R. Schmidt²

¹University of Padova, Padova, Italy

²University of Göttingen, Göttingen, Germany

Synonyms

Fossil resin; Resinite

Definition

Fossilized plant resin from various botanical sources.

Introduction

Amber is a fossilized plant resin from various botanical sources and of different geological ages, ranging from the Carboniferous to the Pliocene, although it is particularly frequent in the Cretaceous and in the Eocene to Miocene. The term “amber” is used most commonly as a synonym for fossil resin of every provenance, geological, and palaeobotanical source. A more reductive meaning of amber has been considered, referring only to *succinite*, the mineralogical species occurring in the Baltic coast deposits and neighboring localities containing significant amounts of succinic acid (up to 8%). However, most commonly, *amber* and *fossil resin* are the terms used interchangeably for indicating macroscopic material (Anderson and Crelling, 1995; Langenheim, 2003). Another term used by coal geologists for fossil resins is *resinite*, which is a microscopic material often found in coal maceral. Amber, fossil resin, and resinite, according to Anderson and Crelling (1995) should therefore be considered “solid, discrete organic material found in coals and other sediments as macroscopic or microscopic particles, which are derived from the resins of higher plants.”

The term amber derives from the Arabic word *anbar*, which was first used to indicate *ambergris* (a waxy substance secreted by the sperm whale, used in perfumery as a fixative) and then also fossil resin, both natural products with the common feature of being carried ashore by sea waves. The Romans called amber *succinum* (from *succus*, literally “juice,” “sap”). The ancient Greek name for amber is *elektron*, that means “shining thing,” a term which was related to the Sun God *Elektor* (the awakener), and the Moon, called *Elektris* (Stoppani, 1886). The modern term *electron* was coined in 1891 by the Irish physicist George Johnstone Stoney, using the Greek word for amber because static electricity could be generated on the surface of rubbed amber. The German word *Bernstein* (or *Börnstein*), the Dutch *Barnsteen*, and the Swedish *bärnsten* mean “burning stone” because amber may burn when exposed to flame.

Geological names are often attributed to fossil resins on the basis of the locality of discovery or of the discoverer, and these names are not based on specific knowledge of the composition; however, they are often used and also quoted in the scientific literature as common names. For example, *Simetite* is for amber collected in Sicily (Italy) near the Simeto river, *Burmite*, for amber found in Burma (Myanmar), and *Rumanite* for that found in Romania; *Beckerite* and *Stantienite* were named for Becker and Stantien, two developers of amber dredging and mining operations in the Samland region in the 1800s. Over a hundred of different fossil resins have been described, so far.

Fossil resins are considered the archetype of a “chemical fossil” (chemical evidence of life processes preserved in the fossil record), and besides their property of

preserving embedded organisms, they can also maintain particular details of their original composition better than any other sedimentary material.

Amber has been known by humans since prehistorical times and most bizarre hypotheses about its origin and composition have been formulated. In the antiquity amber has been considered even as produced by the rays of the Sun (Pliny the Elder, Book 37: *Nicias solis radiorum succum intellegi voluit hoc*) and that was not such a wrong idea, since amber, as a resin of ancient trees, is a metabolite of plants, originated from products of photosynthesis, that is, from the energy of the Sun.

Amber composition and formation

Several different parameters can play relevant roles in amber composition and consequently, they all should be considered in the study and comparison of different amber samples. These are for example:

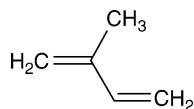
1. *Original environment*: temperature, insolation, and climate influence the resin composition before it is embedded in the sediment (biostratonomic processes);
2. *Age*: complex chemical arrangements occur with time; in general, low molecular weight components disappear with increasing age;
3. *Plant source*: resin produced by different plants exhibit different chemical composition;
4. *Diagenetic history*: following the embedding in the sediment, resin maturation is affected by temperature, pressure and permeating fluids which can modify the chemical composition and the physicochemical properties of fossil resin;
5. *Alteration processes after amber mining* (of relevance especially in archaeological context): direct sunlight and exposition to air and heat may change the color and physical properties (and, consequently, the chemical composition) of the sample.

Resins consist of secondary components of plants, that is, chemicals occurring only in some groups of plants, without an apparent role in primary physiological or metabolic pathways. Most natural resins are derived from *terpenes*, a large and varied class of hydrocarbons, which are chemicals composed by structures based on the isoprene unit (C_5H_8) (Figure 1). The basic molecular formula of terpenes can be expressed as multiples of isoprene, $(C_5H_8)_n$, where n is the number of linked isoprene units. The isoprene units can link together “head to tail” to form linear chains, or alternatively they can arrange to form rings. The overall terpene structure undergoes oxidation or rearrangement of the carbon skeleton, producing compounds, which are generally indicated as *terpenoids* or *isoprenoids*. Mono-, sesqui-, di-, and tri-terpenoids are polymers formed by two, three, four, and six isoprene units, respectively.

The biosynthesis of resins starts from simple molecules, namely mevalonic acid, which can be considered the precursor of isoprene units. Photosynthetically

produced carbohydrates are broken down to give pyruvate, which represents the basis for the synthesis of mevalonic acid. Mevalonic acid is converted into the activated form of isopentenyl pyrophosphate, which gives origin to geranyl pyrophosphate (C₁₀), the basis for the monoterpene synthesis, or can further undergo condensation into farnesyl pyrophosphate (C₁₅) for the production of sesquiterpenes, or geranyl-geranyl pyrophosphate (C₂₀), which yields diterpenes and tetraterpenes, or squalene (C₃₀), which is the basis of triterpenes (Figure 2).

Fluid resins, after their exudation from plant, are characterized by a large volatile fraction, up to 50%, mainly constituted by monoterpenes and sesquiterpenes, while the nonvolatile fraction is composed of di- and tri-terpene acids (carboxylic acids with double bonds and functional groups), with alcohols, aldehydes, and esters. The volatile components of the resins are mainly involved in the defensive role of the resin against pathogens and insects. Evaporation of volatile components, following polymerization of the remaining nonvolatile constituents, mainly presenting dienic functions, causes the hardening of resin, which, if allowed to be embedded in appropriate sediment, poses the basis for transformation into a fossil resin.



Amber, Figure 1 The basic isoprene unit (C₅H₈).

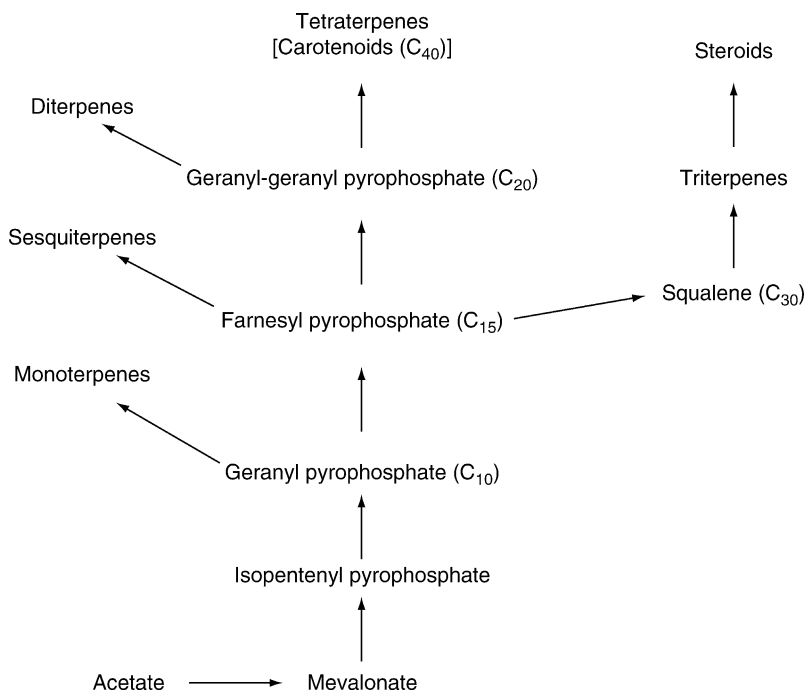
Diterpenoid resins, mainly composed of diterpene acids (C₂₀), are produced by gymnosperms (conifers) and angiosperms (mostly in the Leguminosae family), and they are particularly prone to polymerization and thus being preserved during fossilization into amber.

Gymnosperm diterpenes are mainly represented by diterpene acids of the abietane, pimarane, and labdane types (Figure 3). Abietane- and pimarane-type diterpene acids, such as abietic and pimaric acids, abundant in the Pinaceae family, tend to remain unpolymerized; on the contrary, labdane-type acids (e.g., agathic and communic acids, common in the Cupressaceae family), easily polymerize thanks to conjugated diene compounds (Langenheim, 2003). Resins of the Araucariaceae family may contain all three diterpene groups.

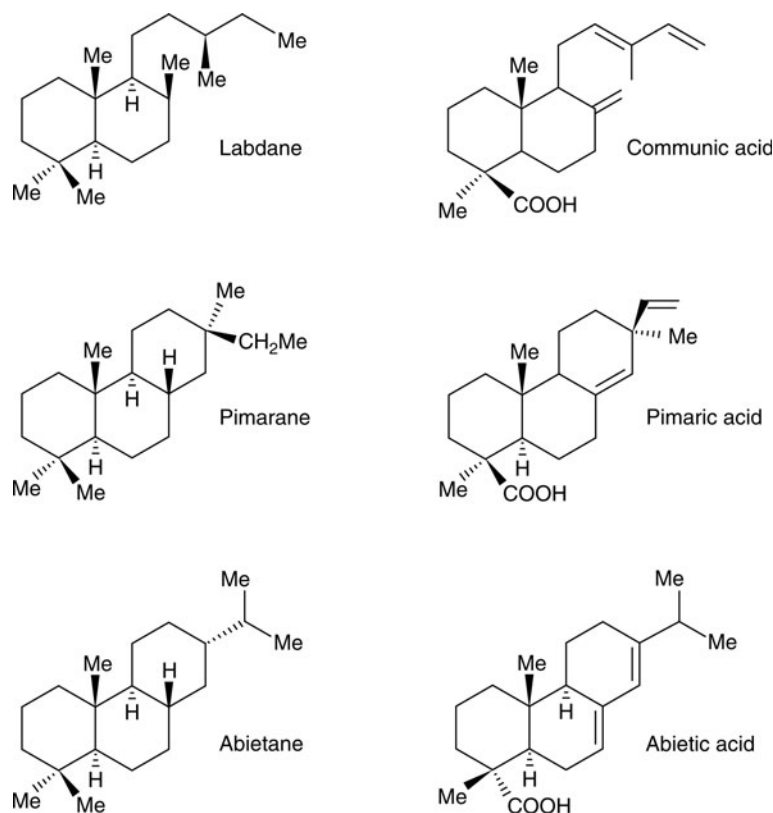
Also the nonvolatile fraction of angiosperm resins, for example, from the genus *Hymenaea* of the Leguminosae (Fabaceae) family, which yields copal, is constituted by diterpenes of the labdadiene group. Resins from other angiosperms, such as Dipterocarpaceae, contain mainly triterpenes.

Therefore, resins containing particular diterpenes, such as abietic acid, cannot form amber *sensu stricto* because the constituent does not contain functional groups that could lead to a cross-linked polymer (Beck, 1999). Also triterpenoid resins (C₃₀), which are mainly produced by broad-leaved trees, generally do not polymerize.

The process of polymerization and resin hardening starts just after resin exudation, and involves free radical mechanisms, which are initiated by exposure to sunlight and air (Langenheim, 2003). Initial polymerization can



Amber, Figure 2 Biosynthesis of terpenoid resins from mevalonate (adapted from Langenheim, 1969).



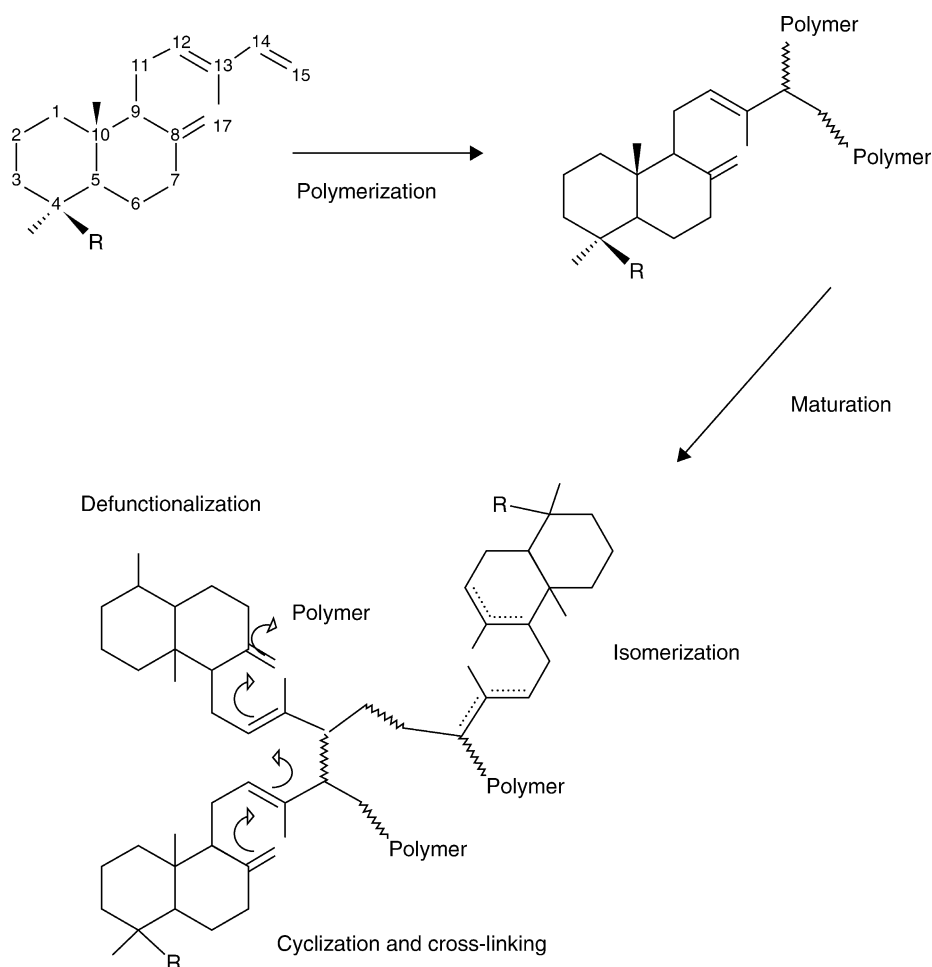
Amber, Figure 3 Chemical structure of major conifer resin components. Left column presents the general skeleton, and right column, an example of specific compounds (for further details, see Langenheim, 2003).

occur involving the terminal groups of a side chain in the molecule of terpenoids, mainly diterpenes with a labdanoid structure (unsaturated labdatriene acids and alcohols, and biformene), leading to the formation of a general polymeric structure (Anderson et al., 1992; Clifford and Hatcher, 1995; Clifford et al., 1997) (Figure 4). As a complex mixture, amber contains also nonpolymerizing compounds such as succinic acid in succinite, or monoterpenes, which are trapped (cross-linked) into the polymer network (Clifford and Hatcher, 1995; Clifford et al., 1997) and function as plasticizers.

Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and burial into the sediment, due to diagenetic and catagenetic processes. The chemical reactions in maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization (Clifford and Hatcher, 1995; Clifford et al., 1997). One typical reaction in polylabdanoid fossil resins is the depletion of exocyclic methylenes (Figure 4) in the C8–C17 double bond of the molecule, and the formation of intramolecular cyclization. The modification of amber structure during its maturation leads to a progressive loss of unsaturated bonds, a decrease in functionalized groups and an increased proportion of aromatized groups (Grimalt et al., 1988).

Although the degree of resin maturation is dependent on the age of the resin, the rate at which this process occurs depends on several geological conditions, including the thermal history of the sample (Anderson et al., 1992) and on the structure and resin composition (Langenheim, 2003). Although a certain direct correlation between resin maturation and the age has also been demonstrated, by means of thermal analysis (Ragazzi et al., 2003), maturity and age cannot always be directly linked, since chemical transformation of resin increases at higher temperatures, and therefore the geothermal variable should be considered. Other physicochemical properties of amber, such as hardness and solvent solubility, may suggest the degree of maturation (Poinar, 1992; Rodgers and Currie, 1999; Langenheim, 2003; Ragazzi et al., 2003).

An alternative approach to define the age of a resin was proposed by Anderson (1997) on the basis of carbon-14 content. Resins that are older than the detection limit of carbon-14, that is, at about 40,000 years, have been considered *fossil resins*, and their age should be determined based on the stratigraphical data of the embedding sediment. Resins of age between 40,000 and 5,000 years are considered *subfossil*, while resins between 5,000 and 250 years are *ancient resins*, and those younger than 250 years are considered as *modern* or *recent*. This



Amber, Figure 4 Schematic representation of the polymeric structure in polylabdanoid fossil resins and of the maturation process (adapted from Clifford and Hatcher, 1995; Clifford et al., 1997).

terminology should be preferred to that referring to “young amber” or “copal,” which is often misleading with respect to the age of the sample.

Physicochemical characteristics of amber

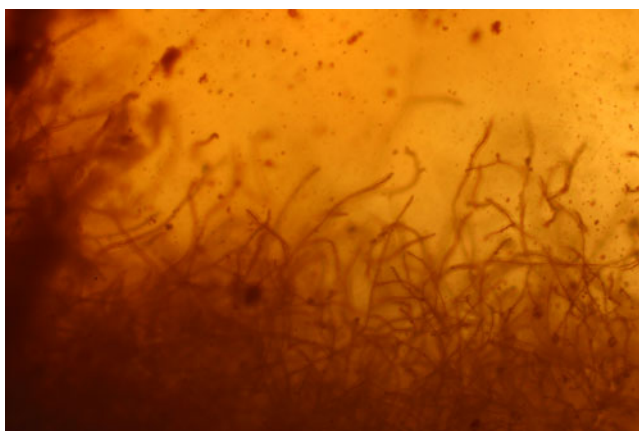
Most amber pieces are minute ranging from millimeter to centimeter size. Resin pieces may be found preserved in their original shape as solidified in the amber forest (e.g., drops, elongate stalactites, lumps with a flattened side which was attached to the bark or to other surfaces). Fossil resin is sometimes still attached to fossil wood, or even *in situ* in wood or twigs which can help to identify the amber-bearing trees of a particular deposit. Amber pieces are more or less rounded after being transported and redeposited.

Amber has been extensively investigated under the physicochemical aspect. The relative hardness according to Mohs' scale is 2–2.5 (corresponding to 199–290 MPa) for most types of amber, indicating that it is slightly harder than gypsum and that it is possible to scratch the surface with calcite or copper. The specific

gravity, that is, the ratio of the density of a substance to the density of water, ranges from about 0.96–1.12. For this reason most amber is nearly floating in sea water (specific gravity of the Baltic sea water is very low, about 1.005) and it sinks to the bottom in quiet water, but it can easily be carried along by disturbed water and washed ashore. Because of its low density, amber floats on a salt solution (about 15 g of NaCl/100 ml), while several types of plastic, often used as amber imitation, sink due to their higher density (e.g., the phenolic plastic bakelite, which has a specific gravity of about 1.4). Amber has resinous luster, and might appear translucent or opaque. Its refractive index ranges between 1.539 and 1.545. The color of translucent amber varies from a honey-like yellow and yellow-orange to light and dark red; the latter color is often caused by weathering. Rare specimens of blue (e.g., some Dominican amber) and green (e.g., some Mexican amber) samples have been found. Resins may be opaque because of microscopic bubbles or tiny detritus particles. Sometimes, densely arranged filaments of bacteria and fungi

that grew into the resin in its liquid stage cause opacity of the outer parts of resin pieces (Figure 5). If exposed to ultraviolet light, amber often produces fluorescence of blue, yellow, or green color. When amber is heated, it becomes soft at 150–180°C and decomposes around 280–300°C, emitting fumes with aromatic odor. After touching amber with a hot needle (hot point test), it produces smoke and burnt-resinous odor; this method is empirically used to distinguish between amber and plastic or copal. Exposed to a flame, amber burns easily. Amber is insoluble in water, and partly soluble in organic solvents,

such as diethyl ether, acetone, and dichloromethane, as well as in mixtures of turpentine oil and acetone. After applying a drop of solvent to the surface, recent resins (copal) or plastic are readily dissolved and the surface becomes sticky, while amber (polymerized fossil resin) is unaffected. Although resin is macroscopically amorphous, some of its constituents may form microscopic drop- or bubble-like and elongated microstructures. Some of these “pseudoinclusions” may resemble enclosed microorganisms but can be distinguished by their variable size and shape and by the absence of diagnostic surface features (Figure 6).



Amber, Figure 5 Filamentous sheathed bacteria of ca 10 μm diameter preserved in French Cretaceous amber after growing into the liquid resin (photograph by Alexander R. Schmidt).

Chemical analyses and classification of amber

Since early chemical investigations in the nineteenth century, amber has been identified as being composed of carbon, hydrogen, and oxygen. It is not possible to indicate a univocal composition formula of amber since it is an amorphous, noncrystalline material. The composition of different types of amber, as a result of elemental analysis, has been indicated approximately in the following range: carbon 75–87%, hydrogen 8.5–11%, oxygen 5–15%, with traces of sulfur (up to 1.7%) and other elements (Kosmowska-Ceranowicz, 1984; Ragazzi et al., 2003).

Since amber is mostly insoluble in organic solvents, a complete characterization of its chemical composition has been obtained using the total solid sample, by means of specific analytical techniques that have become available, such as infrared spectroscopy (IRS), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and nuclear magnetic resonance (NMR) spectroscopy.



Amber, Figure 6 Pseudoinclusions in Cenomanian amber from Fourtou in the French Pyreneans (photograph by Alexander R. Schmidt).

Infrared spectroscopy of powdered samples, since the first application in the study of fossil resins (Beck et al., 1964), has become one of the main techniques for obtaining structural information and identification of amber from different origins (Kosmowska-Ceranowicz, 1999). Infrared spectra allow to define the fingerprinting of any fossil resin, and in particular it has been successful as diagnostic for Baltic amber, observing the presence of the characteristic Baltic shoulder in the 1,150–1,260 cm^{-1} region of the spectrum, attributed to the absorption of ester groups, in particular esterified succinic acid. It is noteworthy that the free succinic acid levels in Baltic amber range between 50 and 400 ppm (Tonidandel et al., 2009), despite the high total succinic acid content of 1–8%.

Anderson et al. (1992; also Anderson and Crelling, 1995), on the basis of various physicochemical methods, mainly Py-GC/MS, proposed a classification of fossil resins into five main classes:

Class I	The macromolecular structures are derived from polymers of primarily labdanoid diterpenes, which include labdatriene carboxylic acids, alcohols, and hydrocarbons
Ia	Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, including communic acid, communol, and succinic acid; example: succinite (Baltic amber)
Ib	Derived from/based on polymers and copolymers of labdanoid diterpenes with regular [1S, 4aR, 5S, 8aR] configuration, often including communic acid, communol, biformene, but not succinic acid; example: New Zealand's fossil kauri resin
Ic	Derived from/based on polymers and copolymers of labdanoid diterpenes with enantio [1S, 4aS, 5R, 8aS] configuration, including ozic acid, ozol, and enantio biformenes; examples: Mexican and Dominican amber, African resinates
Class II	Derived from/based on polymers of bicyclic sesquiterpenoid hydrocarbons, especially cadinene, and related isomers; examples: Utah and Indonesian amber
Class III	Natural (fossil) polystyrene; example: some New Jersey amber, Siegburgite from Germany
Class IV	Nonpolymeric, incorporating sesquiterpenoids based on the cedrane carbon skeleton; example: Moravian amber from the Czech Republic
Class V	Nonpolymeric diterpenoid carboxylic acid, especially based on abietane, pimarane, and isopimarane carbon skeletons; example: Highgate copalite and other retinites in European brown coals

The high-resolution NMR spectra of the carbon nuclei in powdered samples provided detailed information on the types of carbon functionality in the fossil resin, over the entire range of resonances, indicating the presence of

saturated carbons (resonances 0–100 ppm), unsaturated carbons such as alkenic and aromatic (resonances 100–160 ppm), and carbonyl groups (resonances above 160 ppm) (Lambert and Frye, 1982). Using NMR, comparative analysis of samples from different origin permitted to distinguish four major groupings (Group A through D) of fossil resins (Lambert and Poinar, 2002), in good agreement with Anderson's et al. classification based on Py-GC/MS.

Age, global occurrence, and botanical origin of amber

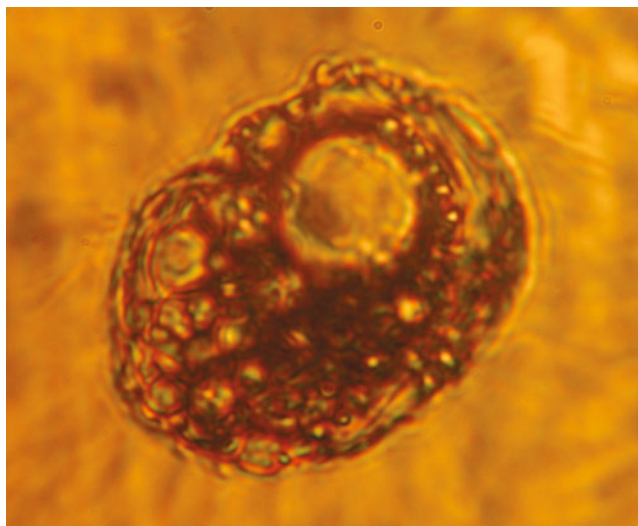
Amber might be found on every continent but most amber deposits have been discovered in the northern hemisphere, so far. A few ambers are known from South America, Africa, Australia, and New Zealand. Many new Mesozoic and Cenozoic amber localities have been described in the last decade (see Poinar, 1992; Grimaldi, 1996; Krumbiegel and Krumbiegel, 2005, for age and global occurrence of ambers).

A few resins were reported from the Carboniferous of Europe and the USA (Bray and Anderson, 2009). The first conifers which produced significant amounts of resin appeared in the Carnian stage of the late Triassic (about 220 million years ago). Remarkable amounts of small Triassic amber pieces have been found in the petrified forests of Arizona (Litwin and Ash, 1991), in the Molteno Formation of Lesotho (Ansorge, 2007) and the Heiligkreuz-Santa Croce Formation of the Italian Dolomites (Roghi et al., 2006, Figure 7). The latter is the oldest fossiliferous amber containing various microorganisms (Schmidt et al., 2006, see Figure 8). The Jurassic is particularly poor in amber and just few samples have been reported from the late Jurassic age of Portugal, Denmark (Bornholm), Germany, and Thailand.

The oldest Cretaceous ambers come from the Wealden Formation of southern England (Nicholas et al., 1993) and from the Kirkwood Formation of South Africa (Gomez et al., 2002), both being Valanginian in age. The most important fossiliferous Cretaceous ambers are (1) the *Middle East ambers* from Lebanon, Israel, and Jordan, ranging from the Hauterivian to the Aptian, (2) the Aptian–Albian *Álava amber* from Peñacerrada in the Basque county of northern Spain, (3) the *Siberian amber* from the Albian to Santonian of the Taimyr Peninsula, (4) the Albian–Cenomanian *ambers from Charente and Charente-Maritime* in southwestern France (see Perrichot et al., 2007, for these newly discovered ambers), (5) the *Burmese amber* from Myanmar which is Albian in age (Grimaldi et al., 2002; Cruickshank and Ko, 2003), (6) the *Atlantic coastal plain ambers* of the USA which comprise several localities of various Upper Cretaceous ages from Maryland and New Jersey to South Carolina, and (7) the Upper Cretaceous *Canadian amber* (Cedar Lake in Manitoba and other localities). Further remarkable Cretaceous resins come from Alaska, from several Albian to Coniacian localities in Japan, and from



Amber, Figure 7 Triassic amber drop from the Italian Dolomites (photograph by Guido Roghi, *Nature*, 444, 835, 2006). The piece is about 3 mm in length.



Amber, Figure 8 Shell of a testate amoeba enclosed in Triassic amber from Italy. This shell is 35 μm in size and morphologically identical to the modern species *Centropyxis hirsuta* (photograph by Alexander R. Schmidt, *Nature*, 444, 835, 2006).

the Aptian age of Golling in Austria, but these ambers yield few fossils.

Cenozoic ambers are largely Eocene to Miocene in age, and some deposits already produced tons of resin. The most well-known fossil resin is the *Baltic amber* which

can be found in the Baltic sea area (especially along the coast of Russia and Poland) in Eocene sediments, washed ashore and also as glacial erratic. The sediments bearing the Baltic amber are considered to be 38–47, some up to 55 million years old (Ritzkowski, 1999). Other fossiliferous Eocene ambers come from the *Paris Basin* in France (50–55 million years old; Nel et al., 1999), from Vastan (Gujarat) in western India (52 million years old; Alimohammadian et al., 2003) and from Fu Shun in the Chinese Liaoning province. The Bitterfeld amber from a coal mine near the city of Bitterfeld in central Germany is uppermost Oligocene in age (24 million years old, see Wimmer et al., 2006) and also produced a plethora of inclusions, mainly arthropods (Knuth et al., 2002). The most fossiliferous Cenozoic New World ambers come from the Dominican Republic and from Chiapas in Mexico (see Poinar, 1992). Recent studies suggest a Miocene age (15–20 million years old) for these Caribbean ambers (Iturralde-Vinent and MacPhee, 1996; Solórzano Kraemer, 2007). Further fossiliferous Miocene ambers were found in western Amazonia in Peru, and in Sicily and Borneo.

Representatives of various gymnosperm and angiosperm families have produced resin in the Earth's history (see Langenheim, 2003; Krumbiegel and Krumbiegel, 2005, for overview). The Italian Triassic amber was possibly produced by members of the Cheirolepidiaceae, an extinct gymnosperm family. Among others, various representatives of the Araucariaceae (Figures 9 and 10) and Cupressaceae are considered to be resin-bearing trees in the Cretaceous. Angiosperm resins are recorded since the Eocene, for example, *Glessite* stems from Burseraceae and *Siegburgite* were produced by Hamamelidaceae. The tropical genus *Hymenaea* (Leguminosae) produced the Dominican and Mexican ambers. The main resin-bearing trees of the Baltic amber forest probably belonged to conifers of the family Sciadopityaceae, as recently suggested by Wolfe et al. (2009).

Embedding and preservation of organisms in resin

Resins are produced by plants as defensive reaction against mechanical damage (injury to the bark, fire, herbivores) and potential invasion by pathogens, such as bacteria and fungi. Also, climatic reasons and ecological disturbance have been discussed to be the reasons for extensive resin production (Henwood, 1993).

The exceptional preservation of organisms included in amber is due to the dehydration environment made possible by the resin and by natural embalming properties due to antiseptic activity of the resin constituents. Components that do not participate in polymerization of the resin, such as some diterpene resin acids (e.g., copalic acid), can remain available for the interactions with biota embedded in resin (Henwood, 1993) and contribute to the preservation even of soft-bodied inclusions. Even cell organelles such as chloroplasts of algae are sometimes well-preserved in amber (Figure 11).

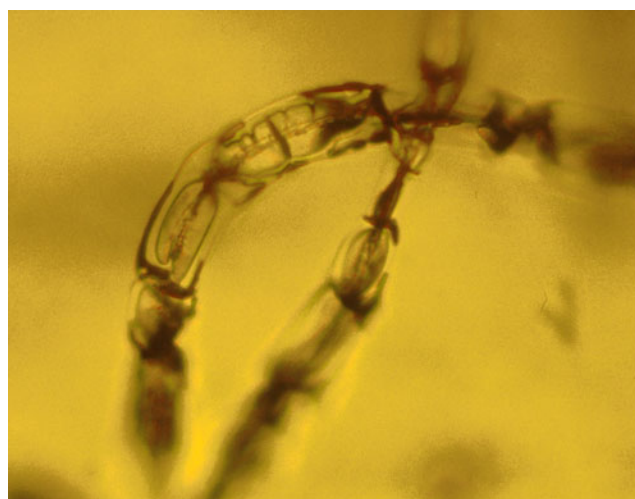


Amber, Figure 9 Resinous forest of *Araucaria columnaris* growing at the coast of Maré, New Caledonia (photograph by Alexander R. Schmidt).



Amber, Figure 10 Extensive resin flows of ~30 cm length induced by fire at the bark of *Araucaria columnaris* in New Caledonia (photograph by Alexander R. Schmidt).

Extraction of ancient DNA from amber has been of particular interest in working with fossils embedded in amber, and in the early 1990s first attempts to establish methods for extraction, amplification, and sequencing of fossil



Amber, Figure 11 Preservation of chloroplasts in the conjugatophyte *Palaeozygnema spiralis* from the Cenomanian resin of Schliersee in the Bavarian Alps. The cells are ~15 μm in diameter (photograph by Alexander R. Schmidt).

DNA from amber were carried out (e.g., Cano et al., 1994; Cano and Borucki, 1995). However, critical reinvestigations suggest that the DNA obtained in these studies was modern contamination and because even the amplification of DNA from copal failed, Austin et al. (1997) concluded that resin is not predestined for the preservation of complex molecules, although morphological structures are recorded very well.

Attached to fresh sticky resin (Figure 10), organisms or parts of them are entirely covered by further resin flows. Amber inclusions are therefore usually found at the surfaces of successive resin flows in amber. Invertebrates of appropriate size, especially arthropods (e.g., diptera, hymenoptera, see Figure 12), and small plant remnants fallen onto the resin are therefore predestined for getting stuck and embedded. Arthropods, plant remnants (flowers, leaves, stellate hairs, wood, pollen, spores) and microorganisms are typical amber inclusions and sometimes, also small vertebrates (frogs, lizards) and parts of larger animals (reptile skin, feathers, hairs) are preserved. Some groups of organisms are only recorded as amber fossils, since few other fossilization processes occur in terrestrial environments which may preserve morphological structures even of soft-bodied organisms very well.

Many life forms and representatives of all trophic levels of a biocoenosis may be found in a single piece of resin: bacteria, algae as producers, protozoans, micrometazoans, molluscs, arthropods as consumers, and fungi as decomposers (Perrichot and Girard, 2009). Although many members of a biocoenosis can potentially become embedded in resin, selective embedding has been observed (Schmidt and Dilcher, 2007). Because of their motility, moving or flying arthropods have a higher probability of encountering and becoming attached to the resin. Therefore, the amount of amber inclusions of a species is not correlated with its abundance in the ancient ecosystem.

Most amber inclusions are fossils of terrestrial organisms which lived at or close to the resin-bearing trees. Much resin solidified at the forest floor, not on the bark (Henwood, 1993) and the term *litter amber* has been proposed by Perrichot (2004) for amber pieces which largely contain soil and litter-dwelling arthropods (Figure 13).



Amber, Figure 12 Hymenoptera (Scelionidae), fossilized in Lower Cenomanian amber of Fouras, Charente-Maritime, southwestern France. The inclusion is 1.8 mm in length (courtesy of Vincent Perrichot).

To find aquatic organisms in tree resin may seem to be highly unlikely but the fossil record provides amber-preserved limnetic arthropods (e.g., water beetles, water striders, crustaceans, larvae of caddisflies and mayflies) and microorganisms (e.g., algae, ciliates, testate amoebae, rotifers). Limnetic organisms may get stuck or enclosed when resin comes into contact with water or even flows into water (Schmidt and Dilcher, 2007).

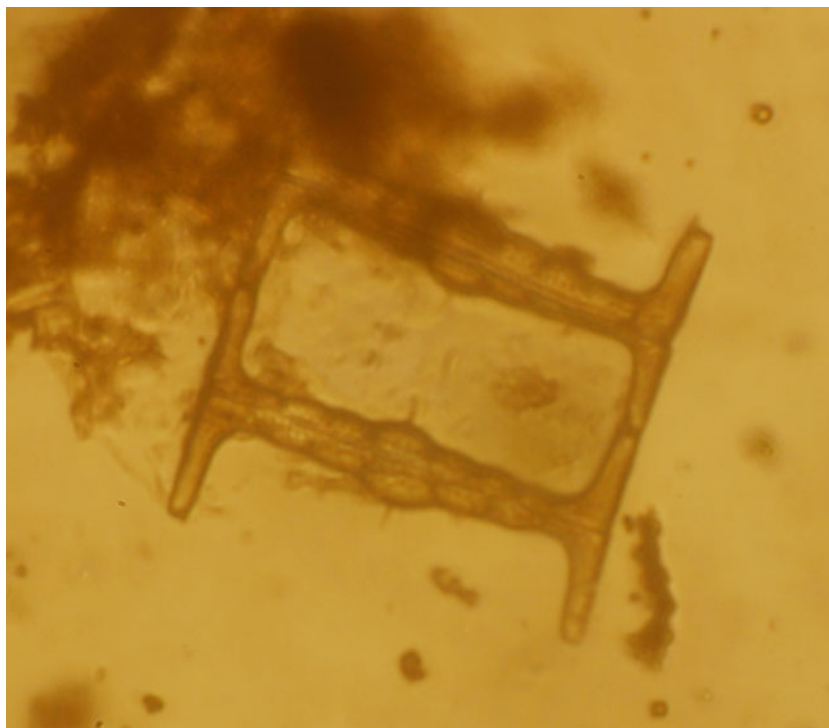
Few amber inclusions of marine organisms (crustaceans, diatoms) have been reported from amber forests which grew directly at the coast (Vonk and Schram, 2007; Girard et al., 2008; see Figure 14). These organisms were probably introduced by wind or spray from beach and sea onto the resin flows in the nearby woods.

Although resin has fungicidal properties, some fungi and bacteria are able to grow into liquid resin and can therefore be found in almost every fossil resin. Usually, these filaments grow in random orientation as long as the resin is liquid. Growth stops when the resin solidifies and the filiform inclusions become well-preserved in the resin. Resinicolous fungi such as ascomycetes of the Mycocaliciales are adapted to their special substrates and are also able to grow on fresh resin. Therefore, they may be found in places where herbivore insects induce long-term resin flows (Rikkinen, 1999; Rikkinen and Poinar, 2000).

Resin which dried at the bark is exposed to the air, and processes of weathering and degradation start rapidly. Long-time preservation of resins and its inclusions and



Amber, Figure 13 *Marchandia magnifica*, a mole cricket from Albian amber from southwestern France. It is 3.8 mm in length (photograph by Vincent Perrichot, courtesy of Elsevier/ Academic Press, *Cretaceous Research*, 23, 307–314, 2002).



Amber, Figure 14 Two frustules of a marine diatom of the genus *Hemiaulus* enclosed in a piece of amber from a coastal Cretaceous forest from Archingeay in south-western France. The cells are ca 40 μm in size (photograph by Alexander R. Schmidt).

formation of amber therefore generally needs redeposition into marine sediments or at least a cover by sediment layers to stop processes of oxidation.

Summary

Amber is fossilized plant resin, which is largely found in the Cretaceous and in the Eocene to Miocene when various representatives of gymnosperms and angiosperms produced considerable amounts of resin. After exudation from plants, the volatile components evaporate and the remaining nonvolatile constituents polymerize which leads to the hardening of the resin. Formation of amber occurs through the process of “maturation,” which is defined as the progressive changes of the resin occurring after the hardening and embedding into the sediment. The chemical reactions in the maturation of the resin to yield fossil resin include cross-linking, isomerization, and cyclization. Several different parameters can play relevant roles in amber composition, such as original environment, age, plant source, diagenetic history, and also alteration processes after amber mining. Inclusions of many life forms may be found in amber, although selective embedding has been observed. The taphocoenosis in a piece of amber may potentially contain organisms of different habitats such as tree bark, soil and litter, freshwater ponds, and littoral.

Bibliography

- Alimohammadian, H., Sahni, A., Patnaik, R., Rana, R. S., and Singh, H., 2003. First record of an exceptionally diverse and well preserved amber-embedded biota from Lower Eocene (~52 Ma) lignites, Vastan, Gujarat. *Current Science*, **89**, 1328–1330.
- Anderson, K. B., 1997. The nature and fate of natural resins in the geosphere – VII. A Radiocarbon (^{14}C) age scale for description of immature natural resins: an invitation to scientific debate. *Organic Geochemistry*, **25**, 251–253.
- Anderson, K. B., and Crelling, J. C., 1995. *Amber, Resinite, and Fossil Resins*. Washington: American Chemical Society.
- Anderson, K. B., Winans, R. E., and Botto, R. E., 1992. The nature and fate of natural resins in the geosphere – II. Identification, classification and nomenclature of resinates. *Organic Geochemistry*, **18**, 829–841.
- Ansorge, J., 2007. Upper Triassic insects and amber from Lesotho (South Africa). In *FossilsX3: Insects, Arthropods, Amber*. Vitoria-Gasteiz, Spain, May 4–9, 2007, Abstracts: p. 52.
- Austin, J. J., Ross, A. J., Smith, A. B., Fortey, R. A., and Thomas, R. H., 1997. Problems of reproducibility – does geologically ancient DNA survive in amber-preserved insects? *Proceedings of the Royal Society of London, Series B*, **264**, 467–474.
- Beck, C. W., 1999. The chemistry of amber. *Estudios del Museo de Ciencias Naturales de Alava*, **14**(Numero especial 2), 33–48.
- Beck, C. W., Wilbur, E., and Meret, S., 1964. Infrared spectra and the origin of amber. *Nature*, **201**, 256–257.
- Bray, P. S., and Anderson, K. B., 2009. Identification of Carboniferous (320 Million Years Old) Class 1c amber. *Science*, **326**, 132–134.

- Cano, R. J., and Borucki, M. K., 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. *Science*, **268**, 1060–1064.
- Cano, R. J., Borucki, M. K., Higby-Schweitzer, M., Poinar, H. N., Poinar, G. O., Jr., and Pollard, K. J., 1994. *Bacillus*-DNA in fossil bees: an ancient symbiosis? *Applied and Environmental Microbiology*, **60**, 2164–2167.
- Clifford, D. J., and Hatcher, P. G., 1995. Structural transformations of polylabdanoid resins during maturation. *Organic Geochemistry*, **23**, 407–418.
- Clifford, D. J., Hatcher, P. G., Botto, R. E., Muntean, J. V., Michels, B., and Anderson, K. B., 1997. The nature and fate of natural resins in the geosphere – VIII. NMR and Py-GC-MS characterization of soluble Labdanoid polymers, isolated from Holocene class I resins. *Organic Geochemistry*, **27**, 449–464.
- Cruickshank, R. D., and Ko, K., 2003. Geology of an amber locality in the Hukawng valley, Northern Myanmar. *Journal of Asian Earth Sciences*, **21**, 441–445.
- Girard, V., Schmidt, A. R., Saint Martin, S., Struwe, S., Perrichot, V., Saint Martin, J.-P., Grosheny, D., Breton, G., and Néraudeau, D., 2008. Evidence for marine microfossils from amber. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17426–17429.
- Gomez, B., Martinez-Delclos, X., Bamford, M., and Philippe, M., 2002. Taphonomy and palaeoecology of plant remains from the oldest African Early Cretaceous amber locality. *Lethaia*, **35**, 300–308.
- Grimaldi, D. A., 1996. *Amber: Window to the Past*. New York: Harry N. Abrams Inc. in Association with the American Museum of Natural History.
- Grimaldi, D. A., Engel, M. S., and Nascimbene, P. C., 2002. Fossiliferous Cretaceous amber from Myanmar (Burma): its rediscovery, biotic diversity, and paleontological significance. *American Museum Novitates*, **3361**, 1–72.
- Grimalt, J. O., Simoneit, B. R. T., Hatcher, P. G., and Nissenbaum, A., 1988. The molecular composition of ambers. *Organic Geochemistry*, **13**, 677–690.
- Henwood, A., 1993. Recent plant resins and the taphonomy of organisms in amber: a review. *Modern Geology*, **19**, 35–59.
- Iturralde-Vinent, M. A., and MacPhee, R. D. E., 1996. Age and paleogeographical origin of Dominican amber. *Science*, **273**, 1850–1852.
- Knuth, G., Koch, T. h., Rappsilber, I., and Volland, L., 2002. Concerning amber in the Bitterfeld Region – geologic and genetic aspects. *Hallesches Jahrbuch für Geowissenschaften*, **24**, 35–46.
- Kosmowska-Ceranowicz, B., 1984. Amber and its origins. In Kosmowska-Ceranowicz, B. (ed.), *Amber in Nature*. Warsaw: PAN Muzeum Ziemi, pp. 17–18.
- Kosmowska-Ceranowicz, B., 1999. Succinite and some other fossil resins in Poland and Europe (deposits, finds, features and differences in IRS). *Estudios del Museo de Ciencias Naturales de Alava*, **14**(Número especial 2), 73–117.
- Krumbiegel, G., and Krumbiegel, B., 2005. *Bernstein. Fossile Harze aus aller Welt*. Wiebelsheim, Germany: Quelle & Meyer.
- Lambert, J. B., and Frye, J. S., 1982. Carbon functionalities in amber. *Science*, **217**, 55–57.
- Lambert, J. B., and Poinar, G. O., Jr., 2002. Amber: the organic gemstone. *Accounts of Chemical Research*, **35**, 628–636.
- Langenheim, J. H., 1969. Amber: a botanical inquiry. *Science*, **163**, 1157–1169.
- Langenheim, J. H., 2003. *Plant Resins. Chemistry, Evolution, Ecology, Ethnobotany*. Portland: Timber Press.
- Litwin, R. J., and Ash, S. R., 1991. First early Mesozoic amber in the western hemisphere. *Geology*, **19**, 273–276.
- Nel, A., De Plöeg, G., Dejaj, J., Dutheil, D., de Franceschi, D., Gheerbrant, E., Godinot, M., Hervet, S., Menier, J. J., Augé, M., Bignot, G., Cavagnetto, C., Duffaud, S., Gaudant, J., Hua, S., Jossang, A., De Lapparent de Broin, F., Pozzi, J. P., Paicheler, J. C., Bouchet, F., and Rage, J. C., 1999. Un gisement sparnacien exceptionnel à plantes, arthropodes et vertébrés (Éocène basal, MP7): Le Quesnoy (Oise, France). *Comptes Rendus de l'Académie des Sciences, Sciences de la Terre et des Planètes*, **329**, 65–72.
- Nicholas, C. J., Henwood, A. A., and Simpson, M., 1993. A new discovery of Early Cretaceous (Wealden) amber from the Isle of Wight. *Geological Magazine*, **130**, 847–850.
- Perrichot, V., 2004. Early Cretaceous amber from south-western France: insight into the Mesozoic litter fauna. *Geologica Acta*, **2**, 9–22.
- Perrichot, V., and Girard, V., 2009. A unique piece of amber and the complexity of ancient forest ecosystems. *Palaaios*, **24**, 137–139.
- Perrichot, V., Néraudeau, D., Nel, A., and De Ploëg, G., 2007. A reassessment of the Cretaceous amber deposits from France and their palaeontological significance. *African Invertebrates*, **48**, 213–227.
- Poinar, G. O., Jr., 1992. *Life in Amber*. Palo Alto: Stanford University Press.
- Ragazzi, E., Roghi, G., Giarretta, A., and Gianolla, P., 2003. Classification of amber based on thermal analysis. *Thermochimica Acta*, **404**, 43–54.
- Rikkinen, J., 1999. Two new species of resinicolous *Chaenothecopsis* (Mycocaliciaceae) from western North America. *The Bryologist*, **102**, 366–369.
- Rikkinen, J., and Poinar, G. O., Jr., 2000. A new species of resinicolous *Chaenothecopsis* (Mycocaliciaceae, Ascomycota) from 20 million year old Bitterfeld amber, with remarks on the biology of resinicolous fungi. *Mycological Research*, **104**, 7–15.
- Ritzkowski, S., 1999. Das geologische Alter der bernsteinführenden Sedimente in Sambia (Bezirk Kaliningrad), bei Bitterfeld (Sachsen-Anhalt) und bei Helmstedt (SE-Niedersachsen). In: *Investigations into Amber. Proceedings of the International Interdisciplinary Symposium: Baltic Amber and Other Fossil Resins*, September 2–6, 1997, Gdansk. The Archaeological Museum in Gdansk, Museum of the Earth, Polish Academy of Sciences, Gdansk, pp. 33–40.
- Rodgers, K. A., and Currie, S., 1999. A thermal analytical study of some modern and fossil resins from New Zealand. *Thermochimica Acta*, **326**, 143–149.
- Roghi, G., Ragazzi, E., and Gianolla, P., 2006. Triassic amber of the southern Alps (Italy). *Palaaios*, **21**, 143–154.
- Schmidt, A. R., and Dilcher, D. L., 2007. Aquatic organisms as amber inclusions and examples from a modern swamp forest. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16581–16585.
- Schmidt, A. R., Ragazzi, E., Coppellotti, O., and Roghi, G., 2006. A microworld in Triassic amber. *Nature*, **444**, 835.
- Solórzano Kraemer, M. M., 2007. Systematic, palaeoecology, and palaeobiogeography of the insect fauna from the Mexican amber. *Palaeontographica Abteilung A*, **282**, 1–133.
- Stoppani, A., 1886. *L'ambra nella storia e nella geologia, con speciale riguardo agli antichi popoli d'Italia nei loro rapporti colle origini e collo svolgimento della civiltà in Europa*. Milan, Italy: Dumolard.
- Tonidandel, L., Ragazzi, E., and Traldi, P., 2009. Mass spectrometry in the characterization of ambers. II. Free succinic acid in fossil resins of different origins. *Rapid Communication in Mass Spectrometry*, **23**, 403–408.
- Vonk, R., and Schram, F. R., 2007. Three new tanaid species (Crustacea, Peracarida, Tanaidacea) from the Lower Cretaceous Álava amber in Northern Spain. *Journal of Paleontology*, **81**, 1502–1509.

- Wimmer, R., Pester, L., and Eissmann, L., 2006. Das bernsteinführende Tertiär zwischen Leipzig und Bitterfeld. *Mauritiana (Altenburg)*, **19**, 373–421.
- Wolfe, A. P., Tappert, R., Muehlenbachs, K., Boudreau, M., McKellar, R. C., Basinger, J. F., and Garrett, A., 2009. A new proposal concerning the botanical origin of Baltic amber. *Proceedings of the Royal Society B*, **276**, 3403–3412.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Molecular Fossils)
 Diatoms
 Fungi and Lichens
 Geomycology
 Protozoa (Heterotroph, Eukaryotic)

ANAEROBIC OXIDATION OF METHANE WITH SULFATE

Katrin Knittel, Antje Boetius
 Max Planck Institute for Marine Microbiology, Bremen,
 Germany

Definition

Anaerobic oxidation of methane (AOM): microbially mediated oxidation of methane to CO₂ by electron acceptors other than oxygen.

Introduction

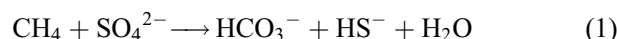
Methane is the most abundant hydrocarbon in the atmosphere, and an important greenhouse gas (see *Methane, Origin*). A great deal of research has focused on the cause and climatic consequences of the variation in fluxes of methane to the atmosphere, throughout the Earth's history. Three key functional groups of microbial organisms play a central role in regulating the fluxes of methane on the Earth, namely the methanogens, the aerobic methanotrophic bacteria, and the more recently discovered anaerobic methanotrophic archaea (ANME). It is estimated that AOM is a major sink for methane on the Earth, and of similar relevance as its photooxidation in the atmosphere (Hinrichs and Boetius, 2002; Reeburgh, 2007).

Today, most methane is produced by methanogenesis, i.e., the final step in the fermentation of organic matter taking place in soils, wetlands, landfills, rice fields, freshwater and marine sediments, as well as in the guts of animals. Almost all of the methane produced in ocean sediments is consumed by AOM within the sulfate penetrated seafloor zones. Hence, the ocean does not contribute significantly to the atmospheric methane budget (<2%; Reeburgh, 2007). The first evidence for the removal of methane within anoxic sediments and seawaters came from

geochemical observations in the 1970s and 1980s (Reeburgh, 1969, 1976; Barnes and Goldberg, 1976; Martens and Berner, 1977). The involvement of sulfate as terminal electron acceptor was demonstrated by radiotracer measurements of methane oxidation and sulfate reduction in parallel incubations of undisturbed sediment samples (Iversen and Jørgensen, 1985). Field observations and experiments led to the hypothesis of a coupled mechanism, in which both methanotrophic archaea and sulfate-reducing bacteria (SRB) could profit from AOM, despite the generally low thermodynamic energy yield from this reaction (Hoehler et al., 1994). Finally, consortia of such microorganisms were detected in sediments from methane seeps, and the substantial depletion of their specific biomarkers in the stable carbon isotope ¹³C suggested that methane or a methane-derived compound is used as carbon source by these organisms (Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001b). Till recently, all known anaerobic methanotrophs were clades related to the methanogenic Euryarchaeota termed ANME. In 2008, bacteria were found capable of oxidizing methane anaerobically with nitrate (Ettwig et al., 2008; 2010). In the past decade, much has been learned about the distribution, activity, and physiology of the ANME, however, not a single member of these groups has been obtained in culture and the biochemical functioning of AOM remains unknown.

The process of AOM

The AOM with sulfate as the final electron acceptor according to



is the major sink of methane in the oceans and hence a significant process in the global carbon cycle and methane budget (Reeburgh, 2007). Aggregated methanotrophic archaea (ANME) and SRB are assumed to act syntrophically (Hoehler et al., 1994; Hansen et al., 1998; Hinrichs et al., 1999; Boetius et al., 2000) where the archaeal partner activates and metabolizes methane, leading to an intermediate that is scavenged as electron donor by the sulfate-reducing partner. So far, there is no evidence for the involvement of extracellular intermediates such as hydrogen, formate, or acetate (Nauhaus et al., 2002; Boetius et al., 2008). The process of AOM with sulfate has been maintained in vitro in the laboratory and investigated with respect to substrate-product stoichiometry and dependence on pressure, temperature, and a variety of other environmental factors (Nauhaus et al., 2002, 2005, 2007; Girguis et al., 2003, 2005). The ANME doubling time was reported to be approximately 7 months, and the molar growth yield of AOM was 0.6 g cell dry weight (mol CH₄ oxidized)⁻¹. Corresponding to this estimate, only 1% of the consumed methane is channeled into the ANME biomass

(Nauhaus et al., 2007). Further steps toward an understanding of the metabolic pathway were the purification of a candidate enzyme (Ni-protein I) that may catalyze methane activation in a reverse terminal methyl-coenzyme M reductase reaction (Krüger et al., 2003) and genome-based observations of nearly all genes involved in methane production present in one specific ANME type (Hallam et al., 2004; Meyerdierks et al., 2009) both supporting the hypothesis that ANME carry out AOM by reverse methanogenesis.

Anaerobic methanotrophs

Discovery of ANME

Hinrichs et al. (1999) discovered a new archaeal group in sediments from a methane seep in the Eel River basin off California, together with characteristic archaeal lipid biomarkers strongly depleted in ^{13}C , indicating that methane must be the carbon source (Hinrichs et al., 1999). The dominant archaeal group was defined as ANME-1 and a second major group associated with was identified later as ANME-2 (Orphan et al., 2001a). Boetius et al. (2000) provided the first microscopic evidence for a syntrophic association of ANME archaea and SRB (Boetius et al., 2000). In both studies specific archaeal lipid biomarkers, i.e., archaeol, hydroxyarchaeol, crocetane, and pentamethylcosane (PMI), and bacterial lipid biomarkers common for SRB, i.e., *iso*- and *anteiso*- C_{15} fatty acids, were strongly depleted in ^{13}C . The first direct link between the identity and metabolic activity of ANME was provided by using a combination of ion microprobe mass spectrometry (SIMS) and fluorescence in situ hybridization (FISH) (Orphan et al., 2001b, 2002). These experiments confirmed the extreme ^{13}C depletion in the aggregate biomass, as predicted from the biomarker extractions. In 2006, a third archaeal ANME group, ANME-3, was discovered at the submarine mud volcano Haakon Mosby in the Barents Sea (Niemann et al., 2006b). Recently, a new clade of ANME-associated archaea (AAA) was identified in a bioreactor inoculated with sediments from a Dutch canal, but their relevance in freshwater and marine sediments remains unknown (Raghoebarsing et al., 2006; Knittel and Boetius 2009).

Diversity and morphology of ANME organisms

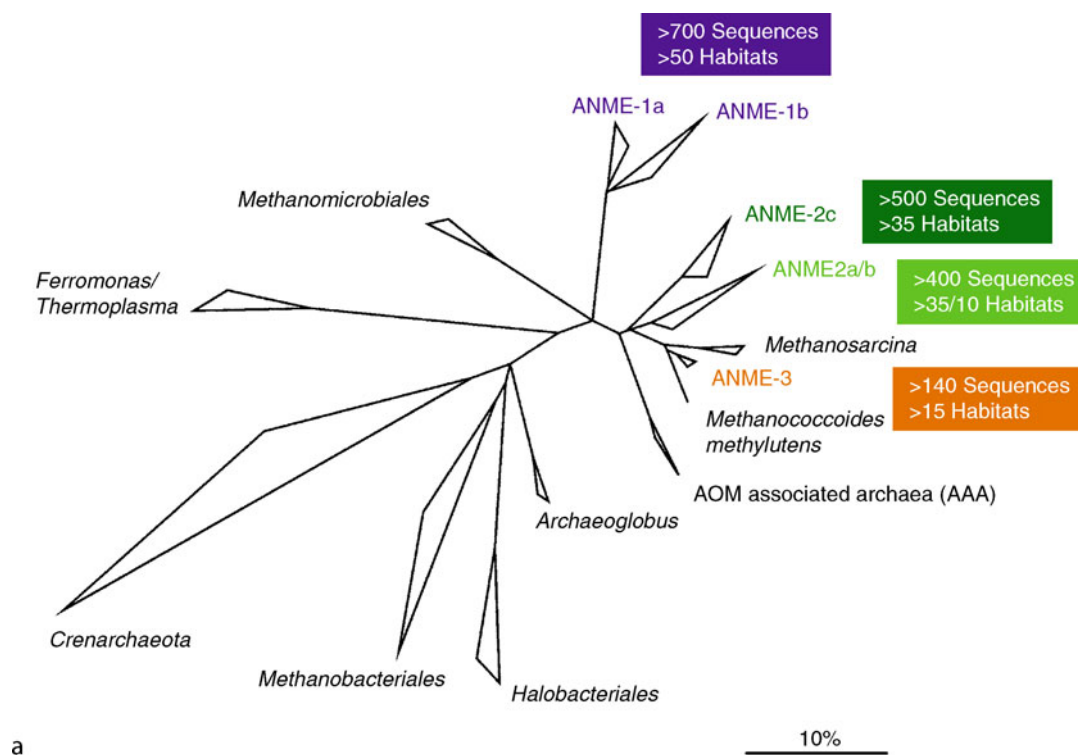
All yet known ANMEs are Euryarchaeota, and they form clusters distantly or closely related to the orders *Methanosarcinales* and *Methanomicrobiales*, which comprise a major part of the cultivated methanogens (Figure 1a).

The phylogenetic distance between the three ANME groups is large with 75–92% sequence similarity. Thus, members of ANME-1 and ANME-2 certainly belong to different orders or families, which have apparently similar physiological properties, i.e., the capability to oxidize methane anaerobically.

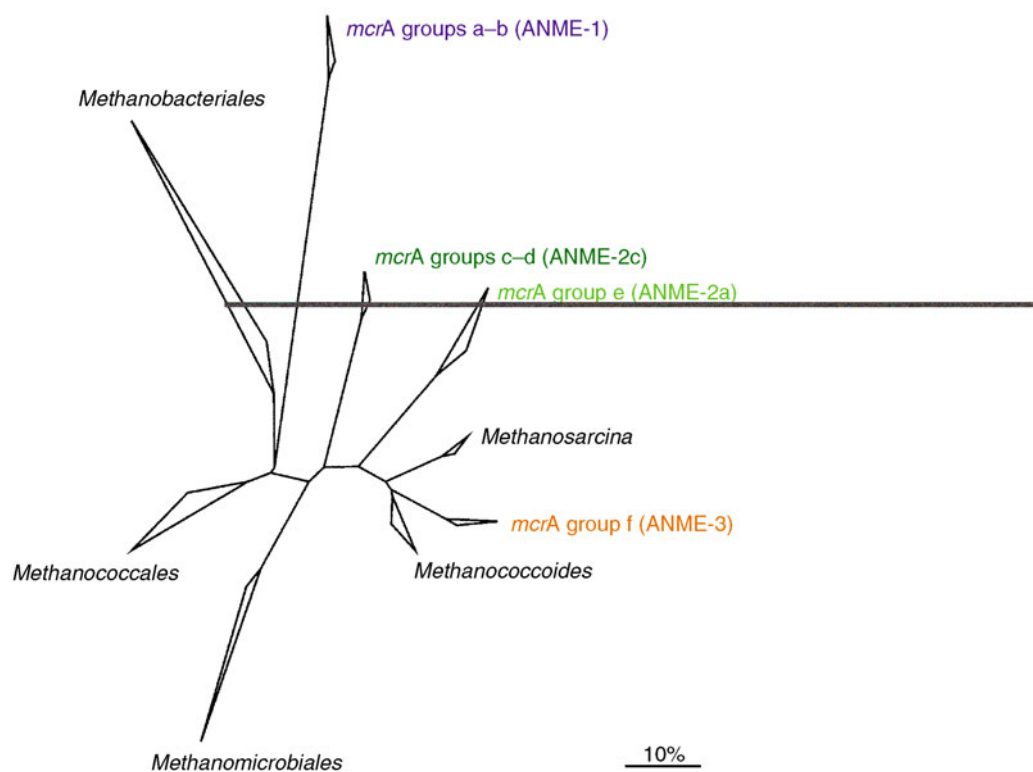
ANME-1 cells have a typical rectangular morphology and are autofluorescent under UV light, a feature characteristic for methanogenic archaea containing coenzyme F_{420} . ANME-1 archaea most often occur as single cells (Figure 2a) or in chains of two to four cells. In the Black Sea, “mat-type” associations of ANME-1 archaea and sulfate-reducing partners (Figure 2b) or ANME-1 and ANME-2 archaea (Figure 2c) form microbial mats (Michaelis et al., 2002; Knittel et al., 2005; Treude et al., 2007).

ANME-2 archaea belong to the order *Methanosarcinales*, but sequence similarity to cultivated representatives is below 90%. Similarity between subgroups, ANME-2a to ANME-2c, varies with an inter- and intra-group similarity of 75–90% and 90–100%, respectively. Diverse forms of associations between ANME-2 and their partner bacteria have been found (Figure 2d–k). Single ANME-2 cells were also reported (Figure 2d, l; Treude et al., 2007) as aggregated ANME-2 without any partner (Figure 2m, n; Orphan et al., 2002; Treude et al., 2007). In addition to the differences in phylogenetic origin, the two subpopulations ANME-2a and -2c could be distinguished from each other by their aggregate morphology. In general, ANME-2a/*Desulfosarcina* (DSS) aggregates represent the “mixed-type.” Here, archaea and SRB are completely mixed and the aggregates are not always spherical (Figure 2f–k). Typical ANME-2c/DSS aggregates represent the common “shell-type” with an inner core of ANME-2 which is partially or fully surrounded by an outer shell of SRB (Figure 2o–x). Sometimes SRB grow into the inner ANME-2c core, but they do not seem to mix completely (Figure 2u–w; Knittel et al., 2005). Microscopical analysis of a growing ANME-2 population showed that population growth occurred both as an increase in the number as well as in size of the shell-type consortia (Nauhaus et al., 2007). Starting from a few archaea and SRB cells, consortia seem to develop from smaller sized consortia to big aggregates of up to 100,000 cells (Holler and Knittel, unpublished data). Larger consortia separate evenly into two or unevenly into more aggregates when SRB grow into the archaeal colonies. The average diameter of consortia is 3–5 μm in situ with the smallest detected aggregate consisting of one ANME and one SRB cell and the largest 20 μm (Boetius et al., 2000; Knittel et al., 2005; Lösekann et al., 2007). Even much larger aggregates have been observed in enrichment cultures with >40 μm in diameter (Nauhaus et al., 2007; Holler, unpublished data). After reaching a specific size they appear to burst, releasing single cells into the environment (Figure 2ab).

The ANME-3 clade also belongs to the order *Methanosarcinales* and is closely related to cultivated species of the genus *Methanococcoides* with 95% 16S rRNA sequence similarity (Figure 1a). They form shell-type aggregates with a *Desulfobulbus* population as sulfate-reducing partner, however, only very few bacterial cells are associated (Figure 2y–z, aa).



a



b

Anaerobic Oxidation of Methane with Sulfate, Figure 1 (Continued)

Identification of ANME by methyl coenzyme M reductase gene phylogeny

The first genomic and proteomic analyses of sediments and microbial mats naturally enriched in the ANME biomass revealed a nickel protein similar to methyl coenzyme M reductase (MCR) as well as genomic fragments coding for nearly all genes typically associated with methane production. Based on these observations, earlier hypotheses on the role of reverse methanogenesis in archaea (Zehnder and Brock, 1979, 1980; Hoehler et al., 1994) appeared in a new light. Subsequently, molecular studies were conducted targeting the enzyme MCR, which catalyzes the terminal step in biogenic methane production (Shima and Thauer, 2005). Investigations have revealed a remarkably high phylogenetic diversity within *mcrA* genes among ANME archaea (Hallam et al., 2003; Inagaki et al., 2004, 2006a; Dhillon et al., 2005; Kelley et al., 2005; Alain et al., 2006; Lloyd et al., 2006; Nunoura et al., 2006; Lösekann et al., 2007) which have been classified into four subgroups (Hallam et al., 2003; Lösekann et al., 2007), group a–b (ANME-1), group c–d (ANME-2c), group e (ANME-2a), and group f (ANME-3) (Figure 1b). These groups are all distinct from those formed by methanogens. ANME *mcrA* gene phylogeny appears to be partially phylogenetically congruent to the 16S rRNA gene (Figure 3; Hallam et al., 2003; Nunoura et al., 2006; Lösekann et al., 2007). Quantification of specific ANME groups based on their *mcrA* gene abundance is now an alternative method to 16S rRNA based FISH to study distributional patterns of anaerobic methanotrophs in AOM zones (Nunoura et al., 2006).

Identification of ANME by their specific lipid biomarkers

Another molecular method, which has been crucial in the investigation of the natural distribution of ANME populations, is the identification of specific lipid biomarkers (see *Biomarkers*) and their stable carbon isotope signatures. This method can integrate phylogenetic information with function (^{13}C signatures indicating methane assimilation) and can be used for comparative analyses of community biomass. All three known ANME groups and their partner bacteria incorporate light (^{13}C -depleted) methane-derived carbon into their membrane lipids (Orphan et al., 2002; Niemann et al., 2006b). Using either naturally ^{13}C depleted methane (Nauhaus et al., 2007) or ^{13}C labeled methane as substrate (Blumenberg et al., 2004) for active populations of ANME in environmental samples have helped to identify typical membrane lipid

profiles. Crocetane is the only biomarker lipid specific for ANME, i.e., not found in methanogen cultures. Glycerol dibiphytanyl glycerol tetraethers (GDGT) were also used as indicator of ANME distribution (Schouten et al., 2003), but the ANME GDGTs appear to show some overlap with those of benthic Crenarchaeota, which often share the same niche in the seabed (Knittel et al., 2005). Recently, the biomarker approach was extended to include intact polar lipids (IPLs) which are of higher taxonomic specificity (level of families to orders) and property to select for living biomass (Sturt et al., 2004; Biddle et al., 2006; Rossel et al., 2008). Characteristic IPL molecular fingerprints have been reported for each specific ANME type: the lipids of ANME-1 archaea were dominated by diglycosidic GDGT derivatives, while IPLs of ANME-2 and ANME-3 archaea are dominated by phosphate-based polar derivatives of archaeol and hydroxyarchaeol (Rossel et al., 2008).

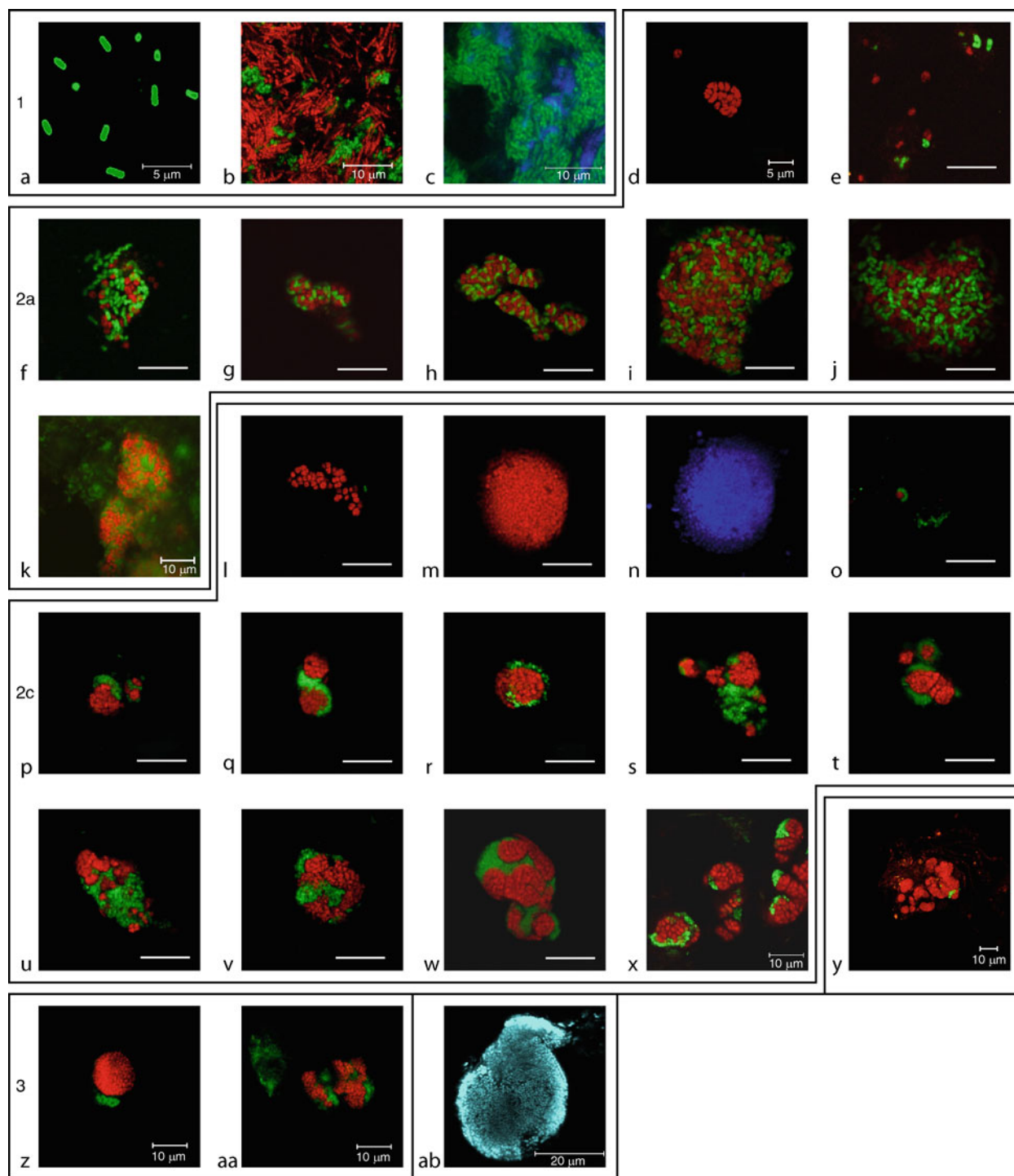
Bacterial partners of ANME

ANME-1 and ANME-2 archaea are usually associated with SRB of the SEEP-SRB I branch, subgroup a, of the *Desulfosarcina/Desulfococcus* (DSS) group (Figure 2; Schreiber et al., 2010).

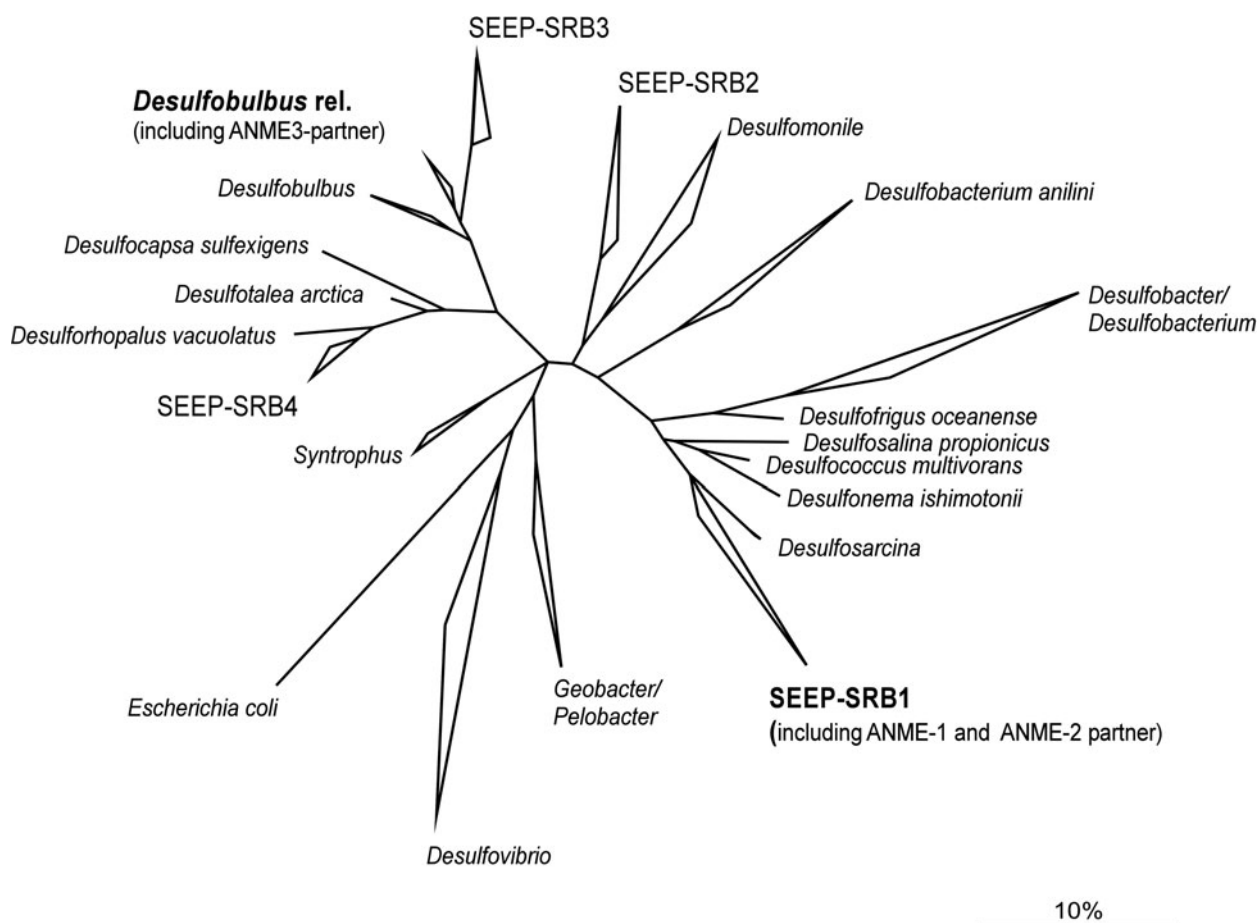
These SRB are physically attached to the ANME-2 archaea, whereas ANME-1 is most often found without close physical association to other cells. The diversity within the SEEP-SRB1 cluster is high, and it is still unknown if the partners of the ANME-2a, -2b, and -2c subgroups belong to the same species. The morphology of the DSS cells varies from cocci (mostly associated with ANME-2c cells, Figure 2p–x) to rods (mostly associated with ANME-2a cells, Figure 2f–j). Other SRB have not yet been identified to be associated with ANME-1 and -2. ANME-3 archaea are most often associated with SRB of the *Desulfobulbus* branch (Figure 2), but have also been detected together with SRB of the *Desulfosarcina/Desulfococcus* group in shallow subsurface sediments of cold seeps from the Hydrate Ridge (Lösekann et al., unpublished data). Single cells of ANME-1, -2, and -3 have also been found in situ as well as in vitro enrichments, indicating that the physical association may be not obligate, but certainly the typical life mode of the anaerobic methanotrophs in most habitats.

There is also some evidence that the diversity of bacteria associated with ANME may be larger than anticipated. The analysis of ANME-2c consortia captured by whole-cell magneto-FISH showed a diversity of bacterial partners of ANME-2 far beyond the Deltaproteobacteria

Anaerobic Oxidation of Methane with Sulfate, Figure 1 Phylogenetic trees showing the affiliations of (a) anaerobic methanotrophic archaea (ANME) 16S rRNA gene sequences to selected reference sequences of the domain archaea. The tree was calculated with nearly full-length sequences (>1,300 bp) by maximum-likelihood analysis in combination with filters excluding highly variable positions. Data in colored boxes give information about the distribution and abundance of sequence retrieval and (b) ANME gene sequences coding for the alpha subunit of methyl coenzyme M reductase (*mcrA*) to selected sequences of the domain archaea. The tree was generated from deduced amino acid sequences (>247 amino acids) by neighbor-joining analysis with a 30% amino acid frequency filter. *mcrA* tree by courtesy of Lösekann. Bar, 10% estimated sequence divergence.



Anaerobic Oxidation of Methane with Sulfate, Figure 2 Epifluorescence micrographs of different ANME single cells and aggregates visualized by fluorescence in situ hybridization (FISH) or CARD-FISH. (a) Single ANME-1 cells living in a microbial mat from the Black Sea; (b) mat-type consortium formed by ANME-1 (red) and DSS cells (green); (c) aggregated ANME-1 (green) and ANME-2 cells (blue) in a microbial mat from the Black Sea; (d) single ANME-2a cells in an enrichment culture from Hydrate Ridge sediments; (e–k) mixed-type consortia of ANME-2a (red) and DSS (green) cells observed in different seep sediments; (l) single ANME-2a cells; (m) monospecific ANME-2a aggregate; (n) corresponding DAPI staining in an enrichment culture from Hydrate Ridge sediments; (o–x) shell-type consortia of ANME-2c (red) and DSS (green) cells of different sizes and structure observed in different seep sediments; (y–aa): ANME-3; (y) monospecific aggregation of ANME-3 at Haakon Mosby mud volcano, (z, aa) ANME-3/*Desulfobulbus* consortia; and (ab): DAPI staining of a large ANME-2c aggregate. Unless otherwise indicated, scale bar 10 μm.



Anaerobic Oxidation of Methane with Sulfate, Figure 3 Phylogenetic tree showing the affiliations of ANME-sulfate-reducing bacteria partner 16S rRNA gene sequences to selected reference sequences of the *Deltaproteobacteria*. The tree was calculated with nearly full-length sequences (>1,450 bp) by maximum-likelihood analysis in combination with filters excluding highly variable positions. Groups known as ANME partners are printed in boldface. Scale bar gives 10% estimated sequence divergence.

(Pernthaler et al., 2008). Lösekann et al. found evidence for ANME-3 aggregates with unidentified partners showing a mixed-type morphology in sediments from the Arctic mud volcano Haakon Mosby (Lösekann et al., 2007).

Habitats of ANME

Since their discovery, the distribution of the ANME organisms has been studied intensively, mainly based on 16S rRNA gene phylogeny. More than 1800 16S rRNA gene sequences are available now from more than 50 different marine methane seeps (Hinrichs et al., 1999; Orphan et al., 2001a; Aloisi et al., 2002; Tourova et al., 2002; Mills et al., 2003, 2004, 2005; Inagaki et al., 2004; Heijs et al., 2005, 2007; Knittel et al., 2005; Lanoil et al., 2005; Stadnitskaia et al., 2005; Arakawa et al., 2006a, b; Fang et al., 2006; Lloyd et al., 2006; Martinez et al., 2006; Niemann et al., 2006a; Nunoura et al., 2006; Reed et al., 2006; Lösekann et al., 2007), vents (Takai and Horikoshi, 1999; Teske et al., 2002; Kelley et al., 2005; Brazelton et al., 2006; Inagaki et al., 2006a), and “sulfate–methane

transition zone” (SMTZ) (Thomsen et al., 2001; Niemann et al., 2005; Parkes et al., 2007) differing in e.g., temperature, methane flux, salinity, and pH. Furthermore, the presence of ANME organisms in the water column (Black Sea; Vetriani et al., 2003; Schubert et al., 2006a) and in a terrestrial mud volcano in the Carpathian mountains (Romania; Alain et al., 2006) has also been reported. A comparison of these reports shows that globally, the two most abundant and diverse phylogenetic groups of methane-oxidizing archaea are the ANME-1 and ANME-2 clades. The distribution of ANME-2a and ANME-2c is as wide as for ANME-1, but only few sequences have been reported belonging to subgroup ANME-2b. ANME-2b, originally defined on the basis of few sequences as a separate group (Orphan et al., 2001a), now seem to be rather a subgroup of ANME-2a than a monophyletic group (Figure 1a).

Today it is known that ANME are present wherever methane and sulfate co-occur, at a wide range of environmental conditions (Knittel and Boetius, 2009). However,

usually only one specific ANME group dominates a habitat and seems to be responsible for most of the measured AOM (Knittel et al., 2005). High rates of methane oxidation, sulfate reduction, and/or sulfide production can be used as an indicator for high densities of ANME populations (Boetius et al., 2008). Continuous high fluxes of both AOM substrates methane and sulfate at mM concentrations sustain high cell densities of $>10^{10}$ ANME cells cm^{-3} in the environment and can even form some of the densest and largest cell accumulations known to exist in nature (Michaelis et al., 2002).

Seep ecosystems

Cold seeps form where tectonic or gravitational forces advect free gas, methane-rich porewater and/or mud upward into the sulfate-penetrated surface sediments and sometimes into the hydrosphere (Judd and Hovland, 2007; see *Cold Seeps*). The increased availability of dissolved methane at higher hydrostatic pressures increases the energy yield of AOM and leads to a natural enrichment of ANME populations. The products of AOM, sulfide and carbonate accumulate in the seabed, forming the typical features of cold seep ecosystems such as carbonate precipitates and high sulfide fluxes. The sulfide is oxidized chemically and microbially with oxygen, nitrate, or iron close to the seafloor surface. One of the most striking visual features of submarine cold seep ecosystems are mats of thiotrophic bacteria covering the seafloor. These mats are usually associated with high AOM rates and dense ANME communities inhabiting the underlying sediments (Sahling et al., 2002; Treude et al., 2003; Joye et al., 2004; Niemann et al., 2006b). Also, a variety of thiotrophic microbe–animal symbioses profit from AOM, such as siboglinid tubeworms and mytilid as well as vesicomyid bivalves (Sibuet and Olu, 1998). Thiotrophic mats and invertebrate symbioses may enhance the growth of ANME populations by rapidly removing the toxic endproduct sulfide, and by replenishing sulfate in the sediments (Treude et al., 2003; Cordes et al., 2005; Niemann et al., 2006b). In active cold seep sediments, AOM rates of $500\text{--}5,000\text{ nmol CH}_4\text{ cm}^{-3}\text{ day}^{-1}$ ($>2\text{ mol m}^{-2}\text{ year}^{-1}$) are reached, associated with very dense ANME populations of $>10^{10}$ cells cm^{-3} (Boetius et al., 2000; Knittel et al., 2005; Niemann et al., 2006b).

Cold seep ecosystems from Eel River Basin (Hinrichs et al., 1999; Orphan et al., 2001b), Hydrate Ridge (Boetius et al., 2000; Knittel et al., 2003, 2005), Black Sea (Thiel et al., 2001, 2003, 2007; Michaelis et al., 2002; Blumenberg et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007), Gulf of Mexico (Orcutt et al., 2005; Lloyd et al., 2006; Martinez et al., 2006), the Tommeliten and Gullfaks area in the North Sea (Wegener et al., 2008), and mud volcanoes from the Mediterranean Sea (Omorgie et al., 2008) and the Barents Sea (Niemann et al., 2006b; Lösekann et al., 2007) have been intensively studied. With the exception of cold seeps in the Black Sea (see next) most of the studies indicate a

dominance of ANME-2 or ANME-3. A prominent example is the gas hydrate bearing sediment from the Hydrate Ridge. Hot spots of ANME-2 are surface layers just above the gas hydrates with up to 10^8 aggregates cm^{-3} . Interestingly, ANME-2 subgroups revealed preferences for either *Beggiatoa* (ANME-2a) or *Calyptogenia* (ANME-2c) fields (Knittel et al., 2005) indicating that different environmental conditions select for different ANME groups.

Two seep systems are known yet where other ANMEs dominate: (1) sediments overlaying a brine pool methane seep in the Gulf of Mexico are dominated by ANME-1b archaea. Here, the ANME-1b community was found in the sulfate-methane interface, where low methane concentrations of ca. 100–250 μM are present and coexist with sulfate concentrations of ca. 10 mM; and (2) microbial mats from Black Sea cold seeps. These seeps are unique ecosystems and AOM is important both in sediments and in the water column (Pimenov et al., 1997; Wakeham et al., 2003). Tall reef-like structures composed of porous carbonates and microbial mats are found on the seafloor. These mats have been shown to mediate AOM and consist mainly of densely aggregated ANME-1 cells and SRB (Michaelis et al., 2002; Blumenberg et al., 2004, 2005; Knittel et al., 2005; Reitner et al., 2005a, b; Treude et al., 2005a, 2007). Treude et al. (2007) combined radiotracer incubations, beta-microimaging, SIMS, and CARD-FISH to locate hot spots of methanotrophy. Incorporation of ^{14}C from radiolabeled CH_4 indicated a hot spot for methanotrophy close to the mat surface associated with a dominance of ANME-1 archaea (Treude et al., 2007). The mats, however, are very heterogeneous and also provide niches for ANME-2. Black nodules from the top of the reef seem to be dominated by ANME-2 as shown by specific ^{13}C depleted lipids and FISH (Blumenberg et al., 2004). Reitner et al. found intracellular precipitation of iron sulfide (greigite) by bacteria growing in close association with ANME-2 and suggested iron cycling as an additional pathway involved in AOM (Reitner et al., 2005b).

ANME-3 archaea are commonly found at submarine mud volcanoes (Niemann et al., 2006b; Heijs et al., 2007; Lösekann et al., 2007; Omorgie et al., 2008) but also at other cold seeps (Orphan et al., 2001a; Inagaki et al., 2004; Knittel et al., 2005), hydrothermal vents (Brazelton et al., 2006), and recently in subsurface sediments (Parkes et al., 2007).

Sulfate–methane transition zones (SMTZ)

The most common ANME habitat on the Earth is the so-called SMTZ in the seabed (Thomsen et al., 2001; Ishii et al., 2004; Niemann et al., 2005, 2006a; Treude et al., 2005b; Parkes et al., 2007). SMTZ are found in all seabed horizons where the diffusive transport of methane from below and sulfate from above leads to a zone of AOM. Methane is completely consumed in the SMTZ, which may be found at decimeters to tens of meters below the seafloor (D'Hondt et al., 2004), depending on the depth

of the methane production zone, on the transport velocity of methane and sulfate and on their consumption rates. In diffusive seabed systems, the distribution of ANME is restricted to SMTZ. Population densities of ANME are low with $<10^6$ cells cm^{-3} (Niemann et al., 2005) and associated AOM rates are below $10 \text{ nmol cm}^{-3} \text{ day}^{-1}$ ($<0.04 \text{ mol m}^{-2} \text{ year}^{-1}$). The 16S rRNA gene libraries of ANME populations associated with SMTZ zones are very similar to those of cold seeps. The in situ dominance of ANME groups varies between the habitats. In SMTZ, sediments of Eckernförde Bay (German Baltic) ANME-2 archaea dominated (Treude et al., 2005b) as in the Skagerrak off the Danish coast (Parkes et al., 2007) or in a tidal sand flat of the German Wadden Sea (Ishii et al., 2004) while in the SMTZ of the Tommeliten seep area or at Hydrate Ridge ANME-1 were dominant (Niemann et al., 2005; Orcutt et al., unpublished data). Most likely, the SMTZ ANME archaea represent the seed populations for cold seep communities. It remains unknown whether SMTZ ANME possess special physiological adaptations to their energetically less favorable habitat compared to those at cold seeps.

So far, no ANME-related sequences have been published from the deep subsurface (D'Hondt et al., 2004; Newberry et al., 2004; Biddle et al., 2006; Inagaki et al., 2006b; Sorensen and Teske, 2006; Webster et al., 2006). Even in an extensive study by Inagaki et al. sequencing of several thousands of clones obtained from methane hydrate sites from Peru and Cascadia Margin did not result in the retrieval of ANME sequences (Inagaki et al., 2006b). Instead, archaea of the Marine Benthic Group B (MBGB, synonym Deep Sea Archaeal Group, DSAG) and the Miscellaneous Crenarchaeotal Group (MCG) were consistently the dominant phylotypes. Sediments lacking methane hydrates showed no or few MBGB phylotypes. The study by Biddle et al. supported these findings and suggested, based on stable isotopic compositions and intact archaeal lipids that these archaea assimilate organic carbon other than methane (Biddle et al., 2006).

Hydrothermal vents

High methane fluxes are also found at hydrothermal vents of mid ocean ridges (see *Hydrothermal Environments, Marine*), but these ecosystems may not offer many niches for ANME populations. The seafloor of mid ocean ridges consists of basalts and lacks a sediment cover, hence, the habitats for ANME-related organisms are limited to small anoxic zones within vent chimneys (Schrenk et al., 2004; Kelley et al., 2005; Brazelton et al., 2006). Furthermore, the fluids of most hydrothermal vents are sulfate free, and the seawater, which could provide sulfate, is oxygen rich – and hence toxic for ANME organisms. Temperatures range from $<40^\circ\text{C}$ to 90°C at pH 9 to 11. By contrast, sedimentary hydrothermal systems such as the Guaymas basin offer suitable habitats within the surface seafloor similar to the cold seep systems (Teske et al.,

2002; Schouten et al., 2003) although AOM accounts for $<5\%$ of sulfate reduction (Kallmeyer and Boetius, 2004). Another extreme environment in which ANME have been recently found is the CO_2 vented sediments of the Yonaguni Knoll hydrothermal field, southern Okinawa Trough, with an in situ pH <5 (Inagaki et al., 2006a).

Water column

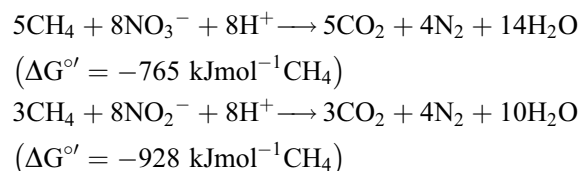
The efflux of methane from cold seeps on the continental margin and mud volcanoes on the abyssal plain make the Black Sea the world's largest surface water reservoir of dissolved methane (Schubert et al., 2006b). Methane concentrations of up to $12 \mu\text{M}$ and AOM rates in the range of nM day^{-1} have been measured (Reeburgh et al., 1991). ANME-1 and ANME-2 archaea are suggested to be responsible for pelagic AOM in the Black Sea (Vetriani et al., 2003; Schubert et al., 2006a, b) accounting each for 3–4% of total cells (Durisch-Kaiser et al., 2005). ANME-1 and ANME-2 appear as single cells in anoxic water, compared to the sediment, where they most often form cell aggregates. Similar to the findings for sediments, one ANME-group dominates, i.e., below approximately 600 m water depth ANME-1 archaea and above 600 m ANME-2 archaea are suggested to be responsible for AOM (Schubert et al., 2006a).

Terrestrial habitats

The presence of ANME has also been reported for terrestrial habitats. ANME-2a archaea are the organisms responsible for AOM activity in a mud volcano field located in the Carpathian Mountains (Romania). Here, thermal alteration of sedimentary organic compounds leads to the release of methane and higher hydrocarbons into the environment (Alain et al., 2006). Additionally, presence of ANME-1 and -2 archaea in a freshwater lake has been reported (Lake Plußsee; northern Germany (Eller et al., 2005)). Long anoxic periods during the summer together with a high organic load in Lake Plußsee favor anaerobic processes in the sediment. In a distinct layer in the anoxic water body, low numbers ($<1\%$) of single ANME archaea were found.

Methane oxidation coupled to denitrification

Methane oxidation could theoretically also be coupled to electron acceptors such as NO_3^- , Fe(III) , and Mn(IV) . Recently, Raghoebarsing et al. (2006) demonstrated AOM coupled to denitrification of nitrate and nitrite in an enrichment culture according to



Anoxic sediment from the Twente canal in the Netherlands was used as inoculum to grow these organisms. The canal contained nitrate concentrations of up to 1 mM and the sediment was saturated with methane, which is typical for freshwater habitats receiving agricultural runoff. The bioreactor biomass was dominated by bacteria of the NC10 clade, a deep branching diverse phylogenetic group (Rappe and Giovannoni, 2003; Raghoebarsing et al., 2006). Recently, the genome of the dominant bacteria *Candidatus Methyloirabilis oxyfera* has been sequenced and surprisingly this apparently anaerobic, denitrifying bacterium encoded, transcribed and expressed the well-established aerobic pathway for methane oxidation, whereas it lacked known genes for dinitrogen production. Isotopic labelling experiments indicated that 'M. oxyfera' bypassed the denitrification intermediate nitrous oxide by the conversion of two nitric oxide molecules to dinitrogen and oxygen, which was used to oxidize methane (Ettwig et al., 2010).

Summary

The AOM is a key biogeochemical process on the Earth, and represents a globally important sink for methane. ANME are present in all environments where methane and sulfate overlap. Unfortunately, no member of the ANME groups or of their sulfate-reducing partner bacteria has been cultivated yet, and the biochemical process is not understood. As long as pure biomass of the archaea is missing, culture-independent molecular tools are the most helpful techniques to investigate the functioning of AOM, including metagenomics and proteomics. Protein patterns of different enrichments help in elucidating the most abundant and hence the most relevant proteins for both partners. Questions to the genome of both partners include for example, whether the ANME themselves may possess the genes for sulfate reduction, and what carbon and energy sources the sulfate reducers could utilize. Answering such questions from metagenomic data sets, however, remains difficult due to the relatively high diversity in the environmental enrichments, and the high proportion of open reading frames in the metagenomes which cannot be assigned to a certain functional category. Here, single cell technologies may provide solutions in the future.

Bibliography

- Alain, K., Holler, T., Musat, F., Elvert, M., Treude, T., and Krüger, M., 2006. Microbiological investigation of methane- and hydrocarbon-discharging mud volcanoes in the Carpathian Mountains, Romania. *Environmental Microbiology*, **8**, 574–590.
- Aloisi, G., Bouloubassi, I., Heijs, S. K., Pancost, R. D., Pierre, C., Damsté, J. S. S., Gottschal, J. C., Forney, L. J., and Rouchy, J.M., 2002. CH₄-consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters*, **203**, 195–203.
- Arakawa, S., Sato, T., Sato, R., Zhang, J., Gamo, T., Tsunogai, U., Hirota, A., Yoshida, Y., Usami, R., Inagaki, F., and Kato, C., 2006a. Molecular phylogenetic and chemical analyses of the microbial mats in deep-sea cold seep sediments at the northeastern Japan Sea. *Extremophiles*, **10**, 311–319.
- Arakawa, S., Sato, T., Yoshida, Y., Usami, R., and Kato, C., 2006b. Comparison of microbial diversity in cold-seep sediments from different depths in the Nankai Trough. *Journal of General and Applied Microbiology*, **52**, 47–54.
- Barnes, R. O., and Goldberg, E. D., 1976. Methane production and consumption in anoxic marine sediments. *Geology*, **4**, 297–300.
- Biddle, J. F., Lipp, J. S., Lever, M. A., Lloyd, K. G., Sorensen, K. B., Anderson, R., Fredricks, H. F., Elvert, M., Kelly, T. J., Schrag, D. P., Sogin, M. L., Brechley, J. E., Teske, A., House, C. H., and Hinrichs, K.-U., 2006. Heterotrophic Archaea dominate sedimentary subsurface ecosystems off Peru. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 3846–3851.
- Blumenberg, M., Seifert, R., Reitner, J., Pape, T., and Michaelis, W., 2004. Membrane lipid patterns typify distinct anaerobic methanotrophic consortia. *Proceedings of the National Academy of Sciences of the United States of America United States of America*, **101**, 11111–11116.
- Blumenberg, M., Seifert, R., Nauhaus, K., Pape, T., and Michaelis, W., 2005. In vitro study of lipid biosynthesis in an anaerobically methane-oxidizing microbial mat. *Applied and Environmental Microbiology*, **71**, 4345–4351.
- Boetius, A., Ravensschlag, K., Schubert, C., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Boetius, A., Holler, T., Knittel, K., Felden, J., and Wenzhöfer, F., 2008. The seabed as natural laboratory: lessons from uncultivated methanotrophs. In Epstein, S. S. (ed.), *Uncultivated Microorganisms*. Heidelberg, Germany: Springer.
- Brazelton, W. J., Schrenk, M. O., Kelley, D. S., and Baross, J. A., 2006. Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Applied and Environmental Microbiology*, **72**, 6257–6270.
- Cordes, E. E., Arthur, M. A., Shea, K., Arvidson, R. S., and Fisher, C. R., 2005. Modeling the mutualistic interactions between tubeworms and microbial consortia. *PLoS Biology*, **3**, 1–10.
- Dhillon, A., Lever, M., Lloyd, K. G., Albert, D. B., Sogin, M. L., and Teske, A., 2005. Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (mcrA) genes in hydrothermal sediments of the Guaymas Basin. *Applied and Environmental Microbiology*, **71**, 4592–4601.
- D'Hondt, S., Jørgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K.-U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegand, J., Padilla, C. N., and Acosta, J. L. S., 2004. Distributions of microbial activities in deep seafloor sediments. *Science*, **306**, 2216–2221.
- Durisch-Kaiser, E., Klauser, L., Wehrli, B., and Schubert, C., 2005. Evidence of intense archaeal and bacterial methanotrophic activity in the Black Sea water column. *Applied and Environmental Microbiology*, **71**, 8099–8106.
- Eller, G., Kanel, L. K., and Krüger, M., 2005. Cooccurrence of aerobic and anaerobic methane oxidation in the water column of Lake Plusssee. *Applied and Environmental Microbiology*, **71**, 8925–8928.
- Ettwig, K. F., Shima, S., van de Pas-Schoonen K. T., Kahnt, J., Medema, M. H., Op den Camp, H. J., Jetten, M. S., Strous, M., 2008. Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. *Environ. Microbiol.* **10**, 3164–3173.
- Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M., Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H. J. C. T., van Alen, T., Luesken, F., Wu, M. L., van de Pas-Schoonen, K. T., Op den

- Camp, H. J. M., Janssen-Megens, E. M., Francoijs, K.-J., Stunnenberg, H., Weissenbach, J., Jetten, M. S. M., Strous, M., 2010. Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature*, **464**, 543–548.
- Fang, J. S., Shizuka, A., Kato, C., and Schouten, S., 2006. Microbial diversity of cold-seep sediments in Sagami Bay, Japan, as determined by 16S rRNA gene and lipid analyses. *FEMS Microbiology Ecology*, **57**, 429–441.
- Girguis, P. R., Orphan, V. J., Hallam, S. J., and DeLong, E. F., 2003. Growth and methane oxidation rates of anaerobic methanotrophic archaea in a continuous-flow bioreactor. *Applied and Environmental Microbiology*, **69**, 5472–5482.
- Girguis, P. R., Cozen, A. E., and DeLong, E. F., 2005. Growth and population dynamics of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria in a continuous-flow bioreactor. *Applied and Environmental Microbiology*, **71**, 3725–3733.
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F., 2003. Identification of methyl coenzyme M reductase A (*mcrA*) genes associated with methane-oxidizing archaea. *Applied and Environmental Microbiology*, **69**, 5483–5491.
- Hallam, S. J., Putnam, N., Preston, C. M., Deter, J. C., Rokhsar, D., Richardson, P. M., and DeLong, E. F., 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science*, **305**, 1457–1462.
- Hansen, L. B., Finster, K., Fossing, H., and Iversen, N., 1998. Anaerobic methane oxidation in sulfate depleted sediments: effects of sulfate and molybdate additions. *Aquatic Microbial Ecology*, **14**, 195–204.
- Heijs, S. K., Sinninghe Damsté, J. S., and Forney, L. J., 2005. Characterization of a deep-sea microbial mat from an active cold seep at the Milano mud volcano in the Eastern Mediterranean Sea. *FEMS Microbiology Ecology*, **54**, 47–56.
- Heijs, S. K., Haese, R. R., van der Wielen, P., Forney, L. J., and van Elsas, J. D., 2007. Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microbial Ecology*, **53**, 384–398.
- Hinrichs, K.-U., and Boetius, A., 2002. The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In Wefer, G., Billett, D., Hebbeln, D., Jørgensen, B. B., Schlüter, M., and van Weering, T. (ed.), *Ocean Margin Systems*. Berlin, Heidelberg: Springer, pp. 457–477.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S., 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate reducer consortium. *Global Biogeochemical Cycles*, **8**, 451–463.
- Inagaki, F., Tsunogai, U., Suzuki, M., Kosaka, A., Machiyama, H., Takai, K., Nunoura, T., Nealson, K. H., and Horikoshi, K., 2004. Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, Southern Ryukyu Arc, by analyzing *pmoA*, *mmoX*, *mxrA*, *mcrA*, and 16S rRNA genes. *Applied and Environmental Microbiology*, **70**, 7445–7455.
- Inagaki, F., Kuypers, M. M. M., Tsunogai, U., Ishibashi, J., Nakamura, K., Treude, T., Ohkubo, S., Nakaseama, M., Gena, K., Chiba, H., Hirayama, H., Nunoura, T., Takai, K., Jørgensen, B. B., Horikoshi, K., and Boetius, A., 2006a. Microbial community in a sediment-hosted CO₂ lake of the southern Okinawa Trough hydrothermal system. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 14164–14169.
- Inagaki, F., Nunoura, T., Nakagawa, S., Teske, A., Lever, M., Lauer, A., Suzuki, M., Takai, K., Delwiche, M., Colwell, F. S., Nealson, K. H., Horikoshi, K., D'Hondt, S., and Jørgensen, B. B., 2006b. Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean margin. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 2815–2820.
- Ishii, K., Mußmann, M., MacGregor, B. J., and Amann, R., 2004. An improved fluorescence in situ hybridization protocol for the identification of bacteria and archaea in marine sediments. *FEMS Microbiology Ecology*, **50**, 203–212.
- Iversen, N., and Jørgensen, B. B., 1985. Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). *Limnology and Oceanography*, **30**, 944–955.
- Joye, S. B., Boetius, A., Orcutt, B. N., Montoya, J. P., Schulz, H. N., Erickson, M. J., and Lugo, S. K., 2004. The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. *Chemical Geology*, **205**, 219–238.
- Judd, A., and Hovland, M. (eds.), 2007. *Seabed Fluid Flow*. Cambridge: Cambridge University Press, p. 475.
- Kallmeyer, J., and Boetius, A., 2004. Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Applied and Environmental Microbiology*, **70**, 1231–1233.
- Kelley, D. S., Karson, J. A., Fruh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- Knittel, K., and Boetius, A., 2009. The anaerobic oxidation of methane - progress with an unknown process. *Annual Reviews of Microbiology*, **63**, 311–334.
- Knittel, K., Boetius, A., Lemke, A., Eilers, H., Lochte, K., Pfannkuche, O., Linke, P., and Amann, R., 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, OR). *Geomicrobiology Journal*, **20**, 269–294.
- Knittel, K., Lösekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea (ANME) at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Krüger, M., Meyerdierks, A., Glöckner, F. O., Amann, R., Widdel, F., Kube, M., Reinhardt, R., Kahnt, J., Böcher, R., Thauer, R. K., and Shima, S., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature*, **426**, 878–881.
- Lanoil, B. D., La Duc, M. T., Wright, M., Kastner, M., Nealson, K. H., and Bartlett, D., 2005. Archaeal diversity in ODP legacy borehole 892b and associated seawater and sediments of the Cascadia Margin. *FEMS Microbiology Ecology*, **54**, 167–177.
- Lloyd, K. G., Lapham, L., and Teske, A., 2006. An anaerobic methane-oxidizing community of ANME-1b archaea in hypersaline Gulf of Mexico sediments. *Applied and Environmental Microbiology*, **72**, 7218–7230.
- Lösekann, T., Knittel, K., Nadalig, T., Fuchs, B., Niemann, H., Boetius, A., and Amann, R., 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby mud volcano, Barents Sea. *Applied and Environmental Microbiology*, **73**, 3348–3362.
- Martens, C. S., and Berner, R. A., 1977. Interstitial water chemistry of Long Island sound sediments, I, dissolved gases. *Limnology and Oceanography*, **22**, 10–25.
- Martinez, R. J., Mills, H. J., Story, S., and Sobecky, P. A., 2006. Prokaryotic diversity and metabolically active microbial populations in sediments from an active mud volcano in the Gulf of Mexico. *Environmental Microbiology*, **8**, 1783–1796.

- Meyerdierks, A., Kube, M., Kostadinov, I., Teeling, H., Glöckner, F. O., Reinhardt, R., and Amann, R., 2009. Metagenome and mRNA expression analyses of anaerobic methanotrophic archaea of the ANME-1 group. *Environmental Microbiology*, **12**, 422–439.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., and Gulin, M. B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Mills, H. J., Hodges, C., Wilson, K., MacDonald, I. R., and Sobecky, P. A., 2003. Microbial diversity in sediments associated with surface-breaching gas hydrate mounds in the Gulf of Mexico. *FEMS Microbiology Ecology*, **46**, 39–52.
- Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A., 2004. Identification of members of the metabolically active microbial populations associated with Beggiatoa species mat communities from Gulf of Mexico cold-seep sediments. *Applied and Environmental Microbiology*, **70**, 5447–5458.
- Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A., 2005. Characterization of microbial community structure in Gulf of Mexico gas hydrates: comparative analysis of DNA- and RNA-derived clone libraries. *Applied and Environmental Microbiology*, **71**, 3225–3247.
- Nauhaus, K., Boetius, A., Krüger, M., and Widdel, F., 2002. In vitro demonstration of anaerobic oxidation of methane coupled to sulphate reduction in sediment from a marine gas hydrate area. *Environmental Microbiology*, **4**, 296–305.
- Nauhaus, K., Treude, T., Boetius, A., and Krüger, M., 2005. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I- and ANME-II-communities. *Environmental Microbiology*, **7**, 98–106.
- Nauhaus, K., Albrecht, M., Elvert, M., Boetius, A., and Widdel, F., 2007. In vitro cell growth of marine archaeal-bacterial consortia during anaerobic oxidation of methane with sulfate. *Environmental Microbiology*, **9**, 187–196.
- Newberry, C. J., Webster, G., Cragg, B. A., Parkes, R. J., Weightman, A. J., and Fry, J. C., 2004. Diversity of prokaryotes and methanogenesis in deep subsurface sediments from the Nankai Trough, ocean drilling program leg 190. *Environmental Microbiology*, **6**, 274–287.
- Niemann, H., Elvert, M., Hovland, M., Orcutt, B., Judd, A., Suck, I., Gutt, J., Joye, S., Damm, E., Finster, K., and Boetius, A., 2005. Methane emission and consumption at a North Sea gas seep (Tommeliten Area). *Biogeosciences*, **2**, 335–351.
- Niemann, H., Duarte, J., Hensen, C., Omorgie, E., Magalhaes, V. H., Elvert, M., Pinheiro, L. M., Kopf, A., and Boetius, A., 2006a. Microbial methane turnover at mud volcanoes of the Gulf of Cadiz. *Geochimica et Cosmochimica Acta*, **70**, 5336–5355.
- Niemann, H., Lösekann, T., DeBeer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E. J., Schlüter, M., Klages, M., Foucher, J. P., and Boetius, A., 2006b. Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature*, **443**, 854–858.
- Nunoura, T., Oida, H., Toki, T., Ashi, J., Takai, K., and Horikoshi, K., 2006. Quantification of *mcrA* by quantitative fluorescent PCR in sediments from methane seep of the Nankai Trough. *FEMS Microbiology Ecology*, **57**, 149–157.
- Omorgie, E. O., Mastalerz, V., de Lange, G., Straub, K. L., Kappler, A., et al. 2008. Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Chefred Mud volcano (Nile Deep Sea Fan, Eastern Mediterranean). *Applied and Environmental Microbiology*, **74**, 3198–3215.
- Orcutt, B., Boetius, A., Elvert, M., Samarkin, V., and Joye, S. B., 2005. Molecular biogeochemistry of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. *Geochimica et Cosmochimica Acta*, **69**, 4267, 5633–5633.
- Orphan, V. J., Hinrichs, K.-U., Ussler, W. III, Paull, C. K., Taylor, L. T., Sylva, S. P., Hayes, J. M., and DeLong, E. F., 2001a. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. *Applied and Environmental Microbiology*, **67**, 1922–1934.
- Orphan, V. J., House, C. H., Hinrichs, K.-U., McKeegan, K. D., and DeLong, E. F., 2001b. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., House, C. H., Hinrichs, K.-U., McKeegan, K. D., and DeLong, E. F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7663–7668.
- Parkes, R. J., Cragg, B. A., Banning, N., Brock, F., Webster, G., Fry, J. C., Hornibrook, E., Pancost, R. D., Kelly, S., Knab, N., Jørgensen, B. B., Rinna, J., and Weightman, A. J., 2007. Biogeochemistry and biodiversity of methane cycling in subsurface marine sediments (Skagerrak, Denmark). *Environmental Microbiology*, **9**, 1146–1161.
- Pernthaler, A., Dekas, A. E., Brown, C. T., Goffredi, S. K., Embaye, T., and Orphan, V. J., 2008. Diverse syntrophic partnerships from deep-sea methane vents revealed by direct cell capture and metagenomics. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 7052–7057.
- Pimenov, N. V., Rusanov, I. I., Poglavzova, M. N., Mityushina, L. L., Sorokin, D. Y., Khmelenina, V. N., and Trotsenko, Y. A., 1997. Bacterial mats on coral-like structures at methane seeps in the Black Sea. *Microbiology (translated from Mikrobiologiya)*, **66**, 354–360.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J. P., Ettwig, K. F., Rijpstra, W. I. C., Schouten, S., Damsté, J. S. S., Op den Camp, H. J. M., Jetten, M. S. M., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 918–921.
- Rappe, M. S., and Giovannoni, S. J., 2003. The uncultured microbial majority. *Annual Review of Microbiology*, **57**, 369–394.
- Reeburgh, W. S., 1969. Observations of gases in Chesapeake Bay sediments. *Limnology and Oceanography*, **14**, 368–375.
- Reeburgh, W. S., 1976. Methane consumption in Cariaco trench waters and sediments. *Earth and Planetary Science Letters*, **28**, 337–344.
- Reeburgh, W. S., 2007. Oceanic methane biogeochemistry. *Chemical Reviews*, **107**, 486–513.
- Reeburgh, W. S., Ward, B. B., Whalen, S. C., Sandbeck, K. A., Kilpatrick, K. A., and Kerkhof, L. J., 1991. Black Sea methane geochemistry. *Deep Sea Research*, **38** (Supplement 2), S1189–S1210.
- Reed, A. J., Lutz, R. A., and Vetriani, C., 2006. Vertical distribution and diversity of bacteria and archaea in sulfide and methane-rich cold seep sediments located at the base of the Florida escarpment. *Extremophiles*, **10**, 199–211.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005a. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005b. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean Shelf (Black Sea). *Facies*, **51**, 66–79.
- Rossel, P., Fredricks, H., Lipp, J., Arnds, J., Boetius, A., Elvert, M., and Hinrichs, K.-U., 2008. Intact polar lipids of anaerobic methanotrophic archaea and associated bacteria. *Organic Geochemistry*, **39**, 992–999.

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schouten, S., Wakeham, S. G., Hopmans, E. C., and Sinninghe Damsté, J. S., 2003. Biogeochemical evidence that thermophilic archaea mediate the anaerobic oxidation of methane. *Applied and Environmental Microbiology*, **69**, 1680–1686.
- Schreiber, L., Holler, T., Knittel, K., Meyerdierks, A., Amann, R. (in press). Identification of the dominant sulfate-reducing bacterial partner of anaerobic methanotrophs of the ANME-2 clade. *Environmental Microbiology*.
- Schrenk, M. O., Kelley, D. S., Bolton, S. A., and Baross, J. A., 2004. Low archaeal diversity linked to seafloor geochemical processes at the Lost City hydrothermal field, Mid-Atlantic Ridge. *Environmental Microbiology*, **6**, 1086–1095.
- Schubert, C. J., Coolen, M. J. L., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E. C., Sinninghe Damsté, J. S., Wakeham, S., and Kuypers, M. M. M., 2006a. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**, 1844–1856.
- Schubert, C. J., Durisch-Kaiser, E., Holzner, C. P., Klauser, L., Wehrli, B., Schmale, O., Greinert, J., McGinnis, D. F., DeBastis, M., and Kipfer, R., 2006b. Methanotrophic microbial communities associated with bubble plumes above gas seeps in the Black Sea. *Geochemistry, Geophysics, Geosystems*, **7**, Q04002, doi:10.1029/2005GC001049.
- Shima, S., and Thauer, R. K., 2005. Methyl-coenzyme M reductase and the anaerobic oxidation of methane in methanotrophic archaea. *Current Opinion in Microbiology*, **8**, 643–648.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep-Sea Research II*, **45**, 517–567.
- Sorensen, K. B., and Teske, A., 2006. Stratified communities of active archaea in deep marine subsurface sediments. *Applied and Environmental Microbiology*, **72**, 4596–4603.
- Stadnitskaia, A., Muyzer, G., Abbas, B., Coolen, M. J. L., Hopmans, E. C., Baas, M., van Weering, T. C. E., Ivanov, M. K., Poludetkina, E., and Sinninghe Damsté, J. S., 2005. Biomarker and 16S rDNA evidence for anaerobic oxidation of methane and related carbonate precipitation in deep-sea mud volcanoes of the Sorokin Trough, Black Sea. *Marine Geology*, **217**, 67–96.
- Sturt, H. F., Summons, R. E., Smith, K., Elvert, M., and Hinrichs, K.-U., 2004. Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. *Rapid Communications in Mass Spectrometry*, **18**, 617–628.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Hinrichs, K.-U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S. P., Sogin, M. L., and Jannasch, H. W., 2002. Microbial diversity of hydrothermal sediments in the Guaymas basin: evidence for anaerobic methanotrophic communities. *Applied and Environmental Microbiology*, **68**, 1994–2007.
- Thiel, V., Peckmann, J., Richnow, H. H., Luth, U., Reitner, J., and Michaelis, W., 2001. Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat. *Marine Chemistry*, **73**, 97–112.
- Thiel, V., Blumenberg, M., Pape, T., Seifert, R., and Michaelis, W., 2003. Unexpected occurrence of hopanoids at gas seeps in the Black Sea. *Organic Geochemistry*, **34**, 81–87.
- Thiel, V., Heim, C., Arp, G., Hahmann, U., Sjövall, P., and Lausmaa, J., 2007. Biomarkers at the microscopic range: ToF-SIMS molecular imaging of archaea-derived lipids in a microbial mat. *Geobiology*, **5**, 413–421.
- Thomsen, T. R., Finster, K., and Ramsing, N. B., 2001. Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. *Applied and Environmental Microbiology*, **67**, 1646–1656.
- Tourova, T. P., Kolganov, T. V., Kuznetsov, B. B., and Pimenov, N. V., 2002. Phylogenetic diversity of the archaeal component in microbial mats on coral-like structures associated with methane seeps in the Black Sea. *Microbiology (translated from Mikrobiologiya)*, **71**, 230–236.
- Treude, T., Boetius, A., Knittel, K., Wallmann, K., and Jørgensen, B. B., 2003. Anaerobic oxidation of methane above gas hydrates at Hydrate Ridge, NE Pacific Ocean. *Marine Ecology Progress Series*, **264**, 1–14.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005a. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**, 6375–6378.
- Treude, T., Krüger, M., Boetius, A., and Jørgensen, B. B., 2005b. Environmental control on anaerobic oxidation of methane in the gassy sediments of Eckernförde Bay (German Baltic). *Limnology and Oceanography*, **50**, 1771–1786.
- Treude, T., Orphan, V., Knittel, K., Gieseke, A., House, C., and Boetius, A., 2007. Consumption of methane and CO₂ by methanotrophic microbial mats from gas seeps of the anoxic Black Sea. *Applied and Environmental Microbiology*, **73**, 2271–2283.
- Vettriani, C., Tran, H. V., and Kerkhof, L. J., 2003. Fingerprinting microbial assemblages from the oxic/anoxic chemocline of the Black Sea. *Applied and Environmental Microbiology*, **69**, 6481–6488.
- Wakeham, S. G., Lewis, C. M., Hopmans, E. C., Schouten, S., and Sinninghe Damsté, J. S., 2003. Archaea mediate anaerobic oxidation of methane in deep euxinic waters of the Black Sea. *Geochimica et Cosmochimica Acta*, **67**, 1359–1374.
- Webster, G., Parkes, R. J., Cragg, B. A., Newberry, C. J., Weightman, A. J., and Fry, J. C., 2006. Prokaryotic community composition and biogeochemical processes in deep subsurface sediments from the Peru Margin. *FEMS Microbiology Ecology*, **58**, 65–85.
- Wegener, G., Shovviti, M., Knittel, K., Niemann, H., Hovland, M., and Boetius, A., 2008. Gullfaks and Tommeliten: microbiological and geochemical description of two of the most active seepage sites of the North Sea. *Biogeosciences*, **5**, 1127–1144.
- Zehnder, A. J. B., and Brock, T. D., 1979. Methane formation and methane oxidation by methanogenic bacteria. *Journal of Bacteriology*, **137**, 420–432.
- Zehnder, A. J. B., and Brock, T. D., 1980. Anaerobic methane oxidation: occurrence and ecology. *Applied and Environmental Microbiology*, **39**, 194–204.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Biosignatures in Rocks](#)
[Carbon \(Organic, Cycling\)](#)
[Cold Seeps](#)
[Fluorescence in Situ Hybridisation \(FISH\)](#)
[Isotopes and Geobiology](#)
[Methane Oxidation \(Aerobic\)](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Nickel, Biology](#)
[Sulfate-Reducing Bacteria](#)

ANAEROBIC TRANSFORMATION PROCESSES, MICROBIOLOGY

Bernhard Schink

University of Konstanz, Konstanz, Germany

Definition and Introduction

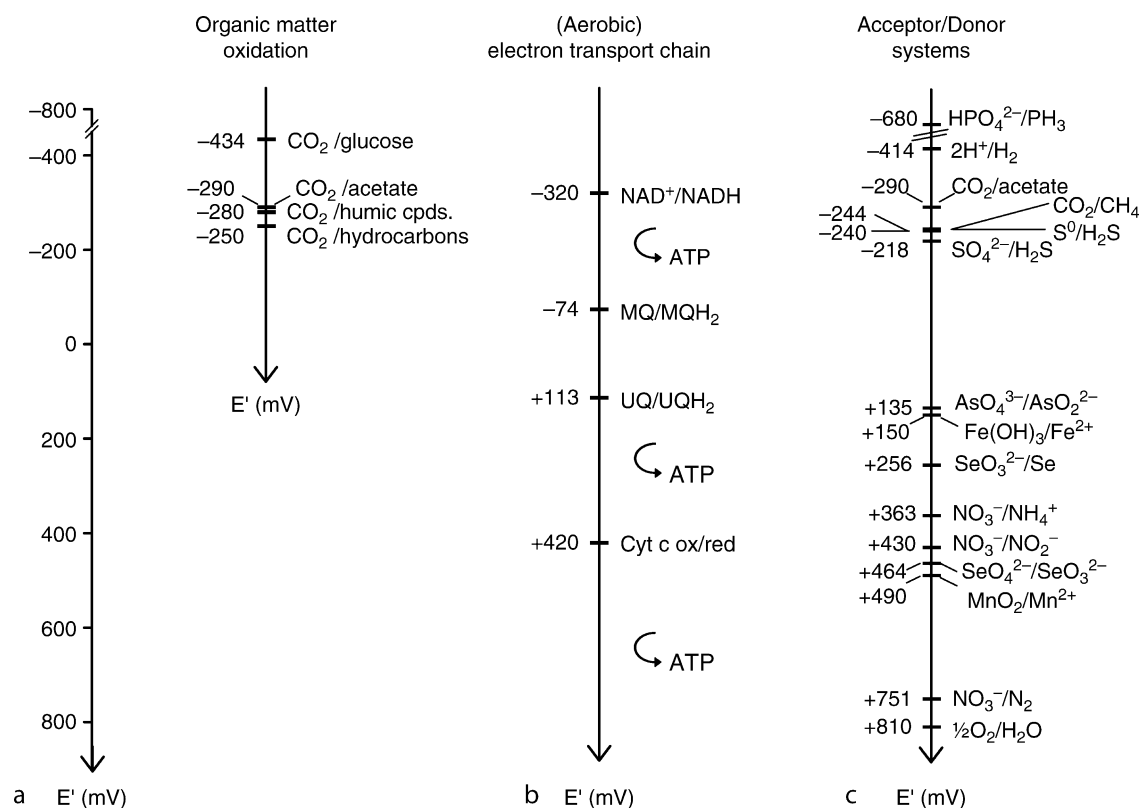
Microbial activities in geochemical processes are most often redox reactions, either between organic matter and inorganic compounds or between different inorganic compounds. In surface soils and lake sediments, organic matter typically derives from primary production by land plants or aquatic plants including algae, provided either directly or after primary digestion by animals. Underground soils and groundwater may receive major amounts of comparably stable organic matter, most often petroleum and its derivatives, through spills and accidents. Microbial oxidation helps to eliminate such contaminants, with concomitant reduction of inorganic electron acceptors. The surprising abundance of bacteria-like structures and even living bacteria in Deep Sea sediments and terrestrial rock material down to several 100 m below surface raised the question of how microbial life can be fuelled in the apparent absence of perceptible external substrate input. The

present overview will try to provide access for geoscientists and other nonbiologists to the concepts of microbial life in the anaerobic world in general.

The terms “anoxic” and “anaerobic” are often used simultaneously with more or less the same meaning. Anoxic means “oxygen-free” and refers to habitats, incubation conditions, etc. The term anaerobic contains the Greek root “bios” (life) and refers to microbes, microbial activities, enzymes, etc.

Organic matter as electron donor in geochemical processes

Organic matter as supplied by plant primary production consists mainly of carbohydrates, proteins, fats, and nucleic acids. Digestion by soil animals decreases the carbohydrate and protein fraction, and increases the content of polyanellated phenolic residues (humic compounds, Stevenson, 1994). The complete oxidation of carbohydrates at pH 7.0, using glucose as a representative molecule, yields electrons at $E_0' = -434$ mV on average (Figure 1a). These electrons are transferred inside microbial cells via specific electron carrier systems, most often via NAD^+/NADH , at $E_0' = -320$ mV. Other carriers operate at lower (e.g., ferredoxins around -420 mV) or higher (flavins, quinones, cytochromes; Figure 1b) redox



Anaerobic Transformation Processes, Microbiology, Figure 1 Redox potentials of (a) organic substrates, (b) biochemical electron carriers, and (c) natural electron acceptor/donor systems. Values taken or calculated from Thauer et al. (1977) and Zehnder and Stumm (1988). MQ menaquinone; UQ ubiquinone.

potentials, and finally all electrons may end up at the $\text{O}_2/\text{H}_2\text{O}$ -couple at $E_0' = +810$ mV (Figure 1c). Electron transport from low to high redox potentials provides chemical energy, which the cell conserves in energy-rich phosphoric acid anhydride linkages in the form of ATP which can be formed in the cell basically by two alternative processes (Thauer et al., 1977): In substrate-level phosphorylation, the transformation of a substrate molecule leads directly to an energy-rich intermediate from which ATP is formed in a stoichiometrically linked reaction. In electron transport phosphorylation, electron transport over a sufficient redox potential difference is coupled to the establishment of a proton (sometimes Na^+ ion) gradient across the cytoplasmic membrane which secondarily drives ATP synthesis via a membrane-bound ATP synthase reaction. Major steps in electron transport phosphorylation are the electron transports between NADH and the quinones, between quinones and cytochrome c, and between cytochrome c and oxygen (Figure 1b and c). In total, oxidation of glucose to 6CO_2 with oxygen as electron acceptor can yield up to 38 mol ATP per mol glucose (Fuchs, 2006).

Partial digestion of biomass leads to less energy-rich derivatives that are less valuable as electron donors. Incomplete oxidation and polymerization of aromatic and aliphatic plant matter residues forms humic compounds with a comparably long residence time of up to 100 years in soils. An average redox potential of humic compounds can be calculated with hydroquinone as a representative constituent, yielding a value at -280 to -300 mV. Fermentation of sugars and proteins produces acetate as the most important primary product; its oxidation yields electrons at -290 mV on average. Oxidation of aliphatic and aromatic hydrocarbons releases electrons at -240 to -270 mV. These examples illustrate that the typical constituents of partly digested or stabilized organic matter are less valuable electron donors in oxidation processes. Moreover, especially humic compounds and hydrocarbons are already comparably inert derivatives. Efficient degradation of these compounds requires molecular oxygen as a co-substrate in the initial oxygenase reactions for activation. Since oxygen-independent alternative reactions of activation of hydrocarbons (Widdel et al., 1993) or polyanellated aromatic compounds (humic compounds) are slow compared to the known oxygen-dependent ones, a major amount of organic matter (e.g., peat) is conserved in the absence of oxygen ("latch hypothesis"; Freeman et al., 2001).

The biogeochemical redox chain

Due to the low solubility of oxygen in water (about $300\text{ }\mu\text{M}$ O_2 in freshwater at air saturation and 10°C), aerobic oxidation of organic matter depends on a continuous supply of oxygen, either by plant photosynthesis or by convective transport from the atmosphere. Therefore, this oxygen supply is restricted to only few millimeters below the top surface in sediments or soils where only diffusive transport is possible. Under such conditions, other

electron acceptors can substitute for oxygen in its function as an electron acceptor, and the sequence of these subsequent redox processes is determined by the standard redox potentials of the individual redox processes (Zehnder and Stumm, 1988; Figure 1c). After oxygen consumption, nitrate is reduced first to nitrite, and afterwards either to N_2 via NO and N_2O , or to ammonia. In any case, nitrite is an important intermediate which may accumulate in laboratory experiments to toxic concentrations. In nature, nitrate concentrations rarely exceed $50\text{ }\mu\text{M}$ and nitrite never becomes toxic, therefore. As the redox potentials listed in Figure 1 indicate, nitrate reduction to N_2 (denitrification) appears to be energetically more feasible than nitrate reduction to ammonia (nitrate ammonification). However, some redox steps in denitrification are rather inefficiently coupled to ATP synthesis, thus wasting a considerable amount of energy and rendering nitrate ammonification the more efficient way of nitrate-dependent energy conservation (Strohm et al., 2007).

Subsequently, manganese(IV) oxides, e.g., MnO_2 (Braunstein), may be reduced to Mn^{2+} ions. The distribution of manganese oxides in soils is rather patchy; in lake sediments, it depends primarily on the chemistry of the catchment area. In Lake Constance, the eastern part of the lake receives ample supply of manganese oxides through the Alpine Rhine river whereas the western part of the lake is nearly devoid of manganese. Different from manganese, iron is a very dominant constituent of rocks and sediments, and can make up up to 1% of the total dry mass. Iron oxides, similar to manganese oxides, are nearly insoluble at neutral pH, and the redox potentials of Fe(III)-oxide reduction to Fe^{2+} -ions depends even on the individual iron(III) mineral involved ($+100$ mV with ferrihydrite, -287 mV with hematite; Widdel et al., 1993; Straub et al., 2005). The main problem in reduction of iron and manganese oxides is their apparent insolubility (Cornell and Schwertmann, 1996) and, with this, the necessity for bacteria to transport electrons to an acceptor outside the cell (see below).

Reduction of sulfate to sulfide is the dominant process of organic matter oxidation in marine sediments because seawater contains sulfate at high concentration (28 mM). Freshwaters contain far less sulfate ($50\text{--}200\text{ }\mu\text{M}$) rendering this process substantially less important at first sight. Nonetheless, sulfide can be reoxidized, e.g., with Fe(III)-oxides (see below) thus making reduction of sulfur compounds probably far more important in freshwater systems than assumed so far. The key problem in sulfate reduction is the initial reduction of the comparably stable sulfate molecule to sulfite which corresponds to a very low redox potential ($E_0' = -527$ mV) that cannot be fed directly with electrons from organic carbon oxidation. The sulfate reducers overcome this problem by ATP investment into this step, thus shifting the resulting redox potential to -60 mV (for details, see Cypionka, *Aerobic Metabolism*). The ATP required for this initial activation step is recovered in the further reduction of sulfite to sulfide.

Elemental sulfur is reduced by specific sulfur-reducing bacteria, but also by sulfate reducers. Elemental sulfur is easy to reduce and does not require prior activation. The problem of sulfur metabolism is again its low solubility in water which may be circumvented by the intermediate formation of water-soluble polysulfides in the presence of the formed hydrogen sulfide.

The terminal process in organic matter degradation in freshwater environments is the formation of methane, either by reduction of CO_2 or by cleavage of acetate (Wolfe, 1992; Thauer, 1998); alternatively, acetate may be formed first by CO_2 reduction, and secondarily be converted to methane (Drake et al., 2002; K. Kuesel, this series). Since the methanogenic archaeobacteria are restricted to the use of only very few substrates (H_2 , C_1 compounds, and acetate) complex organic matter has to go through fermentations by several groups of specialized bacteria (Schink, 1997) before it finally ends up in a mix of CH_4 and CO_2 , i.e., in complete dismutation of the carbon atoms.

Hydrogen is an important product of microbial fermentations of organic matter. It can be formed as well by geochemical processes and may be a source for microbial life in deep sediments and rocks.

The middle axis in Figure 1 (b) positions the key elements of the biochemical electron transport chain between organic matter on the left and the various acceptor systems on the right. This arrangement illustrates the major paths of electron flow in microbial transformation processes. One should emphasize, however, that biological systems are able to shift electrons also against the “normal” electron flow by “reversed electron transport” at the expense of energy supplied by transport of other electrons in the “normal” direction from negative to positive potentials. The scheme illustrates that glucose is comparably a valuable electron donor whereas the electrons derived in degradation of acetate, humic compounds, or hydrocarbons cannot all be transported via NADH, rather, many of them have to enter the transport chain at the level of quinones (either menaquinone or ubiquinone) with the consequence that substantially less ATP is formed. The scheme also illustrates that, e.g., reduction of ferrihydrite can be fed with electrons only at the potential of quinones, and that necessarily the ATP yield of this process is much smaller than, e.g., that of the reduction of nitrate or oxygen. It also becomes evident that phosphate can hardly be reduced to phosphine in a respiratory process, analogous to sulfate or nitrate reduction, but would require substantial amounts of energy rather than yield energy. The origin of traces of phosphine in certain anoxic environments is still unclear; it probably derives from corrosion of iron phosphides in scrap metals (Ding et al., 2005).

Lithotrophic reoxidations

The reduced derivatives formed in the processes above during oxidation of organic matter can act again as electron donors in microbial energy metabolism, e.g., if H_2S ,

reduced Fe^{2+} , or Mn^{2+} diffuses upwards and meets electron acceptors of higher redox potential, thus again creating a redox couple that can yield energy. Such metabolic activities are called “lithotrophic” (Greek *lithos* = rock) because in this metabolism inorganic electron donors serve as electron source. A lithotrophic metabolism is most often, but not necessarily, combined with autotrophic fixation of CO_2 into cell material, i.e., bacterial growth. Obviously, synthesis of organic matter from inorganic sources has to be fueled by energy (ATP) that is derived, in this case, from inorganic redox processes. Therefore, the yield of organic matter, i.e., of microbial cell mass, in these processes is usually small. As the redox chain in Figure 1c illustrates, H_2 , H_2S , and Fe^{2+} are good electron donors whereas nitrite, selenite, and Mn^{2+} are not. Behind every such redox process stand the activities of specialized bacteria and archaea which try to exploit every fraction of energy that can be derived from these redox processes.

An exceptional case is methane oxidation which can proceed either with oxygen as a co-substrate and an electron acceptor, yielding a huge amount of energy or with sulfate as electron acceptor (anaerobic methane oxidation; see Boetius, *Anaerobic Oxidation of Methane with Sulfate*) yielding extremely little energy. Similar problems apply to higher aliphatic or aromatic hydrocarbons (Spormann and Widdel, 2000).

Oxidation of sulfide with ferric oxides should yield energy as well. Nonetheless, this reaction proceeds spontaneously as a chemical reaction (see below), and so far there is no indication for microbial activity exploiting this redox reaction.

Energetics

Bacterial growth requires energy which is derived from substrate conversions in the form of ATP. Irreversible synthesis of the energy-rich acid anhydride linkage of ATP by condensation of ADP with a phosphoryl residue under the conditions prevailing in living cells requires energy (ΔG^0 or Gibbs free energy) in the range of 60–70 kJ per mol (Thauer et al., 1977; Schink, 1997). Nonetheless, metabolic energy can also be converted into ATP at smaller increments. The membrane-bound ATPase enzyme mentioned above catalyzes the synthesis of ATP reversibly with a translocation of protons or Na^+ -ions; the stoichiometry is 3–4 H^+ or Na^+ ions per ATP. Thus, any reaction that can be coupled to the translocation of protons or Na^+ -ions across the membrane can be associated with ATP synthesis, down to reactions transferring only 1 H^+/Na^+ , equivalent to an ΔG^0 in the range of 15–20 kJ per mol. Of course, reactions can proceed also with lower energy yields, but then they cannot feed ATP synthesis and bacterial growth. Growth, on the other hand, is a very important means to maintain a degradative capacity in a certain environment and to adjust its activity to the local needs (different from so-called “co-metabolic” activities which are not positively correlated with bacterial growth; McCarty, 1988).

Extracellular electron transfer

Electron transfer from or into the electron transport chain (Figure 1b) is easy with substrates that can be taken up into the bacterium in a dissolved form. Non-dissolved substrates have to be oxidized or reduced outside the cell, either at the immediate cell surface or via dissolved electron carriers. The reduction of ferric iron oxides can be accomplished in direct contact with the bacterial cells; perhaps, even small cellular appendages can act as “nanowires” for electron transfer (Reguera et al., 2005). Alternatively, either dissolved humic compounds (Lovley et al., 1996) or low-molecular weight quinoid or phenazine compounds may act as electron carriers (Hernandez et al., 2004). As a further alternative, sulfur-reducing bacteria can indirectly reduce iron via H_2S at low (<0.1 mM) concentration (Straub and Schink, 2004). At low H_2S concentration, H_2S delivers electrons to ferric oxides and is converted to a more oxidized species, perhaps thiosulfate or polysulfide, which is reduced again by the bacterium. In this case, a small sulfur cycle acts as an electron delivery system. One should emphasize that in the latter cases, the bacteria do not actually reduce the ferric oxide. They reduce quinoid or sulfur compounds, and the ferric oxides are reduced indirectly in a subsequent unspecific reaction. Such secondary electron transfer reactions may be important also with respect to other reductive transformations such as nitro group reduction, dehalogenation, etc. (Holliger et al., 1997).

The example of indirect ferric iron reduction via sulfur compounds appears to contradict the generally accepted preference of biochemical redox processes according to the sequence mentioned above: since the redox potential of the iron(III)/ Fe^{2+} -couple is higher than that of the sulfate/sulfide system, iron should be reduced before the sulfur compounds. Nonetheless, for bacteria not only the redox potentials but also the bioavailability aspect counts: since the sulfur compounds are easier to access they may be preferred over the insoluble iron oxides.

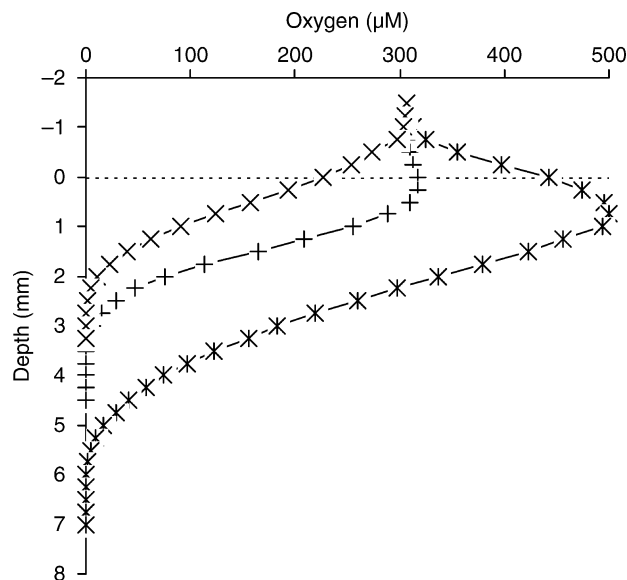
Phototrophic activities

Photosynthesis as performed by green plants and algae is called oxygenic because molecular oxygen is released from water through the activity of two combined light reactions. This biochemical potential was evolved by cyanobacteria (formerly called blue-green algae) and the evolution of oxygenic photosynthesis has changed the chemistry of our planet around 3.5 billion years ago in a most dramatic way from a reducing to an oxygenated atmosphere. The massive precipitation of iron oxides in the so-called “Banded Iron Formations” has for long times been considered to witness the evolution of molecular oxygen in the Earth’s atmosphere, but the oxygen thus evolved would have been consumed to a large extent over several hundred million years in the oxidation of Fe^{2+} to ferric oxides. Anoxygenic phototrophic bacteria perform a simpler, older type of photosynthesis from which oxygenic photosynthesis was later derived. The discovery of

Fe^{2+} -oxidation by anoxygenic phototrophs (Widdel et al., 1993) changed also our understanding of the development of Banded Iron Formations; this process became possible also without the involvement of molecular oxygen, and thus formation of Banded Iron Formations could now testify only the evolution of anoxygenic, rather than of oxygenic photosynthesis (Kappler et al., 2005).

Spatial/seasonal oscillations

Many microbial habitats such as the Deep Sea and its sediments, or rocks in the deep underground are highly stable systems which do not change their properties over many thousands or millions of years. On the other hand, microbial life conditions in a top soil may change dramatically with every rainfall, and bacteria inhabiting the sediment surface in a shallow, light-exposed lake may undergo dramatic life changes in diurnal cycles of light and oxygen supply which are probably the most dramatic changes for living systems at all. As shown in Figure 2, oxygen profiles during light exposure versus darkness show dramatic differences with respect to the oxygen concentration and to oxygen penetration depth into the sediment. Other parameters change over longer time scales, e.g., with seasons. Figure 3 shows a section of the littoral sediment of Lake Constance in January when the water level is low.



Anaerobic Transformation Processes, Microbiology, Figure 2 Distribution profiles of oxygen in littoral sediment of Lake Constance under the influence of different light conditions. (x) 12 h dark, (+) 12 h incubation at low light, equivalent to an overcast day, (*) 12 h incubation at high light intensity, equivalent to a sunny day. Incubation basins were exposed to light under a 12 h-light-dark cycle with three Osram lamps Biolux (L30W/72), Fluora (L30W/77), and Haloline (200 W/R7s) to generate high light intensity of 15 kLux ($250 \text{ E m}^{-2} \text{ s}^{-1}$) at the water surface.



Anaerobic Transformation Processes, Microbiology,

Figure 3 Leaching of ferrous iron from littoral sediment of Lake Constance in Winter 2002. Fe^{2+} ions leach from the upper right part to the left, with continuous chemical/microbial oxidation of Fe(II) to Fe(III) oxides.

The higher regions of the sediment (which are under water through the major part of the year, with annual water level changes of 2.5 m) are exposed to air in wintertime, and the reduced Fe^{2+} leaches out of the sediment to be oxidized to Fe(III) oxides. Of course, such water level fluctuations influence all life forms in the littoral sediment, beyond the normal fluctuations of light and dark in the day cycle, and of temperature through the annual cycle. The microbial community within the sediment either has to adapt to these changes as far as possible or has to migrate within the sediment to maintain a stable life situation.

Conclusions

Microbes are magnificent chemists. They catalyze reactions that are thermodynamically feasible, but enhance them kinetically as any good catalyst does. Beyond that, they can couple exergonic reactions in their energy metabolism with endergonic processes in their assimilatory metabolism in a way that chemical processes could never accomplish. Through bacterial growth, the catalytic potential is adjusted at a certain site to the local needs. Many processes are strongly accelerated by the action of microbes, and the total extent of their activities in Deep Sea sediments and in the deep underground can so far only roughly be estimated. Nonetheless, there is little doubt that microbes in the underground make up a significant part of living protoplasm on Earth in general, ranging in the same order of magnitude as that of plants and animals together (Whitman et al., 1998). The fascinating efficiency with which microbes catalyze chemical processes into impressive dimensions (as, e.g., in the precipitation of the Banded Iron Formations) gives us only a vague idea of the importance of microbial catalysis in the geochemistry of our planet. Geochemists and geomicrobiologists have to join their forces to understand the action of microbes in highly structured environments that appear extremely

poor with respect to utilizable energy sources, in order to assess the contributions of life to geochemistry correctly.

Acknowledgment

The author thanks Dr. Heike Freese, Konstanz, for helping with the design of Figure 1. Experimental work in the author's lab was supported by Deutsche Forschungsgemeinschaft, Bonn, Fonds der Chemischen Industrie, Frankfurt/M., and research funds of Universität Konstanz.

Bibliography

- Cornell, R. M., and Schwertmann, U., 1996. *The Iron Oxides – Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: VCH.
- Ding, L., Wang, X., Zhu, Y., Edwards, M., Glindemann, D., and Ren, H., 2005. Effect of pH on phosphine production and the fate of phosphorus during anaerobic process with granular sludge. *Chemosphere*, **59**, 49.
- Drake, H. L., Küsel, K., and Matthies, C., 2002. Ecological consequences of the phylogenetic and physiological diversities of acetogens. *Antonie Van Leeuwenhoek*, **81**, 203.
- Freeman, C., Ostle, N., and Kang, H., 2001. An enzymic 'latch' on a global carbon store. *Nature (London)*, **409**, 149.
- Fuchs, G. (ed.), 2006. *Allgemeine Mikrobiologie*, begr. von H. G. Schlegel, 8. Aufl. Stuttgart: Thieme, 650 p.
- Hernandez, M. E., Kappler, A., and Newman, D. K., 2004. Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Applied Environmental Microbiology*, **70**, 921.
- Holliger, C., Gaspard, S., Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R. P., and Vazquez, F., 1997. Contaminated environments in the subsurface and bioremediation: organic contaminants. *FEMS Microbiology Reviews*, **20**, 517.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II) -oxidizing bacteria. *Geology*, **33**, 865.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445.
- McCarty, P. L., 1988. Bioengineering issues related to in situ remediation of contaminated soils and groundwater. *Basic Life Science*, **45**, 143.
- Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., and Lovley, D. R., 2005. Extracellular electron transfer via microbial nanowires. *Nature*, **435**, 1098.
- Schink, B., 1997. Energetics of syntrophic cooperations in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, **61**, 262.
- Spormann, A. M., and Widdel, F., 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation*, **11**, 85.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: John Wiley & Sons.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* through electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744.
- Straub, K. L., Kappler, A., and Schink, B., 2005. Enrichment and isolation of ferric iron- and humic acid reducing bacteria. *Methods in Enzymology*, **397**, 58.
- Strohm, T. O., Griffin, B., Zumft, W., and Schink, B., 2007. Growth yields in bacterial denitrification and nitrate ammonification. *Applied Environmental Microbiology*, **73**, 1420.
- Thauer, R. K., 1998. Biochemistry of methanogenesis: a tribute to Marjorie Stephenson. 1998 Marjorie Stephenson prize lecture. *Microbiology*, **144**, 2377.

- Thauer, R. K., Jungermann, K., and Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews*, **41**, 100.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Aßmus, B., and Schink B., 1993. Anaerobic ferrous iron oxidation by anoxygenic phototrophs. *Nature (London)*, **362**, 834.
- Wolfe, R. S., 1992. Biochemistry of methanogenesis. *Biochemical Society Symposia*, **58**, 41.
- Zehnder, A. J. B., and Stumm, W., 1988. Geochemistry and biogeochemistry of anaerobic habitats. In Zehnder, A. J. B. (ed.), *Biology of anaerobic microorganisms*. New York: John Wiley & Sons, pp. 1–38.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Hydrogen](#)
[Iron Sulfide Formation](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Nitrogen](#)
[Photosynthesis](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Terrestrial Deep Biosphere](#)

ANAMMOX

Anammox refers to the microbial **anaerobic oxidation** of **ammonium** by nitrite (NO_2^-), which forms N_2 . See entry “[Nitrogen](#)” for further reading.

ANIMAL BIOCALCIFICATION, EVOLUTION

Gert Wörheide¹, Daniel J. Jackson²

¹Ludwig-Maximilians-Universität München, München, Germany

²University of Göttingen, Göttingen, Germany

Definition

SOM. Soluble organic matrix

IOM. Insoluble organic matrix

GRN. Gene regulatory network

Introduction

One of the major events in the evolution of multicellular animals was the transition from soft-bodied organisms to those that possessed mineralized hard parts for protection

and support. This major evolutionary hallmark supported the rapid diversification of animals and their occupation of a diverse range of novel ecological niches at the dawn of the Phanerozoic, between 560 and 530 million years ago, when mineralized skeletons appeared relatively synchronous in a variety of Phyla during the so-called Cambrian Explosion (Knoll, 2003; Conway Morris, 2006). However, it is still unclear exactly what drove this sudden capacity to construct mineralized structures, be it changes in ocean chemistry (Brennan et al., 2004) or the evolution of more diverse ecologies (Cohen, 2005), including predators (Vermeij, 1989). Investigation of biomineralization processes will provide insight into the molecular and genetic mechanisms that accompanied the appearance of mineralized body parts during the early Cambrian, shedding light on the processes that generate the diversity of life we see around us today.

Until recently, studies of biomineralization processes were mainly restricted to describing the (ultra)structure of the minerals and their interaction with the organic material provided by the organism (Lowenstam and Weiner, 1989; Mann and Webb, 1989). However, the generation and elaboration of biominerals is ultimately controlled by genes (Jackson et al., 2006, 2007a, b), which must in turn be expressed in the appropriate tissue and at an appropriate stage of development. Understanding the evolution of such biomineralizing genes and the gene regulatory networks (GRNs) in which they act is, therefore, vital to the understanding of how animals evolved and will also provide clues for the fabrication of biomimetic materials.

Biomineralization

Biomineralization refers to the processes by which organisms form minerals (e.g., calcium carbonate, calcium phosphate, silica, iron oxides) under strict biological control. These processes are, therefore, defined as *biologically controlled mineralization*, with the skeletons formed being integral, functional parts of the organisms (Lowenstam and Weiner, 1989). There are, however, several other serendipitous processes linked to living organisms (e.g., microbes) or organic substances that result in mineral (mainly calcium carbonate) formation that are not under strict control of organisms. These processes are termed either *biologically induced mineralization*, where inorganic minerals are precipitated by adventitious precipitation resulting from secondary interactions between various metabolic processes and the surrounding environment (Mann, 2001), or *organomineralization*, a term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not (e.g., degrading organic matter) (Trichet and Defarge, 1995).

While both *biologically induced* and *organomineralization* play an important role in the formation of carbonate systems in the geological record (e.g., microbialites, stromatolites, ooids) (Reitner and Neuweiler, 1995; Arp,

1999; Reitner et al., 2000), it is *biologically controlled mineralization* that is responsible for the production of sophisticated biomaterials such as bones, shells, and teeth, some of which show exceptional strength and beauty, often of high commercial value (i.e., pearls).

Biologically controlled mineralization almost always has organic macromolecules associated with the process of mineral formation, which can comprise up to 5% of the total mass of a biomineral and fulfill important functions during initial crystal nucleation, crystal growth, and growth termination, as well as contributing to the biomechanical properties of the mature product (Simkiss, 1986; Simkiss and Wilbur, 1989). Following the removal of the mineral itself, these organic macromolecules are often subclassified as being either water soluble organic matrices (SOMs), or water insoluble organic matrices (IOMs). IOMs are largely neutrally charged, often highly polymerized, and act as frame building matrices akin to collagen, chitin, or silk proteins (Yan et al., 2007; Yano et al., 2007). SOMs are often highly acidic, due to negatively charged carboxylate groups, are typically rich in aspartic acid (asp) and/or glutamic acid (glu), and usually have mean molecular weight of 10–30 kDa (Bédouet et al., 2001, 2006).

While organic macromolecules appear ubiquitous and pivotal in *biologically controlled* biomineralization (with the focus here on biocalcification), an increasing number of studies now show that a transient amorphous (noncrystalline) phase of calcium carbonate (ACC) is frequently involved in the formation of the earliest stages of calcium carbonate biominerals (Aizenberg et al., 2003; Politi et al., 2004; Weiner and Dove, 2003). However, little is known about the ACC as well as the organic macromolecules involved in calcification in most animal taxa.

Several SOM proteins have been sequenced and characterized, mainly in sea urchins, mollusks, and vertebrates (Treccani et al., 2003; Wilt et al., 2003). About 45 soluble proteins were detected by 2D gel electrophoresis in demineralized spicules from the embryo of the sea urchin *Strongylocentrotus purpuratus* (Killian and Wilt, 1996). Several of these proteins were later cloned and sequenced (e.g., SM27, SM30, SM50) (Zhu et al., 2001) and their role and function in the biomineralization process confirmed by immunohistochemical methods (Ameye et al., 1999; Urry et al., 2000), in situ hybridization (Illies et al., 2002), or by using antisense oligonucleotides directed against spicule matrix proteins (Peled-Kamar et al., 2002). All of the cloned spicule matrix proteins of *Strongylocentrotus* share as common features a C-type lectin domain and proline rich repeat regions. Furthermore, the first 35 amino acids of SpSM32 and SM50 are identical, probably representing splicing variants of the same transcript, although this has yet to be confirmed (Wilt et al., 2003). The precise functions of any of these proteins are unknown (Wilt et al., 2003) and their evolutionary origin and relationships remain largely unresolved (but see Bottjer et al., 2006). An increasing number of reports are, however, elucidating the functional roles of various biomineralizing proteins. For example, the starmaker

protein is known to influence crystal size and lattice formation in otolith biomineralization (Söllner et al., 2003), while the nacre protein perlucin (Mann et al., 2000; Weiss et al., 2000) is known to nucleate the growth of aragonitic calcium carbonate crystals (Blank et al., 2003).

Identification and characterization of the genes directly involved in the process of biocalcification, and the ways in which these “structural genes” are regulated, is essential for our understanding of the evolution of metazoan biocalcification. One of the most advanced systems currently used to study the complexity of the GRNs involved in specifying biomineralizing cell types is the sea urchin. The manipulability of urchin embryos and the availability of a high-quality genome sequence have allowed researchers to identify specific transcription factors (genes that activate the expression of target genes) that activate biomineralizing genes and proteins (Ettensohn et al., 2003, 2007). Furthermore, recent work has identified the nodes in biomineralizing GRNs that are responsible for specifying cells involved in larval skeletogenesis and adult skeletogenesis (Gao and Davidson, 2008), providing insight into the evolution of the metazoan biphasic lifecycle. Such endeavors are also highly relevant to issues of global climate change and ocean acidification – by assessing how resilient marine calcifying organisms are to fluctuations in ocean chemistry, highly species-rich marine ecosystems such as coral reefs may be effectively managed.

The evolution of biocalcification

In the geological record, major animal phyla began biomineralizing shortly before, or during, the so-called Cambrian Explosion, about 530 million years ago, when a highly diverse group of animals appeared suddenly in the fossil record (Knoll, 2003). As most of these phyla are thought to have diverged well before this biomineralization event (Peterson et al., 2008), various causes for this relatively synchronous adoption of biomineralizing strategies have been postulated and remain an area of continuing debate. These include the evolutionary driving force of predation (Vermeij, 1989) or the result of a response to changes in ocean chemistry with greater than previous levels of calcium, requiring the need for effective cellular calcium-detoxification mechanisms (Degens et al., 1985; Kazmierczak et al., 1985).

The vast majority of newly appearing biomineral products in the Cambrian was based on calcium (either as calcium carbonate or calcium phosphate) (Lowenstam and Margulis, 1980). Precise control of intracellular calcium levels is necessary for the formation of microtubules which are needed by eukaryotic cells. Based on this, Lowenstam and Margulis suggested that calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization. CaCO_3 biomineral formation can be useful for the organism because this process allows the elimination of a cell-toxic surplus of Ca^{2+} through enzymes such as calmodulin and membrane

bound Ca^{2+} -ATPase, which directly transports calcium to loci where it is used as a physiological control factor (Degens, 1979).

The question of whether the ability to biocalcify evolved several times independently among various animal groups, possibly to produce armor in response to increased pressure from predators (Marshall, 2006), or whether preexisting calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization (Lowenstam and Margulis, 1980), still remains open to debate. If calcium carbonate biomineralization evolved as a physiological response (mutual to many taxa) to increasing calcium levels in the late Precambrian ocean, then an evolutionary link between the proteins and the regulatory elements involved in skeletogenesis can be postulated among metazoan groups. This evolutionary connection could either be due to massive horizontal gene transfer in ancestral Precambrian organisms or as a result of the exaptation of a preexisting genetic makeup into a new role (biocalcification) in many disparate animal lineages (Kirschvink and Hagadorn, 2000). The latter leads to the hypothesis that the last common ancestor of the metazoa (LCAM) provided a “genetic biomineralization toolkit,” a core set of conserved biomineralization genes to regulate and initiate the cellular functions required for the assembly of calcium carbonate biominerals (Ca^{2+} , HCO_3^-). Subsequently, this core gene cassette and associated regulatory elements have been elaborated upon to produce the range of biomineralized structures we observe in the Earth’s history. One major process by which complex biological systems evolve is by taking an existing genetic pattern evolved for a certain function, duplicating it, and adapting it for a new role (e.g., Carroll et al., 2001). The nascent system is gradually debugged and improved through the process of random mutation and natural selection. This powerful hypothesis, in the context of biomineralization, still awaits an expanded program of comparative geobiological research (Knoll, 2003).

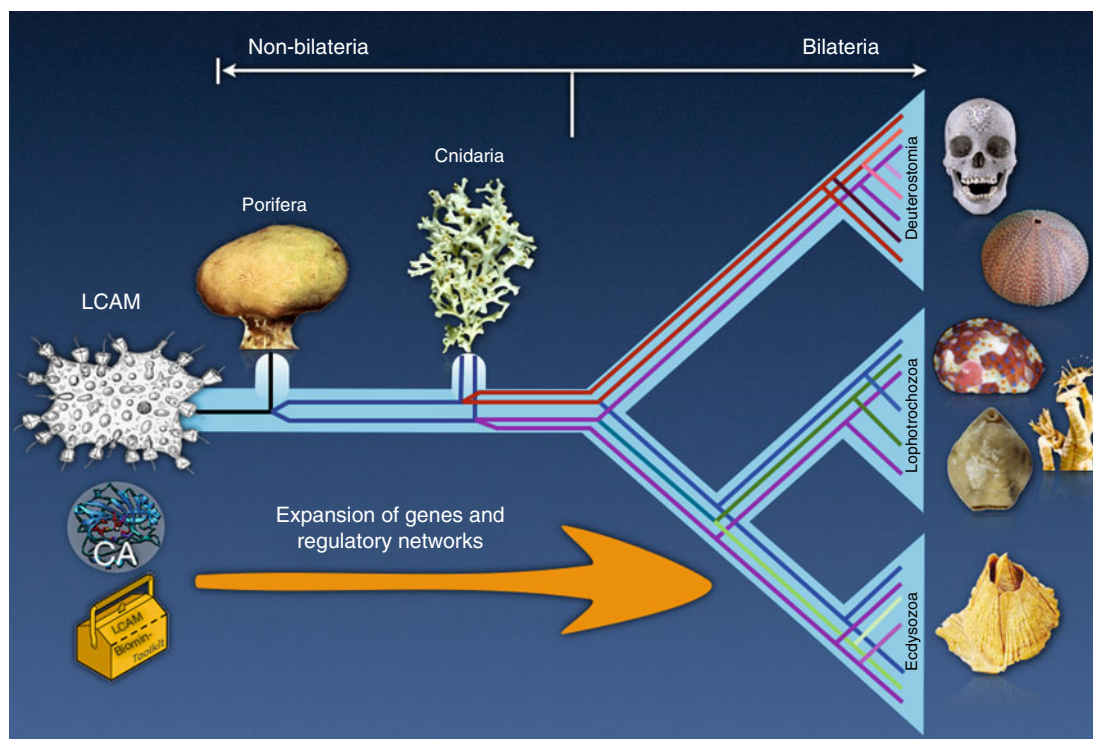
However, evidence for conserved biocalcification mechanisms is beginning to emerge. Some homologies between certain molluscan (Weiss et al., 2001) or sea urchin (Ettensohn et al., 2003) and vertebrate matrix proteins have already been postulated. Ettensohn et al. (2003), for example, found homologies of their sea urchin Sp/LvAlx1 protein, which is an essential component of the gene network that controls downstream genes required for biomineralization, with members of the vertebrate Cart1/Alx3/Alx4 protein family. Members of this gene family have been implicated to be instrumental in the formation of the limb skeleton and the neural crest-derived skeleton of the face and neck in vertebrates. Further, there seems to be some fundamental underlying immunological similarity between macromolecules involved in vertebrate hydroxyapatite and invertebrate aragonite formation (i.e., molluscan nacre), because freshly ground nacre did not result in an immune response when injected into human jaw bones, but in fact

stimulated bone regeneration (Marin and Westbroek, 1998; Lopez et al., 1992). Coral skeletons are already used as bone implants with a similar (albeit weaker) effect on bone regeneration (Demers et al., 2002). Although bone, nacre, and coral skeletons are not homologous structures as such, parts of the complex underlying physiological machinery that controls biomineral formation probably are – a hypothesis strongly supported by the above mentioned results. However, these homologous parts may have served a different function than biomineralization in the (nonskeletonized) common ancestor of corals, mollusks, and vertebrates, later being co-opted for producing biominerals when the necessity arose.

If, as outlined above, calcium carbonate biomineralization systems in the major metazoan phyla evolved from the core skeletogenic toolkit of the last common ancestor of animals, then ancestral metazoan systems should be used as an anchor for a paleogenomic approach to reconstruct this toolkit and explore the evolutionary road map of diversification by comparison with more derived animal taxa. Paleogenomics analyzes the genomes of contemporary taxa to reconstruct ancient genomes and genomic features and to infer the function of ancestral genes.

Recent paleogenomic studies go beyond the focus on classical matrix proteins. Expressed sequence tags (ESTs), cDNA libraries, and recently sequenced genomes have been utilized to examine the inheritance of ancestral toolkits that were involved in skeletogenesis. By using a calcifying coralline sponge of a “living fossil,” *Astrosclera willeyana*, a key component of the ancestral skeletogenic toolkit – α -carbonic anhydrase – has been identified (Jackson et al., 2007b). Duplication of this gene is likely to have allowed biocalcification to evolve in many clades of metazoans. In contrast, the recently sequenced genome of the sea urchin *Strongylocentrotus purpuratus* has revealed that genes essential for the construction of the echinoderm skeleton (the stereome) are echinoderm-specific and are likely to be the same as those in the earliest echinoderms and their direct ancestors more than 520 million years ago (Livingston et al., 2006). Similarly, in gastropods (snails; *Haliotis asinina* and *Lottia scutum*) an unexpected level of genetic novelty and complexity in the skeletogenic toolkit suggests that there are significant molecular differences in the ways in which gastropods synthesize their shells (Jackson et al., 2006). These features, along with the modular design of the molluscan mantle, contribute to the spectacular morphological and mineralogical variety we observe among contemporary mollusk shells (Jackson et al., 2007a).

From the limited data available, we can develop the following scenario as a working hypothesis (Figure 1): the last LCAM possessed a core biomineralization toolkit that at least consisted of an α -carbonic anhydrase, providing the core capacity to regulate the reversible hydration of CO_2 , necessary for biocalcification. Other, as yet undiscovered, parts of this toolkit most likely contained genes that regulate Ca^{2+} trafficking, scaffolding genes for organic matrix production, as well as regulatory



Animal Biocalcification, Evolution, Figure 1 Conceptual summary. The last common ancestor of all Metazoa (LCAM) possessed a core gene toolkit for the regulation of Ca_2^+ and an alpha-carbonic anhydrase to catalyse the reversible hydration of CO_2 . During subsequent metazoan evolution, parts of this toolkit (genes and regulatory elements) were exapted to new roles including calcium carbonate fabrication. Novel genes and biosynthetic pathways were added to the conserved biocalcification toolkit to produce the variety of skeletal forms we observe among extant animal lineages.

elements. During subsequent animal evolution, genes in this core set were duplicated in different lineages, resulting in a relatively simple genetic repertoire in basal metazoans, but in a highly diverse gene array in the various lineages of the Bilateria (Ecdysozoa, Lophotrochozoa, and Deuterostomia). A high degree of lineage-specific innovation on top of a conserved gene-set then allowed for the diversification of the numerous skeletal architectures we observe since the dawn of the Cambrian.

Conclusion

While the structural processes of biomineralization, and in particular biocalcification, are receiving vigorous attention from various international groups, a far more detailed characterization of the core ancestral skeletogenic toolkit as well as lineage specific innovations are needed before we understand how the impressive diversity and beauty of metazoan skeletal structures originated and evolved. As this knowledge is acquired, we can anticipate the construction of new high-performance biomimetic materials and the measurement and prediction of the resilience of marine calcifying organisms to rapidly changing environmental parameters, such as ocean acidification.

Bibliography

- Aizenberg, J., Weiner, S., and Addadi, L., 2003. Coexistence of amorphous and crystalline calcium carbonate in skeletal tissues. *Connective Tissue Research*, **44**, 20–25.
- Ameye, L., Hermann, R., Killian, C., Wilt, F., and Dubois, P., 1999. Ultrastructural localization of proteins involved in sea urchin biomineralization. *Journal of Histochemistry and Cytochemistry*, **47**, 1189–1200.
- Arp, G., 1999. *Calcification of Non-Marine Cyanobacterial Biofilms (USA, PR China, Indonesia, Germany) – Implications for the Interpretation of Fossil Microbialites*. PhD Thesis, Mathematisch-Naturwissenschaftliche Fakultät, Georg-August-Universität Göttingen, Göttingen, p. 118.
- Bédouet, L., Schuller, M. J., Marin, F., Milet, C., Lopez, E., and Giraud, M., 2001. Soluble proteins of the nacre of the giant oyster *Pinctada maxima* and of the abalone *Haliotis tuberculata*: extraction and partial analysis of nacre proteins. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, **128**, 389–400.
- Bédouet, L., Rusconi, F., Rousseau, M., Duplat, D., Marie, A., Dubost, L., Le Ny, K., Berland, S., Péduzzi, J., and Lopez, E., 2006. Identification of low molecular weight molecules as new components of the nacre organic matrix. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, **144**, 532–543.
- Blank, S., Arnoldi, M., Khoshnavaz, S., Treccani, L., Kuntz, M., Mann, K., Grathwohl, G., and Fritz, M., 2003. The nacre protein

- perlucin nucleates growth of calcium carbonate crystals. *Journal of Microscopy*, **212**, 280–291.
- Bottjer, D. J., Davidson, E. H., Peterson, K. J., and Cameron, R. A., 2006. Paleogenomics of echinoderms. *Science*, **314**, 956–960.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Carroll, S. B., Grenier, J. K., and Weatherbee, S. D., 2001. *From DNA to Diversity—Molecular Genetics and the Evolution of Animal Design*. Blackwell: Malden, MA.
- Cohen, B. L., 2005. Not armour, but biomechanics, ecological opportunity and increased fecundity as keys to the origin and expansion of the mineralized benthic metazoan fauna. *Biological Journal of the Linnean Society*, **85**, 483–490.
- Conway Morris, S., 2006. Darwin's dilemma: the realities of the Cambrian "explosion." *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **361**, 1069–1083.
- Degens, E., 1979. Why do organisms calcify. *Chemical Geology*, **25**, 257–269.
- Degens, E. T., Kazmierszak, J., and Ittekkot, V., 1985. Cellular response to Ca_2^+ stress and its geological implications. *Acta Palaeontologica Polonica*, **30**, 115–135.
- Demers, C., Hamdy, C. R., Corsi, K., Chellat, F., Tabrizian, M., and Yahia, L., 2002. Natural coral exoskeleton as a bone graft substitute: a review. *Bio-Medical Materials and Engineering*, **12**, 15–35.
- Ettensohn, C. A., Illies, M. R., Oliveri, P., and De Jong, D. L., 2003. Alx1, a member of the Cart1/Alx3/Alx4 subfamily of paired-class homeodomain proteins, is an essential component of the gene network controlling skeletogenic fate specification in the sea urchin embryo. *Development*, **130**, 2917–2928.
- Ettensohn, C. A., Kitazawa, C., Cheers, M. S., Leonard, J. D., and Sharma, T., 2007. Gene regulatory networks and developmental plasticity in the early sea urchin embryo: alternative deployment of the skeletogenic gene regulatory network. *Development*, **134**, 3077–3087.
- Gao, F., and Davidson, E., 2008. Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6091–6096.
- Illies, M. R., Peeler, M. T., Dechtiaruk, A. M., and Ettensohn, C. A., 2002. Identification and developmental expression of new biomineralization proteins in the sea urchin *Strongylocentrotus purpuratus*. *Development Genes and Evolution*, **212**, 419–431.
- Jackson, D. J., McDougall, C., Green, K., Simpson, F., Wörheide, G., and Degnan, B. M., 2006. A rapidly evolving secretome builds and patterns a sea shell. *BMC Biology*, **4**, 40.
- Jackson, D., Wörheide, G., and Degnan, B. M., 2007a. Dynamic expression of ancient and novel molluscan shell genes during ecological transitions. *BMC Evolutionary Biology*, **7**, 160.
- Jackson, D. J., Macis, L., Reitner, J., Degnan, B. M., and Wörheide, G., 2007b. Sponge paleogenomics reveals an ancient role for carbonic anhydrase in skeletogenesis. *Science*, **316**, 1893–1895.
- Kazmierczak, J., Ittekkot, U., and Degens, E. T., 1985. Biocalcification through time: environmental challenge and cellular response. *Paläontologische Zeitschrift*, **59**, 15–33.
- Killian, C. E., and Wilt, F. H., 1996. Characterization of the proteins comprising the integral matrix of *Strongylocentrotus purpuratus* embryonic spicules. *Journal of Biological Chemistry*, **271**, 9150–9159.
- Kirschvink, J. L., and Hagadorn, J. W., 2000. A grand unifying theory of biomineralization. In Bäuerlein, E. (ed.), *The Biomineralization of Nano- and Micro-Structures*. Weinheim: Wiley, pp. 139–150.
- Knoll, A. H., 2003. Biomineralization and evolutionary history. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization*. Washington, DC: The Mineralogical Society of America/Geochemical Society, pp. 329–356.
- Livingston, B. T., Killian, C. E., Wilt, F., Cameron, A., Landrum, M. J., Ermolaeva, O., Sapojnikov, V., Maglott, D. R., Buchanan, A. M., and Ettensohn, C. A., 2006. A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus*. *Developmental Biology*, **300**, 335–348.
- Lopez, E., Vidal, B., Berland, S., Camprasse, S., Camprasse, G., and Silve, C., 1992. Demonstration of the capacity of nacre to induce bone-formation by human osteoblasts maintained invitro. *Tissue & Cell*, **24**, 667–679.
- Lowenstam, H. A., and Margulis, L., 1980. Evolutionary prerequisites for early phanerozoic calcareous skeletons. *Biosystems*, **12**, 27–41.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. Oxford: Oxford University Press.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. Oxford: Oxford University Press.
- Mann, S., and Webb, J., 1989. *Biomineralization*. Weinheim: VCH.
- Mann, K., Weiss, I. M., Andre, S., Gabius, H. J., and Fritz, M., 2000. The amino-acid sequence of the abalone (*Haliotis laevis*) nacre protein perlucin. *European Journal of Biochemistry*, **267**, 5257–5264.
- Marin, F., and Westbroek, P., 1998. A marriage of bone and nacre. *Nature*, **392**, 861–862.
- Marshall, C. R., 2006. Explaining the Cambrian "explosion" of animals. *Annual Review of Earth and Planetary Sciences*, **34**, 355–384.
- Peled-Kamar, M., Hamilton, P., and Wilt, F. H., 2002. Spicule matrix protein LSM34 is essential for biomineralization of the sea urchin spicule. *Experimental Cell Research*, **272**, 56–61.
- Peterson, K. J., Cotton, J. A., Gehling, J. G., and Pisani, D., 2008. The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **363**, 1435–1443.
- Politi, Y., Arad, T., Klein, E., Weiner, S., and Addadi, L., 2004. Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. *Science*, **306**, 1161–1164.
- Reitner, J., and Neuweiler, F. C., 1995. Mud mounds: a polygenetic spectrum of fine-grained carbonate buildups. *Facies*, **32**, 1–70.
- Reitner, J., Thiel, V., Zankl, H., Michaelis, W., Wörheide, G., and Gautret, P., 2000. Organic and biogeochemical patterns in cryptic microbialites. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 149–160.
- Simkiss, K., 1986. The process of biomineralization in lower plants and animals – an overview. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals. The Systematics Association Special Volume 30*. Oxford: Clarendon, pp. 19–38.
- Simkiss, K., and Wilbur, K. M., 1989. *Biomineralization – Cell biology and Mineral Deposition*. San Diego: Academic.
- Söllner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., and Nicolson, T., 2003. Control of crystal size and lattice formation by starmaker in otolith biomineralization. *Science*, **302**, 282–286.
- Treccani, L., Khoshnavaz, S., Blank, S., Roden, K. v., Schulz, U., Weiss, I. M., Mann, K., Radmacher, M., and Fritz, M., 2003. Biomineralizing proteins, with emphasis on invertebrate-mineralized structures. In Fahnestock, S. R., and Steinbüchel, A. (eds.), *Biopolymers*. Berlin: Wiley, Vol. 8, pp. 289–321.

- Trichet, J., and Defarge, C., 1995. Non-biologically supported organomineralization. *Bulletin de l'Institut Océanographique Monaco*, **14**, 203–236.
- Urry, L. A., Hamilton, P. C., Killian, C. E., and Wilt, F. H., 2000. Expression of spicule matrix proteins in the sea urchin embryo during normal and experimentally altered spiculogenesis. *Developmental Biology*, **225**, 201–213.
- Vermeij, G., 1989. The origin of skeletons. *PALAIOS*, **4**, 585–589.
- Weiner, S., and Dove, P., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–29.
- Weiss, I. M., Kaufmann, S., Mann, K., and Fritz, M., 2000. Purification and characterization of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevis*. *Biochemical and Biophysical Research Communications*, **267**, 17–21.
- Weiss, I. M., Gohring, W., Fritz, M., and Mann, K., 2001. Perlustrin, a *Haliotis laevis* (Abalone) nacre protein, is homologous to the insulin-like growth factor binding protein N-terminal module of vertebrates. *Biochemical and Biophysical Research Communications*, **285**, 244–249.
- Wilt, F. H., Killian, C. E., and Livingston, B. T., 2003. Development of calcareous skeletal elements in invertebrates. *Differentiation*, **71**, 237–250.
- Yan, Z., Jing, G., Gong, N., Li, C., Zhou, Y., Xie, L., and Zhang, R., 2007. N40, a novel nonacidic matrix protein from pearl oyster nacre, facilitates nucleation of aragonite in vitro. *Biomacromolecules*, **8**, 3597–3601.
- Yano, M., Nagai, K., Morimoto, K., and Miyamoto, H., 2007. A novel nacre protein N19 in the pearl oyster *Pinctada fucata*. *Biochemical and Biophysical Research Communications*, **362**, 158–163.
- Zhu, X., Mahairas, G., Illies, M., Cameron, R. A., Davidson, E. H., and Ettensohn, C. A., 2001. A large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo. *Development*, **128**, 2615–2627.

Cross-references

Animal Skeletons, Advent
 Calcium Biogeochemistry
 Carbonate Environments
 Critical Intervals in Earth History
 Ediacaran Biota
 Microbial Biomineralization
 Organomineralization
 Reefs
 Soda Ocean Hypothesis
 Sponges (Porifera) and Sponge Microbes
 Trace Fossils: Neoproterozoic

ANIMAL SKELETONS, ADVENT

Guoxiang Li, Maoyan Zhu, Zhe Chen
 Chinese Academy of Sciences, Nanjing, China

Synonyms

Early biomineralization event; Small shelly fossils

Definition

Animal skeleton. Biologically, an animal skeleton is a hard or rigid framework that could provide protection and

support in many types of animals. Most of the animal skeletons are mineralized, but some of them may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, excluding hydroskeletons. The “skeletons” of resistant organic biopolymers are not included herein.

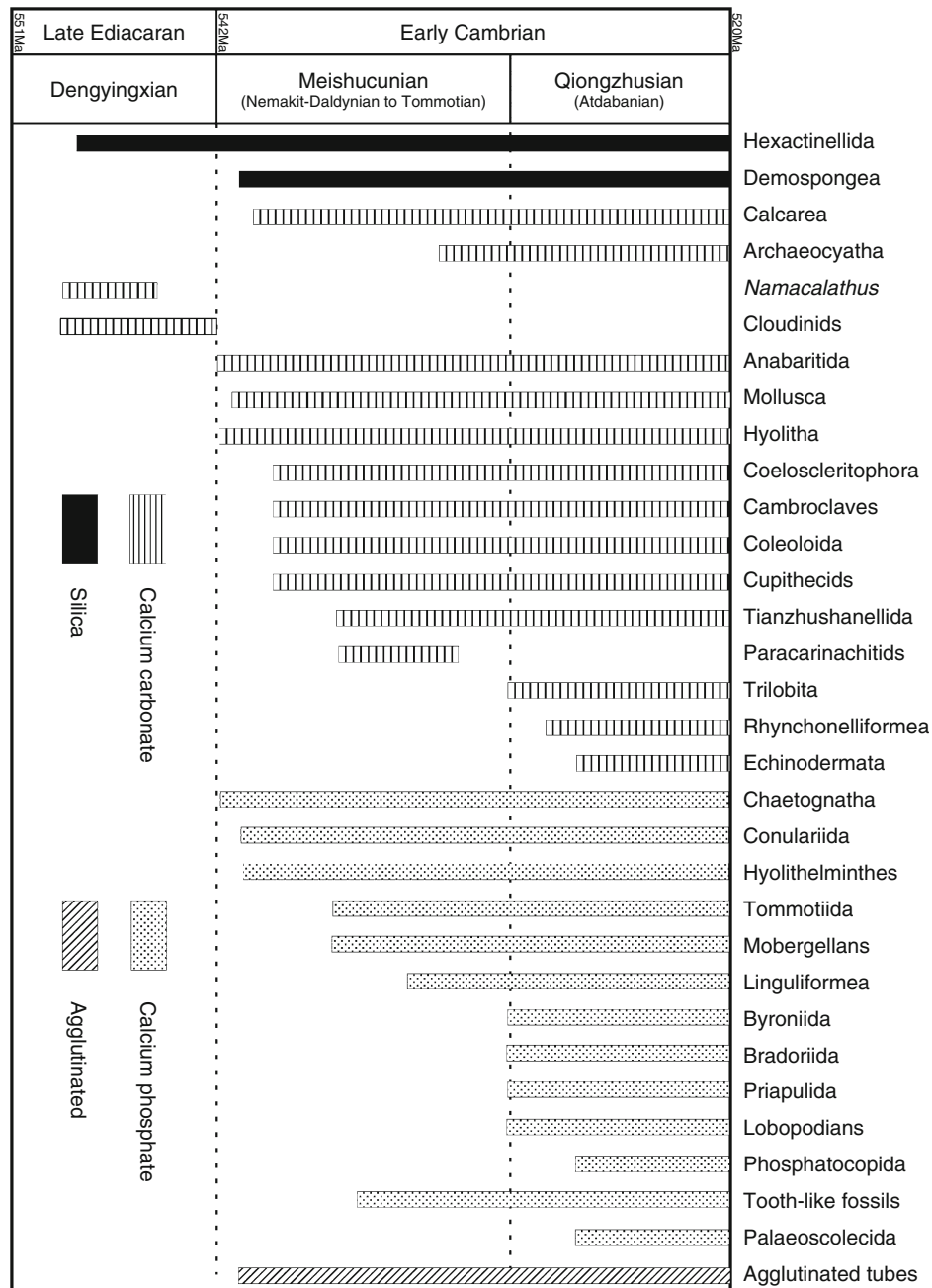
Exoskeleton. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites.

Endoskeleton. Endoskeletons are internal hard parts (typical of many vertebrates). Most of the endoskeletons are generally surrounded by skin and musculature.

Small shelly fossils (SSF or SSFs). A non-taxonomic term was first used by Matthews and Missarzhevsky in the title of their review paper in 1975 to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites.

Introduction

Skeletal biomineralization (skeletonization) was part of the initial diversification of early animals. The fossil record provides an important clue to the origin of biomineralization in the animal phyla (Kirschvink and Hagadorn, 2000). The earliest occurrence of animal skeletons was in the late Ediacaran, e.g., *Cloudina*, *Sinotubulites*, and *Namapoikia*, although the generic diversity was quite low and the biomineralization of these calcareous skeletons was not well controlled biologically (Grant, 1990). The early Cambrian (542–525 Ma) saw the largest burst of diversified animal skeletons, represented in the fossil record by poriferans, cnidarians, many extant bilaterian phyla, and some problematic taxa (Figure 1) (Bengtson, 2004). In terms of structural and mineralogical diversity of animal skeletons, the early Cambrian represents a quantum jump (Bengtson and Conway Morris, 1992). The abrupt advent of diversified skeletal fossils not only has been taken as a major piece of evidence for the Cambrian radiation but also was envisioned the biomineralization event. Since most of these skeletal fossils are typically small in size, and lots of them represent disarticulated sclerites and are enigmatic with respect to their biological affinities, they are commonly referred to as “small shelly fossils (SSFs)” in the literature (Matthews and Missarzhevsky, 1975; Qian, 1999). But some of the early skeletal fossils, such as the sponge-like archaeocyaths, trilobites, and bivalved arthropods, are rarely described under this nontaxonomic term, while some fossils described under the term of SSFs, such as embryo fossils and cnidarian polyps, may have been originally composed of resistant organic membranes or tubes without biomineralization, and the skeleton-like appearance is mainly due to the secondary phosphatization.



Animal Skeletons, Advent, Figure 1 Distribution of major skeletal animal taxa (biomineralized and agglutinated) from the late Ediacaran to the early Cambrian.

Mineralogy of early animal skeletons

Animals use a variety of minerals to fabricate their skeletons (Lowenstam and Weiner, 1989). However, there are only three principal classes of minerals being widely used by organisms, including calcium carbonates, calcium phosphates, and silica. Calcium, carbonate, and phosphate ions are abundant in the oceans, and are ready for use in skeletons (Kirschvink and Hagadorn, 2000), despite

skeletogenesis being a biologically controlled, complex process. Animals rarely switched their mineralogy after their first skeletogenesis, indicating that, for most lineages, environment does not strongly influence skeleton composition, although the selection of skeletal mineralogy during the first acquisition of skeletons in a clade may be somewhat related to ambient seawater chemistry (Knoll, 2003; Porter, 2007; Zhuravlev and Wood, 2008).

It is in most cases not apparent to identify the original minerals of early fossil skeletons because of diagenetic alterations. Especially, there was a very significant phosphogenic event around the Ediacaran-Cambrian transition (Cook and Shergold, 1984), and thus secondary phosphatization of early skeletal fossils was quite common (Qian and Bengtson, 1989). The original composition of fossil skeletons can be learnt by inference and comparative analysis. The fossil record shows that early animals mainly used calcium carbonates, apatites, and silica to build their skeletons (Figure 1).

Several polymorphs of calcium carbonates are the major skeleton-forming minerals, including calcite (high-Mg calcite, low-Mg calcite), aragonite, and vaterite. Vaterite is an extremely unstable polymorph of CaCO_3 and is rarely used as a biomineral. Aragonite is an unstable mineral and it is easily transformed to low-Mg calcite during diagenesis; hence, the original mineralogy of early calcareous fossils is difficult to identify in many cases (Bengtson and Conway Morris, 1992). The earliest animal skeletons during the late Ediacaran-Tommotian interval were composed exclusively of aragonite and high-Mg calcite, being consistent with an aragonite sea (Porter, 2007; Zhuravlev and Wood, 2008). Since the early Atdabanian, there occurred many clades of animals using low-Mg calcite to make skeletons, implying the onset of a calcite sea.

Apatite is another major mineral for early animals to make skeletons (Figure 1), and it is diagenetically the most stable among the major skeleton-building minerals (Bengtson and Conway Morris, 1992). Phosphatic skeletons are usually a combination of phosphate and organic material and hence are called organo-phosphatic. Comparative study indicates that phosphatic skeletons in the

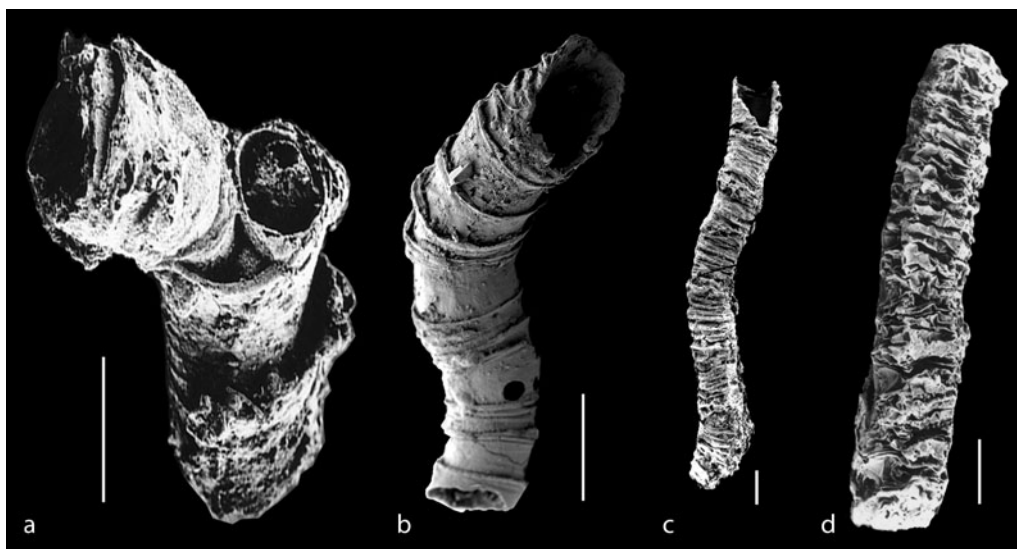
earliest metazoans appear to have been more widespread than today (Runnegar and Bengtson, 1990). The early phosphatic skeletal metazoans include brachiopods (Linguliformea), cnidarians (hyolithelminths, byroniids, and conulariids), bradoriids, lobopodian, protoconodonts, and conodont-like fossils, tomotiids, etc. The high diversity of phosphatic skeletons is somewhat related to wide distribution of phosphate deposits (phosphogenesis event) in various parts of the world during the early Cambrian (Cook and Shergold, 1984).

Siliceous (opal) skeletons differ in distribution from those made of CaCO_3 since silica biomineralization is limited to intracellular precipitation. Therefore, in animals, siliceous skeletons are limited to sponge spicules and other minor occurrences (Bengtson and Conway Morris, 1992). Opal has been used by sponges (including Demospongea and Hexactinellida) to build their skeletons since the late Ediacaran (Gehling and Rigby, 1996). Biogenic opal is unstable and it usually recrystallizes to quartz during diagenesis.

Agglutinated conchs are occasionally found in the Lower Cambrian rocks such as *Salterella*, *Volborthella*, and *Platysolenites*. Both *Salterella* and *Volborthella* are metazoans (Yochelson and Kisselev, 2003), but their biological affinities are uncertain. They have a calcareous sheath partially filled with detrital grains, agglutinating into laminations. *Platysolenites* represents an agglutinating foraminiferan (Glaessner, 1963).

Morphological variety of early animal skeletons

The morpho-types of the animal skeletons in the late Ediacaran are relatively few. They are mainly represented by weakly mineralized calcareous tubes (e.g., *Cloudina*,



Animal Skeletons, Advent, Figure 2 Examples of late Ediacaran skeletal fossils. (a, b) *Cloudina*, an enigmatic tubular fossil, possibly related to annelids; (c, d) *Sinotubulites*, a tubular fossil of unknown affinity. All from the Dengying Formation in southern Shaanxi, China. Scale bars = 500 μm .



Animal Skeletons, Advent, Figure 3 Examples of early Cambrian isolated sclerite fossils. (a) *Sinosachites*, a sclerite of halkieriids; (b1, b2) *Halkieria*, cultrate and palmate sclerites; (c) *Rhombocorniculum*, a tooth-like sclerite; (d) *Protohertzina*, a tooth-like sclerite of protocondonts (Chaetognatha). (e) *Scoponodus*, a sclerite of unknown affinity; (f1, f2) *Siphononuchites*, sclerites of dorsal and ventral-lateral views; (g) *Microdictyon*, a net-like plate; (h) *Cambroclavus*, a nail-like sclerite of unknown affinity; (i) *Archiasterella*, a sclerite of cancelloriids. (j1, j2) *Tannuolina*, mitral and sellate sclerites (tommotiids); (k) *Lapworthella*, a sclerite (tommotiids). All from the Lower Cambrian of South China. (d–f2: After Qian and Bengtson, 1989). Scale bars = 100 μm , except in j2 = 500 μm .

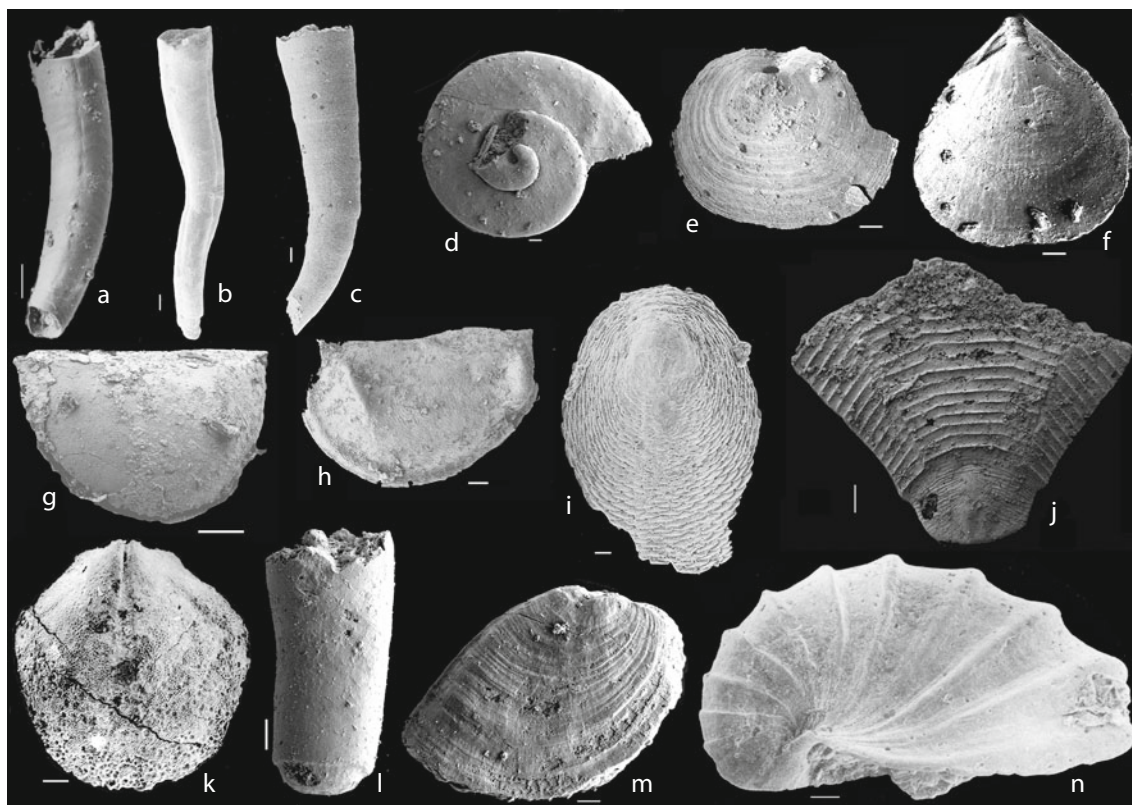
Sinotubulites) (Figure 2) (Hua et al., 2005), modular skeletons (e.g., *Namapoikia*) (Wood et al., 2002), sponge spicules (Brasier et al., 1997) or a reticulating net of spicules (e.g., *Paleophragmodictya*) (Gehling and Rigby, 1996), and goblet-shaped *Namacalathus* (Grotzinger et al., 2000).

Various types of the animal skeletons abruptly occurred in the early Cambrian. Using the theoretical “Skeleton Space” concept, Thomas et al. (2000) made an analysis of Cambrian animal skeletons. Their analysis showed that about 50% of skeletal design elements recognized among living and extinct marine metazoans were exploited in the early Cambrian (Tommotian) and about 80% exploited in the middle Cambrian Burgess Shale animals. The early Cambrian animal skeletons include univalved (Figure 4d, i, n) and bivalved shells (Figure 4e, f), arthropod carapaces (Figure 4g, h), diverse tubes (Figure 4a–c, l), spicules, diverse external sclerites (Figure 3), tooth-like structures (Figure 3c–e), calcareous massive reinforcements (e.g., archeocyathans), echinoderm ossicles, etc.

Late Ediacaran skeletal metazoans

Prior to the Cambrian, the evidence for animal life was almost the soft-bodied Ediacaran fauna and the advent of skeletal animals had been considered to be in the lowermost Cambrian until Germs (1972) described the first Precambrian skeletal fossil *Cloudina* from the Nama Group in Namibia. Since then, biomineralized fossil assemblages have been widely found in the latest Ediacaran over the world, although the biodiversity is very low. Up to now, there are only several skeletal fossil genera being discovered from the Upper Ediacaran rocks, including *Cloudina*, *Sinotubulites*, *Namapoikia*, *Namacalathus*, and *Paleophragmodictya*. Most genera became extinct at the base of the Cambrian, suggesting that a mass extinction occurred at the latest Ediacaran (Amthor et al., 2003).

The worldwide distributed *Cloudina* is a calcareous conical fossil consisting of funnel-like, apically flaring cones nested with one another (Figure 2a, b). The zoological affinities of *Cloudina* are uncertain, but occasional evidence for budding supports either a cnidarian interpretation



Animal Skeletons, Advent, Figure 4 Examples of early Cambrian tube-dwelling and shell-bearing animals. (a) *Torellella*, a tube-like fossil of unknown affinity; (b) *Anabarites*, a tube-like fossil of cnidarian. (c) *Conotheca*, a hyolith; (d) *Aldanella*, a possible gastropod; (e) A ventral valve of *Eohardrotreta*, acrotretid brachiopods; (f) A ventral valve of *Palaeobolus*, lingulid brachiopods; (g) *Dabashanella*, a phosphatocopid; (h) *Kunmingella*, a bradoriid; (i) *Canopoconus*, a possible mollusk; (j) *Hexangulaconularia*, a conulariid (Cnidaria); (k) *Lathamella*, a stem-group brachiopod; (l) *Cupitthea*, a tube-like fossil of unknown affinity; (m) *Watsonella*, a rostroconch (Mollusca); (n) *Archaeospira*, a gastropod. All from the Lower Cambrian of South China (b, c, i, n: After Qian and Bengtson, 1989). Scale bars = 100 μ m.

(Grant, 1990) or an annelid relation (Hua et al., 2005). The *Cloudina* tubes contain the earliest evidence of predatorial borings (Figure 2b). The enigmatic *Sinotubulites* (Figure 2c, d) is a cylindrical fossil, and its tube is composed of several thin layers and characterized by “tube-in-tube” structure (Chen et al., 2007). It has been only documented from the Dengying Formation in South China and co-occurred with *Cloudina*. The irregular wrinkle ornamentation of outer layer shows the *Sinotubulites* tubes being plastic, implying that the tube wall was dominantly organic with aragonitic mineralization (Chen et al., 2007).

Namapoikia is a late Ediacaran, fully biomineralized metazoan from the Nama Group, Namibia, and it is up to 1 m in diameter and bears a complex and robust calcareous skeleton (Wood et al., 2002). It resembles chaetid sponges or simple colonial cnidarians. *Namacalathus* is another calcified animal from the late Ediacaran, and it is goblet shaped up to 2 cm across (Grotzinger et al., 2000). It is more likely to be a cnidarian than a sponge. Both *Namapoikia* and *Namacalathus* co-occur with *Cloudina*.

Hexactinellid sponges have also been found in the late Ediacaran, including the body fossil *Paleophragmodictya*

(with a reticulating net of spicules) (Gehling and Rigby, 1996) as well as clusters of siliceous spicules (Brasier et al., 1997).

Early Cambrian skeletal metazoans

The great watershed in skeletal biomineralization was the early Cambrian, where the diversity of skeletal animals increases nearly exponentially over the interval. Compared with the limited diversity of skeletal fossils in the Ediacaran, the Lower Cambrian is marked by an extraordinary burst of skeletal fossils. Most skeletal metazoans (extant and extinct) appeared for the first time during the early Cambrian (Figure 1). Among these skeletal fossils, many “skeletons” were not integrated and consisted of numerous discrete sclerites, e.g., cambroclaves, tomotiids, halkieriids, cancelloriids, etc. (Figure 3).

Although the advent of animal skeletons in the early Cambrian is quite abrupt or explosive in relation to the long geologic history, the first appearances of different clades in the early Cambrian are sequential (Li et al., 2007). Anabaritids, hyoliths, maikhanellids (mollusks),

and protoconodonts (chaetognaths) first occurred in the early Meishucunian, coeloscleritophorans, tommotiids in the middle Meishucunian, while crown group brachiopods, skeletal arthropods, and lobopods first occurred in the early Qiongzhusian (Figure 1). The skeletal fossil record shows that the clades occurring in the pre-trilobitic interval (Meishucunian) include many extant phyla such as Porifera, Cnidaria, Annelida, Mollusca, Chaetognatha, Brachiopoda (Lingulids), and a number of extinct taxa such as Hyolitha, stem-group brachiopods, tommotiids, coeloscleritophorans, cambroclaves, coleoloids, paracarinachitids, etc. The pre-trilobitic skeletal fossils were dominated by helcionellids (Mollusca), hyoliths, other problematic sclerites, cap-shaped fossils, and tube-like fossils. They are distinctively different from the “Cambrian Evolutionary Fauna,” and were christened the “Tommotian Fauna” (Sepkoski, 1992).

In the Qiongzhusian (Atdabanian) stage, besides many extinct clades, nearly all extant skeletal invertebrate phyla occurred (except Byrozozoa with first occurrence in the early Ordovician). The dominant and characteristic skeletal fossils of this interval include arthropods, brachiopods, and echinoderms, representing an initial stage of the “Cambrian Evolutionary Fauna.”

Summary

The earliest skeletal fossils came from the late Ediacaran rocks, but the early Cambrian witnessed the largest burst of animal skeletons. The abrupt advent of diverse skeletal fossils is an important part of the Cambrian explosion, and biomineralization of animal hard parts provides many advantages for metazoan diversification. Although the trigger for this early biomineralization event has been a subject of extensive debate in literature, it should be an integrating result of extrinsic and intrinsic (biological) factors. The fossil record also exhibits that, along with the rapid diversification of metazoans in the early Cambrian, the metazoans progressively exploited more ecological space. Most of the pre-trilobitic skeletal faunas came from the shallow-water deposits, but during the Qiongzhusian, there already existed a distinct differentiation of shallow water and deep water habitats: a strong linkage between the occurrences of the skeletal fossils and sedimentary environments.

Bibliography

Amthor, J. E., Grotzinger, J. P., Schröder, S., Bowring, S. A., Ramezani, J., Martin, M. W., and Matter, A., 2003. Extinction of *Cloudina* and *Namacalathus* at the Precambrian-Cambrian boundary in Oman. *Geology*, **37**, 431–434.

Bengtson, S., 2004. Early skeletal fossils. In Lipps, J. H., and Waggoner, B. M. (eds.), *Neoproterozoic-Cambrian Biological Revolutions. Paleontological Society Papers*, **10**, 67–78.

Bengtson, S., and Conway Morris, S., 1992. Early radiation of biomineralizing phyla. In Lipps, J. H., and Signor, P. W. (eds.), *Origin and Early Evolution of the Metazoa*. New York and London: Plenum Press, pp. 447–481.

Brasier, M., Green, O., and Shields, G., 1997. Ediacarian sponge spicule clusters from southwestern Mongolia and the origins of the Cambrian fauna. *Geology*, **25**, 303–306.

Chen, Z., Bengtson, S., Zhou, C. M., Hua, H., and Yue, Z., 2007. Tube structure and original composition of *Sinotubulites*: shelly fossils from the late Neoproterozoic in southern Shaanxi, China. *Lethaia*, **41**, 37–45.

Cook, P. J., and Shergold, J. H., 1984. Phosphorous, phosphorites and skeletal evolution at the Precambrian-Cambrian boundary. *Nature*, **308**, 231–236.

Gehling, J. G., and Rigby, K. J., 1996. Long expected sponges from the Neoproterozoic Ediacara fauna of South Australia. *Journal of Paleontology*, **70**, 185–195.

Germis, G. J. B., 1972. New shelly fossils from Nama Group, South west Africa. *American Journal of Science*, **272**, 752–761.

Glaessner, M. F., 1963. Major trends in the evolution of the Foraminifera. In von Koenigswald, G. H. R. (ed.), *Evolutionary Trends in Foraminifera*. Amsterdam: Elsevier, pp. 9–24.

Grant, S. W. F., 1990. Shell structure and distribution of *Cloudina*, a potential index fossil for the terminal Proterozoic. *American Journal of Science*, **290A**, 261–294.

Grotzinger, J. P., Watters, W. A., and Knoll, A. H., 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology*, **26**, 334–359.

Hua, H., Chen, Z., Yuan, X. L., Zhang, L. Y., and Xiao, S. H., 2005. Skeletogenesis and asexual reproduction in the earliest biomineralizing animal *Cloudina*. *Geology*, **33**, 277–280.

Kirschvink, J. L., and Hagadorn, J. W., 2000. A grand unified theory of biomineralization. In Bäuerlein, E. (ed.), *The Biomineralisation of Nano- and Micro-Structures*. Weinheim: Wiley-VCH Verlag, pp. 139–150.

Knoll, A. H., 2003. Biomineralization and Evolutionary History. *Reviews in Mineralogy and Geochemistry*, **54**, 329–355.

Li, G. X., Steiner, M., Zhu, X. J., Yang, A. H., Wang, H. F., and Erdtmann, B.-D., 2007. Early Cambrian metazoan fossil record of South China: Generic diversity and radiation patterns. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 229–249.

Lowenstam, H. A., and Weiner, S., 1989. *On biomineralization*. Oxford: Oxford University Press.

Matthews, S. C., and Missarzhevsky, V. V., 1975. Small shelly fossils of late Precambrian and early Cambrian age: a review of recent work. *Journal of the Geological Society*, **131**, 289–304.

Porter, S. M., 2007. Seawater chemistry and early carbonate biomineralization. *Science*, **316**, 1302.

Qian, Y., 1999. *Taxonomy and Biostratigraphy of Small Shelly Fossils in China*. Beijing: Science Press. (In Chinese with English abstract).

Qian, Y., and Bengtson, S., 1989. Palaeontology and biostratigraphy of the Early Cambrian Meishucunian Stage in Yunnan Province, South China. *Fossils and Strata*, **24**, 1–156.

Runnegar, B., and Bengtson, S., 1990. Origin of hard parts—early skeletal fossils. In Briggs, D. E. G., and Crowther, P. R. (eds.), *Palaeobiology—A Synthesis*. Oxford: Blackwell, pp. 17–30.

Sepkoski, J. J. (Jr.), 1992. Proterozoic-Early Cambrian diversification of metazoans and metaphytes. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 553–561.

Thomas, R. D., Shearman, R. M., and Stewart, G. W., 2000. Evolutionary exploitation of design options by the first animals with hard skeletons. *Science*, **288**, 1239–1242.

Wood, R. A., Grotzinger, J. P., and Dickson, J. A. D., 2002. Proterozoic Modular Biomineralized Metazoan from the Nama Group, Namibia. *Science*, **296**, 2383–2386.

- Yochelson, E. L., and Kisselev, G. N., 2003. Early Cambrian *Salterella* and *Volborthella* (Phylum Agmata) re-evaluated. *Lethaia*, **36**, 8–20.
- Zhuravlev, A. Yu., and Wood, R. A., 2008. Eve of biomineralization: Controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Animal Biocalcification, Evolution
 Breakup of Rodinia
 Ediacaran Biota
 Gondwanaland, Formation
 Origins of the Metazoa
 Trace Fossils: Neoproterozoic

ARCHAEA

Volker Thiel
 University of Göttingen, Göttingen, Germany

Synonyms

Archaeobacteria (term abandoned)

Definition

The Archaea are single-celled or filamentous prokaryotes that constitute the third phylogenetic domain of life, besides the Bacteria and the Eukarya. The word “Archaea” (singular archaeum, archaeon) is derived from the Greek word for “the old ones”.

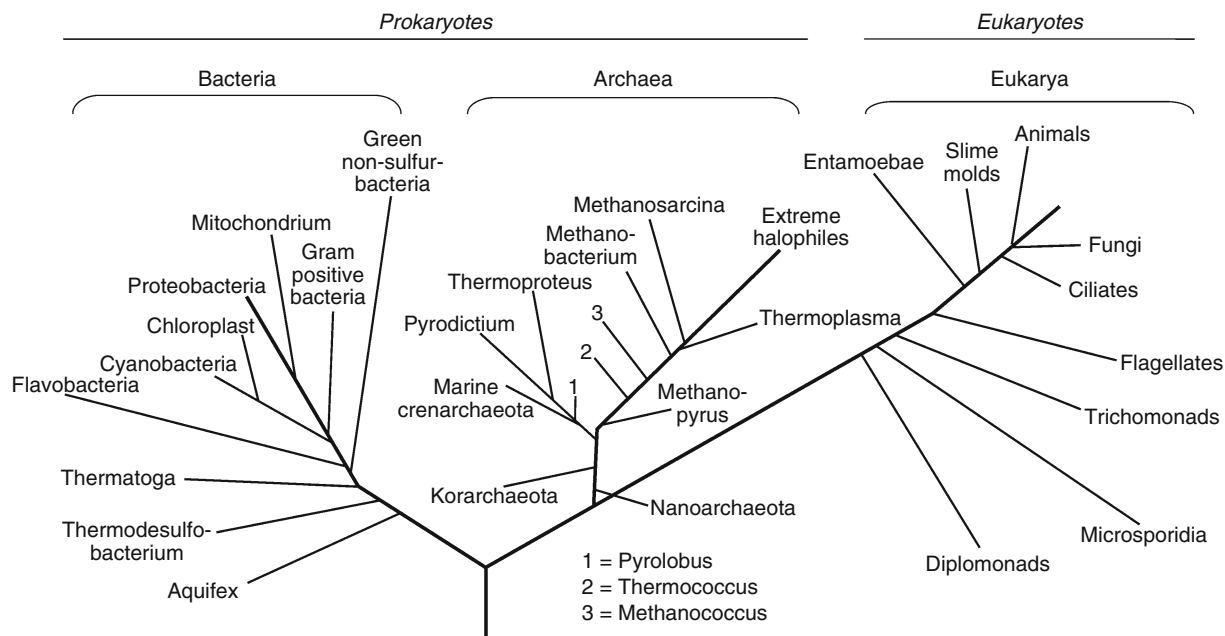
History

The discovery of the Archaea dates back to 1976 when Carl Woese, at his laboratory at Illinois University, compared prokaryotic small subunit ribosomal RNA sequences using oligonucleotide catalogs (Woese, 2007). Woese recognized *Methanobacterium thermoautotrophicum* as the first member of a fundamentally distinct group of prokaryotes that clustered away from all other bacteria. Consequently, Woese and Fox (1977) established the concept of two separate prokaryotic “urkingdoms,” Eubacteria and Archaeobacteria. Later, the term “Archaeobacteria” was changed to “Archaea” to emphasize the fundamental differences between both groups. Based on these discoveries, Woese and his coworkers proposed the now-accepted division of life into the three domains of Archaea, Bacteria, and Eukarya (Figure 1; Woese et al., 1990).

Specific traits

Like the Bacteria, most Archaea show a cell size between 0.5 and 2 μm and lack a cell nucleus, internal membranes, and organelles. However, a number of traits distinguish the Archaea from members of the other domains of life:

1. The genetic machinery of Archaea is different from Bacteria and Eukarya. The distinctive archaeal “signature” contains more than 1,000 archaeal protein-encoding genes. These signature genes account for a significant portion (9–15%) of archaeal genomic DNA (Graham et al., 2000). The archaeal RNA polymerase, an enzyme producing RNA chains from DNA gene templates, consists of eight (methanogens



Archaea, Figure 1 The phylogenetic tree of life. (Redrawn after Madigan and Martinko, 2006.)

- and halophiles) or ten (hyperthermophiles) individual polypeptides. These polymerases are quite different from those of the Bacteria (four polypeptides), but somewhat similar to those of the Eukarya (10–12 polypeptides; Madigan and Martinko, 2006).
2. Unlike bacteria, the cell walls of the Archaea contain no peptidoglycan (a polymer consisting of sugars and amino acids, also known as murein). Rather, archaeal cell walls exhibit a variety of compositions (Kandler and König, 1998). They may consist of the murein analog pseudomurein, polysaccharides, proteins, or glycoproteins (Madigan and Martinko, 2006).
 3. Archaea possess glycerol-based phospholipids as building blocks of their cell membranes, as do bacteria and eukaryotes. However, some features of the archaeal membrane lipids set them apart, namely (a) the stereochemistry of the glycerol moiety (L instead of R), (b) the linkage between the alkyl chains and the glycerol molecules (ether instead of ester bonds), and (c) the design of the alkyl chains (isoprene-based instead of acyl-based; DeRosa and Gambacorta, 1988). In many archaea, these chains are long enough (C₄₀) to span the whole membrane, forming a monolayer instead of the lipid bilayer typical for other organisms (Koga et al., 1993). These isoprenoid lipids, or degradation products thereof, are excellent tracers for archaea-derived organic matter inputs and biogeochemical processes in modern and ancient sediments (Hopmans et al., 2000; Kuypers et al., 2001; Peckmann and Thiel, 2004; see entry *Biomarkers*).

Phylogeny

Most Archaea, and all cultured species, belong to two major, well-established subdivisions, the Euryarchaeota, which include methanogens and extreme halophiles, and the Crenarchaeota, which encompass predominantly hyperthermophiles and anaerobic respirers (Figure 1). Further lineages, namely Korarchaeota (Barns et al., 1996), Nanoarchaeota (Huber and Rachel, 2007), and the Ancient Archaeal Group (Takai and Horikoshi, 1999), have been proposed based on 16S rDNA amplification from environmental samples and/or co-cultivation in mixed laboratory enrichments (Gribaldo and Brochier-Armanet, 2006; Schleper, 2007). However, most of these newly proposed groups still await detailed characterization.

It is as yet unclear at which time in Earth history the domain Archaea arose (Gribaldo and Brochier-Armanet, 2006). Isotopic signatures indicate the presence of methane-cycling archaea 2.8–2.6 billion years ago. Other molecular estimates placed the divergence between Euryarchaeota and Crenarchaeota even 4.1 billion years ago, but this was inferred by using the plant/animal divergence as a calibration point (Battistuzzi et al., 2004). Unequivocal archaeal biosignatures in Precambrian rocks are therefore urgently needed to provide reliable calibration

points for the molecular dating of Archaea and prokaryotes in general (Gribaldo and Brochier-Armanet, 2006).

Ecology

Until recently, it had been thought that archaea are largely restricted to particular ecological niches. Indeed, many archaea are extremophiles (see entry *Extreme Environments*), with growth optima at the most extreme pH values, ranging from pH 1 (*Sulfolobus acidocaldarius*) to pH 12 (*Natronococcus*). Moreover, numerous species are hyperthermophiles, with growth optima above 80°C. The current record for growth temperatures (122°C) is held by a methanogen, *Methanopyrus kandleri* (Takai et al., 2008). Likewise, many halophilic archaea can tolerate extremely high salt concentrations, even at the saturation limit for NaCl (5.5 M; Barns and Nierzwicki-Bauer, 1997; see entry *Halobacteria – Halophiles*). However, ongoing research employing culture-independent molecular techniques and biomarker studies revealed that archaea are by no means restricted to extreme environments. Rather, they are globally distributed and occur in virtually all “normal” settings as well, including soils and freshwater environments (Schleper, 2007), marine planktonic communities (>20% of the total rRNA, >14% of the total cell counts in sea water; Giovannoni and Rappé, 2000), and marine sediments (Lipp et al., 2008). Archaea are also found as partners in symbioses, e.g., with other archaea (Huber and Rachel, 2007), bacteria (Boetius et al., 2000; Stams and Plugge, 2009), ciliates (van Hoek et al., 2000), sponges (Margot et al., 2002), ruminants (Wright et al., 2006), and even in human intestines (Eckburg et al., 2005; see entry *Symbiosis*).

Metabolism

Some examples for biochemical pathways used by the Archaea are given in Table 1.

The Archaea exhibit a wide range of metabolic capacities, however, all members are chemotrophic. Phototrophy, in a strict sense, does not occur among the Archaea, although some halophilic archaea produce pigments, bacteriorhodopsin-like, light driven proton, or ion pumps that allow them to utilize sunlight for biosynthesis, but in a way very different from phototrophic organisms (Madigan and Martinko, 2006). *Chemoheterotrophic* archaea utilize organic substances, particularly metabolic products of other microbes formed during organic matter degradation (Barns and Nierzwicki-Bauer, 1997). These substrates usually consist of simple C₁ and C₂ compounds, such as methanol or acetic acid. Methane and CO₂ are typical metabolic products of archaeal chemoheterotrophy. *Chemolithotrophic* archaea fix inorganic carbon, with hydrogen gas derived from geochemical processes or microbial metabolism being widely used as an energy source (Pedersen, 1997; Berg et al., 2010).

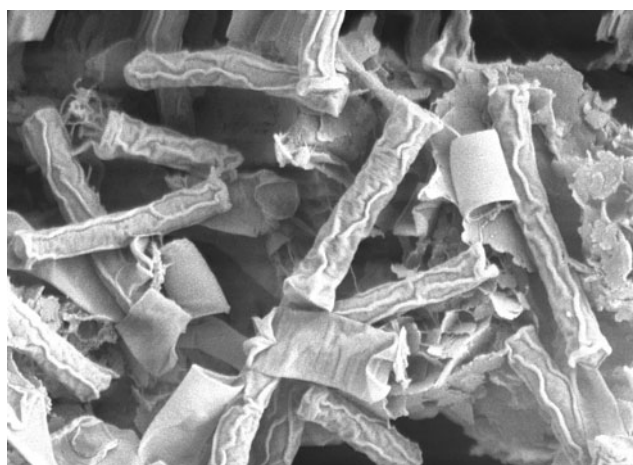
Some archaea perform iron reduction using Fe(III) as an electron acceptor and hydrogen gas or organic compounds as an electron donor (Bond and Lovley, 2002;

Archaea, Table 1 Some examples for biochemical pathways used by archaea

Reaction	
$2\text{H}_2 + \text{O}_2 \rightarrow 2\text{H}_2\text{O}$	Hydrogen oxidation
$\text{H}_2 + \text{S}^0 \rightarrow \text{H}_2\text{S}$	Sulfur reduction
$4\text{H}_2\text{S} + 2\text{O}_2 \rightarrow 4\text{H}_2\text{SO}_4$	Sulfide oxidation
$2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 2\text{H}_2\text{SO}_4$	Sulfur oxidation
$\text{H}_2 + \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$	Nitrate reduction
$\text{H}_2 + 2\text{Fe}^{3+} \rightarrow 2\text{Fe}^{2+} + 2\text{H}^+$	Iron reduction
$\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	Methanogenesis (autotrophic)
$4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O}$	Methanogenesis (heterotrophic)
$4\text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow 3\text{CH}_4 + \text{HCO}_3^-$	Methanogenesis ("acetoclastic," heterotrophic)
Organic compound + $\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{CO}_2$	Aerobic respiration

Kashefi et al., 2002; see entry *Fe(III)-Reducing Prokaryotes*). Other members of the Archaea drive pathways of the nitrogen cycle, such as ammonia oxidation, nitrate respiration and denitrification, and assimilatory pathways like N_2 fixation and nitrate assimilation (Cabello et al., 2004; see entry *Nitrogen*). In addition, sulfur is a central component for reactions performed by numerous thermophilic archaea. Elemental sulfur (S^0), thiosulfate ($\text{S}_2\text{O}_3^{2-}$), sulfite (SO_3^-), or sulfate (SO_4^{2-}) may be used as electron acceptors, and elemental sulfur (S^0) or H_2S as electron donors, depending on species and environmental conditions (Barns and Nierzwicki-Bauer, 1997, see entry *Sulfur Cycle*).

Methanogenic archaea (methanogens) are anaerobic euryarchaeotes that produce methane as a metabolic end product of various reactions (Table 1, see entry *Methane, Origin* for details). For several reasons, these archaea are of particularly interest from a geobiological perspective. (a) Chemoorganotrophic methanogens thriving on simple metabolic products of other (micro)organisms such as methanol or acetate (acetoclastic methanogenesis, Table 1) are key players in the degradation of organic matter in sediments (see entry *Carbon (Organic, Degradation)*). (b) Chemolithotrophic methanogens, utilizing only hydrogen gas and CO_2 may thrive in dark and extremely organic-lean environments. (c) Many methanogens are thermophiles (growth temperatures $>60^\circ\text{C}$) or hyperthermophiles (growth temperatures $>80^\circ\text{C}$) with some species being even capable of withstanding temperatures above 100°C (Huber et al., 1990; see entry *Extreme Environments*). Archaea are therefore predestined to pioneering life in extreme settings such as the Deep Biosphere (Hallbeck and Pedersen, 2008), Hot Springs, and Hydrothermal Environments (see these entries). Third, the product of archaeal methanogenesis, "biogenic" methane, may accumulate in sedimentary reservoirs either as free natural gas or, under certain low-temperature/high-pressure conditions, frozen as solid gas hydrates (see entry *Cold Seeps*). Sedimentary methane reservoirs play a crucial role as an energy resource for man, but when released into the atmosphere, methane may act as a strong driving force of global change (Mascarelli, 2009). In as much as methane is an effective



Archaea, Figure 2 Cylindrical cells of archaea belonging to the ANME-1 group of methanotrophic archaea. Individual cells are about $0.5\ \mu\text{m}$ in diameter. (Image courtesy of J. Reitner.)

greenhouse gas being more than 20 times as potent as CO_2 per molecule, it presently contributes more than 20% to the current global greenhouse effect (IPCC, 2007). Several dramatic events in Earth history, such as the Paleocene/Eocene thermal maximum, have been attributed to sudden increases of atmospheric methane concentrations, possibly induced by rapid methane release due to gas hydrate dissolution (Zachos et al., 2008).

Methanotrophic archaea (ANME, *anaerobic methane oxidizers*, Figure 2), organisms that reversed the methanogenic metabolism, have recently been specified as members of sedimentary microbial communities in anoxic sediments. Living together in a syntrophic consortium with sulfate reducing bacteria, ANME oxidize methane by sulfate or nitrate (Raghoebarsing et al., 2006; Knittel and Boetius, 2009; see entry *Anaerobic Oxidation of Methane with Sulfate*).

Biom mineralization

In methane-rich environments, ANME are key players in the formation of calcium carbonate (CaCO_3) deposits, the so-called seep carbonates or methane-derived



Archaea, Figure 3 A Miocene (~16–20 million years old) carbonate deposit that formed as a result of archaeal methanotrophy (Terzi et al., 1994). Croce della Moggiona, Lower Vicchio Formation, Tuscan Apennines, Italy. (Image courtesy of J. Peckmann.)

carbonates (Figure 3; Peckmann and Thiel, 2004; Reitner et al., 2005; see entry *Cold Seeps*). This biologically induced mineralization process is driven by an increase in *Alkalinity* resulting from the anaerobic oxidation of methane. The anaerobic oxidation of methane may also be involved in dolomite ($\text{Ca, Mg}(\text{CO}_3)_2$) precipitation in marine sediments (Moore et al., 2004). Likewise, methanogens have been linked to dolomite formation in shallow groundwater aquifers (Roberts et al., 2004). Recent laboratory experiments identified acetoclastic and autotrophic methanogenesis associated with dolomite formation (Kenward et al., 2009; see entry *Dolomite, Microbial*).

Summary

The Archaea are a group of unicellular prokaryotes representing the third domain of life, in addition to Bacteria and Eukarya. The Archaea differ from the latter by aspects of their genomic machinery, cell wall composition, and membrane lipid structure. Although the timing of their emergence has not been determined, it is believed that the Archaea have been key players within the carbon, nitrogen, and sulfur cycles for the major part of Earth history. Regarding their ecophysiology, individual archaeal groups display unique capacities, such as methanogenesis,

extreme salt tolerance, and survival at temperatures even higher than 100°C. However, environmental surveys revealed the Archaea to be abundant and metabolically versatile not only in extreme environments, but also in “normal” habitats such as soil, oceans, and freshwater. Archaea involved in methane cycling have also been identified as drivers of biologically induced mineralization resulting in (carbonate) rock formation.

Bibliography

- Barns, S. M., Delwiche, C. F., Palmer, J. D., and Pace, N. R., 1996. Perspectives on archaeal diversity, thermophily and monophyly from environmental rRNA sequences. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 9188–9193.
- Barns, S. M., and Nierzwicki-Bauer, S. A., 1997. Microbial diversity in ocean, surface and subsurface environments. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals. Reviews in Mineralogy and Geochemistry*. Washington, DC: Mineralogical Society of America and Geochemical Society, Vol. 35, pp. 35–79.
- Battistuzzi, F. U., Feijao, A., and Hedges, S. B., 2004. A genomic timescale of prokaryote evolution: insights into the origin of methanogenesis, phototrophy, and the colonization of land. *BMC Evolutionary Biology*, **4**, 44–58.
- Berg, I. A., Kockelkorn, D., Ramos-Vera, W. H., Say, R. F., Zarzycki, J., Hügler, M., Alber, B. E., and Fuchs, G., 2010. Autotrophic carbon fixation in Archaea. *Nature Reviews Microbiology*, **8**, 447–460.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Giesecke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Bond, D. R., and Lovley, D. R., 2002. Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinines. *Environmental Microbiology*, **4**(2), 115–124.
- Cabello, P., Roldán, M. D., and Moreno-Vivián, C., 2004. Nitrate reduction and the nitrogen cycle in archaea. *Microbiology*, **150**, 3527–3546.
- DeRosa, M., and Gambacorta, A., 1988. The lipids of Archaeobacteria. *Progress in Lipid Research*, **27**, 153–175.
- Eckburg, P. B., Bik, E. M., Bernstein, C. N., et al., 2005. Diversity of the human intestinal microbial flora. *Science*, **308**, 1635–1638.
- Giovannoni, S., and Rappé, M., 2000. Evolution, diversity and molecular ecology of marine prokaryotes. In Kirchman, D. L. (ed.), *Microbial Ecology of the Oceans*. New York: Wiley, pp. 47–84.
- Graham, D. E., Overbeek, R., Olsen, G. J., and Woese, C. R., 2000. An archaeal genomic signature. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 3304–3308.
- Gribaldo, S., and Brochier-Armanet, C., 2006. The origin and evolution of Archaea: a state of the art. *Philosophical Transactions of the Royal Society B*, **361**, 1007–1022.
- Hallbeck, L., and Pedersen, K., 2008. Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. *Applied Geochemistry*, **23**, 1796–1819.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S., 1994. Field and laboratory studies of methane oxidation in an anoxic marine sediment: evidence for a methanogen-sulfate

- reducer consortium. *Global Biogeochemical Cycles*, **8**, 451–463.
- Hopmans, E. C., Schouten, S., Pancost, R. D., van der Meer, M. T. J., and Sinninghe Damsté, J. S., 2000. Analysis of intact tetraether lipids in archaeal cell material and sediments by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Communications in Mass Spectrometry*, **14**, 585–589.
- Huber, H., and Rachel, R., 2007. Nanoarchaeota. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 51–59.
- Huber, R., Stoffers, P., Cheminee, J. L., Richnow, H. H., and Stetter, K. O., 1990. Hyperthermophilic archaeobacteria within the crater and open-sea plume of erupting Macdonald Seamount. *Nature*, **345**, 179–181.
- Intergovernmental Panel on Climate Change (IPCC), Fourth Assessment Report 2007 (AR4); Working Group I Report “The physical science basis”, Ch. 2.
- Kandler, O., and König, H., 1998. Cell wall polymers in Archaea (Archaeobacteria). *Cellular and Molecular Life Sciences*, **54**, 305–308.
- Kashefi, K., Tor, J. M., Holmes, D. E., Gaw Van Praagh, C. V., Reysenbach, A. L., and Lovley, D. R., 2002. *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *International Journal of Systematic and Evolutionary Microbiology*, **52**, 719–728.
- Kenward, P. A., Goldstein, R. G., Gonzalez, L. A., and Roberts, J. A., 2009. Precipitation of low-temperature dolomite from an anaerobic microbial consortium: the role of methanogenic Archaea. *Geobiology*, **7**, 556–565.
- Knittel, K., and Boetius, A., 2009. Anaerobic oxidation of methane: progress with an unknown process. *Annual Review of Microbiology*, **63**, 311–334.
- Koga, Y., Nishihara, M., Morii, H., and Akagawa-Matsushita, M., 1993. Ether polar lipids of methanogenic bacteria: structures, comparative aspects, and biosyntheses. *Microbiological Reviews*, **57**, 164–182.
- Kuypers, M. M. M., Blokker, P., Erbacher, J., Kinkel, H., Pancost, R. D., Schouten, S., and Sinninghe Damsté, J. S., 2001. Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. *Science*, **293**, 92–94.
- Lipp, J. S., Morono, Y., Inagaki, F., and Hinrichs, K. U., 2008. Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature*, **454**, 991–994.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock Biology of Microorganisms*, 11th edn. Englewood Cliffs NJ: Prentice Hall.
- Margot, H., Acebal, C., Toril, E., Amils, R., and Fernandez Puentes, J. L., 2002. Consistent association of crenarchaeal Archaea with sponges of the genus *Axinella*. *Marine Biology*, **140**, 739–745.
- Mascarelli, A. L., 2009. A sleeping giant? *Nature Reports, Climate Change*, **3**, 46–49.
- Moore, T. S., Murray, R. W., Kurtz, A. C., and Schrag, D. P., 2004. Anaerobic methane oxidation and the formation of dolomite. *Earth and Planetary Science Letters*, **229**, 141–154.
- Nicol, G. W., and Schleper, C., 2006. Ammonia-oxidising Crenarchaeota: important players in the nitrogen cycle? *Trends in Microbiology*, **14**, 207–212.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Pedersen, K., 1997. Microbial life in deep granitic rock. *FEMS (Federation of European Microbiological Societies) Microbiology Reviews*, **20**, 399–414.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J., Ettwig, K. F., Rijpstra, W. I., Schouten, S., Damsté, J. S., Op den Camp, H. J., Jetten, M. S., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 878–879.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 66–79.
- Roberts, J. A., Bennett, P. C., Gonzalez, L. A., Macpherson, G. L., and Milliken, K. L., 2004. Microbial precipitation of dolomite in methanogenic groundwater. *Geology*, **32**, 277–280.
- Schleper, C., 2007. Diversity of uncultivated Archaea: perspectives from microbial ecology and metagenomics. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 39–53.
- Stams, A. J. M., and Plugge, C. M., 2009. Electron transfer in syntrophic communities of anaerobic bacteria and Archaea. *Nature Reviews Microbiology*, **7**, 568–577.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., and Horikoshi, K., 2008. Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10949–10954.
- Terzi, C., Aharon, P., Ricci Lucchi, F., and Vai, G. B., 1994. Petrography and stable isotope aspects of cold-vent activity imprinted on Miocene-age “calcarei a Lucina” from Tuscan and Romagna Apennines, Italy. *Geo-Marine Letters*, **14**, 177–184.
- van Hoek, A. H. A. M., van Alen, T. A., Sprakel, V. S. I., Leunissen, J. A. M., Brigge, T., Vogels, G. D., and Hackstein, J. H. P., 2000. Multiple acquisition of methanogenic archaeal symbionts by anaerobic ciliates. *Molecular Biology and Evolution*, **17**, 251–258.
- Woese, C. R., 2007. The birth of the Archaea: a personal retrospective. In Garrett, R., and Klenk, H.-P. (eds.), *Archaea: Evolution, Physiology and Molecular Biology*. Oxford: Blackwell, pp. 1–15.
- Woese, C. R., and Fox, G. E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, **74**, 5088–5090.
- Woese, C. R., Kandler, O., and Wheelis, M., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 4576–4579.
- Wright, A. D. G., Toovey, A. F., and Pimm, C. L., 2006. Molecular identification of methanogenic archaea from sheep in Queensland, Australia reveal more uncultured novel archaea. *Anaerobe*, **12**, 134–139.
- Zachos, J. C., Dickens, G. R., and Zeebe, R. E., 2008. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature*, **451**, 279–283.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Anaerobic Transformation Processes, Microbiology
 Bacteria
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Carbon (Organic, Degradation)
 Chemolithotrophy

[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Dolomite, Microbial](#)
[Extreme Environments](#)
[Halobacteria – Halophiles](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Methane, Origin](#)
[Microbial Biomineralization](#)
[Nitrogen](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

ARSENIC

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Arsenic, element 33, is a metalloid in row VB of the periodic table. The main oxidation states are arsine (−3) elemental As(0), arsenite (+3), and arsenate (+5). Arsenate, As(V), and arsenite, As(III), are most common, with HAsO_4^{2-} and $\text{H}_2\text{AsO}_4^{1-}$ predominating under oxic conditions and H_3AsO^0 and $\text{H}_2\text{AsO}_3^{2-}$ prevalent in anoxic environments. Arsenic is found in minerals such as arsenosulfides (e.g., arsenopyrite, realgar), iron hydroxides and aluminosilicates (e.g., clays), fossil fuels, geothermal sources, and weathered volcanic and evaporitic deposits. Anthropogenic inputs may also be considerable as organoarsenicals such as roxarsone (3-nitro-4-hydroxy benzene arsonic acid) and monosodium and disodium methyl arsonate continue to see wide spread use. The toxicology of arsenic is well known; As(V) is an analog of phosphate and can disrupt oxidative phosphorylation, while As(III) binds to sulfhydryl groups and can inhibit respiratory enzymes. Nevertheless, arsenic is readily metabolized by microorganisms, undergoing redox reactions involved in resistance or energy generation. Organarsenicals are used for energy generation or as a carbon source. Environments with elevated concentrations can support a diverse arsenic metabolizing microbial community and a robust biogeochemical cycle (Oremland and Stolz, 2003).

As(III)-oxidizing prokaryotes are found among the alpha-, beta-, and gamma-proteobacteria, Deinocci (i.e., *Thermus*), and Crenarchaeota. Physiologically diverse, they include heterotrophic and chemolithoautotrophic species. The oxidation of arsenite involves the arsenite oxidase (AoxAB or AroBA), a member of the molybdenum family of enzymes. The gene sequence of the catalytic subunit (*aoxB* or *aroA*) is highly conserved, and oligonucleotide primers have been used to investigate the occurrence and distribution of As(III)-oxidizing

species. The dissimilatory reduction of As(V) has been demonstrated in Crenarchaeota, thermophilic bacteria, gamma-, delta-, and epsilon-proteobacteria, and low and high mole %GC gram positive bacteria. The enzyme, ArrAB, is also a molybdoenzyme, but differs significantly from AoxBA. The *arrA* gene has also been used as an environmental probe. Arsenic resistance involves a completely different type of arsenate reductase (ArsC, ACR2). Three different but comparable resistance systems (e.g., *E. coli*, *Staphylococcus*, *Saccharomyces*) have been described. ArsC catalyzes the reduction of arsenate to arsenite, while an arsenite-specific efflux pump (ArsB, ACR3) expels As(III) from the cell. Additional components include an ATPase (ArsA) and regulatory elements (ArsR, ArsD). Far less is known about arsenic methylation and demethylation, however, a SAM-dependent methylase has recently been described (Stolz et al., 2006).

Different microbially mediated processes can result in arsenic contaminated aquifers including (1) the oxidation of arsenic-containing pyrites, (2) the release of As(V) from reduction of iron oxides by autochthonous organic matter, (3) the reduction of iron oxides by allochthonous organic matter from dissolved organics in recharging waters, (4) the exchange of adsorbed As(V) with fertilizer phosphates, and (5) recharge with water contaminated with inorganic arsenic derived from the application or production of wood preservatives, pesticides (e.g., calcium arsenate), and organoarsenicals (e.g., roxarsone). Thus organisms that metabolize arsenic can impact the speciation, mobility, and toxicity of arsenic in the environment (Oremland and Stolz, 2005).

Bibliography

- Oremland, R. S., and Stolz, J. F., 2003. The ecology of arsenic. *Science*, **300**, 939–944.
 Oremland, R. S., and Stolz, J. F., 2005. Arsenic, microbes, and contaminated aquifers. *Trends in Microbiology*, **13**, 45–49.
 Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

Cross-references

[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Geomycology](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Shewanella](#)
[Sulfide Mineral Oxidation](#)

ASTEROID AND COMET IMPACTS

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The impact of an extraterrestrial object (either an asteroid or comet) with the Earth's surface.

Introduction

There are now over 170 asteroid and comet impact craters that have been identified on the surface of the Earth (Table 1). These craters represent a small subset of the total impacts that have occurred during the history of life. Today, impact craters probably represent something on the order of 50,000 km² of Earth's surface. Although this is a small habitat area, impacts are the only extraterrestrial mechanism capable of causing localized ecological disturbance. Because impacts are a mechanism capable of delivering a pulse of energy into ecosystems, from a geobiological point of view they present an important addition to the overall picture of how ecosystems are disturbed and how they recover through time. Comparison to other processes involving ecological disturbance and recovery including volcanism, glaciation/deglaciation, storm damage, fire, landslides, and coastal erosion allows for a more complete picture of the role of catastrophic change in shaping the geobiology of the Earth.

Effects of impacts on the local environment

The kinetic energy associated with the collision of an asteroid or comet with the Earth is enormous. The collision of a rock with a 10-km diameter could release the equivalent energy of 10 million tonnes of TNT. An event on such a scale is expected to occur, on average, once in every 100 million years, and may be capable of causing global-scale disruption and extinction. Such an event is implicated in the Cretaceous–Paleogene extinctions. It is perhaps not surprising, therefore, that the biological destructiveness of impact events has overwhelmingly influenced our view of these objects and their role in ecological and evolutionary processes. However, it is important to realize that the great majority of impacts only cause effects at the local scale, although they may distribute material from the point of impact over wide areas. This contribution will focus on the local geobiological effects of impacts.

The impact of an asteroid and comet with the surface of the Earth has two profound effects that are relevant for life. First, the impact delivers energy into the target area with important geological consequences and second, it rearranges the hydrologic cycle, with implications for the availability of liquid water. Temperatures generated in

the impact cratering process can exceed several thousand degrees centigrade (Melosh, 1989). Most biologically important macronutrients volatilize at temperatures much lower than those associated with temperatures generated in the crater during impact (e.g., nitrates, 200°C; organic phosphorus, 350°C; inorganic phosphorus, 750°C; sulphur, 450°C; potassium, 570°C; and sodium, 880°C). In some parts of the crater, the substrate will not only be sterilized (made devoid of life), but will also be rendered oligotrophic (i.e., lacking in nutrients). The process and/or the extent of recolonization on melted and shocked substrates might be very different to that taking place on unprocessed substrate either inside or outside the crater and it might be very different from that associated with other agents of local ecological disturbance.

Recovery from impacts

Following the impact, there will be a well-defined sequence of biological recovery within the crater (Cockell and Lee, 2002). As there has been no large scale impact event in recorded human history, there has been no opportunity to observe these changes directly unlike post-volcanic recovery. However, observations on the present-day colonization of impact craters, which are in various states of preservation, can yield insights into the likely sequence of events (Cockell and Lee, 2002) and particularly the long-term influence of impacts on the geobiology of a region. Some general phases can be identified that are likely to occur. Following impact, the thermal pulse delivered by the impactor will heat the surrounding rock. High-temperature hydrothermal fluids generated in the modified thermal environment (Osinski et al., 2001) may, in the early stages following impact, provide a habitat for thermophilic and hyperthermophilic (heat-loving) microorganisms. For example, in the Houghton impact structure, a 24-km diameter structure on Devon Island in the Canadian High Arctic, the hydrothermal system may have lasted for at least 10,000 years. The crater cavity formed during the impact acts as a hydrologic depression and will provide a new habitat for aquatic life (either freshwater on land or marine if the impact occurred in the ocean and excavated a new salt-water filled environment).

Following impact, intracrater lacustrine ecosystems become established, depending upon the size of the structure. Even today, many ancient impact craters support lakes. The biota associated with impact crater lakes has now been studied in several cases. For example, the low biomass and poor diversity of the 3.4-km-diameter New Quebec Crater in Canada and the 18-km-diameter El'gygytyn crater in Siberia (Gronlund et al., 1990; Cremer and Wagner, 2003), contrast with the rich, abundant bacterial and algal ecosystem associated with the 1-km-diameter Tswaing impact crater in South Africa (Ashton and Schoeman, 1983; Schoeman and Ashton, 1982) (Figure 1). The Tswaing crater is situated in South African bushveld and receives organic input from the rich

Asteroid and Comet Impacts, Table 1 Information on the craters discussed in this article

Name of crater	Country	Size of crater (km)	Age of crater (Myr)
Chesapeake	Virginia, USA	90	35.5 ± 0.3
El'gygytyn	Russia	18	3.5 ± 0.5
Houghton	Canada	24	39.0
Lockne	Sweden	7.5	455
Mjølner	Norway	40	142.0 ± 2.6
New Quebec	Quebec, Canada	3.44	1.4 ± 0.1
Ries	Germany	24	15.1 ± 0.1
Tswaing	South Africa	1.13	0.22 ± 0.05



Asteroid and Comet Impacts, Figure 1 Impact crater lakes. Two examples of lakes formed in the cavity excavated by two impact events. El'gygytgyn in Siberia (left) and New Quebec in Canada (right).

vegetation that grows within the impact crater cavity. The area is characterized by erratic rainfall (between 400 and 750 mm a year) and summer temperatures that vary between a mean minimum and maximum of 14.2 and 35.2°C, respectively. The New Quebec crater is in a cold environment; temperatures are relatively constant at approximately 2.5–3°C down to the bottom (267 m) of the lake. Although the biota that takes advantage of an intra-crater lake will be influenced by the local climatic regimen, these examples show how impact excavation provides benefits for life by forming a cavity, in which water collects.

Once the crater begins to cool, exposed melt rocks can provide a substrate for primary succession (Cockell et al., 2001). The drainage of the lake by the breach of the crater wall will expose impact lithologies which then provide a substrate on which colonization can occur. Thus the sequence of geobiological events that occur after impact will be influenced by the general characteristics of impact craters (e.g., presence of a thermal pulse, an impact cavity) and the specifics of any given crater (e.g., duration of thermal pulse, local climate, size and depth of intracrater lake, substrate into which impact occurs, and the scale of the impact).

Ultimately a crater will become buried or eroded to point where the internal ecology may be indistinguishable from the exterior ecology.

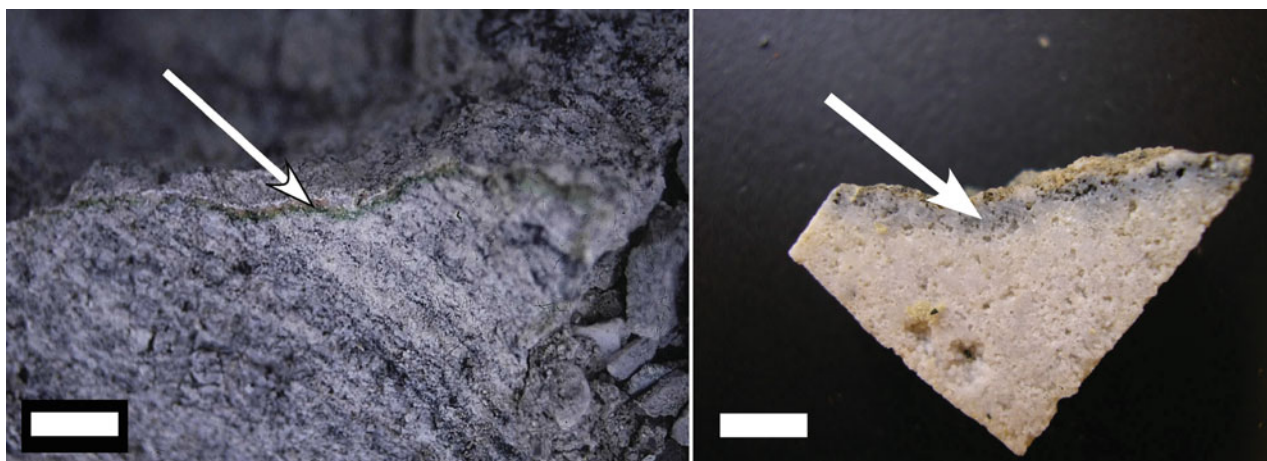
As three-quarters of the Earth is ocean, most impacts occur in water and one initial effect is the large-scale movement of water bodies. Investigations of strata in a corehole 30 km northeast of the Mjølner crater, formed in the late Jurassic by the impact of an object into the 300–400-m deep paleo-Barents Sea, suggest that the impact event mixed water masses and caused a large introduction of nutrients into the Sea (Smelror et al., 2002). High concentrations of marine algae suggest that the increase in the availability of ammonia, nitrates, phosphates, and iron contributed to a postimpact bloom. The backwash of a tsunami, caused by the impact, is postulated

to be the reason for the high concentrations of freshwater algae in the strata. The mixing of the water, caused directly by impact, would also have been exacerbated by the collapse of the crater after formation.

Geomicrobiological influence of impacts

In the sense that impacts merely create a “hole in the ground,” their aquatic ecosystems may be indistinguishable from any topographic low in a similar location. However, it is evident that asteroid and comet impacts can have an important influence on the target lithology that is quite specific to impact and can alter the habitat for rock-dwelling microorganisms, making impact craters an important site for geomicrobiological investigations (Cockell et al., 2002). The temperatures and pressures of impact commonly increase the fracture space within rocks, which provide a greater abundance of habitats for endoliths, i.e., organisms which inhabit cracks within rocks. In some instances, the increase in permeable space within the target material in combination with an increase in the translucence of the rock in the visible part of the spectrum can yield new habitats suitable for phototrophs. Impact-shocked Precambrian gneiss in the Haughton crater has been shown to preferentially offer cryptoendolithic habitats for photosynthetic cyanobacterial colonists (Figure 2). The rock is not otherwise a suitable habitat for them (Cockell et al., 2002). This finding constituted empirical evidence for the formation of a microbial habitat specifically by an asteroid or comet impact.

The fate of highly porous substrates versus low porosity substrates during impact is different (Kieffer, 1971). In the former case more of the impact energy must be taken up in pore collapse. Thus, an examination of the effects of asteroid and comet impacts on the colonization of porous sedimentary target lithologies can provide a model with which to understand more generally the effects of impacts on the colonization potential of rocks (Cockell et al., 2005). In a study of impact-shocked sandstones from the



Asteroid and Comet Impacts, Figure 2 Cyanobacterial communities living in impact-shocked gneiss from the Houghton impact crater, Devon Island, Canadian High Arctic (left) and within class 3 shocked sandstones from the same crater (right). Scale bars 1 cm.

Houghton impact structure, Canadian High Arctic, it was shown that shock pressures from 0 to ~ 10 GPa caused the pore structure to collapse, thus rendering the material less amenable to microbial colonization. However, as shock pressures are increased to ~ 50 GPa the material becomes successively better for colonization on account of the formation of vesicular regions in the rock from rock fracturing and melting, improving conditions for colonization. At higher shock pressures colonization potential is again decreased as a solid glass is formed which is impermeable, although potentially still accessible to rock-boring microorganisms.

Thus, in summary, we can say that in general impact events may have the effect of turning lithic habitats on their head. Habitats that were previously impermeable become better sites for colonization as the rocks are fractured. Habitats that were good sites for colonization are rendered less permeable because of pore collapse, although, as the sandstones of Houghton illustrate, the effects on any given lithology tend to be complex.

Within and around impact craters new minerals can be generated in hydrothermal systems that later become habitats for life. Gypsum deposits laid down in the Houghton crater have become a habitat for cyanobacteria which inhabit the inter-sheet spaces between the sulphate crystals (Parnell et al., 2004). The extent to which these organisms are active is uncertain, but they must certainly be halotolerant.

During impact, the target lithology, especially if it is stratified and diverse, will be fragmented and mixed, leading to a heterogeneous substrate in which diverse geochemical sources of nutrients are brought into close proximity. A study of the weathering of a tsunami surge deposit in the Lockne impact crater in Sweden (formed ~ 455 million years ago) showed how lichens preferentially first colonized the soft chlorite clasts incorporated into the impactite (Cockell et al., 2007). The dissolution of the calcium carbonate clasts by fungi and other

organisms creates cavities in the rock, enhancing weathering and causing the rock to exfoliate in sheets. Previous studies on the chemical weathering of impact-shocked rocks from the Ries crater, Germany, showed that the deformation features introduced into quartz were sites of preferential weathering (Leroux, 2005). Diverse impact-generated lithologies can be used as a model to understand microbial weathering of other mixed sedimentary lithologies such as greywackes and microbe–mineral interactions in extremely disturbed geological settings in general.

Most of the geobiological studies conducted to date pertain to the surface environment. The first drill core retrieved from an impact crater with robust microbiological contamination control came from the Chesapeake Bay Impact Crater and was retrieved during the ICDP (International Continental Drilling Project)-USGS (United States Geological Survey) deep drilling project in 2005 (Gohn et al., 2008). A core of length 1.8 km was retrieved. The sequence included a 600 m sequence of postimpact sediments laid down since the impact in the late Eocene and 1.2 km of postimpact tsunami and “fall-back” deposits laid down in the crater cavity immediately after impact. This work has shown that the microbial diversity and abundance of microorganisms correlates to the lithologies in the crater cavity, showing that impacts can significantly disrupt the characteristics of the deep biosphere.

Impact events and astrobiology

The study of the geomicrobiology of impact craters provides useful clues to the opportunities for life on the Archean Earth since the emergence of life ~ 3.5 Gya ago, when the impact flux were higher than today. The formation of hydrothermal systems and the fracturing of rocks are two such opportunities that impact craters might have offered to early life. Insofar as asteroid and comet

impacts are universal (in that no solar system-forming process is known that is completely free from remnant material), habitats created in craters yield insights into the possible habitats for life on other planets, such as Mars. In particular, the likelihood that impacts melt permafrost, or generate deep fracturing into regions of a planet where liquid water is available, suggests that they offer intriguing possibilities as sites to examine the potential habitability of Mars, past and present.

Summary

Asteroid and comet impacts can cause global environmental perturbations through gross changes in atmospheric chemistry. However, much more commonly they alter geobiology at local scales. Through local disruptions of hydrology and geology, they generate new habitats and substrates, the most conspicuous being intracrater lakes. The energy delivered during impacts can also change habitats at the micron scale through changes in target lithology creating entirely novel geomicrobiological environments which, through pressure and temperature effects, are distinguishable from volcanic and other disturbed environments. The geobiological study of asteroid and comet impacts completes our picture of the role of catastrophic disturbance in influencing life on Earth alongside terrestrially mediated mechanisms of environmental change.

Bibliography

- Ashton, P. J., and Schoeman, F. R., 1983. Limnological studies on the Pretoria Salt Pan, a hypersaline maar lake. 1. Morphometric, physical and chemical features. *Hydrobiologia*, **99**, 61–73.
- Cockell, C. S., and Lee, P., 2002. The biology of impact craters – a review. *Biological Reviews*, **77**, 279–310.
- Cockell, C. S., Lee, P., Schuerger, A., Hidalgo, L., Jones, J., and Stokes, D., 2001. Microbiology and vegetation of micro-oases and polar desert, Haughton impact crater, Devon Island, Canadian High Arctic. *Arctic, Alpine and Antarctic Research*, **33**, 306–318.
- Cockell, C. S., Lee, P., Osinski, G., Horneck, G., and Broady, P., 2002. Impact-induced microbial endolithic habitats. *Meteoritics and Planetary Science*, **37**, 1287–1298.
- Cockell, C. S., Lee, P., Broady, P., Lim, D. S. S., Osinski, G. R., Parnell, J., Koeberl, C., Pesonen, L., and Salminen, J., 2005. Effects of asteroid and comet impacts on habitats for lithophytic organisms – a synthesis. *Meteoritics and Planetary Science*, **40**, 1901–1914.
- Cockell, C. S., Kennerley, N., Lindstrom, M., Watson, J., Ragnarsdottir, V., Sturkell, E., Ott, S., and Tindle, A. G., 2007. Geomicrobiology of a weathering crust from an impact crater and a hypothesis for its formation. *Geomicrobiology Journal*, **24**, 425–440.
- Cremer, H., and Wagner, B., 2003. The diatom flora in the ultra-oligotrophic Lake El'gygytgyn, Chukotka. *Polar Biology*, **26**, 105–114.
- Gohn, G., Koeberl, C., Miller, K. G., Reimold, U., Browning, J. C., Cockell, C. S., Horton, J. W., Kenkman, T., Kulpecz, A. A., Powars, D. S., Sanford, W. E., and Voytek, M. A., 2008. Deep drilling into the Chesapeake Bay impact structure. *Science*, **320**, 1740–1745.
- Gronlund, T., Lortie, G., Guilbault, J. P., Bouchard, M. A., and Saanisto, M., 1990. Diatoms and arcellaceans from lac du Cratere du Nouveau-Quebec, Ungava, Quebec, Canada. *Canadian Journal of Botany*, **68**, 1187–1200.
- Kieffer, S. W., 1971. Shock metamorphism of the Coconino sandstone at Meteor crater, Arizona. *Journal of Geophysical Research*, **76**, 5449–5473.
- Leroux, H., 2005. Weathering features in shocked quartz from the Ries impact crater: Germany. *Meteoritics and Planetary Sciences*, **40**, 1347–1352.
- Melosh, H. J., 1989. *Impact Cratering: A Geologic Process*. Oxford: Oxford University Press.
- Osinski, G. R., Spray, J. G., and Lee, P., 2001. Impact-induced hydrothermal activity within the Haughton impact structure: generation of a transient, warm, wet oasis. *Meteoritics and Planetary Science*, **36**, 731–745.
- Parnell, J., Lee, P., Cockell, C. S., and Osinski, G. R., 2004. Microbial colonization in impact-generated hydrothermal sulphate deposits, Haughton impact structure, and implications for sulphates on Mars. *International Journal of Astrobiology*, **3**, 247–256.
- Schoeman, F. R., and Ashton, P. J., 1982. The diatom flora of the Pretoria Salt Pan, Transvaal, Republic of South Africa. *Bacillaria*, **5**, 63–99.
- Smelror, M., Dypvik, H., and Mørk, A., 2002. Phytoplankton blooms in the Jurassic-cretaceous boundary beds of the Barents Sea possibly induced by the Mjølner impact. In Buffetaut, E., and Koeberl, C. (eds.), *Geological and Biological Effects of Impact Events*. New York: Springer-Verlag, pp. 69–83.

Cross-references

[Astrobiology](#)
[Biological Volcanic Rock Weathering](#)
[Cyanobacteria](#)
[Deep Biosphere of Sediments](#)
[Endoliths](#)
[Extreme Environments](#)
[Hydrothermal Environments, Terrestrial](#)
[Mass Extinctions, Phanerozoic](#)
[Meteoritics](#)

ASTROBIOLOGY

Jack D. Farmer
 Arizona State University, Tempe, AZ, USA

Synonyms

Bioastronomy; Exobiology

Definition

Astrobiology is the interdisciplinary science that studies the origin, evolution, distribution, and future of life in the Universe. It is a highly interdisciplinary field that engages many scientific disciplines, including biology, biogeochemistry, paleontology, earth and atmospheric sciences, planetology, and astrophysics among others.

Historical perspective

Astrobiology as a term was first coined in the 1940s to encompass early scientific ideas about how to explore for extraterrestrial life (Dick and Strick, 2004). During the 1960s, astrobiological research was focused more



Astrobiology, Figure 1 The ALH8400 meteorite was discovered in 1984, in the Allan Hills of Antarctica, where it had emerged from glacial ice. In the early 1990s, the meteorite was subjected to a battery of studies by a team at NASA Johnson Spaceflight Center (McKay et al., 1996). The group suggested that they had discovered evidence in the meteorite that was consistent with the presence of fossilized microorganisms. Most of the evidence for a Martian biology was associated with orange-colored globules of iron carbonate shown in this image (the image is about 5.5 cm across). Subsequent studies by the broader science community have shown that the suggested lines of evidence for life in the meteorite are more likely to be a result of inorganic processes that mimic the signatures of life.

narrowly than today, with an emphasis on the origin of life, and programs funding these activities developed under the umbrella term “exobiology.” This early period of activity culminated in the Viking mission to Mars, which carried life detection experiments designed to explore for life in Martian surface materials (Klein et al., 1976). With the failure of the Viking experiments to detect unambiguous evidence for life on Mars, interest in the search for life elsewhere in the solar system waned. Then in 1995, Dr. Wesley Huntress, who was the National Aeronautics and Space Administration’s (NASA) Associate Administrator for Space Science at the time, reintroduced astrobiology to refer to a broad range of scientific activities that had begun to coalesce around questions of the potential for habitable environments and life beyond Earth. The following year, growth of the field of astrobiology was accelerated by the announcement by a team of scientists at NASA Johnson Space Center of possible signs of life in Martian meteorite, ALH84001 (McKay et al., 1996) (Figure 1). With this came a renewed interest in the search for Martian life. Over the next few years, NASA expanded research to develop more reliable approaches to extraterrestrial life detection, which helped to revitalize the field of astrobiology.

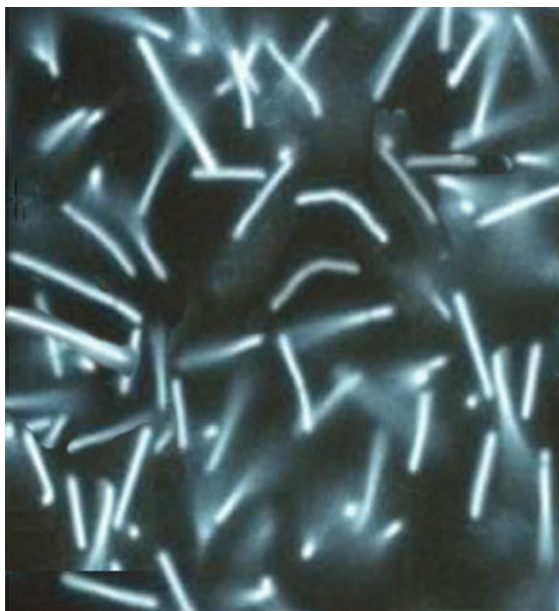
In 1998, NASA helped promote the growth of astrobiology by creating the NASA Astrobiology Institute (NAI), a virtual research organization comprised of interdisciplinary teams of scientists from NASA Research Centers and universities around the United States. An

important goal of the NAI has been to promote the growth of astrobiology through the sponsorship of collaborative, transdisciplinary research that could provide a scientific and technology development framework for future space missions. Following the establishment of the NAI, the growing community of scientists converged on a strategic plan that would help guide astrobiological research, including a scientific roadmap, with well-defined goals and objectives, and technology developments that would be needed to enable missions (Des Marais et al., 2008). Since its inception, the astrobiology roadmap has been revised and updated every few years to reexamine scientific priorities in the light of new scientific discoveries. The NAI has also embraced partnerships with other astrobiology institutions in Spain, Australia, and Great Britain. This includes international cosponsorship of yearly workshops designed to promote collaborative research and train the next generation of astrobiologists. Maturing of the field is also apparent in the development of two international journals for Astrobiology, international conferences each year and the establishment of an Astrobiology Society.

In 2008, the National Research Council reviewed the growth of the science of astrobiology (NRC, 2007a), summarizing the important collaborative efforts and scientific discoveries that had occurred since the inception of the NAI in 1998. Studies of the deep geological record of Earth have helped refine our knowledge of the early evolution of life on Earth, based on the fossil record, while providing new constraints on the nature and evolution of early environments. Studies of prebiotic organic chemical systems have revealed important new insights into the steps that may have preceded the emergence of terrestrial life, as well as the environments where life may have emerged (Russell et al., 1993; Deamer et al., 2002; Ricardo et al., 2004; Ferris, 2005).

Environmental limits for life

Biological studies have also significantly expanded our knowledge of the environmental limits of life on Earth (see Rothschild and Mancinelli, 2001), while the application of new tools of environmental molecular microbiology have revealed the presence of an extraordinarily diverse microbial biosphere, with an equally impressive array of metabolic strategies for extracting energy from Earth’s environments (e.g., Staley and Reysenbach, 2001). Life has been shown to occupy a broad range of temperature (-15°C up to 122°C ; see Kashefi and Lovely, 2003; also, Figure 2 from Takai et al., 2008) and nearly the full range of pH from acidic to alkaline ($\text{pH} \sim 1.0$ to 13.0), over a range of salinities from freshwater to hypersaline brines and water activities >0.6 . We have also learned of the importance of the deep subsurface biosphere, which may account for possibly half or more of the Earth’s biomass (Gold, 1998). The discovery of lithoautotrophs that can use the simple chemical compounds, such as hydrogen and methane (CH_4), released

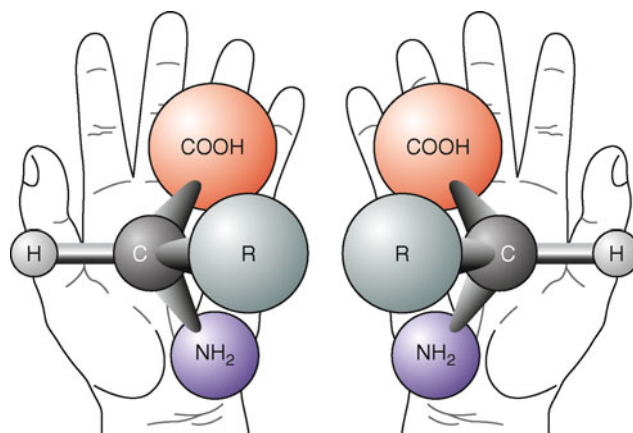


Astrobiology, Figure 2 Life is known to exist over a broad range of physical and chemical conditions and uses an equally impressive array of metabolic processes for extracting energy from the environment. This image was obtained using a fluorescence microscope. The form shown is known as *Methanopyrus kandleri* and is a hyperthermophile (growth optimum $>80^{\circ}\text{C}$) that uses carbon dioxide and hydrogen for its metabolism, producing methane as a by-product. The species was discovered at a submarine hydrothermal vent in the Gulf of California, living at temperatures between 84°C and 110°C . Strain 116 of this species has been shown to grow up to 122°C and survive up to $\sim 130^{\circ}\text{C}$. This is currently the highest temperature organisms known (Kashefi and Lovely, 2003; Takai et al., 2008). Each cell in this image is only a few microns in length.

during the low-temperature inorganic weathering of rocks or sulfide (H_2S) formed by hydrothermal processes has opened up opportunities for the survival of microbial life in the deep subsurface, independent of photosynthesis (Stevens, 1997). These discoveries and others in the fields of biology and paleontology have helped broaden the search space for habitable environments and life elsewhere in our Solar System and beyond.

Defining life

Earth has the distinction of being the only place in the Universe where life is known to exist. Thus, terrestrial life provides a logical starting point for evaluating the prospects for the existence of life elsewhere and identifying strategies for astrobiological exploration. For exploration, we need to identify basic attributes of living systems that can be used to inform exploration strategies to search for life beyond the Earth. Ideally, we would like to follow universal criteria that are not tied to the particular circumstances found on Earth. But, universal definitions of life have proven elusive and challenging to apply



Astrobiology, Figure 3 In nature, amino acids come in right- and left-handed forms called isomers that are identical in composition and differ only in structure. On Earth, amino acid isomers occur in nature in equal abundances (i.e., racemic mixtures). However, when making proteins, organisms select only left-handed amino acids. While the origins of chirality remain unclear, some have suggested that this unique property of terrestrial life may provide a useful signature for identifying life on other worlds.

(Cleland and Chyba, 2002). As a practical approach to exploration, the search for life begins with what is known of terrestrial life, following a terracentric definition that combines features like a water-based carbon chemistry, and self-replication, with cellular systems that evolve through processes of Darwinian natural selection to adapt to environmental changes. Although such attributes are regarded as essential properties of life, many inorganic systems display similar features, such that a single property, while necessary, is not regarded as sufficient to define life. Methods of life detection for astrobiological exploration of other planetary environments are still in their infancy but presently combine several instrumental techniques that can independently measure life's attributes, as a basis for detection. An example is the Pasteur instrument, which combines the detection of amino acids in soils and ices, with measurements of the chirality (right- versus left-handedness) in amino acid building blocks of proteins (Skelley et al., 2005). Terrestrial life selects left-handed amino acids when making proteins, and this selectivity has been suggested to be a biosignature that could be used to explore for life elsewhere (Figure 3).

Exploring for life in the Solar System

In exploring for life in the Solar System, NASA has adopted a simple approach that emphasizes the search for those environmental factors that are known requirements of terrestrial life. At the top of the list is liquid water, which is required by all known life-forms. In their exploration of the Solar System and beyond, NASA has pursued an exploration strategy designed to "Follow the Water."

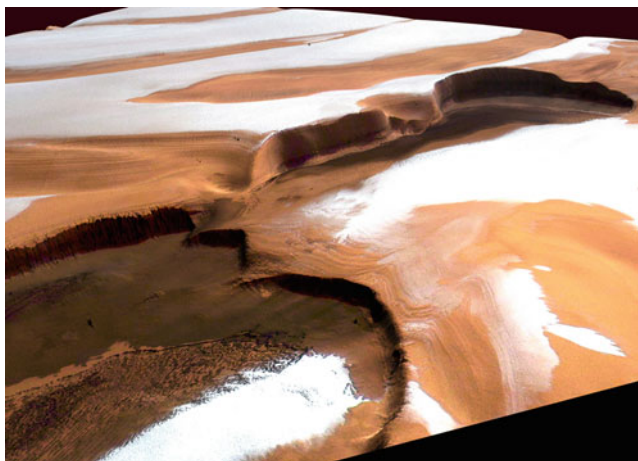
Liquid water plays an essential role in living systems as a medium of biochemical transport and exchange during metabolic reactions. The unique solvent properties of liquid water are primarily derived from the dipolar charge distribution of water molecules and the ease with which hydrogen bonds may be formed and broken in solution. These properties also determine many of the unique physical properties of water, including the broad temperature range, over which it remains liquid and the decrease in density that occurs upon freezing. This has the interesting consequence that the crystalline structure of water expands and density decreases during freezing, so that the ice floats at the surface instead of sinking to the bottom. Thus, oceans freeze from the top down, thereby enhancing long-term habitability. Terrestrial examples that illustrate the importance of this for habitability include perennially ice-covered lakes of the “dry valleys” of Antarctica. High salinity (which depresses the freezing point), combined with the self-insulating properties of the frozen surface of the lake, promotes the persistence of habitable zones of liquid water environments, even in the winter when air temperatures have fallen far below the freezing point (Andersen et al., 1995). On Mars, one consequence of this is that ice-covered lakes could have persisted on Mars long after the planet began to lose its atmosphere, thus rendering liquid water at the surface unstable (Wharton et al., 1995; McKay and Davis, 1991). Recent climatic variations on Mars mediated by

changes in the obliquity and recorded in polar deposits, suggests the possibility that transient habitable conditions could have developed at the Martian surface very recently in the planet’s history (Figure 4).

It is also widely appreciated that living systems require more than liquid water. Habitable environments are also sources of the biological elements (C, H, N, O, P, S), inclusive of about two-dozen transition metals that are critical for enzyme function (Crabb and Moore, 2009), and sources of energy for sustaining cellular metabolism, growth, and replication (Hoehler et al., 2007). Thus, the exploration for potentially habitable zones in the Solar System and beyond has been focused on the search for environments where liquid water coexists with sources of biogenic elements and energy (Figure 5).

Recent progress in astrobiology

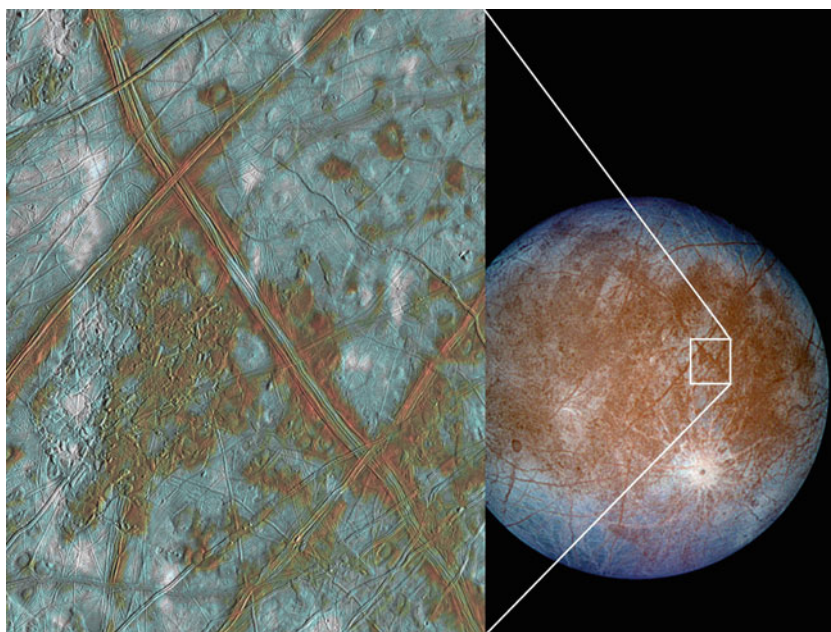
In parallel with the studies of life on Earth, missions to other bodies in our Solar System have revealed many examples of potentially habitable environments, with evidence for the past, or present existence of liquid water, biologically important elements, and potential energy sources. The exploration of Mars has identified



Astrobiology, Figure 4 The High Resolution Stereo Camera (HRSC), onboard the European Space Agency’s Mars Express Orbiter, acquired this image of the Chasma Boreale region of the northern polar cap of Mars. Erosive winds carved the large canyon, Chasma Boreale, cutting deeply into polar deposits and exposing a record of past climate changes. The image shows finely layered, ice-rich sedimentary sequences that appear to reflect cyclical obliquity changes in the climate of Mars, occurring over the past few hundred thousand years. This caused the polar caps to wax and wane, affecting the habitability of the planet’s surface.



Astrobiology, Figure 5 In January 2004, the Mars Exploration Rover (MER) mission landed twin rovers on the surface of Mars to explore for evidence of ancient habitable environments. The Opportunity Rover, which landed at Meridiani Planum, discovered unequivocal evidence of past water in the form of sulfate minerals, which only form in the presence of water (Knoll et al., 2005). This image, taken by the MER Opportunity Panoramic Camera (Pancam), shows light-toned slabs of sulfate-rich sedimentary rocks that have been partially buried by migrating sand dunes. (Image Credit: NASA.)

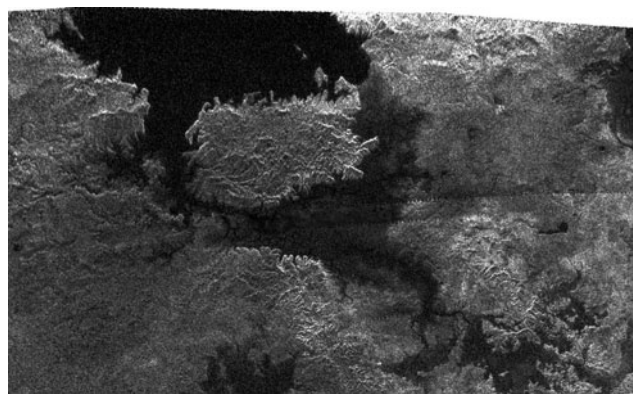


Astrobiology, Figure 6 During a close flyby, the Galileo spacecraft imaged the surface of Europa, one of the moons of Jupiter, showing a pervasively fractured surface composed of water ice that has apparently formed by the upwelling of warm ice and/or brines, from deep within the moon's interior. The internal energy believed to drive melting has been attributed to tidal flexing of the interior due to gravitational forces from Jupiter and the other moons that orbit the planet. Whether there is a global subsurface ocean on Europa, or warm, convecting ice is still uncertain, but magnetometer experiments onboard the Galileo Orbiter suggest the presence of subsurface brines (Kivelson et al., 2000). (Image Credit: NASA.)

widespread geological evidence for climatic changes that could have affected ancient surface water systems, while also strengthening the case for liquid water in the subsurface today (NRC, 2007b). NASA missions have also “followed the water” to the outer Solar System, with the discovery of brines beneath the surfaces of three of the icy Galilean satellites of Jupiter (Europa, Ganymede, and Callisto; Kivelson et al., 2000; Greenberg, 2008), as well as Enceladus, one of the moons of Saturn (Kieffer et al., 2006). In addition, Saturn's moon, Titan, has also been shown to harbor water ice as well as lakes of liquid methane (Mitri et al., 2007) (Figure 6).

The discovery of lakes of methane on Titan has led to a debate over the potential for life to originate within highly reducing, hydrocarbon-rich environments, where liquid water may be replaced by alternative solvents, such as liquid methane or ammonia, as alternative solvents for life processes (e.g., Benner et al., 2004) (Figures 7 and 8).

Finally, discoveries in observational astronomy have shown that planets and planetary systems to be common features of other stars in the nearby Milky Way Galaxy. Since the first confirmed discovery in 1992, more than 450 extrasolar planets have been identified, and the number continues to grow each year (Schneider, 2010). Orbiting planets have been detected by a variety of methods, but primarily by measuring small perturbations in the position of the parent star due to the gravitational tug and pull of an orbiting planet(s), or as a result of slight

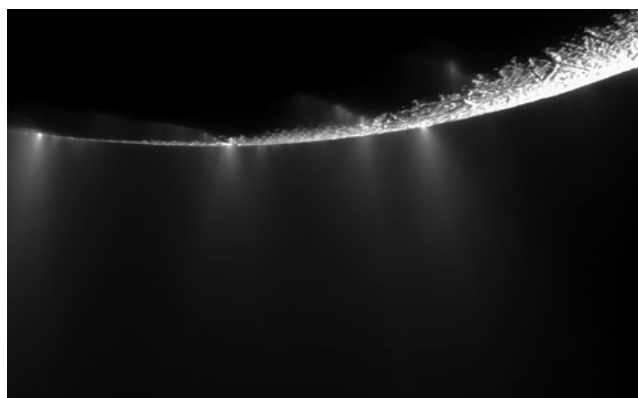


Astrobiology, Figure 7 The Cassini-Huygens mission to Saturn has revealed the presence of hydrocarbon lakes on the surface of Saturn's moon, Titan. Beyond Earth, these are the only other lakes in the Solar System. Yet, they are composed of liquid methane and other hydrocarbons – not water (Mitri et al., 2006). This discovery has led some scientists to consider the possibility that liquid hydrocarbons such as methane or ammonia might provide alternative solvents to sustain life-forms quite different from those found on Earth. (Image Credit: NASA.)

decreases in the stars brightness as an orbiting planetary body passes in front of the star. More rarely, extrasolar planets have also been discovered using direct imaging methods (Figure 9).

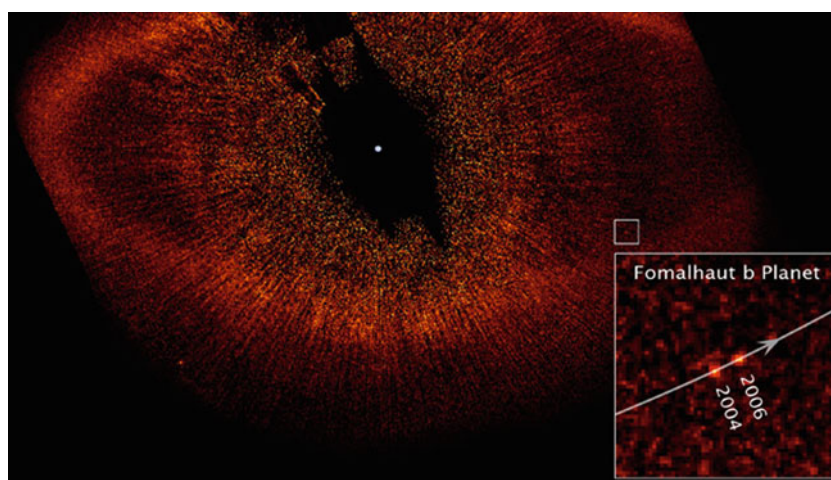
Summary

The emerging field of astrobiology is taking a highly interdisciplinary approach to the basic question: “Are we alone in the Universe?” An answer to this question is being pursued on a large number of fronts both within and beyond our Solar System. For example, studies of biology on Earth has continued to expand the environmental limits for life while revealing a dizzying array of potential energy sources. This has significantly expanded the potential number of habitable zones in the solar system, opening up new opportunities for astrobiological exploration in the solar system. Ongoing exploration of the planet Mars, long regarded as most Earth-like in its potential habitability, has provided abundant evidence for past water on the



Astrobiology, Figure 8 Enceladus, a moon of Saturn, imaged during flybys of the Cassini orbiter, has been shown to actively erupt plumes of water ice, vapor, and organic compounds from the moon’s interior (Kiefer et al., 2006). These eruptions occur along fracture systems located near the south pole. (Image credit: NASA.)

planet’s surface, along with potential building blocks for life and sources of energy to support living systems. Although the present surface of Mars appears to be uninhabitable due to an absence of liquid water, life could still exist in the subsurface (Warner and Farmer, 2010), or its remains be preserved in ancient aqueous sediments exposed at the surface (Farmer, 2000; Farmer and Des Marais, 1999). Future exploration will continue to pursue the search for fossil or extant biosignatures of microbial life on Mars, in the hope of making a transformative and revolutionary discovery of life. But exploration is also pursuing the question of microbial life in potentially habitable icy moons of the outer Solar System, specifically three of the Galilean moons of Jupiter (Europa, Ganymede, and Callisto), as well as Saturn’s moon, Enceladus, where internal zones of liquid water are maintained not by solar radiation but by the internal tidal heating resulting from gravitational flexing of the interior of these moons. In addition, Jupiter’s moon, Titan, has provided access to potentially habitable environments that are fundamentally different from Earth. On Titan, we have discovered alternative environments potentially capable of supporting non-terran forms of life, sustained by alternative solvents (oceans of liquid methane and ammonia) and energy sources. Finally, the discovery of numerous planets and planetary systems orbiting other stars in the nearby galaxy has widened the search for habitable environments beyond the confines of our Solar System. These and other important discoveries have shaped the way astrobiologists evaluate potential for the origin, evolution, and persistence of life elsewhere, contributing to the development of a robust conceptual framework for planning missions and developing the technologies needed to explore for extraterrestrial life (e.g., Mix et al., 2006; Plaxco and Gross, 2006; Sullivan and Baross, 2007).



Astrobiology, Figure 9 Fomalhaut b is a multiple Jupiter-mass planet that was directly imaged in its orbit around the star Fomalhaut, located about 25 light years away from Earth in the constellation Piscus Austrinus (Kalas et al., 2008).

Bibliography

- Andersen, D. T., Doran, P., Bolshiyarov, D., Rice, J., Galchenko, V., Cherych, N., Wharton, R. A., McKay, C. P., Meyer, M., and Garshnek, V., 1995. A preliminary comparison of two perennially ice-covered lakes in Antarctica: Analogs of past martian lacustrine environments. *Advances in Space Research*, **15**, 199–202.
- Benner, S. A., Alonso, R., and Carrigan, M. A., 2004. Is there a common chemical model for life in the Universe? *Current Opinion in Chemical Biology*, **8**, 672–689.
- Cleland, C., and Chyba, C., 2002. Defining life. *Origins of Life and Evolution of the Biosphere*, **32**, 387–393.
- Crabb, E., and Moore, E. A. (eds.), 2009. *Metals and Life*. Cambridge: RSC Publishing.
- Deamer, D., Dworkin, J. P., Sandford, S. A., Bernstein, M. P., and Allamandola, L. J., 2002. The first cell membranes. *Astrobiology*, **2**, 371–382.
- Des Marais, D. J., Nuth, J. A., Allamandola, L. J., Boss, A. P., Farmer, J. D., Hoehler, T. M., Jakosky, B. M., Meadows, V. S., Pohorille, A., Runnegar, B., and Spormann, A. M., 2008. Focus paper: The NASA astrobiology roadmap. *Astrobiology*, **8**, 715–730.
- Dick, S. J., and Strick, J. E., 2004. *The Living Universe: NASA and the Development of Astrobiology*. New Brunswick, New Jersey: Rutgers University Press.
- Farmer, J. D., 2000. Exploring for a fossil record of extraterrestrial life. In Derek Briggs, and Crowther, P. (eds.), *Palaeobiology II*, Oxford: Blackwell Science Publishers, pp. 10–15.
- Farmer, J. D., and Des Marais, D. J., 1999. Exploring for a record of ancient Martian life. *Journ. Geophys. Res.*, **104**(E11), 26977–26995.
- Ferris, J. P., 2005. Mineral catalysis and pre-biotic synthesis: Montmorillonite-catalyzed formation of RNA. *Elements*, **1**, 145–149.
- Gold, T., 1998. *The Hot Deep Biosphere*. New York: Springer.
- Greenberg, R., 2008. *Unmasking Europa: The Search for Life on Jupiter's Ocean Moon*. New York: Springer.
- Hoehler, T. M., Amend, J. P., and Shock, E. L., 2007. A “Follow the Energy” approach for astrobiology. *Astrobiology*, **7**, 819–823.
- Kashefi, K., and Lovley, D. R., 2003. Extending the upper temperature limit for life. *Science*, **301**, 934.
- Kalas, P., Graham, J. R., Chiang, E., Fitzgerald, M. P., Clampin, M., Kite, E. S., Stapelfeldt, K., Marois, C., and Krist, J., 2008. Optical images of an Exosolar Planet 25 light-years from Earth. *Science*, **322**, 1345–1348.
- Kieffer, S. W., Lu, X., Bethke, C. M., Spencer, J. R., Marshak, S., and Navrotsky, A., 2006. A clathrate reservoir hypothesis for Enceladus's south polar plume. *Science*, **314**, 1764–1766.
- Kivelson, M. G., Khurana, K. K., Russell, C. T., Volwerk, M., Walker, R. J., and Zimmer, C., 2000. Galileo magnetometer measurements: A stronger case for a subsurface ocean at Europa. *Science*, **289**, 1340–1343.
- Klein, H. P., Lederberg, J., Rich, A., Horowitz, N. H., Oyama, V. I., Levin, G. V., 1976. The viking mission search for life on Mars. *Nature*, **262**, 24–27.
- Knoll, A., Carr, M., Clark, B., Des Marais, D., Farmer, J., Fischer, W., Grotzinger, J., Hayes, A., McLennan, S., Malin, M., Schröder, C., Squyres, S., and Wdowiak, T., 2005. An astrobiological perspective on Meridiani Planum. *Earth and Planetary Science Newsletters*, **240**, 179–189.
- McKay, D. S., Gibson, E. K. Jr., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chilliier, X. D. F., Maechling, C. R., and Zare, R. N., 1996. Search for past life on Mars: Possible relic biogenic activity in Martian Meteorite ALH 84001. *Science*, **273**, 924–930.
- McKay, C. P., and Davis, W. L., 1991. Duration of liquid water habitats on early Mars. *Icarus*, **90**, 214–221.
- Mitri, G., Showmana, A. P., Lunine, J. I., and Lorenz, R. D., 2007. Hydrocarbon Lakes on Titan. *Icarus*, **186**, 385–394.
- Mix, L. J., Armstrong, J. C., Mandell, A. M., Mosier, A. C., Raymond, J., Raymond, S. N., Stewart, F. J., von Braun, K., and Zhaxybayeva, O., (eds.), 2006. The astrobiology primer: An outline of general knowledge—Version 1. *Astrobiology*, **6**, 735–813.
- National Research Council (NRC), 2007a. Assessment of the NASA Astrobiology Institute, ISBN: 0–309–11498–5, 100 p.
- National Research Council (NRC), 2007b. *An Astrobiology Strategy for the Exploration of Mars*. Washington: National Academies Press.
- Plaxco, K. W., and Gross, M., 2006. *Astrobiology: A Brief Introduction*. Baltimore: Johns Hopkins University Press.
- Ricardo, A., Carrigan, M. A., Olcott, A. N., and Benner, S. A., 2004. Borate minerals stabilize ribose. *Science*, **303**, 196.
- Rothschild, L., and Mancinelli, R., 2001. Life in extreme environments. *Nature*, **409**, 1092–1101.
- Russell, M. J., Daniel, R. M., and Hall, A. J., 1993. On the emergence of life via catalytic iron sulfide membranes. *Terra Nova*, **5**, 343–347.
- Staley, J. T., and Reysenbach, A. L., 2001. *Biodiversity of Microbial Life: Foundation of Earth's Biosphere*. New Jersey: Wiley.
- Stevens, Todd., 1997. Lithoautotrophy in the subsurface. *FEMS Microbiology Reviews*, **20**(3–4), 327–337.
- Schneider, J., 2010. Interactive extra-solar planets catalog. Website: <http://exoplanet.eu/catalog.php>.
- Skelley, A. M., Scherer, J. R., Aubrey, A. D., Grover, W. H., Ivester, R. H. C., Ehrenfreund, P., Grunthaner, F. J., Bada, J. L., and Mathies, R. A., 2005. Development and evaluation of a microdevice for amino acid biomarker detection and analysis on Mars. *Proceedings of the National Academy of Sciences, USA*, **102**, 1041–1046.
- Sullivan, W. T., III, and Baross, J., 2007. *Planets and Life: The Emerging Science of Astrobiology*. Cambridge, United Kingdom: Cambridge University Press.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Miyazaki, J., Hirayama, H., Nakagawa, S., Nunoura, T., and Horikoshi, K., 2008. Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences, USA*, **105**, 10949–10954.
- Warner, N.H., Farmer, J.D., 2010. Sub-glacial hydrothermal alteration minerals in Jokulhlaup deposits in southern Iceland, with implications for detecting past or present habitable environments on Mars. *Astrobiology*, Volume 10, Number 5, 2010; DOI: 10.1089/ast.2009.0425.
- Wharton, R. A., Jr., Crosby, J. M., McKay, C. P., and Rice, J., Jr., 1995. Paleolakes on Mars. *Journal of Paleolimnology*, **13**, 267–283.

Cross-references

[Asteroid and Comet Impacts](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biosignatures in Rocks](#)
[Cosmic Molecular Clouds](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Meteoritics](#)
[Origin of Life](#)
[Raman Microscopy \(Confocal\)](#)

rapidly in this vast new array of oxygenated marine habitats (Knauth, 2005).

Summary

Salinity is not fixed by any equilibria, feedbacks, or geochemical cycles and appears to have been generally higher in past times. It is apparently a free variable dependent upon the vagaries of intermittent salt (and brine) sequestration on long-lived continental cratons. Ocean salinity declines when the salt and brine accumulate and rises when they are eroded back into the sea. Sequestration seems to have been enhanced following breakup of the two supercontinents in Earth history. These breakups are, in turn, driven by the vagaries of mantle convection. The combination of processes therefore necessary to lower the initial, pre-continent high salinity and to affect the rise and fall of ocean salinity involve a long chain of independent variables. The role of chance and contingency in biologic evolution has long been recognized. The low odds that a rerun of the tape of evolution would yield the same results have been duly noted (Gould, 1990). The same might be said about the evolution of oceanic salinity. Salinity history should therefore probably be added to all those chance occurrences that have likely affected the course of biologic evolution.

Bibliography

- Gomes, R., Levison, H. F., Tsiganis, K., and Morbidelli, A., 2005. Origin of the cataclysmic Late Heavy Bombardment period of the terrestrial planets. *Nature*, **435**, 466–469.
- Gould, S. J., 1990. *Wonderful Life: The Burgess Shale and the Nature of History*. New York: W.W. Norton.
- Hay, W. W., Migdisov, A., Balukhovskiy, A. N., Wold, C. N., Fogel, S., and Soding, E., 2006. Evaporites and the salinity of the ocean during the Phanerozoic: implications for climate, ocean circulation and life. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **240**, 3–46.
- Holland, H. D., 1978. *The Chemistry of the Atmosphere and Oceans*. New York: Wiley.
- Johnson, W. J., and Goldstein, R. H., 1993. Cambrian sea water preserved as inclusions in marine low-magnesium calcite cement. *Nature*, **362**, 335–337.
- Knauth, L. P., 1988. Origin and mixing history of brines, Palo Duro Basin, Texas, USA. *Applied Geochemistry*, **3**, 455–474.
- Knauth, L. P., 2005. Temperature and salinity history of the Precambrian ocean: implications for the course of microbial evolution. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 53–69.
- Land, L. S., 1995. The role of saline formation water in crustal cycling. *Aquatic Chemistry*, **1**, 137–145.
- Rogers, J. J. W., 1996. A history of continents in the past three billion years. *The Journal of Geology*, **104**, 91–107.
- Sonnenfeld, P., 1984. *Brines and Evaporites*. Orlando, FL: Academic Press.

Cross-references

[Critical Intervals in Earth History](#)
[Evaporites](#)
[Hypersaline Environments](#)
[Pore Waters](#)
[Soda Ocean, Hypothesis](#)

SCANNING PROBE MICROSCOPY (INCLUDES ATOMIC FORCE MICROSCOPY)

Michael Hoppert

University of Göttingen, Göttingen, Germany

Synonyms

Atomic force microscopy; Atomic probe microscopy; Force microscopy; Scanning force microscopy

Definition

Scanning probe microscopy uses a nanoscopic probe that is scanned over a solid surface. The interaction between the probe and the surface may be a mechanical or electromagnetic force. The force signal is enhanced and then composed to a force diagram of the surface. Under ideal conditions, an atomic-scale resolution may be achieved.

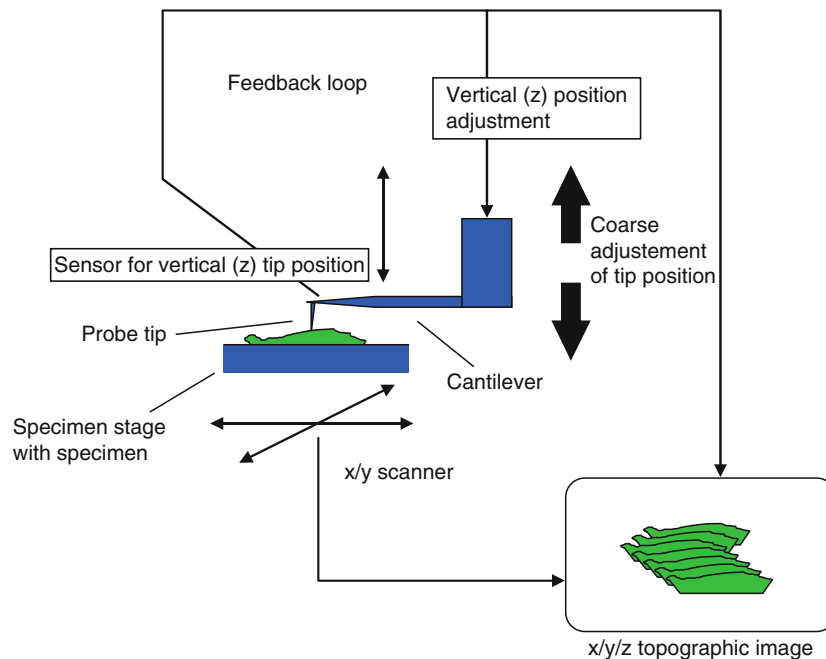
Introduction

Since their development during the 1970s and early 1980s, scanning probe microscopes (SPMs) have developed into instruments suitable for the analysis of surface topography (and other surface properties) down to near-atomic resolutions. All instruments belonging to this family contain essential components as depicted in [Figure 1](#). In the scanning process, surface material properties are mapped in all three directions. The essential parts of the system are the sharp scanning probe tip, which moves in the *z* direction, a piezoelectric scanner that moves the specimen, or, alternatively, the tip, in the *x/y* directions, and a sensor for tip positioning. Sensor and tip are connected in a feedback loop for the correction of *z* positioning. The *x/y/z* positions are used to generate a topographic image of the specimen. The resolution of SPMs is only limited by the size of the probe tip (or the probe-sample interaction volume). Since the tip apex ends up in one single atom and the piezoelectric actuators execute motions down the range of angstroms, resolutions down to atomic scale are possible. In contrast to electron microscopes, the sample can be observed under ambient conditions, which makes the technique potentially attractive for life science, including geobiological research.

The scanning tunneling microscope (STM)

The scanning tunneling microscope (STM) is the ancestor of all SPMs (Binnig et al., 1982). The specimen needs to be conductive or semiconductive. STM is predominantly used in materials sciences and chemistry. Though not ideally suited for biological specimens, the technique has also been used in life sciences (see, e.g., Guckenberger et al., 1988). The instrument provides a surface topography down to atomic resolution.

STMs use a sharpened conducting tip with a bias voltage (in mV to V-range) applied between the tip and the sample. If the distance between the tip and the sample is large, no current flows. When, however, the tip is brought



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 1 Basic setup of a scanning force microscope.

very close (1 nm), but without physical contact, a small current (pico- to nano-amperes) flows across the gap between the tip and the sample. This current is the result of the overlapping of wave functions between the tip atoms and the surface atoms. Electrons can “tunnel” across the vacuum barrier separating the tip and sample in the presence of the bias voltage. The magnitude of the tunneling current increases exponentially when the tip approaches the specimen surface. This results in high system sensitivity: when the separation between the tip and the specimen changes by 10%, the tunneling current changes by an order of magnitude. This provides, in the end, surface images with lateral atomic resolution. The image of the tunneling current depicts approximately the topography of the sample. Actually, rather than physical topography, the STM measures a surface of constant electron tunneling probability.

Atomic force microscopy and related techniques

Atomic force microscopy (AFM) comprises several derivatives and is applicable for measurement of numerous physical and chemical parameters of sample surfaces. AFM in the strict sense (without a modified probe tip) is used to study the material properties of specimens (stiffness, plasticity, and viscosity; see, e.g., Rotsch et al., 1997). For biological specimens, on the cellular level, the properties of the cytoskeleton and (induced) cytoskeletal changes become visible (see Fung, 1993; Goldmann et al., 1998 for some applications). With the aid of chemically modified tips, AFM is useful for quantification of binding forces between receptors and ligands, protein

complexes and cells (Lehenkari and Horton, 1999; Yip, 2001; Allison et al., 2002; Dufrene, 2008). Since AFM is generally suited for solid surfaces, it is especially a versatile tool for the observation of biomineral formation down to nanoscale in situ (see, e.g., Hildebrand et al., 2008).

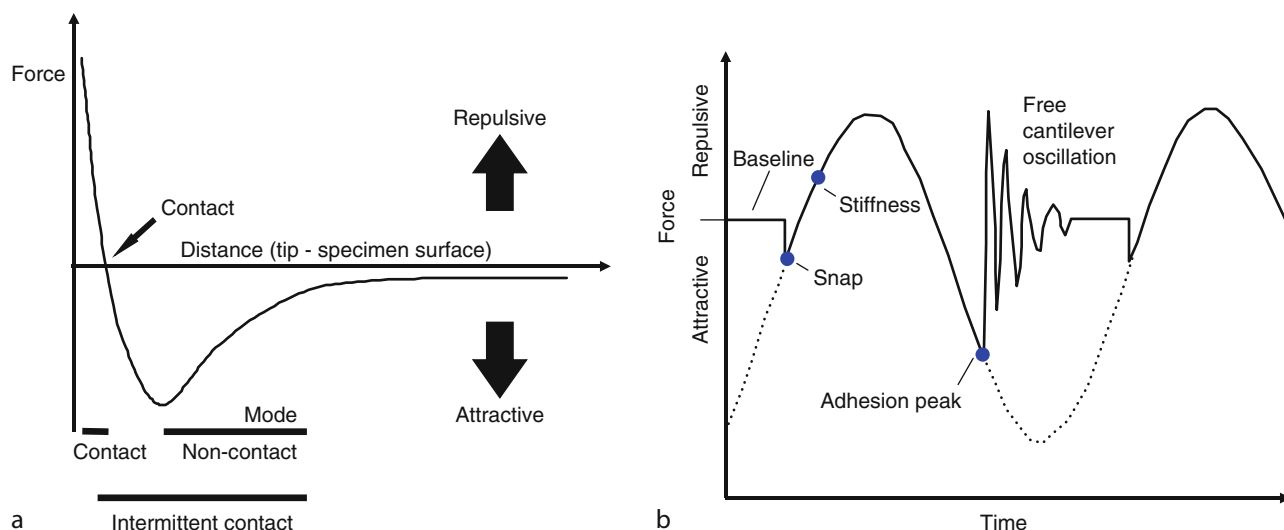
Image formation in AFM is generally determined by sensing of interatomic forces, making the technique applicable to all solid specimens. Similar to STM, the AFM probes the surface of a sample with a sharp tip, less than 10 nm in diameter. The tip is located at the free end of a 100–500 μm long cantilever. The cantilever is deflected by forces between the tip and the sample surface, and a detector measures the cantilever deflection as the tip is scanned over the sample (or the sample is scanned under the tip). The measured cantilever deflections are used to generate a map of surface topography (Figure 1). Conductors, semiconductors, and insulators may all be scanned. Resolution in the atomic range requires rigid specimens; hence, for most biological samples (except for biominerals), maximum resolution is not achievable.

The topographic data set necessary for image formation can be directly obtained from the measurement of cantilever deflection. When a laser beam is projected onto the cantilever, the position of the reflected beam is shifted, which results in detection of even sub-nm movement at the tip surface. Other methods of signal enhancement are based upon optical interference (e.g., by a Michelson interferometer). A STM tip may also be used to read the cantilever deflection. If the cantilever is made from a piezoresistive material, its deflection can also be detected electrically.

When the cantilever deflection is detected, this can be used to generate the topographic data set directly. This mode (constant height) is often used for producing atomic-scale images of atomically flat surfaces, for which the cantilever deflections and variations in applied force are small. The constant height-mode is also essential for recording real-time images of changing surfaces, which requires high scan speeds. Alternatively, the deflection of the cantilever can be used in a feedback loop that moves the scanner up and down in the z direction, responding to the topography, by keeping the cantilever deflection constant. In this case, the image is generated from the scanner's motion. With the cantilever deflection held constant, the total force applied to the sample is constant. This mode (constant force) is generally preferred for most applications.

Figure 2a depicts the typical force–distance curve for AFM. As the atoms of the tip and the specimen surface are gradually brought together, they first weakly attract each other by van der Waals forces. This attraction increases until the electron clouds of the atoms are so close together that their electrostatic repulsion progressively weakens the attractive force. The force becomes zero when the distance between the atoms reaches a couple of ångströms, about the length of a chemical bond. In the *contact mode*, the cantilever is held less than a few ångströms from the sample surface, and the interatomic force between the cantilever and the sample is repulsive. For biological (i.e., soft and wet) objects, the contact mode may induce several artifacts. This is avoided by application of modes in which less force is applied to the specimen. However, smaller forces also have to be detected, which in practice reduce the final resolution. In the *noncontact mode*, the cantilever is held of the order of tens to hundreds of ångströms from the sample surface, and the

interatomic force between the cantilever and sample is attractive (largely as a result of long-range van der Waals interactions). In this mode, the cantilever is oscillated with a frequency of 100–400 kHz (the resonance frequency of the cantilever) with an amplitude of a few tens of nanometers. The resonance frequency of the cantilever varies with the force gradient acting on the cantilever. These variations can be used as a measure of changes in the force gradient, which in turn reflects the changes in the tip-to-sample distance, or sample topography. The resonant frequency or vibrational amplitude of the cantilever is monitored and kept constant with the aid of the feedback loop that moves the scanner up and down in the z direction. The system also keeps the average tip-to-sample distance constant. As in contact AFM (in constant-force mode), the motion of the scanner is used to generate the topographic data set. It thus detects changes in the resonant frequency or vibration amplitude as the tip comes near the sample surface. *Intermittent-contact* (or tapping mode) AFM is similar to noncontact AFM, but the vibrating tip is brought closer to the sample in such a way that the sample is intermittently contacted or “tapped.” As in noncontact AFM, the oscillation amplitude of the tip also changes in response to the distance between tip and sample. Intermittent-contact AFM is also less likely to damage the sample than contact AFM. Though it touches the specimen, lateral forces (friction or drag) between the tip and the sample are minimal. The *pulsed force mode* (PFM) is used to monitor, besides sample topography, stiffness and adhesion simultaneously (Krotil et al., 1999). In PFM, the probe scans the surface in contact mode. When the cantilever is oscillated with a frequency of about 1 kHz, it is possible to analyze the force output as a function of the distance between the tip and the surface. The force output is depicted in Figure 2b. During an oscillation



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 2 (a) Force–distance curve in atomic force microscopes. (b) Force curve in the pulsed force mode (PFM). Important features of the curve are marked with blue dots.

cycle the probe tip is attracted by the surface and “snaps” into contact at a certain distance. The tip is then pushed further to the sample, the force signal increases, whereby the slope of the curve depends on the specimen stiffness. When the distance increases, force signal decreases, the tip loses contact (adhesion peak) and oscillates freely until the signal is damped to the baseline. Characteristic points of these curves are taken for processing to generate simultaneous topographic, stiffness (deformation of the specimen under an applied force), and adhesion maps. In the lateral force mode (LFM), lateral deflections (twisting) of the cantilever are measured. These are caused by forces on the cantilever parallel to the plane of the sample surface. LFM studies are used to detect surface friction arising from inhomogeneity in surface material; an artificial enhancement of edges in the image may also be achieved (Overney et al., 1992). In the force modulation mode (FMM), the elastic properties of a specimen are mapped. The cantilever tip is scanned in contact with the sample surface. As in constant-force contact AFM, the feedback loop uses the cantilever deflection signal to maintain a constant force between the tip and the sample and to generate a topography image. When a periodic oscillation is applied to the tip or the sample, the sample surface resists and the cantilever bends according to the elastic properties of the sample surface. The system generates an image, a map of sample stiffness, from the changes in the amplitude of cantilever modulation (Troyon et al., 1997).

Scanning force microscopy is a highly variable technique, because forces between a specimen and an AFM tip may be caused by a multitude of underlying factors. A modified scanning tip may therefore be used to measure a variety of parameters other than the described surface forces. In life sciences, the detection of chemical properties on biological surfaces is of outstanding interest. Some derivatives of AFM, mainly based on a modified scanning tip, are listed below.

Magnetic force microscopy (MFM) is used for measuring the magnetic force between the tip and the sample. For MFM, the tip is coated with a thin ferromagnetic film. The system mostly operates in noncontact mode and detects magnetic domains down to a resolution of approximately 20 nm (de Lozanne, 2006).

Electrostatic force microscopy (EFM) measures the forces originating from an electrical field between the AFM tip and the sample. When a voltage is applied between the tip and the sample (the tip is not in contact with the surface), the cantilever deflects when it scans over charged particles on the sample surface. In this way, EFM maps locally charged domains on the sample surface (Girard, 2001).

Scanning thermal microscopy (SThM) measures the specimen temperature and thermal conductivity of the sample surface. The SThM tip may be made from a thermocouple temperature sensor: a pair of dissimilar metals joined at the tip (platinum/silver Wollaston wires). The temperature difference between the tip and the open end generates a proportional net voltage. In this way, thermal

data and surface topography (with the conventional AFM force feedback mechanism) can be measured at the same time. Probes made of Wollaston wires also operate as active heat sources. The probe resistance is proportional to the probe temperature. Changes in the current required to keep the probe at constant temperature produce a thermal conductivity map. When the tip is held at a constant current, the specimen temperature is mapped by changes in the probe resistance (Price et al., 1999).

Photothermal microspectroscopy (PTMS) combines scanning probe microscopy at a submicron spatial resolution with an IR interferometer. IR radiation from a Fourier-transform spectrometer is focused on a specimen. IR absorption causes stretching and bending of molecular bonds. Upon relaxation of these molecular vibrations, evanescent thermal waves are emitted. This signal is detected with a Wollaston wire (SThM) probe. The resulting temperature fluctuations are converted to electrical signals, recorded as a function of time and amplified to produce an “interferogram” which can be transformed back to give a conventional spectrum as in other forms of FTIR spectroscopy. In PTMS, resolutions down to 10 μm are achievable, whereas in conventional FTIR microspectrometric devices, only 100 μm are possible (Grude et al., 2007).

Phase detection microscopy (PDM) is another variation for mapping of surface properties such as elasticity, adhesion, and friction. Unlike FMM (see above), the technique operates in the noncontact mode, which is more suitable for less rigid (biological) samples. The technique monitors the phase lag between the signal causing the cantilever to oscillate and the cantilever oscillation output signal. Changes in the phase lag reflect changes in the mechanical properties of the sample surface. The feedback loop in PDM operates in the usual manner, using changes in the cantilever’s deflection or vibration amplitude to measure sample topography. The phase lag is monitored while the topographic image is being taken, so that images of the topography and material properties can be collected simultaneously.

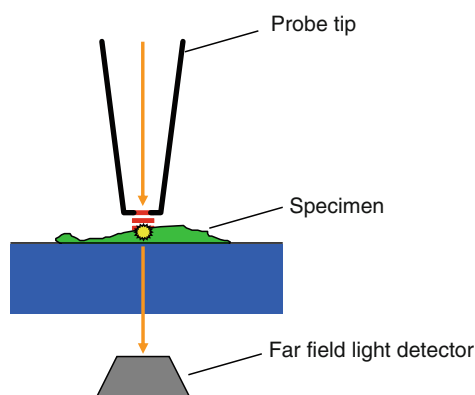
Scanning near-field acoustic microscopy (SNAM) utilizes an ultrasonic quartz oscillator as resonator and at the same time as a sensor of the damping force exerted by the specimen. Damping increases when the tip approaches the surface. The instruments have been especially developed for scanning of larger areas, with a surface relief in the millimeter range. For low resolution, wide probing tips are used. At resolutions of 100 nm, both hard and soft specimens are well suited for mapping. Higher resolution is obtained with sharp AFM tips (Murdfield et al., 1996).

With chemical force microscopy (CFM) it is possible to obtain modified force curves based on specific interactions between molecules on the specimen surface and a sensor molecule on the probe tip. The tip (normally made from silicon or silicon nitride) is modified by coating a very thin metallic film (normally a 5 nm thick chromium layer followed by a 50 nm thick gold layer) onto the probe, followed by immersion of the probe in a solution of an

organic thiol. The molecules form a monolayer, with the thiol group bound to the gold surface. The distal end of thiol molecule contains an appropriate functional group for interaction with the sample surface. Besides small methyl, carboxyl or amino groups, macromolecules may also be used as specific probes. Coupled enzyme proteins may be used to measure enzyme–substrate interactions. In a similar experimental setup, even whole bacterial cells, coupled to the cantilever, were used to measure distance–force curves between the cell surface and biominerals (Lower et al., 2001; Dufrene, 2008 and references therein).

Near-field scanning optical microscopes (NSOMs) are based upon the AFM operating in the noncontact mode. At the same time, the probe tip generates nonpropagating (evanescent) electromagnetic wave fields to excite a specimen at a small (down to tens of nm) surface spot. Most NSOM setups use an aluminum-coated glass fiber with a sub-light wavelength aperture (25–100 nm in diameter) at the tip of an AFM-like scanning probe (Figure 3). Light, guided through the fiber optics, causes an evanescent wave field that decays exponentially with distance from its origin. Evanescent waves interact with the specimen surface, e.g., by excitation of fluorophores. Fluorescence light emitted from the specimen is scattered from the sample, but only from the evanescent wave field region (at distances from the probe tip of approximately one fifth of the light wavelength). The fluorescence signal is conventionally propagating far-field light. This signal may be collected by a conventional optics below the specimen (e.g., an inverted microscope). Because of the extremely small excitation volume determined by the evanescent wave, together with the high-resolution topographical map generated by the AFM tip, a high-resolution map of the fluorescent targets in the specimen is achieved.

With NSOM, the AFM topographic information is combined with optical properties of a specimen, such as



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 3 Hollow fiber tip in a near-field scanning optical microscope (NSOM). At the tip, the evanescent wave field (red) excites a fluorophore marker (yellow). The resulting (far-field) light signal is detected by an external detector. Propagating far-field light (orange arrows).

refraction index, reflectivity, transparency, polarization Raman shift, and fluorescence (Gheber et al., 1998; de Lange et al., 2001; Edidin, 2001).

Conclusion

Scanning probe microscopy introduced a purely surface-related technique with atomic resolution into the field of material sciences and also life science. For the first time it was possible to visualize macromolecules directly, under physiological conditions. Even more important is the application potential for mapping all kinds of intermolecular interactions. The fact that just signals from a surface can be collected may be a drawback for the observation and documentation of most biological processes because they are inside cells and other compartments. Most mechanisms in material science, chemistry and biology are, however, surface-related. It is just the question how a surface, which may also be a membrane or the interface of another compartment, can be prepared in a way that it is reachable by an SPM.

Other limitations are the low scanning speed of most modes and the small obtainable image area. Hence, it is straightforward to combine scanning probe microscopy with other imaging techniques. Correlation of low-resolution techniques including fast image acquisition (fluorescence light microscopy or confocal microscopy) with the high-resolution AFM image may be versatile for a multitude of applications, especially concerning the interaction of organisms with solid surfaces (e.g., Arnold et al., 2004; overview in Haupt et al., 2006).

Bibliography

- Allison, D. P., Hinterdorfer, P., and Han, W., 2002. Biomolecular force measurements and the atomic force microscope. *Current Opinion in Biotechnology*, **13**, 47–51.
- Arnold, J. W., Boothe, D. H., Suzuki, O., and Bailey, G. W., 2004. Multiple imaging techniques demonstrate the manipulation of surfaces to reduce bacterial contamination and corrosion. *Journal of Microscopy*, **216**, 215–221.
- Binnig, G., Rohrer, H., Gerber, C., and Weibel, E., 1982. Surface studies by scanning tunneling microscopy. *Physical Review Letters*, **49**, 57–61.
- de Lange, F., Cambi, A., Huijbens, R., de Bakker, B., Rensen, W., Garcia-Parajo, M., van Hulst, N., and Figdor, C. G., 2001. Cell biology beyond the diffraction limit: near-field scanning optical microscopy. *Journal of Cell Science*, **114**, 4153–4160.
- de Lozanne, A., 2006. Application of magnetic force microscopy in nanomaterials characterization. *Microscopy Research and Technique*, **69**, 550–562.
- Dufrene, Y. F., 2008. Towards nanomicrobiology using atomic force microscopy. *Nature Reviews Microbiology*, **6**, 674–680.
- Edidin, M., 2001. Near-field scanning optical microscopy, a siren call to biology. *Traffic*, **2**, 797–803.
- Fung, Y. C., 1993. *Biomechanics: Mechanical Properties of Living Tissues*. New York: Springer.
- Gheber, L., Hwang, J., and Edidin, M., 1998. Design and optimization of a near-field scanning optical microscope for imaging biological samples in liquid. *Applied Optics*, **37**, 3574–3581.
- Girard, P., 2001. Electrostatic force microscopy: principles and some applications to semiconductors. *Nanotechnology*, **12**, 485–490.

- Goldmann, W. H., Galneder, R., Ludwig, M., Xu, W., Adamson, E. D., Wang, N., and Ezzell, R. M., 1998. Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Experimental Cell Research*, **239**, 235–242.
- Grude, O., Hammiche, A., Pollock, H., Bentley, A. J., Walsh, M., Martin, F. L., and Fullwood, N. J., 2007. Near-field photothermal microspectrometry for adult stem-cell identification and characterization. *Journal of Microscopy*, **228**, 366–372.
- Guckenberger, R., Kösslinger, C., Gatz, R., Breu, H., Levai, W., and Baumeister, W., 1988. A scanning tunneling microscope (STM) for biological applications. Design and performance. *Ultramicroscopy*, **25**, 795–802.
- Haupt, B. J., Pelling, A. E., and Horton, M. A., 2006. Integrated confocal and scanning probe microscopy for biomedical research. *The Scientific World Journal*, **6**, 1609–1618.
- Hildebrand, M., Doktycz, M. J., and Allison, D. P., 2008. Application of AFM in understanding biomineral formation in diatoms. *Pflügers Archiv-European Journal of Physiology*, **456**, 127–137.
- Krottil, H.-U., Stifter, T., Waschipky, H., Weishaupt, K., Hild, S., and Marti, O., 1999. Pulsed force mode: a new method for the investigation of surface properties. *Surface and Interface Analysis*, **27**, 336–340.
- Lehenkari, P. P., and Horton, M. A., 1999. Single integrin molecule adhesion forces in intact cells measured by atomic force microscopy. *Biochemical and Biophysical Research Communications*, **259**, 645–650.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Schewanella* and α -FeOOH. *Science*, **292**, 1360–1363.
- Murdfeld, T., Fischer, U. C., and Fuchs, H., 1996. Acoustic and dynamic force microscopy with ultrasonic probes. *Journal of Vacuum Science and Technology B*, **14**, 877–881.
- Overney, R. M., Meyer, E., Frommer, J., Brodbeck, D., Lüthi, R., Howald, L., Güntherodt, H.-J., Fujihira, M., Takano, H., and Gotoh, Y., 1992. Friction measurements on phase-separated thin films with a modified atomic force microscope. *Nature*, **359**, 133–134.
- Price, D. M., Reading, M., Hammiche, A., and Pollock, H. M., 1999. Micro-thermal analysis: scanning thermal microscopy and localised thermal analysis. *International Journal of Pharmaceutics*, **192**, 85–96.
- Rotsch, C., Braet, F., Wisse, E., and Radmacher, M., 1997. AFM imaging and elasticity measurements on living rat liver macrophages. *Cell Biology International*, **21**, 685–696.
- Troyon, M., Wang, Z., Pastre, D., Lei, H. N., and Hazotte, A., 1997. Force modulation microscopy for the study of stiff materials. *Nanotechnology*, **8**, 163–171.
- Yip, C. M., 2001. Atomic force microscopy of macromolecular interactions. *Current Opinion in Structural Biology*, **11**, 567–572.

Cross-references

[Raman Microscopy \(Confocal\)](#)

SEDIMENT DIAGENESIS – BIOLOGICALLY CONTROLLED

Kurt O. Konhauser¹, Murray K. Gingras¹, Andreas Kappler²

¹University of Alberta, Edmonton, Alberta, Canada

²University of Tübingen, Tübingen, Germany

Definition

The biological processes that modify unconsolidated sediment after initial deposition and burial.

Overview

Throughout the sediment column, up to the temperature threshold of life, microbial activity plays an integral role in diagenesis. It is widely recognized that through their various chemoheterotrophic pathways, microorganisms are ultimately responsible for the conversion of organic carbon to CO₂ and CH₄ at temperatures <100°C. Some aerobic bacteria use hydrolytic enzymes to break down complex molecules into simple monomers such as sugars, amino acids, and fatty acids that they can then utilize, while most anaerobes are restricted to simple organic compounds (e.g., acetate, lactate, H₂) that are the by-products of fermentation (Lovley and Chapelle, 1995). Typically, the more labile materials (e.g., proteins, carbohydrates) are degraded in near-surface sediments on time scales of days to years, and more refractory materials (e.g., lipids) are broken down deeper in the sediment on time scales of decades, while the most resistant materials (lignins) are transformed only on timescales of thousands to millions of years. The amount of refractory material left after microbial attack will depend on the nature of the primary producers and the degree of processing that has taken place in the river catchment area or oceanic water column. In the end, for example, <1% of the original material buried into marine sediment may ultimately contribute to the sedimentary organic geochemical record (Emerson and Hedges, 1988).

Pore water and mineralogical changes during diagenesis are directly related to bacterial reduction of soluble (O₂, NO₃[−], SO₄^{2−}, CO₂) or solid-phase (MnO₂ and ferric oxyhydroxides) components in the sediment. The terminal electron accepting processes (TEAPs) that occur at any given layer depends on oxidants that are available and, in the situation when multiple electron acceptors are present (as in the uppermost sediment layers), on the free energy yield of the specific reaction. Thus, the decomposition of freshly deposited organic material in sediments proceeds in a continuous sequence of redox reactions, with the most electropositive oxidants being consumed at, or near the surface, and progressively energetically poorer oxidants being consumed at depth until the labile organic fraction is exhausted and the deeper sediments are left with a composition very different from that of the sediments originally deposited (Figure 1). Inorganic by-products of chemoheterotrophy (e.g., HCO₃[−], Mn²⁺, Fe²⁺, NH₄⁺, NO₂[−], HS[−], HPO₃^{2−} and CH₄) seldom accumulate. Physical and macrofaunal processes will cause the net transport of these reduced species from the deeper layers toward the sediment surface, where residing anaerobic and aerobic chemolithoautotrophic bacteria use them as metabolic reactants. Alternatively, their presence in pore waters may trigger important abiotic reactions between the solid and dissolved phases leading to secondary mineral formation, i.e., cementation.

Oxic zone reactions

The sediment–water interface is the site of most intense heterotrophic activity. Aerobic respiration (Reaction 1) is

B

BACTERIA

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms

Eubacteria (term abandoned)

Definition

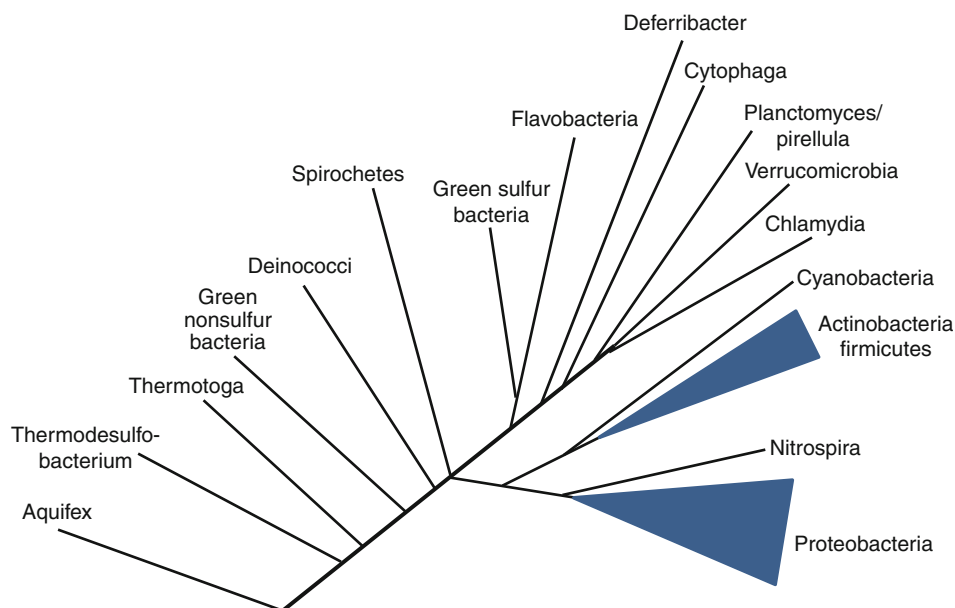
Prokaryotes that constitute, besides Archaea and Eukarya, a domain of life. According to (old Greek) bakterion “small rod.”

History

During the nineteenth century, several fundamental discoveries defined the beginning of modern microbiology (cf. Schlegel, 1999). Louis Pasteur (1822–1895) found that growth of bacteria in nutrient broths is not due to spontaneous generation and that fermentation is caused by the growth of microorganisms. Ferdinand Cohn (1828–1898) could state that bacteria must belong to a phylogenetic group separated from other unicellular plants or animals, due to their size, shape, the mode of cell division, and their metabolic properties. Robert Koch (1842–1910) developed the concept of infectious diseases. The final proof that bacteria must be distinct from plants and animals could be shown not until the distinct compartmentation of bacterial cells was noticed by electron microscopy, in the 1960s. Especially, the absence of a nuclear membrane was a clear indicator for some fundamental differences in cellular processes. Hence, the term “prokaryote” for bacteria (including Archaea) was introduced. The separation of *Archaea* from Bacteria (as phylogenetic groups) was achieved by Woese and Fox (1977).

Besides the missing nuclear membrane as a common cytological feature, Bacteria and Archaea are structurally and physiologically distinct. The Bacteria are the phylogenetically most diverse domain, with a rapidly growing number of phyla. Currently, more than 80 phyla could be identified (Pace, 2009). About two-third of these phyla are just represented by rDNA sequences from environmental samples. Twenty-five phyla are represented by known species. Among them, twelve phyla contain the majority of currently known and well-characterized species. The well-known representatives of phyla, however, stand for an incomplete and subjective view on the bacterial domain. Human pathogenic species are, for example, overrepresented, because of their importance for human medicine. The significance of several groups relevant for environmental processes, such as Planctomycetes or the *Roseobacter* clade has just been addressed recently. General knowledge on the physiology of these groups is still limited. Most in-depth analyses on microbial physiology and molecular biology has been performed with selected species from a small number of phyla, such as *Escherichia coli*, *Salmonella*, and *Pseudomonas* species (Proteobacteria) or *Bacillus* (Firmicutes). Some organisms like *Ralstonia* (an aerobic hydrogen oxidizing Proteobacterium) or *Rhodobacter* (a photosynthetic bacterium) are model organisms for environmentally relevant processes.

The phylogenetic tree (Figure 1), based upon 16S-rDNA data shows an overview of these major, well-characterized lineages (Cole et al., 2009; Madigan et al., 2009). In-depth analysis based upon whole genomes may allow, in future, a more accurate positioning of genes and organisms in phylogenetic trees (Ciccarelli et al., 2006; Wu and Eisen, 2008). Though the major phyla have been confirmed, some repositioning may be necessary. Some reports imply that Firmicutes are descendants of



Bacteria, Figure 1 Phylogenetic tree of Bacteria, derived from 16S rDNA sequences. (Redrawn after Madigan et al., 2009.)

the stem group, other than expected from rDNA analysis (Ciccarelli et al., 2006).

In the following chapter, a short survey on the hitherto well-investigated groups will illustrate the metabolic diversity of the organisms. Terms denoting defined phyla are capitalized.

Diversity in phylogenetic lineages

Important metabolic key processes are unique to bacteria and have just been overtaken by endosymbiosis to eukaryotes, such as chlorophyll-dependent photosynthesis and mitochondrial respiration. Thus, metabolism in every eukaryote is based upon bacterial metabolism. Eukaryotes, however, received just a small range of the highly various metabolic features from bacteria. Virtually, every reduced organic compound and numerous reduced inorganic molecules can be oxidized in a bacterial metabolism that may be either respiratory, that is, generating a membrane potential, driven by an electron flow toward an oxidized acceptor (oxygen, nitrate, and others) or fermentative (generation of ATP independent of a membrane potential). These metabolic features are unsuitable to discriminate between large groups because they are mostly not specific for only one phylum (Tables 1–4; Schlegel and Jannasch, 2006; Zinder and Dworkin, 2006). Some strategies and bacterial life-styles are, however, suitable to discriminate between certain subgroups within a phylum. In the following, some key features that may be confined to the respective groups are specified. The groups are assigned to phyla according to the “classical” phylogenetic scheme, obtained by

16S-rDNA sequence analysis (Cole et al., 2008; cf. Madigan et al., 2009).

Ancient phyla

Several representatives of the ancient bacterial groups clustering deep in the phylogenetic tree are hyperthermophilic, some of them are chemolithotrophs. The hyperthermophilic genus *Aquifex* is an obligately hydrogen oxidizing, microaerophilic, chemolithotrophic (see *Chemolithotrophy*) bacterium (Huber and Eder, 2006). The fixation of carbon dioxide does not depend on the Calvin cycle (ribulose biphosphate carboxylase as key enzyme), like in further derived phyla and green plants, but, instead, on a reversed citrate cycle. The organisms are not capable to grow on organic molecules, that is, they are obligate chemolithotrophs utilizing molecular hydrogen or reduced sulfur compounds as electron donors, at optimum growth temperatures of 85°C. Thus, the environmental conditions for the ecological niche of *Aquifex* are equivalent to the reducing conditions of the earth’s early atmosphere. The related genera *Hydrogenobacter* and *Thermocrinis* share most of *Aquifex*’ properties, but are aerobic and also utilize organic compounds.

Several important cellular features that are found in most bacterial phyla are already present in this ancient lineage: the cytoplasmic membrane is surrounded by another membrane of lipid bilayer type (outer membrane). Thus, the membranes enclose an additional compartment, the periplasmic space. The periplasmic space contains the rigid murein (peptidoglycan) sacculus. Another feature found in all, including the most ancient bacterial phyla is the bacterial flagellum (Figure 2a). Though analogous

Bacteria, Table 1 Groups of chemolithotrophic bacteria (After Schlegel and Jannasch, 2006, with modifications)

Typical species	Lineage	Metabolic process	Electron donor	Electron acceptor	Product
<i>Aquifex</i>	Aquificales	Hydrogen oxidation	H ₂	O ₂	H ₂ O
		Sulfur oxidation	S ₂ O ₃ ²⁻ , S ₀	O ₂	SO ₄ ²⁻
<i>Ralstonia eutropha</i>	Beta-Proteobacteria	Hydrogen oxidation	H ₂	O ₂	H ₂ O
<i>Pseudomonas carboxydovorans</i>	Gamma-Proteobacteria	CO oxidation	CO	O ₂	CO ₂
<i>Nitrosomonas europaea</i>	Beta-Proteobacteria	Ammonium oxidation	NH ₄ ⁺	O ₂	NO ₂ ⁻
<i>Nitobacter</i>	Alpha-Proteobacteria	Nitrite oxidation	CO ₂	O ₂	NO ₃ ⁻
<i>Thiobacillus thiooxidans</i>	Beta-Proteobacteria	Sulfur oxidation	H ₂ S, S ₀	O ₂	SO ₄ ²⁻
<i>Thiobacillus ferrooxidans</i>	Beta-Proteobacteria	Iron oxidation	Fe ²⁺	O ₂	Fe ³⁺
<i>Beggiatoa alba</i>	Gamma-Proteobacteria	Sulfur oxidation	H ₂ S, S ₀	O ₂ , NO ₃ ⁻	SO ₄ ²⁻ , N ₂
<i>Acetobacterium woodii</i>	Firmicutes	Acetogenesis	H ₂	CO ₂	Acetate

Bacteria, Table 2 Groups of phototrophic bacteria (After Schlegel and Jannasch, 2006, with modifications)

Bacterial group	Lineage	Key genus	Chemoorganotrophic growth	Electron donors for photosynthesis
Purple non-sulfur bacteria	Alpha-/beta-Proteobacteria	<i>Rhodospirillum</i>	+	H ₂ , organic, (S ²⁻)
Purple sulfur bacteria	Gamma-Proteobacteria	<i>Chromatium</i>	—	S ²⁻ , S ⁰ , S ₂ O ₃ ²⁻ , H ₂
Green sulfur bacteria	Chlorobi	<i>Chlorobium</i>	—	S ²⁻ , S ⁰ , S ₂ O ₃ ²⁻ , H ₂
<i>Chloroflexus</i> group	Chloroflexi	<i>Chloroflexus</i>	+	Organic (S ²⁻)
Heliobacteriaceae	Firmicutes	<i>Heliobacterium</i>	—	Organic
Aerobic phototrophic Proteobacteria	Alpha-Proteobacteria	<i>Roseobacter</i>	—	Organic
Cyanobacteria	Cyanobacteria	<i>Synechococcus</i> <i>Oscillatoria</i>	—	H ₂ O H ₂ O (S ²⁻)

Bacteria, Table 3 Bacterial groups and their electron acceptors for respiratory processes (After Schlegel and Jannasch, 2006, with modifications)

Physiological group	Typical species	Lineage	Respiratory process	Electron acceptor	Reduction products
Denitrifiers	<i>Pseudomonas denitrificans</i>	Gamma-Proteobacteria	Aerobic respiration nitrite respiration	O ₂ , NO ₃ ⁻	N ₂ , N ₂ O, NO ₂ ⁻
Sulfate reducers	<i>Desulfovibrio vulgaris</i>	Delta-Proteobacteria	Sulfate respiration	SO ₄ ²⁻	S ²⁻
Sulfur reducers	<i>Desulfuromonas acetoxidans</i>	Delta-Proteobacteria	Sulfur respiration	S ⁰	S ²⁻
Acetogenic bacteria	<i>Acetobacterium woodii</i>	Firmicutes	Carbonate respiration	CO ₂	acetate
Succinogenic bacteria	<i>Wolinella succinogenes</i>	Epsilon-Proteobacteria	Fumarate respiration	fumarate	succinate
Iron reducer	<i>Pseudomonas GS-15</i>	Gamma-Proteobacteria	Iron respiration	Fe ³⁺	Fe ²⁺

motility structures are also found in Archaea (and in Eukarya), they are phylogenetically completely unrelated. For other types of appendages (bacterial pili, Figure 2a), homologues in Archaea could be described (Szabo et al., 2007).

Thermotoga and related genera also comprise chemoorganotrophic hyperthermophilic representatives. Comparative genomics revealed that more than 20% of the *Thermotoga* genome shares strong homologies with genes from various hyperthermophilic archaeal species, accounting for an extensive inter-domain horizontal gene transfer (Nelson et al., 1999).

Chloroflexi and Chlorobi

(Bacterio)chlorophyll-dependent photosynthesis (see *Photosynthesis*) is unique for bacterial organisms (Table 2; Overmann and Garcia-Pichel, 2006). Ancestors of green nonsulfur bacteria (Chloroflexi) were very likely the first photosynthetic organisms of the bacterial domain (Oyaizu et al., 1987). The key genus *Chloroflexus* is a thermophilic representative of the group. Besides photoautotrophic growth, *Chloroflexus* is also able to use organic compounds. Carbon dioxide fixation is still Rubisco independent and is carried out by transfer of hydrogen carbonate to activated acetate and propionate

Bacteria, Table 4 Groups of fermentative bacteria

Fermentative process	Typical species	Lineage	Preferred substrates	Fermentation products (minor products)
Ethanol fermentation	<i>Zymomonas mobilis</i>	Alpha-Proteobacteria	Glucose	Ethanol (CO ₂)
Lactate fermentation – homofermentative – heterofermentative	<i>Lactobacillus casei</i> <i>Leuconostoc mesenteroides</i>	Firmicutes	Glucose, lactose Glucose, lactose	Lactate Lactate (ethanol, CO ₂)
Butyrate fermentation	<i>Clostridium butyricum</i> <i>Clostridium acetobutylicum</i> <i>Clostridium klyuveri</i>	Firmicutes Firmicutes Firmicutes	Glucose Glucose Ethanol+acetate	Butyrate (acetate, H ₂ , CO ₂) Butyrate, butanol (acetone, 2-propanol)
Homoacetate fermentation	<i>Clostridium acetivum</i>	Firmicutes	Fructose	Acetate
Propionate and succinate fermentation	<i>Propionibacterium pentosaceum</i> <i>Veillonella alcalescens</i> <i>Bacteroides ruminicola</i>	Actinobacteria Firmicutes	Sugars, Lactate Lactate Sugars	Propionate (succinate) Propionate (acetate, H ₂ , CO ₂) Propionate
Mixed acid and butanediol fermentation	<i>Escherichia coli</i> <i>Enterobacter aerogenes</i>	Gamma-Proteobacteria	Glucose, lactose Glucose	Lactate, ethanol acetate (formate, succinate, H ₂ , CO ₂) 2,3 butanediol, ethanol (formate, H ₂ , CO ₂)
Amino acid fermentation	<i>Clostridium tetanomorphum</i> <i>Clostridium sticklandii</i> <i>Clostridium oroticum</i>	Firmicutes Firmicutes Firmicutes	Glutamate Lysine Orotate	Butyrate (acetate, CO ₂ , NH ₃) Butyrate (acetate, NH ₃) Acetate (CO ₂ , NH ₃)

(acetyl-coenzyme A, propionyl-coenzyme A) in the hydroxypropionate pathway. Green sulfur bacteria (Chlorobi) represent a separate lineage of anoxygenic photosynthetic bacteria. Similar to photosynthetic Proteobacteria, the organisms use reduced sulfur compounds (H₂S, S₀) as well as organic compounds as electron donors. The organisms use a reverse citric acid cycle for carbon dioxide fixation. A remarkable structural feature, the chlorosome, consisting of densely stacked self-assembled antenna bacteriochlorophylls, is a simple but very effective light harvesting complex. This arrangement transfers light energy (excitons) effectively to the reaction centre in the cytoplasmic membrane. The organisms are, hence, adapted to very low light conditions. Chlorosome genes were transferred, by horizontal gene transfer, to some phototrophic lineages, including some genera of the *Chloroflexus* group (cf. Hanada and Pierson, 2006).

Spirochetes

Spirochetes are rather structurally than metabolically remarkable (Charon et al., 1992). The spirochete endoflagellum is of the standard bacterial type, but is located in the periplasmic space. In all other bacterial groups, the flagellum (if present) traverses all layers of the envelope. The endoflagellum is helically wound around the flexible protoplast and gives the cell its unique, helically coiled appearance. The movement of the flagellum drives the cell body in a drill-like manner. By this way, movement of the cell in highly viscous media like tissue is facilitated. In fact, numerous symbiotic and

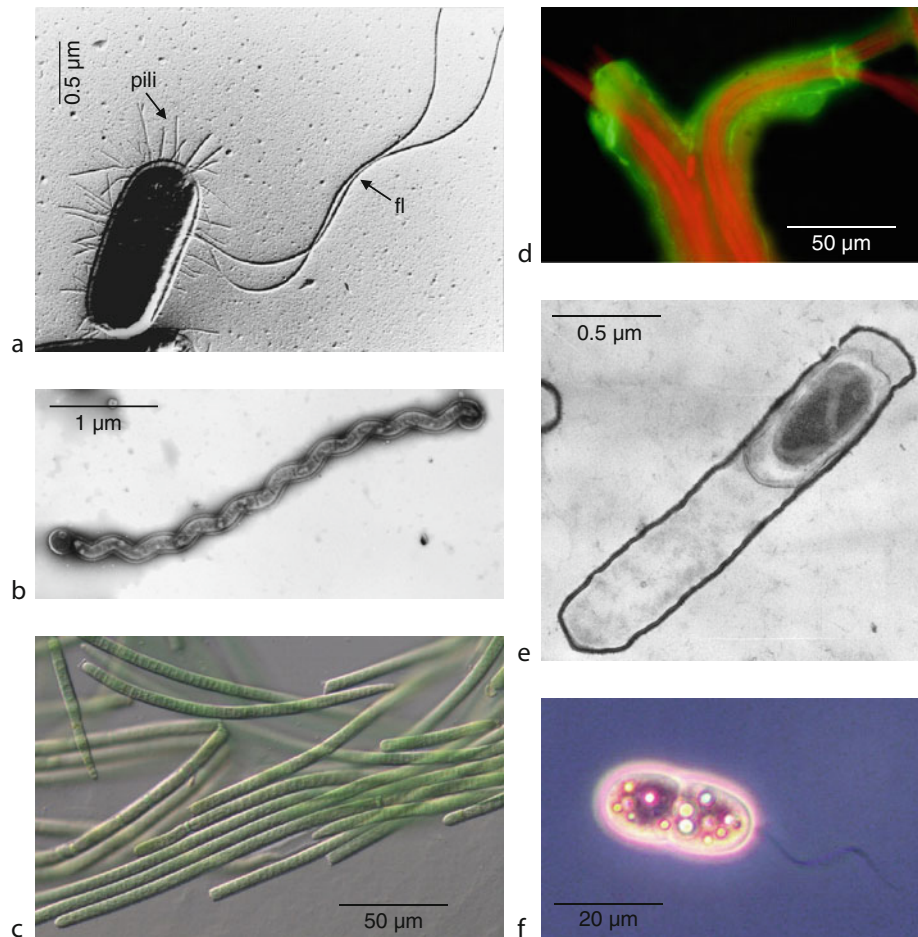
pathogenic organisms belong to this group (*Teponema*, *Borrelia*, *Leptospira*).

Deinococcus/Thermus

Another separate lineage of extremophilic bacteria is represented by the key genera *Deinococcus* and *Thermus*. The organisms mainly oxidize carbohydrates and proteins. *Thermus* species are thermophilic and grow between 50°C and 80°C. The remarkable members of the *Deinococcus* group are adapted to desiccation and ionizing or ultraviolet radiation. The composition and thickness of the cell wall, DNA compaction, and DNA repair systems are special protective features (Da Costa et al., 2006).

Flavobacteria/Cytophaga

The Flavobacteria/Cytophaga phylum comprises diverse, mostly heterotrophic, anaerobic or aerobic bacteria (Bernardet and Nakagawa, 2006). Within this group, the members of the genus *Bacteroides* represent the dominant bacteria in the human large intestine. The second key genus *Flavobacterium* is mostly found in freshwater and marine habitats. The Acidobacteria with the genera *Acidobacterium*, *Geothrix*, and *Halophaga* are heterotrophic soil inhabitants. It is known from phylogenetic analysis of environmental DNA that uncultured members of the genera are widespread in other terrestrial and aquatic habitats and may thus play an important ecological role. Several members of *Cytophaga* and related genera are important degraders of cellulose and other polysaccharides. Cells move by gliding, a surface motility mechanism that is also observable in other bacterial lineages.



Bacteria, Figure 2 Some cytological features of bacteria. (a) The bacterial flagellum (fl) is a unique motility structure of the bacterial kingdom. The short spikelike appendages (pili) are also found in Archaea (electron micrograph of a metal shadowed *Escherichia coli* cell). (b) The corkscrew-like *Spirochete* morphology (here, *Spirochaeta thermophila*) is determined by the flagellum ("axial filament") wound around the elongate cell body. The axial filament propels the whole cell in a drill-like manner. (c) Aquatic organisms tend to form long filaments (filamentous cyanobacterium *Phormidium* sp.). Light micrograph, differential interference contrast. (d) Several filaments of the cyanobacterium *Microcoleus*, frequently found in terrestrial habitats, are surrounded by a polysaccharide sheath (green). The filaments (red) glide out of the tubelike sheath. Sheaths are found in various bacterial groups. Fluorescence light micrograph. (e) *Bacillus* mother cell with endospore. Electron micrograph of an ultrathin section. (f) Purple sulfur bacterium (*Chromatium*) with intracellular sulfur granula. Light micrograph, phase contrast.

The gliding motility of *Cytophaga* and relatives, however, is based on a unique, not yet understood, cell envelope-associated mechanism.

Planctomyces/Verrucomicrobia

The *Planctomyces/Verrucomicrobia* lineages comprise morphologically uncommon groups of uncertain phylogeny. Planctomycetes develop protein stalks as attachment structures and divide by budding (instead of binary fission, the common way of bacterial and archaeal cell division). Peptidoglycan is missing. Planctomycetes and the related phylum of Verrucomicrobia also show a highly compartmentalized cytoplasm (Lee et al., 2009). Endocytosis-like processes, a typical feature of eukaryotes, could also be observed (Lonhienne et al., 2010).

In *Gemmata* spp. the nucleoid is surrounded by an intracytoplasmic unit membrane. The anammoxosome in *Brocadia anammoxidans* is a structurally similar compartment, but appears to be involved in the compartmentation of anaerobic oxidation of ammonia, which is a unique feature of this group. The genera *Verrucomicrobium* and *Prostheco bacter* possess lobe-like cellular extensions. The organisms are mostly found in freshwater habitats. The lobed appendages increase the cell surface and facilitate the uptake of substrate molecules in an oligotrophic life-style.

Cyanobacteria

Cyanobacteria and prochlorophytes differ from all other phototrophic bacteria in fundamental ways (Overmann

and Garcia-Pichel, 2006). The oxygenic photosynthesis, conducted by cyanobacteria, requires photosystems acting over a wide redox span. To split the water molecules, the reduction potential of chlorophyll in photosystem II must be more positive than that of the O_2/H_2O couple. Excited electrons are transferred, via several redox carriers, to a second photosystem (PS I), capable of NAD(P)H regeneration. The reducing power is particularly needed for carbon dioxide fixation (for fixation of 1 mol CO_2 , 2 mol NADPH is required), carried out by the Calvin cycle. A unique feature for Cyanobacteria are phycobilins as accessory (light harvesting) pigments, an adaptation to low light conditions. Like plastids, cyanobacteria contain intracytoplasmic thylakoid membranes as additional compartments for the membrane-bound photosystems.

Cyanobacteria are the morphologically most diverse group of bacteria, with some organizational levels analogous to eukaryotic algae (Figure 2c; Waterbury, 2006). Many species are filamentous. They are present in marine and freshwater, as well as in terrestrial habitats (Figure 2c, d). Some live in symbiotic relationship to higher plants (owing to the cyanobacterial nitrogen fixing activity). Several filamentous species carry out nitrogen fixation in specialized cells (heterocysts), lacking the oxygen evolving photosystem II.

Since cyanobacteria are responsible for oxygenation of the early atmosphere, the group has left the possibly deepest impact ever on earth's ecosystems. They are still of global importance for carbon cycling. Marine species are responsible for an estimated 20–40% of carbon dioxide fixation in oceans (e.g., Campbell and Vault, 1993). Since recent Cyanobacteria and the endosymbiotic plastids of green plants have common ancestors, most of the photosynthetic processes are basically due to the activity of Cyanobacteria and their ancestors.

Firmicutes/Actinobacteria

Next related to Cyanobacteria are the Firmicutes/Actinobacteria phyla (Stackebrandt and Schumann, 2006; Madigan et al., 2009). A common feature of all species within these groups is the lack of an outer membrane. Most members of the groups are chemoheterotroph and exhibit a thick peptidoglycan layer, resulting in a positive reaction of the Gram stain. Thus, the organisms are also referred to as “Gram-positive.” The thick cell wall may be also considered as an adaptation to the high osmotic stresses in terrestrial ecosystems (i.e., soil). The genera *Bacillus* and *Clostridium*, but especially the filamentous Actinobacteria (the genus *Streptomyces*, in particular) are important groups in soil habitats. Interestingly, also a small group of photosynthetic genera belongs to the Firmicutes. *Heliobacterium* and related genera are anaerobic, photoheterotrophic soil bacteria, with a minimal photosynthetic apparatus and incapable of carbon dioxide fixation (Asao and Madigan, 2010). *Frankia* genera are important nitrogen-fixing symbionts in alder roots. The production of different antibiotic agents

is another important feature, especially in Actinobacteria and *Bacillus*. Another unique feature within this lineage is the formation of endospores (Figure 2e).

Key genera of endospore formers are *Bacillus* and *Clostridium*. Whereas bacilli are aerobic respiratory organisms, clostridia lack a respiratory chain and are obligate anaerobic, fermenting organisms. They are important for anaerobic degradation of organic compounds, mostly producing organic acids (Table 4). Several clostridia as well as related acetogenic bacteria (e.g., *Acetobacterium*, *Acetogenium*) grow anaerobically either by using carbon dioxide as the terminal electron acceptor in anaerobic respiration with hydrogen as substrate (i.e., chemolithoautotrophically), or by fermenting carbohydrates. In either case, the acetyl-CoA pathway is used to reduce carbon dioxide to acetate. In the course of carbon dioxide reduction to acetate, a sodium ion gradient (instead of a proton gradient) across the membrane is generated that drives ATP synthesis.

Lactic acid bacteria obtain energy solely by fermentative processes. They typically have limited biosynthetic abilities, and need external sources of amino acids, vitamins, purines, and pyrimidines. This life-style is an adaptation to commensalism with other organisms: lactic acid bacteria mainly live on (decaying) plant leaves and fruit, the skin surface and the intestinal mucosa of mammals. Due to their ability to produce high amounts of lactic acid from carbohydrates (including lactose), their habitats are difficult to colonize by competing microorganisms. This also excludes pathogens from mucosal tissue. Another beneficial effect is the conservation of food (especially dairy products) by lactic acid fermentation.

Interestingly, some members of the Gram-positive lineage lost their peptidoglycan layer. These cell wall-less bacteria (key genera *Mycoplasma*, *Spiroplasma*) live as parasites on animal and plant tissue. The lack of a cell wall facilitates the flow of substrates from the host to the bacterium.

Proteobacteria

The most diverse bacterial lineage, the Proteobacteria, is divided into five subdivisions (alpha, beta, gamma, delta, and epsilon). Within most subdivisions, all metabolic key processes, ranging from chemo- and photoautotrophy to chemoheterotrophy, respiration, and fermentation (Tables 1–4), are common (Kersters et al., 2006; Madigan et al., 2009). Here, just the ecologically most important life-styles are pointed out. At least in three subdivisions, photoautotrophs are present (Overmann and Garcia-Pichel, 2006). All of them carry out anoxygenic photosynthesis with one photosystem; different types of intracytoplasmic membranes (lamellar, spheroidal) can be found (Tucker et al., 2010). Electron donors are reduced sulfur compounds (such as H_2S) in purple sulfur bacteria (Figure 2f) and mostly organic compounds (H_2S also in low concentrations) in purple non-sulfur bacteria. Purple non-sulfur bacteria are photoheterotrophic

organisms that utilize external carbon sources, if available. Most of them also fix carbon dioxide by the Calvin cycle.

Chemolithoautotrophy occurs in all proteobacterial subdivisions. Interestingly, ammonia and nitrite oxidation is mainly confined to the Proteobacteria. In four subdivisions (and one additional, recently discovered lineage, related to Proteobacteria) obligate chemolithotrophic nitrifying bacteria, oxidizing either ammonia to nitrite or nitrite to nitrate are present. Like phototrophs, also nitrifying bacteria contain internal membranes. Oxidation of sulfur and iron is represented by several groups in four proteobacterial subdivisions. Sulfur-oxidizing genera have been found in four proteobacterial subdivisions. Some genera are acidiphilic (e.g., *Acidithiobacillus*, *Thiomonas*). Among sulfur oxidizing chemolithotrophs (thiotrophic bacteria), exceptionally large morphotypes were described (*Beggiatoa*, *Thioploca*, *Thiomargarita*). Several sulfur oxidizers, especially from the gamma-subgroup, live in symbiosis with marine mollusks (Dubilier et al., 2008). Oxidation of hydrogen, already mentioned for some bacterial stem lineages, is also widespread in Proteobacteria. All hydrogen oxidizers are facultative chemolithotrophs, that is, they are also able to grow on organic compounds (Kelly and Wood, 2006).

Up to now, the aerobic methanotrophy is mainly confined to Proteobacteria, though recently, a methanotrophic verrucomicrobial bacterium could be found (Chistoserdova et al., 2009). Methylotrophic bacteria oxidize a variety of C₁ compounds (methanol, methylamine, formate). All methanotrophs possess methane monooxygenase as key enzyme for hydroxylation of the chemically inert methane to methanol. Some methanotrophic bacteria are also symbionts in marine animals.

Several genera are well-known model organisms especially for medical microbiology, such as pseudomonads and enterobacteria. Both groups are chemoorganotrophic, respiratory bacteria, enteric bacteria are facultative anaerobes. In global cycles, however, these organisms are largely insignificant. Especially enteric bacteria (within the gamma-subdivision), as commensals and pathogens of diverse higher organisms are well adapted to their hosts.

Sulfate and sulfur reduction is not only restricted to Proteobacteria. *Archaeoglobus*, as an example, is an archaeon, *Thermodesulfobacterium* is a deeply branching genus close to the root of the bacterial phylogenetic tree. Most of the sulfate- and sulfur-reducers are, however, proteobacteria that belong to the delta-subdivision. They are obligate anaerobes using mainly hydrogen, fatty and other organic acids or alcohols as energy sources. Sulfate or elemental sulfur serve as the terminal electron acceptors in an anaerobic respiration with hydrogen sulfide as product. The organic compounds are either oxidized to acetate or to carbon dioxide. These organisms are geochemically important, and are widespread in anaerobic sulfate-rich environments (Barton and Fauque, 2009).

Nitrogen fixation is a feature of multiple bacterial and archaeal genera, only a few groups are important symbiotic organisms. The alpha-proteobacterial Rhizobia

conduct specific symbiosis with legume plants by formation of root nodules.

Several proteobacterial groups exhibit differentiated life cycles: filamentous beta-Proteobacteria like *Spaerotilus* form bundles of sheathed filaments in eutrophic freshwater habitats. Under unfavorable conditions, flagellated swarmer cells are liberated from the open tip of a sheath. Sheaths of *Leptothrix* are involved in the precipitation of Fe³⁺ and oxidation of Mn⁴⁺. In iron-rich waters, mass developments of iron oxide-covered sheaths form large flocculent layers, which are also involved in the formation of bog iron deposits (Crerar et al., 1979). Prosthecae bacteria are primarily found among the alpha-proteobacterial subgroup. Members of this group have many different types of extensions (prosthecae) that contain cytoplasm and are bounded by the cell wall. Similar extensions are formed by the genera *Hyphomicrobium* and *Rhodomicrobium*. Daughter cells arise as growing buds from the hyphal tips. In *Caulobacter*, a stalk attaches the cell to a surface, whereas at the distal cell pole, a flagellated swarmer cell is formed. After division, the swarmer attaches to a new place and will form a new stalk at the formerly flagellated pole (Angert, 2005). The myxobacteria have a complex life cycle, which culminates in the production of fruiting bodies containing resting structures called myxospores. The formation of these structures requires the cooperation and coordinated differentiation of large numbers of cells. Aggregates of cells form through a chemotactic response (Zusman et al., 2007).

Summary

The brief overview points out that bacteria are taxonomically as well as metabolically more diverse than Archaea. Though the phylogenetic relationships among the bacterial phyla is still a matter of debate, several lines of evolutionary developments can be stated.

Chlorophyll-dependent photosynthesis must have been developed early in bacterial evolution, though some lineages may also have acquired genes of the photosynthetic apparatus by horizontal gene transfer. Fixation of carbon dioxide is not conducted via the Calvin cycle in stem groups, but a common feature in Cyanobacteria and Proteobacteria.

Except from Firmicutes/Actinobacteria and Planctomycetes, bacterial cell envelopes consist of two double-layer membranes. It is a matter of debate, if the Firmicutes are a lineage closest to the root of the bacterial phylogenetic tree or if the membrane got lost as a secondary event. The volume enclosed by both membranes, the periplasmic space, is, especially in Proteobacteria, of significance for cellular transport, DNA transfer, and cellular motility. Intracytoplasmic membranes are widespread in Proteobacteria as well as Cyanobacteria, but have not yet been detected in the basal lineages or in Archaea.

Several major phyla contain symbiotic and pathogenic organisms, especially Firmicutes and Proteobacteria

(as far as we know, no Archaeon is pathogenic in a strict sense). Especially within the bacterial “crown group,” complex host–symbiont/pathogen interaction has been developed. Mitochondria have very likely an alpha-proteobacterial ancestor (Gray et al., 1999).

Though biofilms, as an elementary type of multicellular organization, are already found in Archaea, some true multicellular stages in bacteria exhibit elaborate cellular differentiations and complex life cycles. In aquatic habitats, the organisms sometimes switch from planktonic to sessile stages. Several groups of bacteria exhibit diverse adaptations to terrestrial habitats: Firmicutes develop extremely durable and heat-resistant endospores, streptomycetes grow and develop like zygomycete or ascomycete fungal mycelia with branched hyphae and conidia-like exospores. A similar convergence with life cycles to a eukaryote is carried out by myxobacteria. Their life cycle has some striking analogies to cellular slime molds and represents the most complex pattern of bacterial differentiation known so far.

In brief, the development of photosynthesis, the colonization of terrestrial (soil habitats), and the interaction with eukaryotic hosts, are besides the diverse metabolic capabilities, the major achievements of bacteria.

Bibliography

- Asao, M., and Madigan, M. T., 2010. Taxonomy, phylogeny and ecology of the heliobacteria. *Photosynthesis Research*, **104**, 103–111.
- Barton, L., and Fauque, G. D., 2009. Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Advances in Applied Microbiology*, **68**, 41–98.
- Bernadet, J.-F., and Nakagawa, Y., 2006. An introduction to the family *Flavobacteriaceae*. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 455–480.
- Campbell, L., and Vaulot, D., 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). *Deep-Sea Research Part I: Oceanographic Research Papers*, **40**, 2043–2060.
- Charon, N. W., Greenberg, E. P., Koopman, M. B., and Limberger, R. J., 1992. Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. *Microbiological Research*, **143**, 597–603.
- Chistoserdova, L., Kalyuzhnaya, M. G., and Lidstrom, M. E., 2009. The Expanding World of Methylotrophic Metabolism. *Annual Review of Microbiology*, **63**, 477–499.
- Ciccarelli, F. D., Doerks, T., von Mering, C., Creevey, C. J., Snel, B., and Bork, P., 2006. Toward Automatic Reconstruction of a highly resolved tree of life. *Science*, **311**, 1283–1287.
- Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. A., Marsh, T., Garrity, G. M., and Tiedje, J. M., 2008. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research*, **37**, D141–D145.
- Crerar, D. A., Knox, G. W., and Means, J. L., 1979. Biogeochemistry of bog iron in the New Jersey Pine Barrens. *Chemical Geology*, **24**, 111–135.
- Da Costa, M. S., Rainey, F. A., and Nobre, M. F., 2006. The genus *Thermus* and relatives. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 797–812.
- Dubilier, N., Bergin, C., and Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosymbiosis. *Nature Reviews Microbiology*, **6**, 725–740.
- Gray, M. W., Burger, G., and Lang, B. F., 1999. Mitochondrial evolution. *Science*, **283**, 1476–1481.
- Hanada, S., and Pierson, B. K., 2006. The family Chloroflexaceae. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 815–842.
- Huber, R., and Eder, W., 2006. Aquificales. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 925–938.
- Kelly, D. P., and Wood, A. P., 2006. The chemolithotrophic Bacteria. In Balows, et al. (eds.), *The Prokaryotes* 2. Heidelberg: Springer, pp. 441–456.
- Kerstens, K., de Vos, P., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E., 2006. Introduction to Proteobacteria. In Balows, et al. (eds.), *The Prokaryotes* 5. Heidelberg: Springer, pp. 3–37.
- Lee, K. C., Webb, R. I., Janssen, P. H., Sangwan, P., Romeo, T., Staley, J. T., and Fuerst, J. A., 2009. Phylum Verrucomicrobia representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. *BMC Microbiology*, **9**, 5.
- Lonhienne, T. G., Sagulenko, E., Webb, R. I., Lee, K. C., Franke, J., Devos, D. P., Nouwens, A., Carroll, B. J., and Fuerst, J. A., 2010. Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. *Proceedings of the National Academy of Sciences USA*, **107**, 12883–12888.
- Madigan, M. T., Dunlap, P. V., and Clark, D. P., 2009. *Brock Biology of Microorganisms*, 12th edn. San Francisco: Pearson/Benjamin Cummings.
- Nelson, K. E., Clayton, R. A., Gill, S. R. et al., 1999. Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. *Nature*, **399**, 323–329.
- Overmann, J., and Garcia-Pichel, F., 2006. The phototrophic way of life. In Balows, et al. (eds.), *The Prokaryotes* 1. Heidelberg: Springer, pp. 82–85.
- Oyaizu, H., Debrunner-Vossbrinck, B., Mandelco, L., Studier, J. A., and Woese, C. R., 1987. The green non-sulfur bacteria: a deep branching in the eubacterial line of descent. *Systematic and Applied Microbiology*, **9**, 47–53.
- Pace, N. R., 2009. Mapping the tree of life. *Microbiology and Molecular Biology Reviews*, **73**, 565–576.
- Schlegel, H. G., 1999. *Geschichte der Mikrobiologie (Acta Historica Leopoldina 28)*. Halle (Saale): Deutsche Akademie der Naturforscher Leopoldina.
- Schlegel, H. G., and Jannasch, H. W., 2006. Prokaryotes and their habitats. In Balows, et al. (eds.), *The Prokaryotes* 1. Heidelberg: Springer, pp. 137–184.
- Stackebrandt, E., and Schumann, P., 2006. Introduction to the taxonomy of Actinobacteria. In Balows, et al. (eds.), *The Prokaryotes* 3. Heidelberg: Springer, pp. 297–321.
- Szabó, Z., Stahl, A. O., Albers, S. V., Kissinger, J. C., Driessen, A. J., and Pohlschröder, M., 2007. Identification of diverse archaeal proteins with class III signal peptides cleaved by distinct archaeal prepilin peptidases. *J Bacteriol*, **189**, 772–778.
- Tucker, J. D., Siebert, C. A., Escalante, M., Adams, P. G., Olsen, J. D., Otto, C., Stokes, D. L., and Hunter, C. N., 2010. Membrane invagination in *Rhodobacter sphaeroides* is initiated at curved regions of the cytoplasmic membrane, then forms both budded and fully detached spherical vesicles. *Molecular Microbiology*, **76**, 833–847.
- Waterbury, J. B., 2006. The Cyanobacteria – isolation, purification, and identification. In Balows, et al. (eds.), *The Prokaryotes*. Heidelberg: Springer, pp. 1053–1073.
- Woese, C. R., and Fox, G. E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Science USA*, **74**, 5088–5090.
- Wu, M., and Eisen, J. A., 2008. A simple, fast, and accurate method of phylogenomic inference. *Genome Biology*, **9**, R151.

- Zinder, S. H., and Dworkin, M., 2006. Morphological and physiological diversity. In Balows, et al. (eds.), *The Prokaryotes 1*. Heidelberg: Springer, pp. 185–220.
- Zusman, D. R., Scott, A. E., Yang, Z., and Kirby, J. R., 2007. Chemosensory pathways, motility and development in *Myxococcus xanthus*. *Nature Reviews of Microbiology*, **5**, 862–872.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Bacterioplankton](#)
[Cyanobacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fluorescence In Situ Hybridisation \(FISH\)](#)
[Gallionella](#)
[Halobacteria – Halophiles](#)
[Histology](#)
[Hydrogen](#)
[Leptothrix](#)
[Metallogenium](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Communities, Structure, and Function](#)
[Nan\(n\)obacteria](#)
[Piezophilic Bacteria](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

BACTERIOPLANKTON

Thomas Pommier
 CNRS-Université Lyon, Villeurbanne, France

Synonyms

Aquatic bacteria

Definition

Bacterial component of the plankton that drifts in the water column of both seawater and freshwater ecosystems.

Introduction

The name bacterioplankton comes from the association of the Greek word *πλαγκτός* “planktós,” meaning “wanderer” or “drifter,” and bacterium, a word coined in the nineteenth century by Christian Gottfried Ehrenberg (Thurman, 1997). In contrast to land, microbes drive the ecology of the aquatic environments both as producers and consumers of fixed carbon. Their colossal biomass vastly outreaches the ones of all other members of the oceanic and freshwaters biota. Considering its size, the Ocean encompasses more bacteria than the count of known stars (estimated to 10^{21}) in the Universe. Though its role in aquatic ecosystems was for long eluded, the bacterioplankton carries out the largest fraction of the biological activity and occupies a range of ecological niches.

Enumeration and description

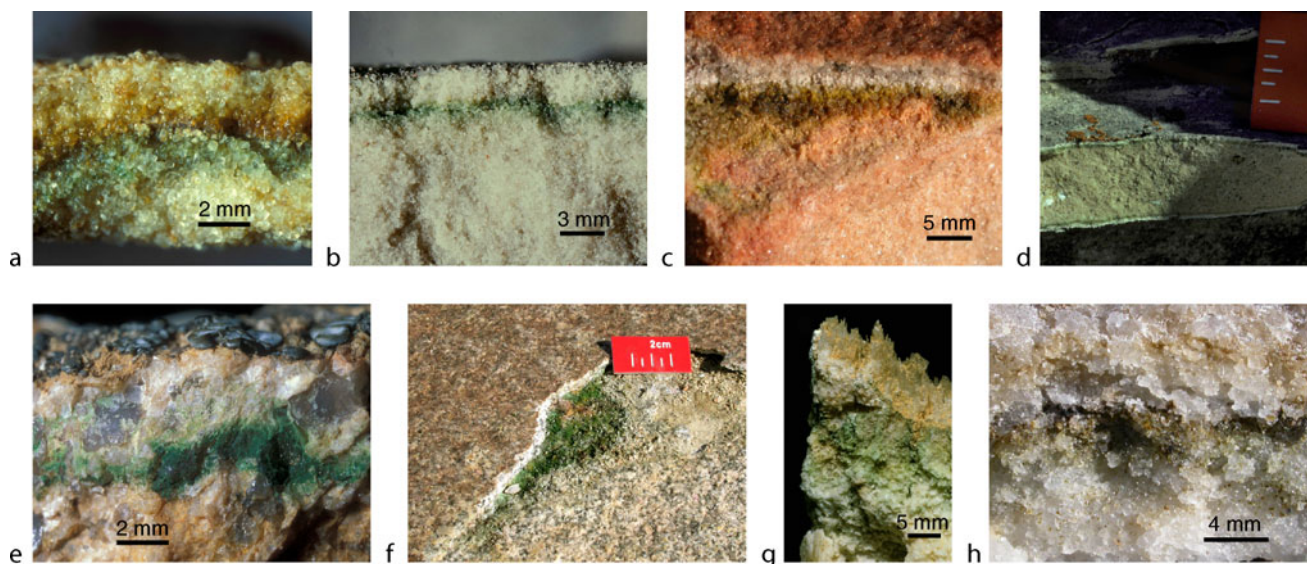
Since the beginning of microbiology, counting the number of bacteria that occur in various aquatic environments has been an exciting subject of investigation. Because of the size and the mobility of the bacterioplankton, early bacteriologists had difficulties to simply enumerate them with a conventional microscope. With the development of appropriate techniques (e.g., size selection on membrane filters and DNA/RNA specific fluorochromes stainings), as well as advances in microscopy, bacterioplanktonic cells could be observed and counted (Hobbie et al., 1977). This method indicated that the bacterioplankton were on an average $\sim 10^6$ cells per milliliter of water. Recent developments in flow cytometry have confirmed and sharpened these counts (Amann et al., 1990). In the water column, the bacterioplankton is also exposed to grazing activity by larger protists and viral infections, but irrespective to variations in primary productivity, these two causes of mortality seem to maintain a relatively constant abundance.

Though it is not always mentioned in the current literature, a clear distinction should be made between the fraction of the bacterioplankton that is attached to drifting particles of organic matters (also called “marine snow” in the marine environment) and the fraction that is free-living in the water column. A common trait for free-living bacterioplankton is their small size, or small cell volume, ranging from 0.1 to $0.22 \mu\text{m}^3$, which is about ten times lower than that of the most attached bacterioplankton in aquatic habitats or in soils (Fenchel et al., 1998) and the most cultured cells.

Bacterioplankton biological activity

Based on both direct observations and indirect measurements of bacterial growth rates in whole seawater (e.g., frequency of dividing cells (Hagström et al., 1979), radioactive thymidine or leucine incorporation (Fuhrman and Azam, 1982; Chin-Leo and Kirchman, 1988)) as well as when bacterial grazers (protozoans) were removed (Vacelet, 1972), generation times (i.e., the time required to double population) of most bacterioplanktons range from 0.049 to 0.080 h^{-1} .

Because a significant proportion of the bacterioplankton (99%) of the total counted cells fail to grow on nutrient-rich agar plates, it has been suggested that most counted cells were either starving cells, dormant (Stevenson and Schmidt, 1998) or even non-viable cells (Kjelleberg et al., 1987). Different in situ measurements of amino acids or glucose uptake by microautoradiography (Hoppe, 1976) indicate that from 5% to >60% of the cells are metabolically active in different aquatic environments (Tabor and Neihof, 1984; Fuhrman and Ferguson, 1986; Smith and del Giorgio, 2003). Whether or not most bacteria in aquatic environment are dormant remains controversial, most scientists will agree however that the bacterial community, as a whole and over



Chroococcidiopsis, Figure 1 *Chroococcidiopsis* endolithic in rocks. (a) Sandstone of the Clarens formation, Golden Gate Highlands National Park, South Africa. (b) Tshipise Sandstone Plateau, Limpopo Province, South Africa. (c) Beacon Sandstone, Dry Valleys, Antarctica. (d) Cocconino Sandstone, Colorado Plateau, Arizona, USA. (e) Granite inselberg, Limpopo Province, South Africa. (f) Granite rock boulder, Dry Valleys, Antarctica. (g) Gypsum from a sebkah, Tunisia. (h) Calcite rock outcrop, Dry Valleys, Antarctica.

Lichinomycetes group (see entry “*Fungi and Lichens*”), mostly growing on rock surfaces of hot deserts. As an endolith, it contributes to biogenic weathering (see entry “*Bioerosion*”) of silicate rocks by photosynthetic alkalization (Büdel et al., 2004).

Bibliography

- Büdel, B., and Rhiel, E., 1985. A new cell wall structure in a symbiotic and a free-living strain of the blue-green alga genus *Chroococcidiopsis* (Pleurocapsales). *Archiv für Mikrobiologie*, **143**, 117–121
- Büdel, B., Weber, B., Kühl, M., Pfanz, H., Sültemeyer, D., and Wessels, D. C. J., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268
- Fewer, D., Friedl, T., and Büdel, B., 2002. *Chroococcidiopsis* and heterocyst-differentiating cyanobacteria are each others’ closest living relatives. *Molecular Phylogenetics and Evolution*, **41**, 498–506
- Geitler, L., 1933. Diagnosen neuer Blaualgen von den Sunda-Inseln. *Archiv für Hydrobiologie*, **4**(Supplement 12 Tropische Binnengewässer), 622–634
- Komárek, J., and Anagnostidis, K., 1998. *Cyanoprokaryota 1. Teil Chroococcales*. Jena: Gustav Fischer.

Cross-references

[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biological Volcanic Rock Weathering](#)
[Cyanobacteria](#)
[Desert Varnish](#)
[Endoliths](#)
[Extreme Environments](#)
[Fungi and Lichens](#)
[Geomycology](#)

CLAY AUTHIGENESIS, BACTERIAL

Kurt O. Konhauser
 University of Alberta, Edmonton, Alberta, Canada

Definition

The formation of clay minerals in situ through the direct or indirect action of bacteria.

Overview

Clays are one of the largest and most diverse assemblage of minerals known. The vast majority of clay minerals are aluminum silicate in composition, with various other cations substituted into the crystal lattice. The replacement of either Si or Al can lead to an excess of negative charge, which is either neutralized by the adsorption of additional cations onto the outer mineral surfaces or within the interlayer spaces.

Within the past 2 decades, field studies have led to the general recognition that bacteria can mediate the formation of clay minerals. Some clays form as replacement products from the alteration of primary minerals. In this regard, the bacterial community growing on the rock surface potentially function initially as the weathering agents (through the production of organic acids or ligands that facilitate mineral dissolution) and later as reactive surfaces for the nucleation of secondary clay phases. For instance, Konhauser et al. (2002) documented that highly altered rocks found within active steam vents at Kilauea Volcano, Hawaii, contained bacteria with small (<500 nm in diameter) grains of smectite $[(\text{Al}_{1.6}\text{Fe}_{0.4})(\text{Si}_{3.8}\text{Al}_{0.2})\text{O}_{10}(\text{OH})_2\cdot\text{K}_{0.2}]$ attached directly to the outer cell walls.

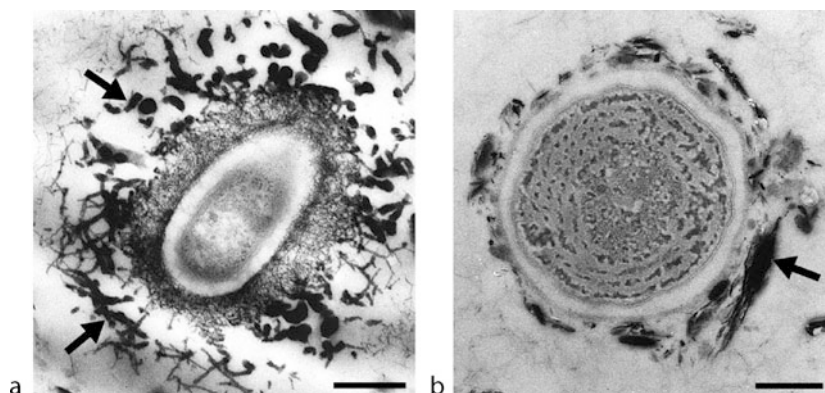
Many of the elements now incorporated into the clays originated when the volcanic glass dissolved and released them into the pore waters. Leveille et al. (2002) similarly documented the formation of kerolite $[\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}]$ in association with microbial mats colonizing the surfaces of walls and ceilings within basaltic sea caves on the north coast of Kauai, Hawaii. That microorganisms were in some way involved in mineral formation was suggested by the lack of kerolite within basalt vesicles near the rock surface or in the carbonate-rich evaporitic coatings found on other exposed surfaces without the presence of microbial mats.

Other clays form in environments far removed from the actual sites of weathering. The best examples come from biofilms retrieved from the sediment–water interface of lakes and rivers. Ferris et al. (1987) initially described complex (Fe,Al)-silicates on bacterial cells growing in metal-contaminated lake sediment in northern Ontario. These precipitates ranged from poorly ordered and uncharacterized phases to crystalline forms of the Fe-rich chlorite, chamosite $[(\text{Fe})_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2]$. Since then, similar clayey precipitates have been reported from various rivers around the world (e.g., Konhauser et al., 1998). What is particularly remarkable about bacterially mediated clays is that they share a number of properties, irrespective of the chemical composition of the waters from which they were sampled: (1) They are generally amorphous to poorly ordered structures. (2) All have grains sized $<1\text{ }\mu\text{m}$, although the majority are $<100\text{ nm}$. (3) The grains have a composition dominated by iron, silicon, and aluminium in varying amounts.

Based on the observations from natural studies, a general sequence of events leading to clay biomineralization can be adduced (Konhauser and Urrutia, 1999). In the initial stages, a bacterium adsorbs any number of different free aqueous iron species, i.e., Fe^{2+} , Fe^{3+} , $\text{Fe}(\text{OH})^{2+}$, and $\text{Fe}(\text{OH})_2^+$, depending on solution chemistry and redox potential. Adsorption can occur on either the cell wall,

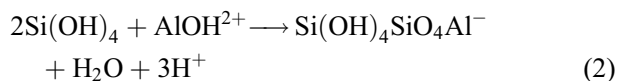
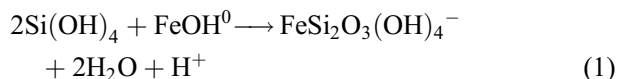
which is frequently lined with anionic carboxyl and phosphate functional groups, or to the more neutrally charged carbohydrate-rich extracellular layers that surround the cell, including sheaths and capsules – the latter are the organic components that make up the bulk of biofilms. If the soluble iron concentration around the cell surface exceeds the solubility product of ferric hydroxide, then the reaction between the bound iron with dissolved iron will lead to the development of small ($\sim 100\text{ nm}$ in diameter), dense, mineral aggregates on the outer cell surface (Figure 1a). Indeed, it has been shown experimentally that a continuum exists between cationic iron sorption and precipitation reactions at bacterial surfaces; with the three stages of (1) sorption, (2) surface site saturation and (3) precipitation, all evident in Langmuir-type isotherms relating the solid-phase concentration of Fe(III) to the equilibrium proton and soluble Fe(III) concentrations (Warren and Ferris, 1998). Alternatively, bacteria can attract preformed nanometer-sized ferric hydroxide particles from suspension (Glasauer et al., 2001), thereby negating the need for the nucleation step. In most solutions, iron is only found in trace amounts compared to other ions, particularly silica. Under those conditions, the adsorbed/particulate iron may instead serve as kinetically favorable site for the development of more complex precipitates of variable clay composition, morphology, and structure (Figure 1b).

Why these clays form is likely as follows. In the pH range of most natural water bodies, negatively charged counter-ions, or those molecules that are neutrally charged but exhibit residual surface electronegativity (e.g., oligomeric and colloidal silica species), accumulate near the solution-adsorbed iron/solid interface to neutralize the net positive charge of iron. Two surface species of iron oxide exist in this pH range; FeOH_2^+ and $\text{Fe}(\text{OH})^0$, but the majority of the surface charge is positive at circumneutral pH. The initial (Fe,Al)-silicate phases then form via hydrogen bonding between the hydroxyl groups



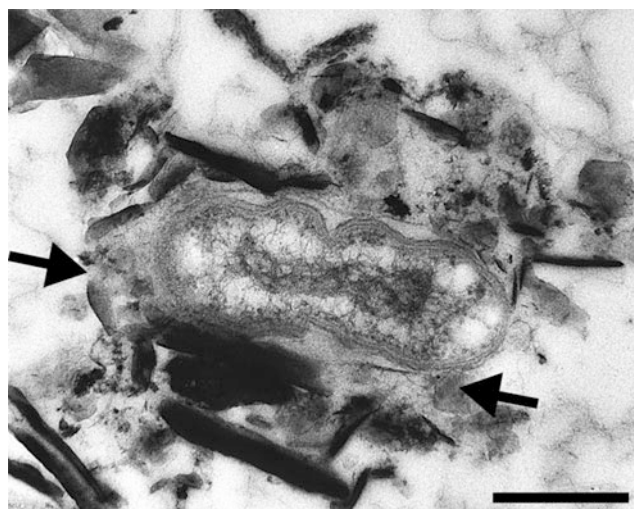
Clay Authigenesis, Bacterial, Figure 1 Transmission electron micrographs (TEM) of bacteria (stained) from the Rio Solimões in Brazil. (a) Fe-mineralized capsule with formation of dense Fe-rich aggregates on the outer edges (arrow). (b) Partially encrusted cyanobacterium with various amorphous clay precipitates (arrow) tangential to the cell wall and surrounding sheath. Scale bars = 500 nm.

associated with the cell-bound iron and the hydroxyl groups in the dissolved silica, aluminum, or aluminosilicate complexes (e.g., Taylor et al., 1997; Davis et al., 2002). Exactly how these reactions occur in nature has not been ascertained, but we do know that dimeric silica (the species that accounts for more than 99% of the oligomeric silica in natural waters) is highly reactive toward iron hydroxide surfaces (Reaction 1), and that oligomeric silica exhibits a strong affinity for dissolved aluminum, forming soluble hydroxy-aluminosilicate ions (e.g., Reaction 2) that subsequently react with cell-bound iron.



This arrangement of ions forms an electric double layer with iron attaching to the bacterial surface as an inner sphere complex, while the silica-aluminosilicate species attach as more diffuse outer layers. The surface charge of these composites is inevitably dependent upon the solution pH, the ionic strength of the solution, and the time of reaction, such that as the particles age more silica (and aluminium) sorb. Interestingly, this mechanism of binding Fe to the bacterial cell surface and subsequent reaction with Si and Al from solution has been shown in experimental systems with *Bacillus subtilis* (e.g., Urrutia and Beveridge, 1994). Accordingly, if the microbial mats are subject to sufficiently concentrated solutions, then continued reaction of the dissolved components to the Fe-bearing solid interface eventually results in the formation of amorphous to poorly ordered clay phases. Often, these reactions lead to the partial and complete encrustation of some bacterial cells as abiological surface reactions accelerate the rate of mineral precipitation: in some biofilms, the density of clayey material surrounding individual cells can extend hundreds of nanometers away from the cell surface (Figure 2). Over time, these hydrous compounds dehydrate, some converting to more stable crystalline forms. This latter material presumably uses some fraction of the precursor surface as a template for its own growth, in effect circumventing the need for direct nucleation (Steeff and van Cappellen, 1990). Once it begins to grow, the more stable clay phase increases its own surface area until it can control the composition of the proximal solution. When this happens, the saturation state of the solution moves below the solubility of the precursor, inhibiting further growth, or even dissolving the precursor.

The reactions above are naturally an oversimplification because a number of inorganic processes are also at play. It is well known that soluble Al–Si complexes precipitate as poorly ordered Al–silicates when a state of supersaturation is achieved (Wada and Wada, 1980). Moreover, Fe ions can readily be incorporated into those structures



Clay Authigenesis, Bacterial, Figure 2 TEM image of highly encrusted bacterium from the Speed River in Ontario, with clay minerals extending over 500 nm away from cell surface. Arrow indicates surrounding capsule in which the clay minerals have formed. Scale bar = 500 nm.

leading to clay-like products (Farmer et al., 1991). All these reactions can conceivably take place in the proximity of bacterial surfaces or within the extensive EPS, particularly since diffusion through the polysaccharide matrix is inherently slow, and a microenvironment can be established that is conducive to mineralization. Additionally, colloidal species of (Fe, Al)-silicate composition that either form initially in the water column or are products of weathering and soil formation, may react directly with the outermost cellular layers. It follows that anything which will neutralize or diminish the charge of the colloids (e.g., bacterial surface if colloids are positively charged or adsorbed iron if the colloids are negatively charged) will cause the particles to flocculate out of solution.

One of the intriguing questions that arises from the observation of bacterial cells completely encrusted in clays is why do they allow for mineralization to occur? It has been established that bacteria have the ability to partially control their surface charge (Doyle, 1989). During metabolism, a membrane-induced proton motive force continuously pumps protons into the wall fabric that effectively compete with metal ions for anionic wall sites (Urrutia et al., 1992). Subsequently, living bacteria may be able to reduce authigenic mineral formation (and detrital mineral adhesion) on their cellular surfaces, unless environmental conditions exceed their capacity to compensate. In most aqueous environments with low dissolved solutes, this condition should not arise. Therefore, the presence of epicellular minerals on bacteria suggests that (1) the cells observed under the electron microscope had lysed prior to mineralization, or (2) that the mineralized matrix is in some way advantageous to the microorganism. Several possibilities for the latter

exist. First, clays provide a good source of exchangeable inorganic nutrients readily available to microbes (Tazaki et al., 1994). Second, clays such as montmorillonite stimulate the respiration of bacteria by maintaining the pH and water content of the environment at a level suitable for growth (Stotzky and Rem, 1966). Third, the formation of an external mineralized matrix may protect the living cells from the detrimental effects of toxin-producing microorganisms (Habte and Barrion, 1984) and predation by grazing protozoans (e.g., Heynen et al., 1988).

Summary

Microorganisms are commonly associated with fine-grained (Fe, Al)-silicates of variable composition. The inorganic phases develop in a predictable manner, beginning with the adsorption of cationic iron to anionic cellular surfaces, supersaturation of the proximal fluid with Fe (III), nucleation and precipitation of a precursor ferric hydroxide phase on the cell surface, followed by reaction with dissolved silica and aluminium, and eventually the growth of an amorphous clay-like phase.

Bibliography

- Davis, C. C., Chen, H. W., and Edwards, M., 2002. Modelling silica sorption to iron hydroxide. *Environmental Science and Technology*, **36**, 582–587.
- Doyle, R. J., 1989. How cell walls of gram-positive bacteria interact with metal ions. In: Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley, pp. 275–293.
- Farmer, V. C., Krishnamurti, G. S. R., and Huang, P. M., 1991. Synthetic allophane and layer-silicate formation in $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-FeO-Fe}_2\text{O}_3\text{-MgO-H}_2\text{O}$ systems at 23°C and 89°C in a calcareous environment. *Clays and Clay Minerals*, **39**, 561–570.
- Ferris, F. G., Fyfe, W. S., and Beveridge, T. J., 1987. Bacteria as nucleation sites for authigenic minerals in a metal-contaminated lake sediment. *Chemical Geology*, **63**, 225–232.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2001. Sorption of Fe Hydr(oxides) to the surface of *Shewanella putrefaciens*: cell-bound fine-grained minerals are not always formed de novo. *Applied and Environmental Microbiology*, **67**, 5544–5550.
- Habte, M., and Barrion, M., 1984. Interaction of *Rhizobium* sp. with toxin producing fungus in culture medium and in a tropical soil. *Applied and Environmental Microbiology*, **47**, 1080–1083.
- Heynen, C. E., van Elsas, J. D., Kuikman, P. J., and van Veen, J. A., 1988. Dynamics of *Rhizobium leguminosarum* biovar *trifolii* introduced in soil; the effect of bentonite clay on predation by protozoa. *Soil Biology and Biochemistry*, **20**, 483–488.
- Konhauser, K. O., and Urrutia, M. M., 1999. Bacterial clay authigenesis: a common biogeochemical process. *Chemical Geology*, **161**, 399–413.
- Konhauser, K. O., Fisher, Q. J., Fyfe, W. S., Longstaffe, F. J., and Powell, M. A., 1998. Authigenic mineralization and detrital clay binding by freshwater biofilms: the Brahmani River, India. *Geomicrobiology Journal*, **15**, 209–222.
- Konhauser, K. O., Schiffman, P., and Fisher, Q. J., 2002. Microbial mediation of authigenic clays during hydrothermal alteration of Basaltic Tephra, Kilauea Volcano. *Geochemistry, Geophysics, and Geosystems*, **3**(12), 1–13.
- Leveille, R. J., Longstaffe, F. J., and Fyfe, W. S., 2002. Kerolite in carbonate-rich speleothems and microbial deposits from Basaltic Sea Caves, Kauai, Hawaii. *Clays and Clay Minerals*, **50**, 514–524.
- Steefel, C. I., and van Cappellen, P., 1990. A new kinetic approach to modeling water–rock interaction: the role of nucleation, precursors, and ostwald ripening. *Geochimica et Cosmochimica Acta*, **54**, 2657–2677.
- Stotzky, G., and Rem, L. T., 1966. Influence of clay minerals on microorganisms. 1. Montmorillonite and kaolinite on bacteria. *Canadian Journal of Microbiology*, **12**, 547–563.
- Taylor, P. D., Jugdaohsingh, R., and Powell, J. J., 1997. Soluble silica and high affinity for aluminium under physiological and natural conditions. *Journal of the American Chemical Society*, **119**, 8852–8856.
- Tazaki, K., Fyfe, W. S., Iizumi, S., Sampei, Y., Watanabe, H., Goto, M., Miyake, Y., and Noda, S., 1994. Clay aerosols and arctic ice algae. *Clays and Clay Minerals*, **42**, 402–408.
- Urrutia, M. M., and Beveridge, T. J., 1994. Formation of fine-grained metal and silicate precipitates on a bacterial surface (*Bacillus subtilis*). *Chemical Geology*, **116**, 261–280.
- Urrutia, M. M., Kemper, M., Doyle, R., and Beveridge, T. J., 1992. The membrane-induced proton motive force influences the metal binding ability of *Bacillus subtilis* cell walls. *Applied and Environmental Microbiology*, **58**, 3837–3844.
- Wada, S. I., and Wada, K., 1980. Formation, composition and structure of hydroxy-aluminosilicate ions. *Journal of Soil Science*, **31**, 457–467.
- Warren, L. A., and Ferris, F. G., 1998. continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Cross-references

Black Shales
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Microbial Silicification – Bacteria (or Passive)
 Microbial Surface Reactivity
 Soils

COCCOLITHOPHORES

Volker Thiel

University of Göttingen, Göttingen, Germany

Coccolithophores (coccolithophorids) are a group of unicellular, marine, planktonic algae belonging to the haptophytes (Prymnesiophyta). The coccolithophores are capable of controlling the intracellular precipitation of calcite onto organic plates and the assembly of the mature carbonate scales at the cell surface. These scales, the coccoliths, are spherical or oval, often intricately patterned and generally less than 20 µm in diameter. Given the abundance of coccoliths in sediments, coccolithophorids have been playing a major role in the global carbon budget. They were very abundant in the Mesozoic era, particularly in the Cretaceous period, in which they became a major component of the “Chalk” lithology (e.g., White Cliffs of Dover, England). Today, coccolithophorids are accounting for about a third of the total marine CaCO_3 production, and coccoliths are a major component of the

- Zinder, S. H., and Dworkin, M., 2006. Morphological and physiological diversity. In Balows, et al. (eds.), *The Prokaryotes 1*. Heidelberg: Springer, pp. 185–220.
- Zusman, D. R., Scott, A. E., Yang, Z., and Kirby, J. R., 2007. Chemosensory pathways, motility and development in *Myxococcus xanthus*. *Nature Reviews of Microbiology*, **5**, 862–872.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Bacterioplankton](#)
[Cyanobacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fluorescence In Situ Hybridisation \(FISH\)](#)
[Gallionella](#)
[Halobacteria – Halophiles](#)
[Histology](#)
[Hydrogen](#)
[Leptothrix](#)
[Metallogenium](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Communities, Structure, and Function](#)
[Nan\(n\)obacteria](#)
[Piezophilic Bacteria](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

BACTERIOPLANKTON

Thomas Pommier
 CNRS-Université Lyon, Villeurbanne, France

Synonyms

Aquatic bacteria

Definition

Bacterial component of the plankton that drifts in the water column of both seawater and freshwater ecosystems.

Introduction

The name bacterioplankton comes from the association of the Greek word *πλαγκτός* “planktós,” meaning “wanderer” or “drifter,” and bacterium, a word coined in the nineteenth century by Christian Gottfried Ehrenberg (Thurman, 1997). In contrast to land, microbes drive the ecology of the aquatic environments both as producers and consumers of fixed carbon. Their colossal biomass vastly outreaches the ones of all other members of the oceanic and freshwaters biota. Considering its size, the Ocean encompasses more bacteria than the count of known stars (estimated to 10^{21}) in the Universe. Though its role in aquatic ecosystems was for long eluded, the bacterioplankton carries out the largest fraction of the biological activity and occupies a range of ecological niches.

Enumeration and description

Since the beginning of microbiology, counting the number of bacteria that occur in various aquatic environments has been an exciting subject of investigation. Because of the size and the mobility of the bacterioplankton, early bacteriologists had difficulties to simply enumerate them with a conventional microscope. With the development of appropriate techniques (e.g., size selection on membrane filters and DNA/RNA specific fluorochromes stainings), as well as advances in microscopy, bacterioplanktonic cells could be observed and counted (Hobbie et al., 1977). This method indicated that the bacterioplankton were on an average $\sim 10^6$ cells per milliliter of water. Recent developments in flow cytometry have confirmed and sharpened these counts (Amann et al., 1990). In the water column, the bacterioplankton is also exposed to grazing activity by larger protists and viral infections, but irrespective to variations in primary productivity, these two causes of mortality seem to maintain a relatively constant abundance.

Though it is not always mentioned in the current literature, a clear distinction should be made between the fraction of the bacterioplankton that is attached to drifting particles of organic matters (also called “marine snow” in the marine environment) and the fraction that is free-living in the water column. A common trait for free-living bacterioplankton is their small size, or small cell volume, ranging from 0.1 to $0.22 \mu\text{m}^3$, which is about ten times lower than that of the most attached bacterioplankton in aquatic habitats or in soils (Fenchel et al., 1998) and the most cultured cells.

Bacterioplankton biological activity

Based on both direct observations and indirect measurements of bacterial growth rates in whole seawater (e.g., frequency of dividing cells (Hagström et al., 1979), radioactive thymidine or leucine incorporation (Fuhrman and Azam, 1982; Chin-Leo and Kirchman, 1988)) as well as when bacterial grazers (protozoans) were removed (Vacelet, 1972), generation times (i.e., the time required to double population) of most bacterioplanktons range from 0.049 to 0.080 h^{-1} .

Because a significant proportion of the bacterioplankton (99%) of the total counted cells fail to grow on nutrient-rich agar plates, it has been suggested that most counted cells were either starving cells, dormant (Stevenson and Schmidt, 1998) or even non-viable cells (Kjelleberg et al., 1987). Different in situ measurements of amino acids or glucose uptake by microautoradiography (Hoppe, 1976) indicate that from 5% to >60% of the cells are metabolically active in different aquatic environments (Tabor and Neihof, 1984; Fuhrman and Ferguson, 1986; Smith and del Giorgio, 2003). Whether or not most bacteria in aquatic environment are dormant remains controversial, most scientists will agree however that the bacterial community, as a whole and over

long period of time, plays a paramount role in global biochemical processes.

The blue-green algae, or *Cyanobacteria*, are the “plants” of the bacterioplankton and carry out photosynthesis, which supports the entire ocean food web. In the open ocean, these phototrophic bacteria are responsible for about 80% of the total oceanic primary production, which represents about half of the planet’s primary production (ranging from 35 to 65 Gt of carbon per year) (del Giorgio and Duarte, 2002). Moreover, a new type of phototrophy has recently been discovered among marine bacterioplankton that carry bacteriorhodopsin molecules, which scavenge light to create an energetic gradient of protons (Beja et al., 2001). Since ~13% of the bacterioplankton cells in the photic zone may carry out proteorhodopsin-based phototrophy (Sabeji et al., 2005), this metabolism surely has a global significance and may be the foremost future investigation field in aquatic microbiology.

However in numbers, marine bacterioplankton are considered mostly heterotrophs, or more precisely saprotrophs, and obtain energy by consuming dissolved organic matter (DOM) released by larger aquatic animals (Smith et al., 1992) or by phytoplankton exudation (Larsson and Hagström, 1979). This activity, known as the “microbial loop” (Azam et al., 1983), is crucial to the aquatic trophic foodwebs because essential inorganic nutrients are recycled through bacterial metabolism into the water column.

Heterotrophic and phototrophic bacterioplankton are continuously grazed by protozoans (i.e., mostly flagellates and ciliates), which are later consumed by larger zooplankton, which are subsequently consumed by fishes. Phages (i.e., viruses infecting bacteria) also affect DOM turnover by lysing cells, which releases organic matter available to surrounding bacterioplankton cells (Proctor and Fuhrman, 1990).

A large fraction of the carbon flow in the aquatic ecosystem is processed by heterotrophic bacterioplankton that respire particulate and dissolved organic carbon (DOC) (del Giorgio and Duarte, 2002). Especially with the rapid increase in global atmospheric CO₂ due to modern human activities, this bacterial process is recognized as a key component in global climate regulation (Falkowski et al., 2000).

Bacterioplankton diversity and global distribution

While growth on agar plates, microscopic observations were intensively used to delineate several types of bacteria, their capacity of differentiation showed quickly to be too restrictive and new methods were required to distinguish between two species with similar cell and colony morphologies. Moreover, a general concern grew among microbiologists when they realized that many bacteria counted under the microscope could not be cultivated on agar plate or in liquid media. For instance, marine microbiologists estimated that only 1% of the cells counted

under the microscope formed colonies on agar plates, and named this issue the “Great plate count anomaly” (Jannasch and Jones, 1959; Kogure et al., 1979).

In the early 1980s, a breakthrough in bacterial taxonomy was achieved by Woese et al., who utilized ribosomal ribonucleic acids (rRNA) to catalogue related bacterial species (Fox et al., 1980; Woese et al., 1985, 1987). Applying this approach to aquatic environmental samples often recovers members from about 18 bacterial clades, mostly represented within the α -*Proteobacteria* and γ -*Proteobacteria* phyla. Moreover, members from the *Cyanobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctobacteria*, *Fibrobacter*, *Chloroflexi* and β -*Proteobacteria* are often retrieved from environmental rRNA genes clone libraries. Interestingly, though some members of the global bacterioplankton community (e.g., the so-called SAR11 clade) are recurrently found in environmental samples, and therefore show a ubiquitous distribution, endemism of rRNA genes is the most common trait of the bacterioplankton, and their diversity follows a latitudinal gradient as observed in the other macro-organisms (Pommier et al., 2007).

The extensive use of the SSU rRNA as phylogenetic marker has actually reached the level where variations in the bacterioplankton composition can be linked to observed changes both in natural (Acinas et al., 1999; Cottrell and Kirchman, 2000; Hollibaugh et al., 2002) and reconstructed environments (i.e., meso- or microcosms) (Riemann et al., 2000). The current view of marine bacterioplankton phylogeny is however being questioned together with the relevance of the SSU rRNA gene to delineate bacterial species.

Conclusions

Though it was for long considered a mass of dormant cells, bacterioplankton has proven to be a key actor in aquatic ecosystems throughout the world. As for many ecosystems, the bacterioplankton drives the whole biological activity of the water foodweb and occupies a huge variety of niches throughout the water column. Further research aims to show the importance of local bacterioplanktonic processes involved in global biogeochemistry.

Summary

The bacterial component of the plankton that drifts in the water column of all aquatic ecosystems is named *Bacterioplankton*. Though it was for long considered solely dormant, this part of the biota is now recognized as a determinant actor on the global recycling of nutrients and at the basis of all aquatic foodwebs. With a million cells per millimeter of water, they represent the most successful organisms, as well as the most diverse biome on our planet. Modern approaches combining molecular biology to epi-fluorescence microscopy have sharpened our knowledge on the abundances, the diversity and the ecology of the bacterioplankton.

Bibliography

- Acinas, S. G., Anton, J., and Rodriguez-Valera, F., 1999. Diversity of free-living and attached bacteria in offshore western mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, **65**, 514–522.
- Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., and Stahl, D. A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology*, **56**, 1919–1925.
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F., 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, **10**, 257–263.
- Beja, O., Spudich, E. N., Spudich, J. L., Leclerc, M., and DeLong, E. F., 2001. Proteorhodopsin phototrophy in the ocean. *Nature*, **411**, 786–789.
- Chin-Leo, G., and Kirchman, D. L., 1988. Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. *Applied and Environmental Microbiology*, **54**, 1934–1939.
- Cottrell, M. T., and Kirchman, D. L., 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. *Applied and Environmental Microbiology*, **66**, 5116–5122.
- del Giorgio, P. A., and Duarte, C. M., 2002. Respiration in the open ocean. *Nature*, **420**, 379–384.
- Eilers, H., Pernthaler, J., Peplies, J., Glockner, F. O., Gerdt, G., and Amann, R., 2001. Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. *Applied and Environmental Microbiology*, **67**, 5134–5142.
- Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Hogberg, P., Linder, S., Mackenzie, F. T., Moore, B. III, Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., and Steffen, W., 2000. The global carbon cycle: a test of our knowledge of earth as a system. *Science*, **290**, 291–296.
- Fenchel, T., King, G. M., and Blackburn, T. H., 1998. *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*. London: Academic.
- Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., et al., 1980. The phylogeny of prokaryotes. *Science*, **209**, 457–463.
- Fuhrman, J. A., and Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology*, **66**, 109–120.
- Fuhrman, J. A., Ferguson, R. I., 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. *Marine Ecology Progress Series*, **33**, 237–242.
- Giovannoni, S. J., Rappé, M. S., Vergin, K. L., and Adair, N. L., 1996. 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 7979–7984.
- González, J. M., and Moran, M. A., 1997. Numerical dominance of a group of marine bacteria in the alpha-subclass of the class proteobacteria in coastal seawater. *Applied and Environmental Microbiology*, **63**, 4237–4242.
- Gordon, D. A., and Giovannoni, S. J., 1996. Detection of stratified microbial populations related to *Chlorobium* and *Fibrobacter* species in the Atlantic and Pacific Oceans. *Applied and Environmental Microbiology*, **62**, 1171–1177.
- Hagström, Å., Larsson, U., Hörstedt, P., and Normark, S., 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. *Applied and Environmental Microbiology*, **37**, 805–812.
- Hobbie, J. E., Daley, R. J., and Jasper, S., 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, **33**, 1225–1228.
- Hollibaugh, J. T., Bano, N., and Ducklow, H. W., 2002. Widespread distribution in polar oceans of a 16S rRNA gene sequence with affinity to *Nitrospira*-like ammonia-oxidizing bacteria. *Applied and Environmental Microbiology*, **68**, 1478–1484.
- Hoppe, H. G., 1976. Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of micro-autoradiography. *Marine Biology*, **36**, 291–302.
- Jannasch, H. W., and Jones, G. E., 1959. Bacterial populations in sea water as determined by different methods of enumeration. *Limnology and Oceanography*, **4**, 128–139.
- Kjelleberg, S., Hermansson, M., Mården, P., and Jones, G. W., 1987. The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. *Annual Review of Microbiology*, **41**, 25–49.
- Kogure, K., Simidu, U., and Taga, N., 1979. A tentative direct microscopic method for counting living marine bacteria. *Canadian Journal of Microbiology*, **25**, 415–420.
- Larsson, U., and Hagström, Å., 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Marine Biology*, **52**, 199–206.
- Morris, R. M., Rappé, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, **420**, 806–810.
- Pernthaler, A., Pernthaler, J., and Amann, R. I., 2002. Fluorescent in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, **68**, 3094–3101.
- Pinhassi, J., and Hagström, Å., 2000. Seasonal succession in marine bacterioplankton. *Aquatic Microbial Ecology*, **21**, 245–256.
- Pinhassi, J., Winding, A., Binnerup, S., Zweifel, U. L., Riemann, B., and Hagström, Å., 2003. Spatial variability in bacterioplankton community composition at the Skagerrak-Kattegat Front. *Marine Ecology Progress Series*, **255**, 1–13.
- Pommier, T., Canbäck, B., Riemann, L., Boström, H. K., Lundberg, P., Tunlid, A., and Hagström, Å., 2007. Global patterns of diversity and community structure in marine bacterioplankton. *Molecular Ecology*, **16**, 867–880.
- Proctor, L. M., and Fuhrman, J. A., 1990. Viral mortality of marine bacteria and cyanobacteria. *Nature*, **343**, 60–62.
- Rappé, M. S., Connon, S. A., Vergin, K. L., and Giovannoni, S. J., 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature*, **418**, 630–633.
- Riemann, L., Steward, G. F., and Azam, F., 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied and Environmental Microbiology*, **66**, 578–587.
- Sabehi, G., Loy, A., Jung, K. H., Partha, R., Spudich, J. L., Isaacson, T., Hirschberg, J., Wagner, M., and Beja, O., 2005. New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *PLOS Biology*, **3**, 1409–1417.
- Salomon, P. S., Janson, S., and Granéli, E., 2003. Molecular identification of bacteria associated with filaments of *Nodularia spumigena* and their effect on the cyanobacterial growth. *Harmful Algae*, **2**, 261–272.
- Smith, E. M., and del Giorgio, P. A., 2003. Low fractions of active bacteria in natural aquatic communities? *Aquatic Microbial Ecology*, **31**, 203–208.
- Smith, D. C., Simon, M., Alldredge, A. L., and Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature*, **359**, 139–142.

- Stevenson, B. S., and Schmidt, T. M., 1998. Growth rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli*. *Journal of Bacteriology*, **180**, 1970–1972.
- Suzuki, M. T., Preston, C. M., Chavez, F. P., and Delong, E. F., 2001. Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey bay. *Aquatic Microbial Ecology*, **24**, 117–127.
- Tabor, P. S., and Neihof, R. A., 1984. Direct determination of activities for microorganisms of Chesapeake bay populations. *Applied and Environmental Microbiology*, **48**, 1012–1019.
- Thurman, H. V., 1997. *Introductory Oceanography*. New Jersey, USA: Prentice Hall College. ISBN 0132620723.
- Vacelet, E., 1972. Generation time measured in minutes for marine bacteria. *Comptes rendus hebdomadaires des séances de l'Académie des sciences. Série D: Sciences naturelles*, **274**, 2083–2085.
- Woese, C. R., 1987. Bacterial evolution. *Microbiological Reviews*, **51**, 221–271.
- Woese, C. R., Stackebrandt, E., Macke, T. J., and Fox, G. E., 1985. A phylogenetic definition of the major eubacterial taxa. *Systematic and Applied Microbiology*, **6**, 143–151.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cyanobacteria
 Fluorescence In Situ Hybridisation (FISH)
 Metals, Acquisition by Marine Bacteria
 Piezophilic Bacteria
 Protozoa (Heterotroph, Eukaryotic)

BANDED IRON FORMATIONS

Nicole R. Posth¹, Kurt O. Konhauser², Andreas Kappler¹

¹University of Tübingen, Tübingen, Germany

²University of Alberta, Edmonton, Alberta, Canada

Synonyms

BIFs

Definition

Sedimentary deposits of alternating iron-rich (~20–40% Fe) and iron-poor, siliceous (~40–50% SiO₂) mineral layers that primarily precipitated throughout much of the late Archean (2.7–2.5 Ga) and Paleoproterozoic (2.5–1.8 Ga), but then remerged in the Neoproterozoic (~0.8 Ga).

BIF characterization, mineralogy, and distribution

BIFs are globally distributed, with some of the larger formations being those of the Hamersley range in Australia and the Transvaal Supergroup in South Africa (Figures 1 and 2). Other major BIFs include Krivoy Rog Supergroup, Ukraine (2.2 Ga); Labrador Trough, Canada; Lake Superior Region, USA; Gunflint and Biwabik, North America

(2.2–2.0 Ga), Carajás Formation (2.6 Ga) and Urucum Region, Brazil (0.8 Ga).

The mineralogy of the least metamorphosed BIFs consists of chert, magnetite, hematite, carbonates (siderite and dolomite–ankerite), greenalite, stilpnomelane, and riebeckite (Klein, 2005): the presence of both ferric and ferrous minerals gives BIF an average oxidation state of Fe^{2.4+} (Klein and Beukes, 1992). It is generally agreed that none of the minerals in BIF are primary, but that instead, the minerals reflect both diagenetic and metamorphic overprinting. For instance, the primary iron minerals were most likely ferric hydroxide (Fe(OH)₃), greenalite ((Fe)₃Si₂O₅(OH)₄), and siderite (FeCO₃) (Klein, 2005).

Based on their depositional environment, BIFs have been categorized as Algoma or Superior type. Algoma type BIFs are generally small in lateral extent and display a volcanic association (Gross, 1965, 1980; Huston and Logan, 2004), while Superior type BIFs are found in shelf environments, are vast, and are without a dominant volcanic influence. The “Superior” type BIFs, including those in the Hamersley Group, Western Australia and the Transvaal Supergroup, South Africa, are hundreds of meters thick, over 10⁵ km² in areal extent, and contain >10¹³ t of iron (Trendall, 2002). They are characteristically laminated, with alternating Fe-rich and Si-rich layers (Figures 2 and 3). Banding can be observed on a wide range of scales, from coarse macrobands (meters in thickness) to mesobands (centimeter-thick units) to millimeter and sub-millimeter layers (Trendall and Blockely, 1970). Among the latter is the wide variety of varve-like repetitive laminae, known as microbands.

BIFs have also been categorized according to their Fe oxide composition, as hematite or magnetite BIFs (Figure 3) (James, 1954). The former refers to BIFs facies consisting principally or entirely of interbedded chert and (primary) hematite, which are in some places oolitic (e.g., the Gunflint range). The latter describes Fe oxide layers of magnetite that may grade imperceptibly into silicate rock in some areas, be mixed with carbonate, or sprinkled with hematite or chert lenses (James, 1954).

A third categorization of BIF is based on the granular versus nongranular nature of the silica layers. Granules, seen in the 2.0 Gyr Gunflint BIF are approximately 0.5 mm in diameter, irregular, and can be within a chert matrix. They can have a greenalite or magnetite core. These granules, however, do not show the concentric layering seen in hematitic oolites. Non-granular BIFs are typically thin-bedded or laminated and may chiefly be composed of minnesotaite with magnetite and carbonate in some layers (James, 1954).

Banded iron formations and the study of the early Earth

The height of BIF deposition was 2.7–2.4 Ga (Trendall, 2002; Klein, 2005), a timeframe which overlaps with the currently estimated age for the rise of oxygen on the Earth ~2.5 Ga (Anbar et al., 2007). As it is agreed that the

- Stevenson, B. S., and Schmidt, T. M., 1998. Growth rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli*. *Journal of Bacteriology*, **180**, 1970–1972.
- Suzuki, M. T., Preston, C. M., Chavez, F. P., and Delong, E. F., 2001. Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey bay. *Aquatic Microbial Ecology*, **24**, 117–127.
- Tabor, P. S., and Neihof, R. A., 1984. Direct determination of activities for microorganisms of Chesapeake bay populations. *Applied and Environmental Microbiology*, **48**, 1012–1019.
- Thurman, H. V., 1997. *Introductory Oceanography*. New Jersey, USA: Prentice Hall College. ISBN 0132620723.
- Vacelet, E., 1972. Generation time measured in minutes for marine bacteria. *Comptes rendus hebdomadaires des séances de l'Académie des sciences. Série D: Sciences naturelles*, **274**, 2083–2085.
- Woese, C. R., 1987. Bacterial evolution. *Microbiological Reviews*, **51**, 221–271.
- Woese, C. R., Stackebrandt, E., Macke, T. J., and Fox, G. E., 1985. A phylogenetic definition of the major eubacterial taxa. *Systematic and Applied Microbiology*, **6**, 143–151.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cyanobacteria
 Fluorescence In Situ Hybridisation (FISH)
 Metals, Acquisition by Marine Bacteria
 Piezophilic Bacteria
 Protozoa (Heterotroph, Eukaryotic)

BANDED IRON FORMATIONS

Nicole R. Posth¹, Kurt O. Konhauser², Andreas Kappler¹

¹University of Tübingen, Tübingen, Germany

²University of Alberta, Edmonton, Alberta, Canada

Synonyms

BIFs

Definition

Sedimentary deposits of alternating iron-rich (~20–40% Fe) and iron-poor, siliceous (~40–50% SiO₂) mineral layers that primarily precipitated throughout much of the late Archean (2.7–2.5 Ga) and Paleoproterozoic (2.5–1.8 Ga), but then remerged in the Neoproterozoic (~0.8 Ga).

BIF characterization, mineralogy, and distribution

BIFs are globally distributed, with some of the larger formations being those of the Hamersley range in Australia and the Transvaal Supergroup in South Africa (Figures 1 and 2). Other major BIFs include Krivoy Rog Supergroup, Ukraine (2.2 Ga); Labrador Trough, Canada; Lake Superior Region, USA; Gunflint and Biwabik, North America

(2.2–2.0 Ga), Carajás Formation (2.6 Ga) and Urucum Region, Brazil (0.8 Ga).

The mineralogy of the least metamorphosed BIFs consists of chert, magnetite, hematite, carbonates (siderite and dolomite–ankerite), greenalite, stilpnomelane, and riebeckite (Klein, 2005): the presence of both ferric and ferrous minerals gives BIF an average oxidation state of Fe^{2.4+} (Klein and Beukes, 1992). It is generally agreed that none of the minerals in BIF are primary, but that instead, the minerals reflect both diagenetic and metamorphic overprinting. For instance, the primary iron minerals were most likely ferric hydroxide (Fe(OH)₃), greenalite ((Fe)₃Si₂O₅(OH)₄), and siderite (FeCO₃) (Klein, 2005).

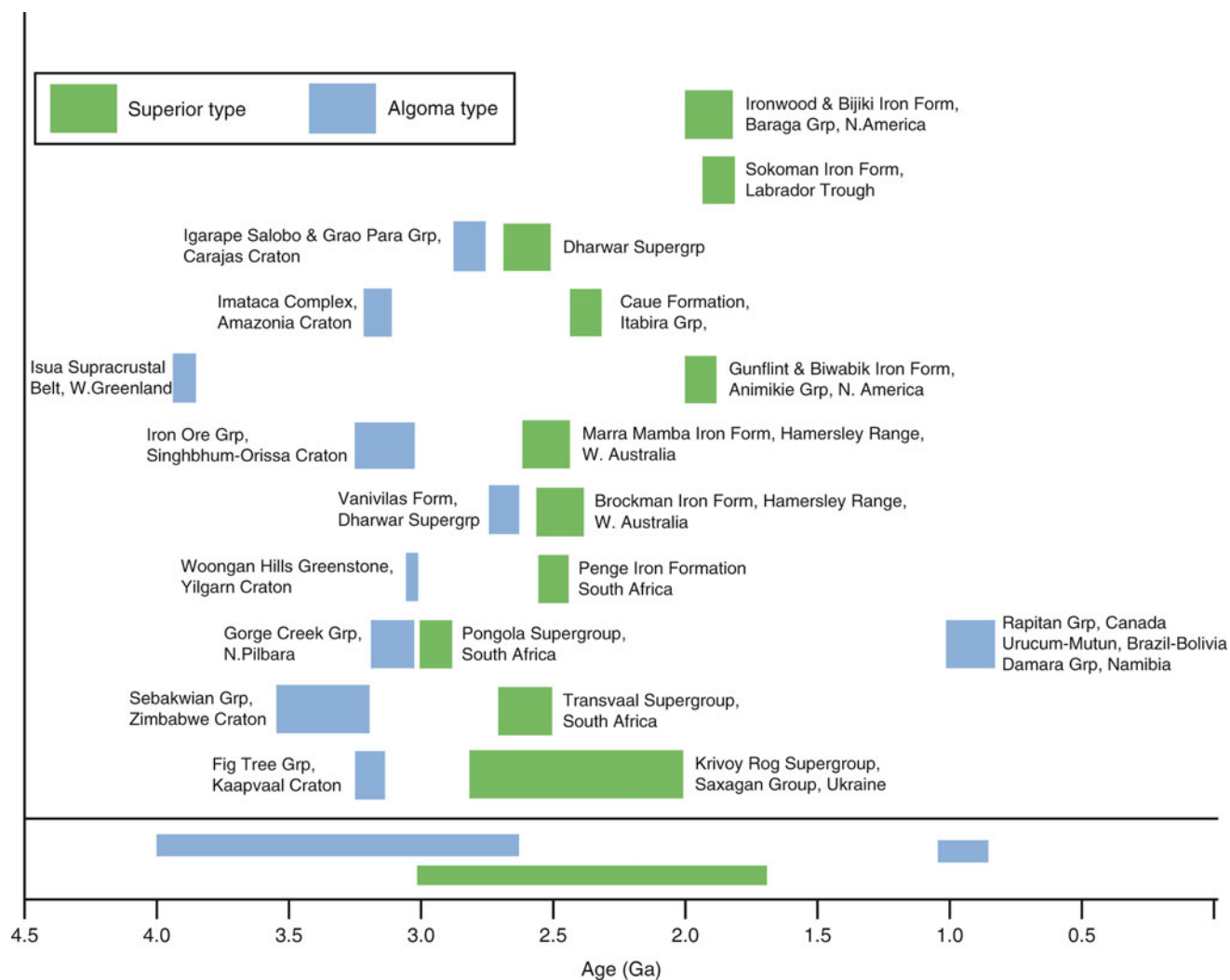
Based on their depositional environment, BIFs have been categorized as Algoma or Superior type. Algoma type BIFs are generally small in lateral extent and display a volcanic association (Gross, 1965, 1980; Huston and Logan, 2004), while Superior type BIFs are found in shelf environments, are vast, and are without a dominant volcanic influence. The “Superior” type BIFs, including those in the Hamersley Group, Western Australia and the Transvaal Supergroup, South Africa, are hundreds of meters thick, over 10⁵ km² in areal extent, and contain >10¹³ t of iron (Trendall, 2002). They are characteristically laminated, with alternating Fe-rich and Si-rich layers (Figures 2 and 3). Banding can be observed on a wide range of scales, from coarse macrobands (meters in thickness) to mesobands (centimeter-thick units) to millimeter and sub-millimeter layers (Trendall and Blockely, 1970). Among the latter is the wide variety of varve-like repetitive laminae, known as microbands.

BIFs have also been categorized according to their Fe oxide composition, as hematite or magnetite BIFs (Figure 3) (James, 1954). The former refers to BIFs facies consisting principally or entirely of interbedded chert and (primary) hematite, which are in some places oolitic (e.g., the Gunflint range). The latter describes Fe oxide layers of magnetite that may grade imperceptibly into silicate rock in some areas, be mixed with carbonate, or sprinkled with hematite or chert lenses (James, 1954).

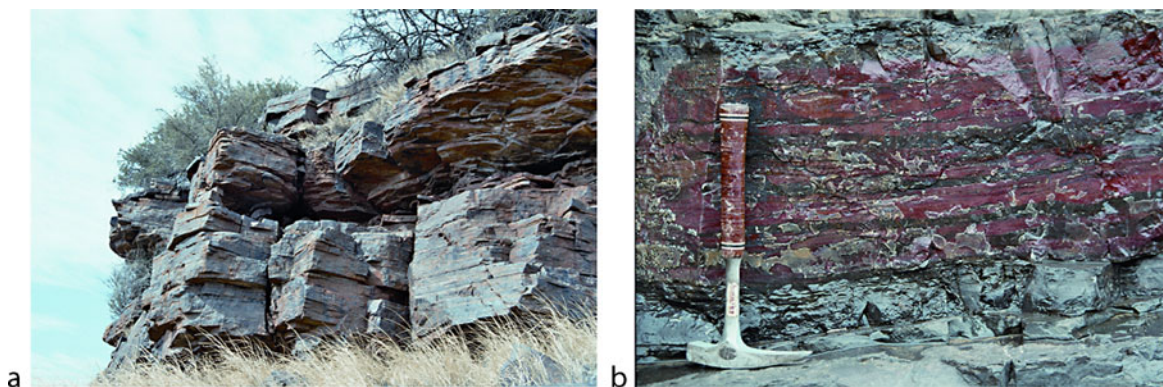
A third categorization of BIF is based on the granular versus nongranular nature of the silica layers. Granules, seen in the 2.0 Gyr Gunflint BIF are approximately 0.5 mm in diameter, irregular, and can be within a chert matrix. They can have a greenalite or magnetite core. These granules, however, do not show the concentric layering seen in hematitic oolites. Non-granular BIFs are typically thin-bedded or laminated and may chiefly be composed of minnesotaite with magnetite and carbonate in some layers (James, 1954).

Banded iron formations and the study of the early Earth

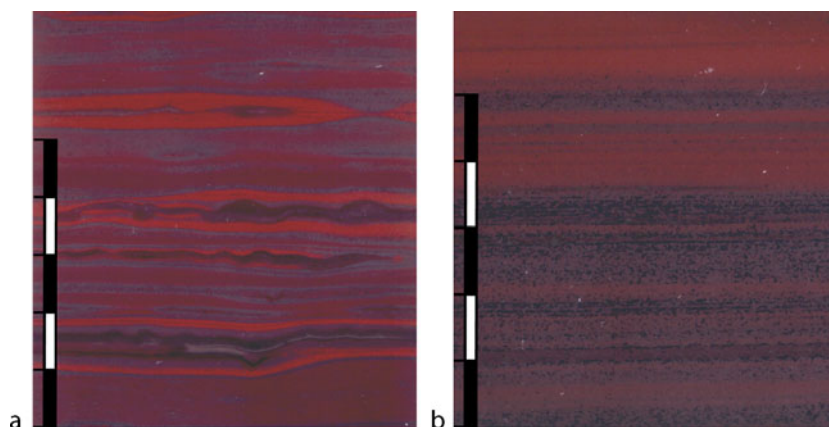
The height of BIF deposition was 2.7–2.4 Ga (Trendall, 2002; Klein, 2005), a timeframe which overlaps with the currently estimated age for the rise of oxygen on the Earth ~2.5 Ga (Anbar et al., 2007). As it is agreed that the



Banded Iron Formations, Figure 1 Appearance of BIFs in geological time and marked as Algoma and Superior types.



Banded Iron Formations, Figure 2 BIF outcrop, Gamoha Hill, South Africa (a), and White Mfolozi Gorge, Kwazulu Natal, South Africa, Jasper and Magnetite, 3.0 billion-years-old (b). Pictures by Nicole Posth.



Banded Iron Formations, Figure 3 Thin sections of banded iron formation, Mamatwan Manganese Mine, Hotazel, northern Cape Province, South Africa. Samples were taken from a depth of 83.4 m (a) and 87.5 m (b).

source of iron for these deposits was Fe(II), the Fe(III) minerals in BIF indicate that an oxidizing mechanism was present. Once oxygen began its rise due to the advent of cyanobacteria, it is plausible that an oxic mechanism formed BIF, perhaps even in pockets or oxygen oases. In the case of BIF dated prior to 2.7 Ga, however, an anoxic oxidizing mechanism is required to explain the formation of these vast deposits. The latest BIFs (0.8 Ga), which seem to represent a revival later in the geologic record, may be archives of major climate changes related to the Snowball Earth (Kirschvink, 1992; Kopp et al., 2005). Due to the intricacies of the depositional mechanisms, BIFs have been studied for decades regarding their potential as archives of the early Earth environment, but may also prove to be a detailed record of the early Earth biosphere.

BIF deposition

Depositional setting

Early studies of BIF mineral association (James, 1954), as well as comparative BIFs studies (Trendall, 1968; Beukes, 1973; Gole and Klein, 1981; Simonson, 1985; to name a few) postulated that these sedimentary deposits likely formed on the margins of cratons in semi-restricted, marine basins with minimal wave action (for a comprehensive review, Klein, 2005). They also laid a foundation for both theoretical and experimental geochemical modeling based on atmospheric (oxygen and carbon dioxide) and oceanic (iron and silica) concentrations, which are largely linked to mineralogical, petrologic, and isotopic analyses and have been invoked to describe the BIF deposition mechanism (Drever, 1974; Cairns-Smith, 1978; Han, 1978; Braterman et al., 1983; Baur et al., 1985; Francois, 1986; Garrels, 1987; Morris, 1993).

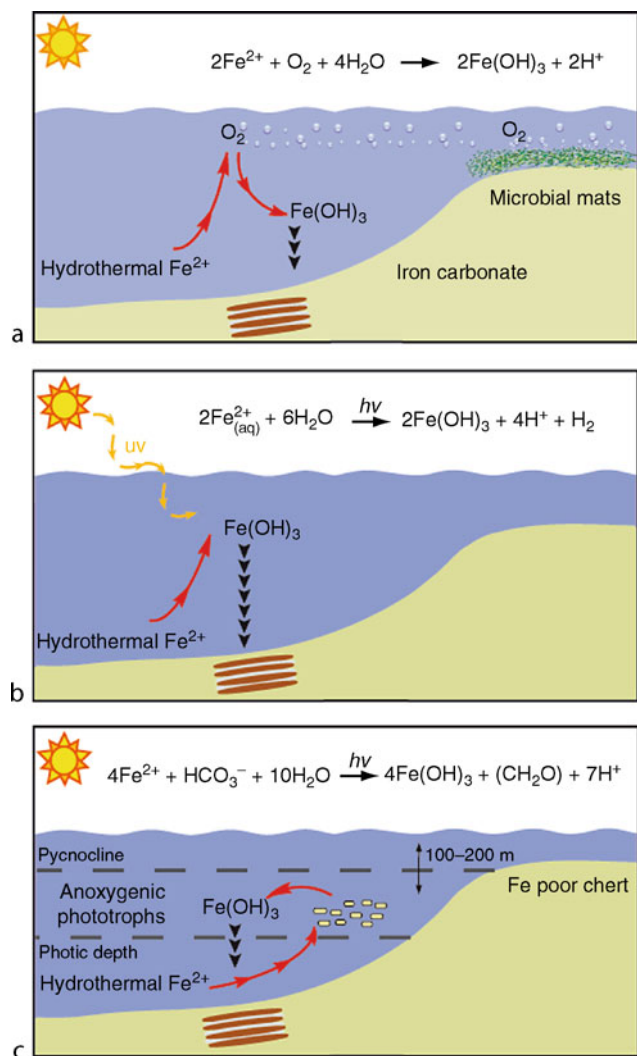
There is ample evidence that the dominant source of Fe (II) into the Archean ocean was hydrothermal (e.g., Jacobsen and Pimentel-Klose, 1988; Bau and Möller,

1993), but it has also recently been suggested that a significant fraction of the Fe in Archean seawater came from the continents (Alexander et al., 2008). The proximity of the Fe(II) source to the site of deposition, however, is still unclear. The estimated Fe(II) concentration for these basins ranges from 0.02 to 0.5 mM (Holland, 1973; Morris, 1993). This Fe(II) may have been delivered from the deep ocean to the outer continental shelf by upwelling currents from a mid-ocean ridge system (e.g., Holland, 1973). Accordingly, BIFs would sediment from below the wave base (free of wave- and storm-induced currents) onto partially submerged platforms of the continental shelves. Alternatively, Fe(II) could have been directly supplied from plumes in a seamount type system (Isley, 1995), which would curtail the difficulties introduced by the high upwelling rates required in the continental shelf model. After deposition, these sediments would undergo diagenesis, with some BIF sediment possibly even being redistributed to deeper waters via turbidity currents (Krapež et al., 2003).

There is significant evidence that Archean oceans also contained elevated concentrations of dissolved silica, possibly even to saturation with respect to amorphous silica (~2.20 mM; Siever, 1992; Maliva et al., 2005). Under such silica-rich conditions, the direct precipitation of amorphous silica could have taken place directly from the water column onto the sea basin floor (Krapež et al., 2003). Moreover, silica could have reacted with dissolved Fe(II) and precipitated as ferrous or ferric-silicates (Konhauser et al., 2007).

Theories of Precambrian BIF deposition

BIF mineralogy dictates that some form of Fe(II) oxidation was necessary for their formation, yet which mechanism(s) dominated is still highly uncertain (Figure 4). While dissolved Fe(II) readily oxidizes in the presence of oxygen at circumneutral pH, to the rise of oxygen on Earth occurred approximately 2.5 Ga (Kopp et al., 2005; Anbar et al., 2007). An alternative, O₂-independent



Banded Iron Formations, Figure 4 Models of banded iron formation deposition: The traditional model of BIF deposition involves the production of oxygen by cyanobacteria, which is released into the water column to chemically react with hydrothermal dissolved $\text{Fe}(\text{II})$ (a). The two proposed mechanisms of deposition in an anoxic ocean water column are the abiotic $\text{Fe}(\text{II})$ photooxidation by UV light, which has recently been discounted (Konhauser et al., 2007) (b), and direct microbial $\text{Fe}(\text{II})$ oxidation via anoxygenic $\text{Fe}(\text{II})$ -oxidizing phototrophy (c).

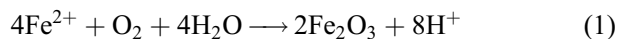
mechanism is therefore necessary to explain the deposition of the earliest BIF.

Both abiotic and biotic mechanisms of BIF deposition have been put forth. The potential role of microorganisms in BIF was first proposed 4 decades ago (Cloud, 1968) and in part addressed the difficulties in explaining these deposits based on geochemical processes alone (Brown et al., 1995). Yet, BIFs contain very little or no organic carbon (0.5% [w/w], Beukes and Klein, 1992) which makes it difficult to constrain the responsible microbial process

simply based on geochemical and petrographic studies. Any fossil information about the presence of life in the Archean has therefore been garnered from contemporaneous formations. For this reason, isotopic, biomarker, and ecophysiological studies with modern analogues have been implemented in order to supplement the geochemical BIF data and test the plausibility of the depositional models and the role of microorganisms.

Oxygenic photosynthesis model

The traditional model of BIF deposition was first postulated by Cloud (1968). Herein, $\text{Fe}(\text{II})$ is abiotically oxidized by photosynthetically produced O_2 , allowing for the indirect biogenic precipitation of ferric hydroxide as shown in Equation 1 (Figure 4a). Under an anoxic atmosphere, this O_2 could have been confined to local “oxygen oases” associated with cyanobacterial blooms in coastal settings (Cloud, 1965, 1973). Cloud (1965, 1973) further proposed that such primitive O_2 -producing photosynthetic bacteria, which lacked suitably advanced oxygen-mediating enzymes, required ferrous iron to detoxify oxygen. If so, these microorganisms would have flourished specifically when $\text{Fe}(\text{II})$ and nutrients were made episodically available. Once oxygen was present, aerobic $\text{Fe}(\text{II})$ oxidizers could also have contributed to biogenic Fe minerals precipitation (Holm, 1989).



The oldest evidence to support the presence of cyanobacteria during the time of BIF deposition came from the 3.45 billion-year-old Apex cherts, Warrawoona, Australia (Schopf, 1993). These findings, however, have since been challenged by Brasier et al. (2002) as being secondary artefacts formed by Fischer–Tropsch type reactions associated with seafloor hydrothermal systems. In fact, Buick (1988) maintained that the chert matrix of the “oldest microfossils” is a secondary hydrothermal deposit of younger age that cross-cuts the primary bedding. The use of kerogen as a marker of past life in these formations has also been challenged by experiments demonstrating the abiotic pathways of kerogen synthesis; simple organic hydrocarbons of abiological origins (e.g., formaldehyde) readily condense onto silica–carbonate inorganic filaments, and subsequently polymerize under gentle heating to yield kerogenous products (García-Ruiz et al., 2003).

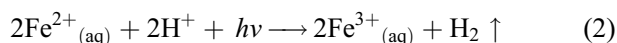
In light of these studies, stronger evidence for the presence of cyanobacteria comes from the stromatolitic assemblages of the 2.7 Gyr Tumbiana Formation, Western Australia. The presence of oxygenic photosynthesis in these formations is based on their likely habitat in sulfate-deficient evaporative lakes (Buick, 1992). This is further supported by the earliest undisputed fossil assemblages of the 2.6 Gyr Campbell Group, South Africa, that include cyanobacterial genera which helped form stromatolitic reefs in shallow subtidal to intertidal settings (Altermann and Schopf, 1995).

Biomarkers, organic compounds derived from more complex precursors which still resemble the original biological molecule after a long burial history, may be used to identify a specific group of organisms in ancient rock. For example, 2 α -methylhopanes are derivatives of prominent lipids in cyanobacteria (methylbacteriohopanepolyols). Abundant amounts were extracted from bitumens of the 2.6 Gyr Marra Mamba Iron Formation and the 2.5 Gyr Mt. McRae Shale of the Hamersley Group, Western Australia (Brocks et al., 1999; Summons et al., 1999). As these compounds were first solely found in cyanobacteria, their presence was first interpreted as diagnostic of oxygenic photosynthesis, however, they have recently been detected in anoxygenic phototrophs (Rashby et al., 2007), which weakens the use of this biomarker as conclusive proof of oxygenic photosynthesis in ancient rock.

Specific steranes are more indicative of this metabolism. These are 28- to 30-carbon isomers and are unique alteration products of sterols known from extant eukaryotic cell membranes. The only prokaryotes known to synthesize sterols have biosynthetic pathways leading to different structural isomers. Importantly, O₂ is required for the biosynthesis of sterols, hence, their extraction from Archean rock suggests that at least some dissolved oxygen (~ 0.002 ml O₂ L⁻¹) was present at the time of their production (Runnegar, 1991). To date, there is no experimental evidence to support the alternative theory that steranes may have been produced via an anaerobic pathway utilizing hydrogen peroxide or organic peroxides (Fischer and Pearson, 2007), which make them a specific biomarker of oxygenic photosynthesis. They are found, for example, in the 2.7 billion-years-old shales of the Jeerinah Formation, Hamersley Group (Brocks et al., 1999). Their presence in these shales is particularly intriguing as other redox-sensitive proxies suggest that oxygen arose much later in earth history (Rye and Holland, 1998; Rasmussen and Buick, 1999; Farquhar et al., 2000). Only the presence of localized oxygen oases can explain these conflicting results.

UV photooxidation model

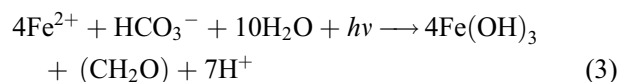
Prior to the rise of atmospheric oxygen and the development of a protective ozone layer, the Earth's surface was also subjected to high levels of ultraviolet radiation. This led to a theory of BIF deposition in which UV radiation drives an abiotic mechanism of Fe²⁺ oxidation and Fe(III) mineral precipitation. Specifically, under conditions of anoxia and high dissolved Fe(II) concentrations, dissolved ferrous iron species, such as Fe²⁺ or Fe(OH)⁺, can absorb radiation in the 200–400 nm range. Dissolved ferric iron is formed (Equation 2), which hydrolyzes to form ferric hydroxide at circumneutral pH (Cairns-Smith, 1978; Braterman et al., 1983) (Figure 4b).



These experiments, however, were recently carried out in complex solution simulating disequilibrium Precambrian ocean water chemistry in which Fe(II)-rich hydrothermal waters react with ambient Si-saturated seawater also containing high HCO₃⁻ concentrations (Konhauser et al., 2007). It was shown that the oxidation effects of either UVA or UVC were negligible in fluids with high dissolved Fe(II), Si, and HCO₃⁻, compared to the precipitation of ferrous-iron-silicates and ferrous carbonate. This suggests that under such conditions Fe²⁺ would have been largely deposited as Fe(II) minerals instead of being oxidized by UV radiation. The fact that Fe(III) minerals are present in BIF therefore suggests that in the absence of O₂ the alternative anoxic depositional mechanism, Fe(II) oxidation by anoxygenic phototrophs, drove Fe oxide deposition.

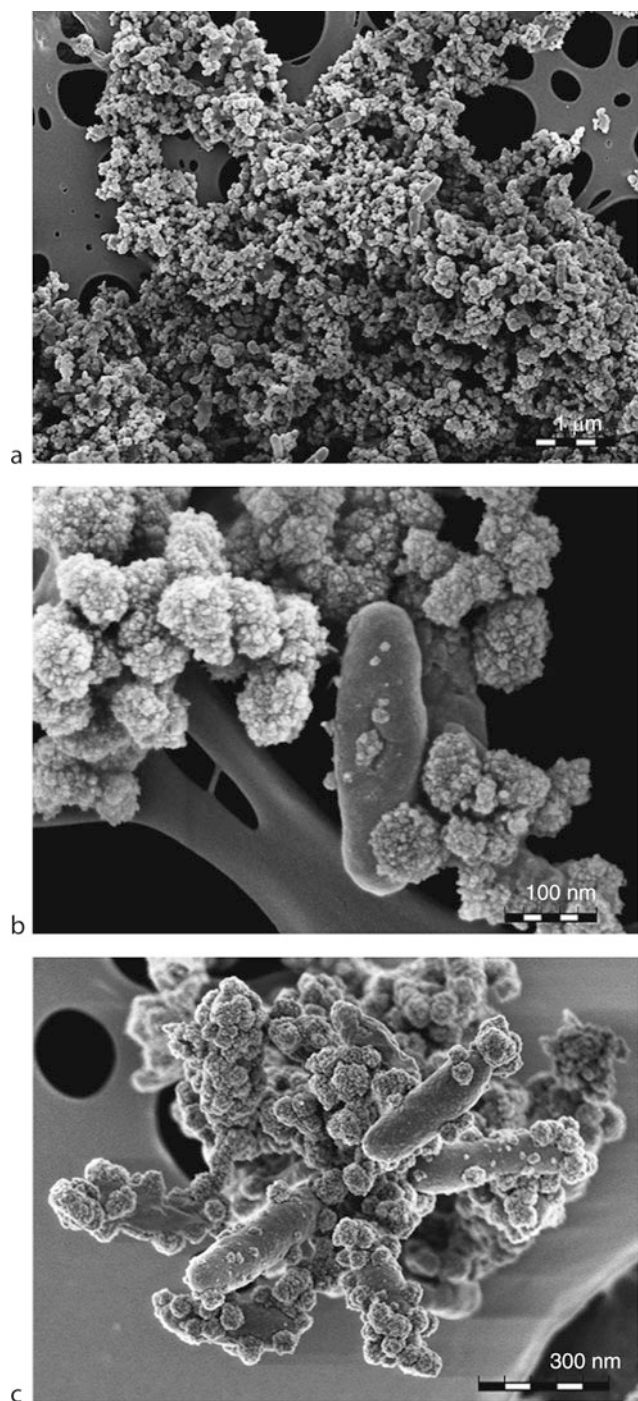
Anoxygenic Fe(II)-oxidizing phototroph model

Anoxygenic photosynthesis was first suggested as a BIF deposition mechanism by Garrels et al. (1973) and Hartman (1984). They proposed that light, not O₂, may have coupled the carbon and iron cycles via photosynthesis that used Fe(II) rather than H₂O as an electron donor and produced Fe(III) rather than O₂ (Figure 2c). It was only 2 decades later that the plausibility of this theory was validated by the discovery of a modern Fe(II)-oxidizing phototroph (Widdel et al., 1993). Seven extant strains of anoxygenic Fe(II)-oxidizing phototrophs are now known to use light energy to catalyze the oxidation of Fe(II) and the corresponding reduction of CO₂, which yields biomass and ferric hydroxide as shown in Equation 3.



In the context of BIFs, anoxygenic Fe(II)-oxidizing photosynthesis (photoferrotrophy) is the only direct biological process that can precipitate Fe(III) minerals in the absence of oxygen.

While no direct evidence exists for the presence of these strains on the Archean Earth, various lines of evidence support the plausibility of anoxygenic phototrophs in BIF deposition. Ecophysiological studies performed with modern anoxygenic Fe(II)-oxidizing phototrophs test the plausibility of this deposition mechanism. It was shown that these strains inhabit a wide range of growth environments; being globally distributed and present in fresh and marine waters (Ehrenreich and Widdel, 1994; Heising et al., 1999; Straub et al., 1999; Croal et al., 2004; Jiao et al., 2005). These organisms do not have a unique form, all being rod-shaped (Figure 5), yet morphological variety is apparent, one example being the presence of vacuoles in one strain, *Thiodictyon* sp. strain F4 (Croal et al., 2004). These strains also utilize a variety of substrates, such as acetate, FeS and H₂, which suggests that they are versatile enough to be an integral group in both



Banded Iron Formations, Figure 5 Anoxic Fe(II)-oxidizing phototrophs "*Rhodobacter ferrooxidans*" sp. strain SW2 (a, b) and *Chlorobium ferrooxidans* sp. strain KoFox (c) form aggregates with the Fe(III) hydroxides produced by their metabolism. Many cells are not encrusted by, but only associated with, the Fe(III) minerals that may have been the primary minerals in BIFs.

ancient and modern environments. Modeling of the photo-synthetic Fe(II) oxidation rates determined in these studies suggest that such microorganisms could have accounted for all of the Fe(III) initially deposited in primary BIF sediment (Kappler et al., 2005). Importantly, while the Fe(II) oxidation rate of these strains is dependent on light intensity, anoxygenic phototrophs can oxidize Fe(II) in light regimes befitting the photic zone of ocean water (of a few hundred meters depth) (Kappler et al., 2005). Also, Fe(II) oxidation is not hindered by high concentrations of dissolved silica (tested at 2 mM Archean ocean concentration) (Siever, 1992; Konhauser et al., 2007).

While modern studies show that cyanobacteria are indeed important in stromatolite building through the trapping and binding of carbonate grains in their filaments and extracellular polymers, several anaerobic pathways, such as sulfate reduction (e.g., Visscher et al., 1998; Paerl et al., 2001), are also integral in carbonate cementation and localized alkalinity generation. Indeed, sequence analysis of small subunit rRNA genes amplified with PCR from genomic DNA showed that anoxygenic phototrophs represent a considerable fraction of biomass in the modern stromatolite communities of Hamelin Pool, Shark Bay, Australia (Papineau et al., 2005). Recently, Bosak et al. (2007) experimentally demonstrated that the anoxygenic phototroph, *Rhodospseudomonas palustris*, stimulates the precipitation of calcite in saturated solutions and builds stromatolite-like structures.

While 2 α -methylhopanes were first considered diagnostic of oxygenic photosynthesis, they may have a structural function and are not related to cell metabolism. Indeed, several facultative and obligate anaerobes possess the genes for hopanoid biosynthesis, and *Geobacter sulfurreducens* produces a wide variety of complex hopanoids structurally related to 2 α -methylhopane under strictly anoxic conditions in pure culture (Härtner et al., 2005; Fischer and Pearson, 2007). Moreover, it was recently demonstrated that an anoxygenic Fe(II)-oxidizing phototroph, *Rhodospseudomonas palustris*, generates substantial quantities of 2-methylhopanoids in the absence of oxygen (Rashby et al., 2007), which renders their presence in ancient rocks alone as an unsatisfactory marker for a specific metabolism.

Pigment biomarkers may offer further evidence of a microbial role in BIF. Okenane, the fossil hydrocarbon biomarker of the carotenoid pigment precursor, okenone, was recently found in the 1.64 billion-year-old Barney Creek Formation in northern Australia, a marine, sub-wavebase base succession. This pigment is exclusively found in purple sulfur bacteria and in recent sediments under euxinic conditions (Brocks, 2005).

Isotopic evidence and the BIF depositional mechanism

The Fe isotope composition of iron minerals in BIFs is markedly different from the homogenous values seen in

igneous rocks and many modern marine sediments ($\delta^{56}\text{Fe}$ $0.00 \pm 0.05\text{‰}$). Specifically, the hematite, magnetite, Fe-carbonate, and pyrite Fe isotope compositions reflect the various processes contributing to BIF formation; equilibrium fractionation of minerals, a variation in the fluid isotope composition from which the minerals precipitated, as well as microbial processes (Johnson et al., 2003; Johnson and Beard, 2005).

In an effort to elucidate the role of microorganisms in the formation of BIF, the Fe isotope fractionation carried out by Fe metabolizing microorganisms was analyzed in controlled laboratory studies of both pure and enrichment cultures. These show that ferric hydroxides are enriched in the heavy isotope relative to Fe(II) for anoxygenic Fe(II)-oxidizing phototrophy ($1.5 \pm 0.2\text{‰}$) (Croal et al., 2004), microbially catalyzed aerobic Fe(II) oxidation at low pH (Balci et al., 2006), aerobic chemical oxidation (Bullen et al., 2001), and UV Fe(II) oxidation (Straton et al., 2006), suggesting that all major processes produce similar Fe isotope fractionations.

The isotopic fractionations reported from laboratory experiments correlate well in part with the values recovered from Archean to early Proterozoic banded rocks of the Transvaal Supergroup, South Africa. $^{56}\text{Fe}/^{54}\text{Fe}$ values in hematite are as high as $+0.75$ to $+1.0\text{‰}$ (Johnson et al., 2003) when compared to the Fe from hydrothermal vents ($\pm 0\text{‰}$). As UV photooxidation was recently ruled out as a significant BIF deposition mechanism (Konhauser et al., 2007), these positive to near-zero $\delta^{56}\text{Fe}$ values are even consistent with (although not proof of) phototrophic Fe(II) oxidation in the early Archean oceans. However, the similarity in values from anoxygenic phototrophs with those of Fe(III) oxides formed by chemical oxidation and microbially catalyzed aerobic Fe(II) oxidation make it difficult to distinguish the key biotic and abiotic processes (Bullen et al., 2001; Balci et al., 2006). Iron isotope fractionation during oxidation itself is obviously independent of the oxidation mechanism (chemical or biological) because of the very rapid isotopic exchange between aqueous Fe^{2+} and Fe^{3+} , allowing isotopic equilibrium to be attained between reduced and oxidized aqueous species. Moreover, in these ancient formations interpretation of the isotopic signature is complicated by the complexity of the depositional environments, the diagenetic processes which have altered the Fe mineralogy, including the infiltration of external fluids during diagenesis and/or low temperature metamorphism which may have erased the primary isotope record (Hoefs, 1997; Johnson et al., 2003). Indeed, the large variation of $\delta^{56}\text{Fe}$ (-2.5 to $+1.0\text{‰}$ relative to the bulk Earth) in late Archean and early Paleoproterozoic BIFs from the Transvaal Supergroup, South Africa incorporates the entire range of values measured on the Earth.

The carbon isotopic record of BIFs is constrained by the low occurrence of organic carbon (0.5% [w/w], Beukes and Klein, 1992) in these structures. Carbon isotope signatures observed in contemporaneous formations may offer information about organisms which were present at the

time of deposition, but whose record was erased, for example, by postdeposition diagenetic processes. In such ancient formations, negative carbon isotope signatures are often interpreted as an indicator of life. Organisms transform inorganic carbon (e.g., CO_2 or HCO_3^-) via autotrophic pathways into organic carbon, preferentially incorporating the lighter isotope, ^{12}C , into the organic phase and producing residual CO_2 enriched in the heavier isotope, ^{13}C . Cyanobacteria, for example, display a $\delta^{13}\text{C}$ range of -4 to -35‰ (Schidlowski, 2000). For example, early photosynthesis has been linked to mat-forming communities in microfossiliferous units within Archean formations from Western Australia and South Africa (e.g., Altermann and Kazmierczak, 2003; Tice and Lowe, 2004) by carbon isotopic values ranging from -20‰ to -35‰ , as well as the $-31.0\text{‰} \pm 4.7$ values from organic carbon in early Proterozoic cherts (Strauss et al., 1992).

Key to this approach is the consideration of overlapping degrees of fractionation produced by various carbon fixation pathways (reductive acetyl-CoA, reductive citric acid, Calvin cycle, and hydroxypropionate). This makes it impossible to determine whether these putative biological residues were remnants of chemolithoautotrophs, evidence for early photosynthesis, or abiological C-isotopic fractionations, such as those associated with Fischer-Tropsch type processes ($\Delta^{13}\text{C}$ between -50‰ and -100‰).

Theories of iron and silica mineral layering

One of the characteristic features of BIF are the alternating Fe-rich (hematite, magnetite, and siderite) and the silicate/carbonate (chert, jasper, dolomite, and ankerite) layers that form bands varying in thickness from the microscale (μm in thickness) to meter-thick units (Trendall, 1968; Klein and Beukes, 1992; Klein, 2005). Some deposits, such as in the Dales Gorge Member, Hamersley Group, Western Australia, show laterally contiguous layers up to a 100 km in distance. This suggests that some unifying trigger of iron and silicate mineral precipitation exists over large areas with some regularity, most likely one which largely subdues the precipitation of one mineral while stimulating that of the other. Furthermore, given the range of band thickness from the micro- to macro-scale, as well as the complex mineralogy, it is most likely that layering in BIF is determined by a multilayered mechanism; one producing primary layering and a second mechanism acting post deposition via diagenesis.

Following this observation, many models attribute the primary genesis of micro and mesobands to seasonal stratification, or to yearly or decadal climatic cycles. Episodic hydrothermal pulsation and upwelling of Fe(II)-rich waters into depositional basins already saturated with dissolved silica have been invoked to explain these bands; silicification is driven by seasonal evaporation of basin waters (Holland, 1973; Garrels, 1987; Jacobsen and Pimentel-Klose, 1988; Siever, 1992). This mechanism, however, does not explain the laterally contiguous fine-scale banding, nor does it resolve why the iron and silica

do not co-precipitate but rather form distinct mesobands (Trendall, 1968; Garrels, 1987; Morris, 1993). Furthermore, in light of increasing evidence for an active microbial component in the Fe cycle (Johnson et al., 2003), the potential importance of microorganisms in BIF deposition models has been suggested. Indeed, a mechanism of alternating Fe and silicate mineral layer deposition with a microbial driver triggered by temperature has been recently presented (Posth et al., 2008).

Diagenesis in BIFs: oxidation and the reduction of Fe(III) minerals

The average oxidation state of $\text{Fe}^{2.4+}$ can be described by the simultaneous deposition of Fe(II) and Fe(III). Yet, this mixed oxidation state can also be explained by partial biotic or abiotic diagenetic re-reduction of precipitated Fe(III) to Fe(II). Experimental studies testing such diagenetic mineral transformations in the presence and in the absence of biomass have not yet been reported. Nonetheless, the presence of magnetite as (1) disseminated grains within but obscuring sedimentary laminae, (2) laminated beds that clearly truncate sedimentary layering, (3) layer-discordant veins, and (4) cleavage fills, suggests such a secondary origin (Ewers and Morris, 1981; Krapež et al., 2003).

The diagenetic or microbial modification of Fe(III) after sedimentation to produce siderite (FeCO_3) and magnetite (Fe_3O_4) requires a reductant. Even if a surface water oxic zone was generated by cyanobacterial activity (Summons et al., 1999), deep waters remained anoxic (e.g., Canfield, 1998). The dominant anaerobic respiratory processes would have oxidized fermentation products in the bottom waters and/or shallow sediments (Rothman et al., 2003). The scarcity of O_2 limited both nitrate and sulfate. This is supported by both negligible sulfur isotopic fractionations between late Archean and early Paleoproterozoic sulfide and sulfate minerals (Strauss, 2003), as well as the absence of pyrite in BIF, except in association with interlayered shaley units (Ewers and Morris, 1981). The significance of manganese pathways is also refuted by the low relative concentrations of Mn(II) released in hydrothermal effluent, as well as the lack of phototrophic or nitrate-independent Mn(II) oxidizing bacteria in modern environments. In contrast, abundant ferric hydroxide deposited as BIF may point to the importance of a microbial process which coupled the oxidation of organic carbon, dead planktonic bacteria, to the reduction of ferric iron producing such reduced iron mineral phases.

Evidence of BIF diagenesis via ancient microbial Fe(III) reduction comes from Fe isotopic ratios in Fe-bearing minerals from Archean sedimentary rocks that closely mimic those observed during modern dissimilatory Fe(III) reduction. For instance, analyses of carbon- and magnetite-rich rocks, from the 2.9 Gyr Rietkuil Formation, Witwatersrand Supergroup, South Africa, revealed $\delta^{56}\text{Fe}$ values as low as -2.3‰ (Yamaguchi et al., 2005). These negative fractionations are very similar to what might be expected from multiple stages of Fe(III)

reduction (Johnson et al., 2004), where each single-step bacterial Fe(III) reduction leads to isotopically light ($\delta^{56}\text{Fe} < 1.2\text{‰}$) aqueous Fe(II) relative to the initial ferric hydroxide substrate (Icopini et al., 2004). The importance of Fe(III) reduction as a means to explain the iron fractionations has, however, been challenged by Rouxel et al. (2005). They suggested that highly variable, but negative values in pyrite from black shales (0.5‰ to -3.5‰) between 2.8 and 2.3 Ga more likely reflect the initial deposition of Fe oxides (e.g., BIFs) which preferentially removed isotopically heavy ^{56}Fe , driving the ocean waters to the negative $\delta^{56}\text{Fe}$ values recorded in pyrite. This interpretation is consistent with the notion that partial biological and abiological processes oxidized the dissolved Fe(II) brought into the shallow waters, but is problematic in light of recent studies showing significant isotopic variations for minerals within close proximity and thus, time of deposition (Johnson et al., 2008). This variation is perhaps most easily explained if the Fe isotopes reflect diagenetic pathways and not the bulk seawater (Johnson et al., 2008).

BIF diagenesis and the Archean marine Fe cycle

BIFs have recently been studied to develop a model of the Archean marine Fe cycle. In doing so, Konhauser et al. (2005) made two assumptions. First, they assumed that the bulk of the Fe(II) component in Fe-rich BIF-type macrobands formed diagenetically through biological Fe(III) reduction, i.e., the magnetite is not primary. Based on a predicted rate of Fe(III) deposition annually (1 mm year^{-1}), they then quantified the electrons that were needed to generate the amount of magnetite as reported in BIF (one third of Fe minerals; Morris, 1993). Second, they quantified the amount of photosynthetic Fe(II)-oxidizer biomass that may have been generated in the photic zone of the water column (based on Kappler et al., 2005), in order to estimate the amount of Fe recycled prior to burial. The results demonstrated that under ideal growth conditions, as much as 70% of the biologically formed Fe(III) could have been recycled back into the water column via fermentation and organic carbon oxidation coupled to microbial Fe(III) reduction. It was also suggested that some of the biomass may have been ultimately consumed via methanogenesis, i.e., coupling the oxidation of acetate or H_2 to methane formation. The hypothesis is to some extent corroborated with the analyses of kerogens (extracted from rocks 2.8 and 2.6 Gyr) with highly negative $\delta^{13}\text{C}$ signatures (between -40‰ and -60‰) that possibly formed as the result of methanogenic ^{12}C -rich gas production, the incorporation of the methane into the biomass of methanotrophic bacteria and inevitably the preservation of ^{12}C -enriched organic matter (Hayes, 1983).

Summary

Banded iron formations (BIFs) have been studied for decades in regard to their importance as an economically

viable source of iron ore, but their significance also extends to their potential as archives of the early Earth environment.

In spite of this effort, the mechanism of their deposition and in particular whether microorganisms drove the precipitation of BIF minerals, is still uncertain. Increasing evidence of a predominantly anoxic Earth until ~2.5–2.4 Ga forces the investigation of O₂-independent mechanisms for BIF deposition. For this reason, recent studies have explored the long-standing proposition that Archean BIFs may have been formed, and diagenetically modified, by anaerobic microbial metabolisms.

Bibliography

- Alexander, B. W., Bau, M., Andersson, P., and Dulski, P., 2008. Continentally-derived solutes in shallow Archean seawater: rare earth element and Nd isotope evidence in iron formation from the 2.9 Ga Pongola Supergroup, South Africa. *Geochimica et Cosmochimica Acta*, **72**, 378–394.
- Altermann, W., and Kazmierczak, J., 2003. Archean microfossils: a reappraisal of early life on Earth. *Research in Microbiology*, **154**, 611–617.
- Altermann, W., and Schopf, J. W., 1995. Microfossils from the Neoproterozoic Campbell Group, Griqualand West Sequence of the Transvaal Supergroup, and their paleoenvironmental and evolutionary implications. *Precambrian Research*, **75**, 65–90.
- Anbar, A. D., Duan, Y., Lyons, T. W., Arnold, G. L., Kendall, B., Creaser, R. A., Kaufman, A. J., Gordon, G. W., Scott, C., Garvin, J., and Buick, R., 2007. A whiff of oxygen before the Great Oxidation event? *Science*, **317**, 1903–1906.
- Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W., 2006. Iron isotope fractionation during microbially stimulated Fe(II) oxidation and Fe(III) precipitation. *Geochimica et Cosmochimica Acta*, **70**, 622–639.
- Barns, S. M., and Nierzwicki-Bauer, S. A., 1997. Microbial diversity in ocean, surface and subsurface environments. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals*. Washington, DC: Mineralogical Society of America, pp. 35–79.
- Bau, M., and Möller, P., 1993. Rare earth element systematics of the chemically precipitated component in Early Precambrian iron-formations and the evolution of the terrestrial atmosphere-hydrosphere-lithosphere system. *Geochimica et Cosmochimica Acta*, **57**, 2239–2249.
- Baur, M. E., Hayes, J. M., Studley, S. A., and Walter, M. R., 1985. Millimeter-scale variations of stable isotope abundances in carbonates from banded iron formations in the Hamersley Group of Western Australia. *Economic Geology*, **80**, 270–282.
- Beukes, N. J., 1973. Precambrian iron-formations of Southern Africa. *Economic Geology*, **68**, 960–1004.
- Beukes, N., 2004. Early options in photosynthesis. *Nature*, **431**, 522–523.
- Beukes, N. J., and Klein, C., 1992. Models for iron-formation deposition. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: University of Cambridge.
- Bosak, T., Greene, S. E., and Newman, D. K., 2007. A likely role for anoxygenic photosynthetic microbes in the formation of ancient stromatolites. *Geobiology*, **5**(2), 119–126.
- Brasier, M. D., Green, O. R., Jephcoat, A. P., Klepepe, A. K., Van Kranendonk, M. J., Lindsay, J. F., Steele, A., and Grassineau, N. V., 2002. Questioning the evidence for Earth's oldest fossils. *Nature*, **416**, 76–81.
- Braterman, P. S., Cairns-Smith, A. G., and Sloper, R. W., 1983. Photo-oxidation of hydrated Fe²⁺ – significance for banded iron formations. *Nature*, **303**, 163–164.
- Brocks, J. J., 2005. Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. *Nature*, **437**, 866–870.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1036.
- Brown, A. D., Gross, G. A., and Sawicki, J. A., 1995. A review of the microbial geochemistry of banded iron formations. *The Canadian Mineralogist*, **33**, 1321–1333.
- Buick, R., 1988. Carbonaceous filaments from North Pole, West Australia: are they fossil bacteria in Archean stromatolites? A reply. *Precambrian Research*, **39**, 311–317.
- Buick, R., 1992. The antiquity of oxygenic photosynthesis: evidence for stromatolites in sulphate-deficient Archean lakes. *Science*, **255**, 74–77.
- Bullen, T. D., White, A. F., Childs, C. W., Vivit, D. V., and Schulz, M. J., 2001. Demonstration of significant abiotic iron isotope fractionation in nature. *Geology*, **29**(8), 699–702.
- Cairns-Smith, A. G., 1978. Precambrian solution photochemistry, inverse segregation, and banded iron formations. *Nature*, **276**, 807–808.
- Campbell, A. C., Palmer, M. R., Klinkhammer, G. P., Bowers, T. S., Edmond, J. M., Lawrence, J. R., Casey, J. F., Thompson, G., Humphris, S., Rona, P., and Karson, J. A., 1988. Chemistry of hot springs on the mid-Atlantic ridge. *Nature*, **335**, 514–519.
- Canfield, D. E., 1998. A new model for Proterozoic ocean chemistry. *Nature*, **396**, 450–453.
- Cloud, P., 1965. Significance of the Gunflint (Precambrian) microflora. *Science*, **148**, 27–35.
- Cloud, P., 1968. Atmospheric and hydrospheric evolution on the primitive Earth. *Science*, **160**, 729–736.
- Cloud, P., 1972. A working model of the primitive earth. *American Journal of Science*, **272**, 537–548.
- Cloud, P., 1973. Paleocological significance of the banded iron-formation. *Economic Geology*, **68**, 1135–1143.
- Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K., 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, **68**, 1227–1242.
- Drever, J. I., 1974. Geochemical model for the origin of Precambrian banded iron formations. *Geological Society of America Bulletin*, **85**, 1099–1106.
- Ehrenreich, A., and Widdel, F., 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Applied and Environmental Microbiology*, **60**(12), 4517–4526.
- Ewers, W. E., and Morris, R. C., 1981. Studies of the Dales Gorge member of the Brockman iron formation, Western Australia. *Economic Geology*, **76**, 1929–1953.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulfur cycle. *Science*, **289**, 756–758.
- Fischer, W. W., and Pearson, A., 2007. Hypotheses for the origin and early evolution of triterpenoid cyclases. *Geobiology*, **5**, 19–34.
- Francois, L. M., 1986. Extensive deposition of banded iron formations was possible without photosynthesis. *Nature*, **320**, 352–354.
- García-Ruiz, J. M., Hyde, S. T., Camerup, A. M., Christy, A. G., Van Kranendonk, M. J., and Welham, N. J., 2003. Self-assembled silica-carbonate structures and detection of ancient microfossils. *Science*, **302**(4), 1194–1197.

- Garrels, R. M., 1987. A model for the deposition of the microbanded Precambrian iron formations. *American Journal of Science*, **287**, 81–105.
- Garrels, R. M., Perry, E. A., and MacKenzie, F. T., 1973. Genesis of Precambrian iron-formations and the development of atmospheric oxygen. *Economic Geology*, **68**, 1173–1179.
- Gole, M. J., and Klein, C., 1981. Banded iron formations through much of Precambrian time. *Journal of Geology*, **89**, 169–183.
- Gross, G. A., 1965. Geology of iron deposits in Canada, Vol. 1. General geology and evaluation of iron deposits. *Geological Survey of Canada Economic Report*, **22**(1), 181 p.
- Gross, G. A., 1980. A classification of iron formations based on depositional environments. *Canadian Mineralogist*, **18**, 215–222.
- Han, T. M., 1978. Microstructures of magnetite as guides to its origin in some Precambrian iron-formations. *Fortschritte der Mineralogie*, **56**(1), 105–142.
- Hartman, H., 1984. The evolution of photosynthesis and microbial mats: a speculation on banded iron formations. In Cohen, Y., Castenholz, R. W., and Halvorson, H. O. (eds.), *Microbial Mats: Stromatolites*. New York: Alan Liss, pp. 451–453.
- Härtner, T., Straub, K. L., and Kannenberg, E., 2005. Occurrence of hopanoid lipids in anaerobic geobacter species. *FEMS Microbiology Letters*, **243**, 59–64.
- Hayes, J. M., 1983. Geochemical evidence bearing on the origin of aerobiosis, a speculative hypothesis. In Schopf, J. W. (ed.), *Earth's Earliest Biosphere, Its Origin and Evolution*. Princeton, NJ: Princeton University Press, pp. 291–301.
- Heising, S., Richter, L., Ludwig, W., and Schink, B., 1999. *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a *Geospirillum* sp. strain. *Arch Microbiology*, **172**, 116–124.
- Hoefs, J., 1997. *Stable Isotope Geochemistry*, 4th edn. Heidelberg: Springer.
- Holland, H. D., 1973. The oceans: a possible source of iron in iron-formations. *Economic Geologist*, **68**, 1169–1172.
- Holm, N. G., 1989. The $^{13}\text{C}/^{12}\text{C}$ ratios of siderite and organic matter of a modern metalliferous hydrothermal sediment and their implications for banded iron formations. *Chemical Geology*, **77**, 41–45.
- Huston, D. L., and Logan, G. A., 2004. Barite, BIFs and bugs: evidence for the evolution of the Earth's early hydrosphere. *Earth and Planetary Science Letters*, **694**, 1–15.
- Icopini, G. A., Anbar, A. D., Ruebush, S. R., Tien, M., and Brantley, S. L., 2004. Iron isotope fractionation during microbial reduction of iron. *Geology*, **32**(3), 205–208.
- Isley, A. E., 1995. Hydrothermal plumes and the delivery of iron to banded iron formation. *Journal of Geology*, **103**, 169–185.
- Jacobsen, S. B., and Pimentel-Klose, M. R., 1988. A Nd isotopic study of the hamersley and michipicoten banded iron formations: the source of REE and Fe in Archean oceans. *Earth and Planetary Science Letters*, **87**, 29–44.
- James, H. L., 1954. Sedimentary facies of iron-formation. *Economic Geology*, **49**(3), 236–294.
- Jiao, Y., Kappler, A., Croal, L., and Newman, D. K., 2005. Isolation and characterization of a genetically tractable photoautotrophic Fe(II)-oxidizing bacterium, *Rhodopseudomonas palustris* strain TIE-1. *Applied and Environmental Microbiology*, **71**(8), 1–10.
- Johnson, C. M., and Beard, B. L., 2005. Biogeochemical cycling of iron isotopes. *Science*, **309**, 1025–1027.
- Johnson, C. M., Beard, B. L., Beukes, N. J., Klein, C., and O'Leary, J. M., 2003. Ancient geochemical cycling in the Earth as inferred from Fe isotope studies of banded iron formations from the Transvaal Craton. *Contributions to Mineralogy and Petrology*, **144**, 523–547.
- Johnson, C. M., Beard, B. L., Klein, C., Beukes, N. J., and Roden, E. E., 2008. Iron isotopes constrain biologic and abiologic processes in banded iron formation genesis. *Geochimica et Cosmochimica Acta*, **72**, 151–169.
- Johnson, C. M., Beard, B. L., Roden, E. E., Newman, D. K., and Nealon, K. H., 2004. Isotopic constraints on biogeochemical cycling of Fe. *Reviews in Mineralogy and Geochemistry*, **55**, 359–408.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, **33**, 865–868.
- Kirschvink, J. L., 1992. Late proterozoic low-latitude global glaciation: The snowball Earth. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: Cambridge University Press, pp. 51–52.
- Klein, C., 2005. Some Precambrian banded iron formations (BIFs) from around the world: their age, geologic setting, mineralogy, metamorphism, geochemistry, and origin. *The American Mineralogist*, **90**, 1473–1499.
- Klein, C., and Beukes, N. J., 1992. Time distribution, stratigraphy, and sedimentologic setting, and geochemistry of Precambrian iron-formations. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: University of Cambridge, pp. 139–146.
- Konhauser, K., Hamade, T., Raiswell, R., Morris, R. C., Ferris, F. G., Southam, G., and Canfield, D. E., 2002. Could bacteria have formed the Precambrian banded iron formations? *Geology*, **30**, 1079–1082.
- Konhauser, K., Newman, D. K., and Kappler, A., 2005. The potential significance of microbial Fe(III) reduction during deposition of Precambrian banded iron formations. *Geobiology*, **3**, 167–177.
- Konhauser, K. O., Amskold, L., Lalonde, S. V., Posth, N. R., Kappler, A., and Anbar, A., 2007. Decoupling photochemical Fe(II) oxidation from shallow-water deposition. *Earth and Planetary Science Letters*, **258**, 87–100.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A., and Nash, C. Z., 2005. The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Science*, **102**(32), 11131–11136.
- Krapež, B., Barley, M. E., and Pickard, A. L., 2003. Hydrothermal and resedimented origins of the precursor sediments to banded iron formation: sedimentological evidence from the Early Paleoproterozoic Brockman supersequence of Western Australia. *Sedimentology*, **50**, 979–1011.
- Lovley, D. R., Kashefi, K., Vargas, M., Tor, J. M., and Blunt-Harris, E. L., 2000. Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms. *Chemical Geology*, **169**, 289–298.
- Maliva, R. G., Knoll, A. H., and Simonson, B. M., 2005. Secular change in the Precambrian silica cycle: insights from chert petrology. *Geological Society of America Bulletin*, **117**, 835–845.
- Morris, R. C., 1993. Genetic modelling for banded iron-formation of the Hamersley Group, Pilbara Craton, Western Australia. *Precambrian Research*, **60**, 243–286.
- Morris, R. C., and Horwitz, R. C., 1983. The origin of the iron-formation-rich Hamersley Group of Western Australia – deposition on a platform. *Precambrian Research*, **21**, 273–297.
- Olson, J. M., and Blankenship, R. E., 2004. Thinking about the evolution of photosynthesis. *Photosynthesis Research*, **80**, 373–386.
- Paerl, H. W., Steppe, T. F., and Reid, R. P., 2001. Bacterially mediated precipitation in marine stromatolites. *Environmental Microbiology*, **3**, 123–130.
- Papineau, D., Walker, J. J., Mojzsis, S. J., and Pace, N. R., 2005. Composition and structure of microbial communities from

- stromatolites of Hamelin Pool in Shark Bay, Western Australia. *Applied and Environmental Microbiology*, **71**(8), 4822–4832.
- Pasteris, J. D., and Wopenka, B., 2003. Necessary, but not sufficient Raman identification of disordered carbon as a signature of ancient life. *Astrobiology*, **3**, 727–738.
- Perry, E. C., Tan, F. C., and Morey, G. B., 1973. Geology and stable isotope geochemistry of the Biwabik iron formation, Northern Minnesota. *Economic Geology*, **68**, 1110–1125.
- Posth, N. R., Hegler, F., Konhauser, K. O. and Kappler, A., 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. *Nature Geoscience*, **1**(10), 703–708.
- Posth, N. R., Konhauser, K. O., and Kappler, A., 2008. Microbiological processes in BIF deposition. In Glenn, C., and Jarvis, I. (eds.), *Authigenic Minerals: Sedimentology, Geochemistry, Origins, Distribution and Applications*. J. Sedimentology IAS Special Publication Series, in press.
- Rashby, S. E., Sessions, A. L., Summons, R. E., and Newman, D. K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of National Academy of Sciences of the United States of America*, **104**(38), 15099–15104.
- Rasmussen, B., and Buick, R., 1999. Redox state of the Archean atmosphere: evidence from detrital heavy metals in ca. 3250–2750 Ma sandstones from the Pilbara Craton, Australia. *Geology*, **27**, 115–118.
- Rothman, D. H., Hayes, J. M., and Summons, R. E., 2003. Dynamics of the Neoproterozoic carbon cycle. *Proceedings of National Academy of Sciences of the United States of America*, **100**, 8124–8129.
- Rouxel, O. J., Bekker, A., and Edwards, K. J., 2005. Iron isotope constraints on the Archaean and Paleoproterozoic. *Science*, **307**, 1088–1091.
- Runnegar, B., 1991. Precambrian oxygen levels estimated from the biochemistry and physiology of early eukaryotes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **71**, 97–111.
- Rye, R., and Holland, H. D., 1998. Paleosols and the evolution of atmospheric oxygen: a critical review. *American Journal of Science*, **298**, 621–672.
- Schidlowski, M., 2000. Carbon isotopes and microbial sediments. In Riding, R., and Awramik, S.M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 84–95.
- Schopf, J. W., 1993. Microfossils of the Early Archean Apex Chert: new evidence of the antiquity of life. *Science*, **260**, 640–646.
- Schopf, J. W., and Packer, B. M., 1987. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Science*, **237**, 70–73.
- Schopf, J. W., Kudryavtsev, A. B., Agresti, D. G., Wdowiak, T. J., and Czaja, A. D., 2002. Laser-Raman imagery of Earth's earliest fossils. *Nature*, **416**, 73–76.
- Siever, R., 1992. The silica cycle in the Precambrian. *Geochimica et Cosmochimica Acta*, **56**, 3265–3272.
- Simonson, B. M., 1985. Sedimentological constraints on the origins of Precambrian iron-formations. *Geological Society of America Bulletin*, **96**, 244–252.
- Straton, S., Amskold, L., Anbar, A., and Konhauser, K. O., 2006. Iron isotope fractionation during Fe(II) photo-oxidation. *Astrobiology Science Conference 4*, Washington, DC.
- Straub, K. L., Rainey, F. R., and Widdel, F., 1999. *Rhodovulum iodolum* sp. nov. and *Rhodovulum robiginosum* sp. nov., two new marine phototrophic ferrous-iron-oxidizing purple bacteria. *International Journal of Systematic Bacteriology*, **49**, 729–735.
- Strauss, H., 2003. Sulphur isotopes and the early Archean sulphur cycle. *Precambrian Research*, **126**, 349–361.
- Strauss, H., Des Marais, D. J., Hayes, J. M., and Summons, R. E., 1992. Concentrations of organic carbon and maturities and elemental compositions of kerogens. In Schopf, J.W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 95–99.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Tice, M. M., and Lowe, D. R., 2004. Photosynthetic microbial mats in the 3,416 Myr old ocean. *Nature*, **431**, 549–552.
- Trendall, A. F., 1968. Three great basins of Precambrian banded iron formation deposition: a systematic comparison. *Geological Society of America Bulletin*, **79**, 1527–1544.
- Trendall, A. F., 2002. The significance of iron-formation in the Precambrian stratigraphic record. *International Association of Sedimentologists. Special Publication*, **33**, 33–66.
- Trendall, A. F., and Blockely, J. G., 1970. The iron formations of the Precambrian Hamersley Group, Western Australia; with special reference to the associated crocidolite. *Western Australia Geological Survey Bulletin*, **119**, 336.
- Van Kranendonk, M. J., 2006. Volcanic degassing, hydrothermal circulation and the flourishing of early life on Earth: a review of the evidence from c. 3490–3240 Ma rocks of the Pilbara Supergroup, Pilbara Craton, Western Australia. *Earth Science Reviews*, **74**, 197–240.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovely, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.
- Visscher, P. T., Reid, R. P., Bebout, B. M., Hoefft, S. E., Macintyre, I. G., and Thompson, J. A., 1998. Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. *American Mineralogist*, **83**, 1482–1493.
- Walker, J. C. G., 1984. Suboxic diagenesis in banded iron formations. *Nature*, **309**, 340–342.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature*, **362**, 834–836.
- Wright, D. T., and Altermann, W., 2000. Microfacies development in Late Archaean Stromatolites and oolites of the Campbellrand Subgroup, South Africa. In Insalco, E., Skelton, P. W., and Palmer, T. J. (eds.), *Carbonate Platform Systems: Components and Interactions*. London: Geological Society, Special Publication, **178**, 51–70.
- Yamaguchi, K. E., Johnson, C. M., Beard, B. L., and Ohmoto, H., 2005. Biogeochemical cycling of iron in the Archean Paleoproterozoic Earth: constraints from iron isotope variations in sedimentary rocks from the Kaapvaal and Pilbara Cratons. *Chemical Geology*, **218**, 135–169.

Cross-references

[Algae \(Eukaryotic\)](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biosignatures in Rocks](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Geobacter](#)
[Hydrothermal Environments, Marine](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

Microbialites, Stromatolites, and Thrombolites
 Nanocrystals, Microbially Induced
 Ores, Microbial Precipitation and Oxidation
 Photosynthesis
 Salinity History of the Earth's Ocean
 Sediment Diagenesis – Biologically Controlled
 Snowball Earth
 Stromatolites

BASALT (GLASS, ENDOLITHS)

Ingunn H. Thorseth
 University of Bergen, Bergen, Norway

Synonyms

Endolithic microorganisms in basaltic glass; Microbial life in glassy basalt

Definition

Basalt endoliths. Microorganisms that colonize and live inside fractures, vesicles, and other cavities in basaltic rocks.

Basalt. The most common volcanic rock formed by eruptions of magma of basaltic composition.

Seafloor basalt. Basalt that is exposed at the seafloor, most commonly in or close to the ocean spreading ridge axes where the crust is young, or at intraplate seamounts.

Subseafloor basalt. Basalt that has been buried by younger lava flows and/or deep-sea sediments, as the crust aged and moved away from the spreading ridges.

Basaltic glass and endolithic microorganisms

Introduction

Basalt is continuously formed during volcanic eruptions along the global mid-ocean ridge system, and constitutes the upper 0.5–1 km thick layer of the ocean crust which covers more than 50% of the Earth's surface. When the basaltic magma is extruded into the cold seawater, the 1–2 cm thick outermost surface layer is cooled so rapidly that its chemical species do not have enough time to be arranged into minerals, but freeze to an amorphous glass rind. These glassy margins become heavily fractured due to cooling-related contraction, and the fracture networks provide an immense surface area for rock-water interactions and colonization by microorganisms. As the basaltic glass dissolves and alters more quickly than the minerals (pyroxene, plagioclase) in the crystalline interior of the lava, the glassy proportion plays an important role in the alteration of the ocean crust and the chemical exchange with the ocean water. The alteration is not only controlled by physiochemical factors but also by microbial processes, implied by the presence of endolithic microorganisms (see *Endoliths*) within the glassy margins. The microbial

activity may, on the other hand, be controlled by the geological environment, which includes a variety of complex fluid temperature and chemistry conditions ranging from seafloor weathering to various hydrothermal systems.

Basaltic glass alteration

Low-temperature alteration of basaltic glass results in the formation of concentrically zoned rims of a yellow-brown, hydrous residual alteration product, frequently called palagonite, along the outer surfaces, and internal vesicles and fractures of the glassy margins of lava flows. The altered glass is amorphous (gel-palagonite) to poorly crystalline with clayish character (fibropalagonite) (e.g., Stroncik and Schmincke, 2001), and is usually highly enriched in oxidized Fe and Ti, and depleted in Si and alkaline and alkaline earth elements compared to the fresh, parental glass. The term palagonite was used for the first time by von Waltershausen in 1845 for the brown groundmass of a tuff from Palagonia, Sicily, and was then thought to be a new mineral phase. Although palagonite was later shown to be a heterogeneous material, the term has commonly been used in the literature. However, several authors now recommend referring to this material as altered glass. During the last 2 decades, various field and laboratory studies have shown that the alteration rate and the chemical and crystalline characteristics of the altered glass strongly depend on the physiochemical and hydrological conditions (e.g., Crovisier et al., 2003). The understanding of how the endolithic microorganisms in the glassy margins of basaltic lava flows influence and interact with the alteration process is, however, still poor.

Evidence of microbial life in glassy basalt

The first in situ observations of endolithic microorganisms in basaltic glass was reported from a scanning electron microscopy (SEM) study of recent (<1 million years old (Ma)) volcanic glass deposits from Iceland (Thorseth et al., 1992). Microbial biofilms together with irregular alteration fronts at the fresh-altered glass boundary lead to the suggestion that the degradation of the volcanic glass could be biocatalyzed by the development of more aggressive microenvironments in the near vicinity of the microbial cells. This was subsequently supported by experimental alteration studies, where development of corrosion marks in the glass surface only was observed in combination with biofilm formation and not in sterile controls (Thorseth et al., 1995a; Staudigel et al., 1995, 1998). Similar alteration textures resembling microbial cell morphologies observed in glassy margins of 6 Ma ocean basalt, sampled 432 m below the seafloor at the Costa Rica Rift during the Ocean Drilling Program (ODP) Leg 148, were then stained using DNA-specific dyes to confirm the presence of DNA within these structures (Thorseth et al., 1995b; Giovannoni et al., 1996; Furnes et al., 1996). Further fluorescence in situ

is then reduced to glyceraldehyde-3-phosphate which can be used to form glucose or other organic compounds. However, only one of six glyceraldehyde-3-phosphate molecules leaves the Calvin cycle while the rest is used for the regeneration of the carbon dioxide acceptor ribulose-1,5-bisphosphate which requires more ATP (Figure 1b; for more details on Calvin cycle, see, e.g., Lawlor, 2001; Heldt, 2003).

Anoxygenic photosynthesis

Anoxygenic photosynthesis, which can be found in purple bacteria, green sulfur bacteria, green non-sulfur bacteria, and heliobacteria, differs from oxygenic photosynthesis in that it requires only one photosystem and, since no splitting of water occurs, no oxygen is produced. The photosystem used by purple bacteria and green nonsulfur bacteria is similar to the PSII of plants and cyanobacteria, but contains bacteriochlorophyll instead of chlorophyll. The electron transport is cyclic since the electrons return to the reaction center after passing through a series of electron carriers. The product of the electron transport in the photosystem is ATP. Purple bacteria and green nonsulfur bacteria must oxidize inorganic or organic compounds such as H_2S , H_2 , succinate, or malate to provide the reduction potential necessary for carbon fixation. By using these compounds as electron donors, NAD^+ (nicotinamide adenine dinucleotide) is reduced to $\text{NADH} + \text{H}^+$ in a process called reverse electron transport. Purple bacteria use the Calvin cycle to fix carbon dioxide while green non-sulfur bacteria use a special process called 3-hydroxypropionate pathway.

Green sulfur bacteria and heliobacteria contain a photosystem that resembles the PSI of oxygenic photosynthetic organisms. It has a cyclic electron flow, but here NAD^+ can be reduced directly. Green sulfur bacteria use the reverse Krebs cycle to fix carbon dioxide, while heliobacteria are photoorganotrophs (for more details on anoxygenic photosynthesis, see, e.g., Whitmarsh and Govindjee, 1999; Madigan et al., 2009).

Summary

The light reactions of photosynthesis use the energy of sunlight to provide chemical energy and reducing power in the form of ATP and $\text{NADPH} + \text{H}^+$, respectively, which can then be used in the light-independent reactions to fix carbon dioxide and to convert it into carbohydrates. During the oxygenic photosynthesis of plants and cyanobacteria, water is split and dioxygen is released, whereas anoxygenic photosynthetic organisms such as purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria do not produce oxygen.

Bibliography

Björn, L. O., and Govindjee, 2009. The evolution of photosynthesis and chloroplasts. *Current Science*, **96**, 1466–1474.
Heldt, H. W., 2003. *Pflanzenbiochemie*. Heidelberg: Spektrum Akademischer Verlag.

Lawlor, D. L., 2001. *Photosynthesis*. Oxford: BIOS Scientific Publishers.

Madigan, M. T., Martinko, J. M., Dunlap, P. V., and Clark, D. P., 2009. *Brock Biology of Microorganisms*. San Francisco: Pearson Benjamin Cummings.

Renger, G., 2010. The light reactions of photosynthesis. *Current Science*, **98**, 1305–1319.

Whitmarsh, J., and Govindjee, R., 1999. The photosynthetic process. In Singhal, G. S., Renger, G., Sopory, S. K., Irrgang, K.-D., and Govindjee, R. (eds.), *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*. Boston: Kluwer, pp. 11–51.

Cross-references

Algae (Eukaryotic)
Bacteria
Banded Iron Formations
Carbon Cycle
Critical Intervals in Earth History
Cyanobacteria
Diatoms
Dinoflagellates
Endosymbiosis
Green Algae

PIEZOPHILIC BACTERIA

Jiasong Fang¹, Li Zhang²

¹Hawaii Pacific University, Kaneohe, HI, USA

²China University of Geosciences, Wuhan Hubei, China

Synonyms

Barophilic bacteria

Definition and introduction

Modern deep-sea microbiology was born in the twentieth century, thanks mainly to the efforts of Claude E. ZoBell, Frank H. Johnson, Richard Y. Morita, Holger W. Jannasch, A. Aristides Yayanos, Koki Horikoshi, and others. ZoBell and coworkers were the first group to study the effect of hydrostatic pressure on microbial activity in the deep sea (ZoBell and Johnson, 1949). They coined the term “barophile” to describe bacteria that have “a requirement for increased pressure for growth, or by increased growth at pressures higher than atmospheric pressure” (Jannasch and Taylor, 1984). Pelagic sediments taken from the Philippine Trench at more than 10,000 m (>100 MPa) contained 10^4 to 10^5 cells of viable bacteria g^{-1} wet weight of various physiological types (ZoBell and Morita, 1956) including nitrate reducers, sulfate reducers, and ammonifiers. Most of these organisms were obligate barophiles, unable to grow at atmospheric pressure (0.1 MPa).

Yayanos and his group were the first to successfully isolate a pressure-adapted bacterium (strain CNPT3) from amphipods collected from the deep sea at 5,700 m in the Pacific (Dietz and Yayanos, 1978). Strain MT41 was probably the first recognized obligate barophile isolated from a decomposing amphipod, *Hirondella gigas*, from a depth of 10,476 m of the Mariana Trench (Yayanos

et al., 1981). Subsequently, numerous deep-sea barophilic bacterial strains have been isolated from the water column, sediments, intestinal tracts, and decaying parts of invertebrates in the deep sea, many of which have been characterized physiologically and phylogenetically (e.g., DeLong et al., 1997).

In 1995, Yayanos (Yayanos, 1995) suggested a change in nomenclature of pressure-loving bacteria, from baro- (Greek word, weight-loving) philes to piezo- (Greek word, pressure-loving) philes. Piezophilic bacteria are now defined as those prokaryotes that display optimal growth at pressures greater than 0.1 MPa or that showing a requirement for increased pressure for growth (Yayanos, 1995). Based on their response to pressure, piezophiles can be classified as piezotolerant (growth from 0.1 to 10 MPa), piezophilic (10–50 MPa), and hyperpiezophilic bacteria (>50 MPa) (Fang and Bazylnski, 2008). This classification is based on the optimal growth pressure, not the maximum growth pressure, of a piezophilic bacterium. Note that the relative growth rate of these organisms decreases with pressure. The upper limits of life with respect to pressure are not yet defined. For example, *Colwellia* strain MT41 grows at pressures greater than 130 MPa, which is more than hydrostatic pressure at any ocean depth (Yayanos, 1986).

Taxonomy and diversity of deep-sea piezophilic bacteria

Most known cultured piezophilic bacteria belong to one of the following five genera of the γ -subdivision of the Proteobacteria: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and a genus containing strain CNPT3, recently identified as *Psychromonas* (Nogi et al., 2002). *Shewanella* includes *S. benthica* and *S. violacea*. *Shewanella* species are particularly widely distributed in the deep sea with a broad range of optimal growth pressure conditions from piezophilic to hyperpiezophilic. Representative species include *S. violacea* strain DSS12; *S. benthica* strain PT99; and strains DB1172F, DB1172R, DB21MT-2, DB5501, DB6101, DB6705, DB6906, WHB46, F1A, PT48. *Moritella* includes *M. japonica*, *M. yayanosii*, *M. profunda*, and *M. abyssi*. *Colwellia* includes *C. hadaliensis* and *C. piezophila* with two hyperpiezophilic strains MT41 and BNL-1. *Photobacterium* includes *P. profundum*. *P. profundum* strain SS9 is one of the most extensively studied piezophilic bacteria whose genomic sequence has been reported recently (Vezzi et al., 2005). *Psychromonas* includes *P. kaikoi* and *P. profunda*. Overall, it seems that there is a limited diversity of the cultured piezophilic bacteria which may be an artifact of the use of rich, heterotrophic growth media. It is likely that diversity of piezophilic bacteria will increase as the use of different types of growth media increases (DeLong et al., 1997). For a more detailed taxonomic description of piezophilic bacteria, see Fang and Kato (2010) and Fang and Bazylnski (2008).

Piezophily, piezophysiology and metabolism of piezophilic bacteria

Temperature (T) and pressure (P) are two interrelated environmental parameters that determine piezophilic bacterial growth over a (P, T)-domain. A bacterial isolate is piezophilic if it has a greater generation time at some high pressure than it does at atmospheric pressure when tested at its habitat temperature (Yayanos, 1998). Generally, deep-sea bacteria show strongest piezophilic response to pressure at their upper temperature for growth (typically 15°C) (Kato et al., 1995). Piezophilic strains become more piezophilic at higher temperatures (Kato et al., 1995). The degree of piezophily increases with increasing collection depth or pressure (Yayanos, 1986). Therefore, each piezophilic isolate would have a single maximum growth rate, $k_{\max}$ ($k = \ln 2/\text{doubling time in h}$) at ($P_{k\max}, T_{k\max}$) on a PTk -diagram (Yayanos, 1986).

Deep-sea piezophilic bacteria are stenothermal (Yayanos, 1998) but “eury-piezotic” (being able to tolerate wide range of pressures). The temperature range ($T_{\max} - T_{\min}$) is roughly 10–20°C (Yayanos, 1986), whereas the pressure range is about 40 MPa for bacteria captured at a depth of less than 3,600 m (Jannasch and Taylor, 1984) and 80 MPa for isolates of depths greater than 5,000 m (e.g., Yayanos and DeLong, 1987).

Laboratory and field studies based on substrate utilization have confirmed piezophilic bacterial activity in deep-sea sediments. Deming and Colwell (1985) examined microbial activity in box cores and sediment trap samples collected in the Demerara abyssal plain in the South Atlantic Ocean (4,470 and 4,850 m). Samples were incubated with low levels (<10% above natural abundance) of [^{14}C] glutamate at 3°C and in situ and atmospheric pressure. In both sediment and sinking particulate samples examined, enhanced microbial utilization of [^{14}C] glutamate was observed at in situ pressure, suggesting that indigenous piezophilic bacteria, not the piezo-sensitive microbes originated in shallow surface waters, were metabolically active and functionally predominant in the cycle of naturally low levels of organic matter in the abyssal sediments (Deming and Colwell, 1985). Similar results were obtained in a study conducted on sediment from the Porcupine Abyssal Plain of the Atlantic Ocean (Eardly et al., 2001). Thus, it is highly likely that piezophilic bacterial activity may be widespread in deep sea. However, the proportion of the metabolically/functionally dominant piezophilic bacterial population over the total microbial population in deep sea is unknown.

It is generally believed that the effect of pressure on metabolic processes is secondary to that of temperature. However, based on thermodynamic calculations, Fang and Bazylnski (2008) demonstrated that pressure does have a significant effect on microbial-mediated redox reactions (Figure 1); high pressures reduce the energy yield of O_2 reduction, NO_3^- reduction, and sulfate reduction and increase the energy yield of the reduction of Fe_2O_3 . The additive effect of temperature and pressure

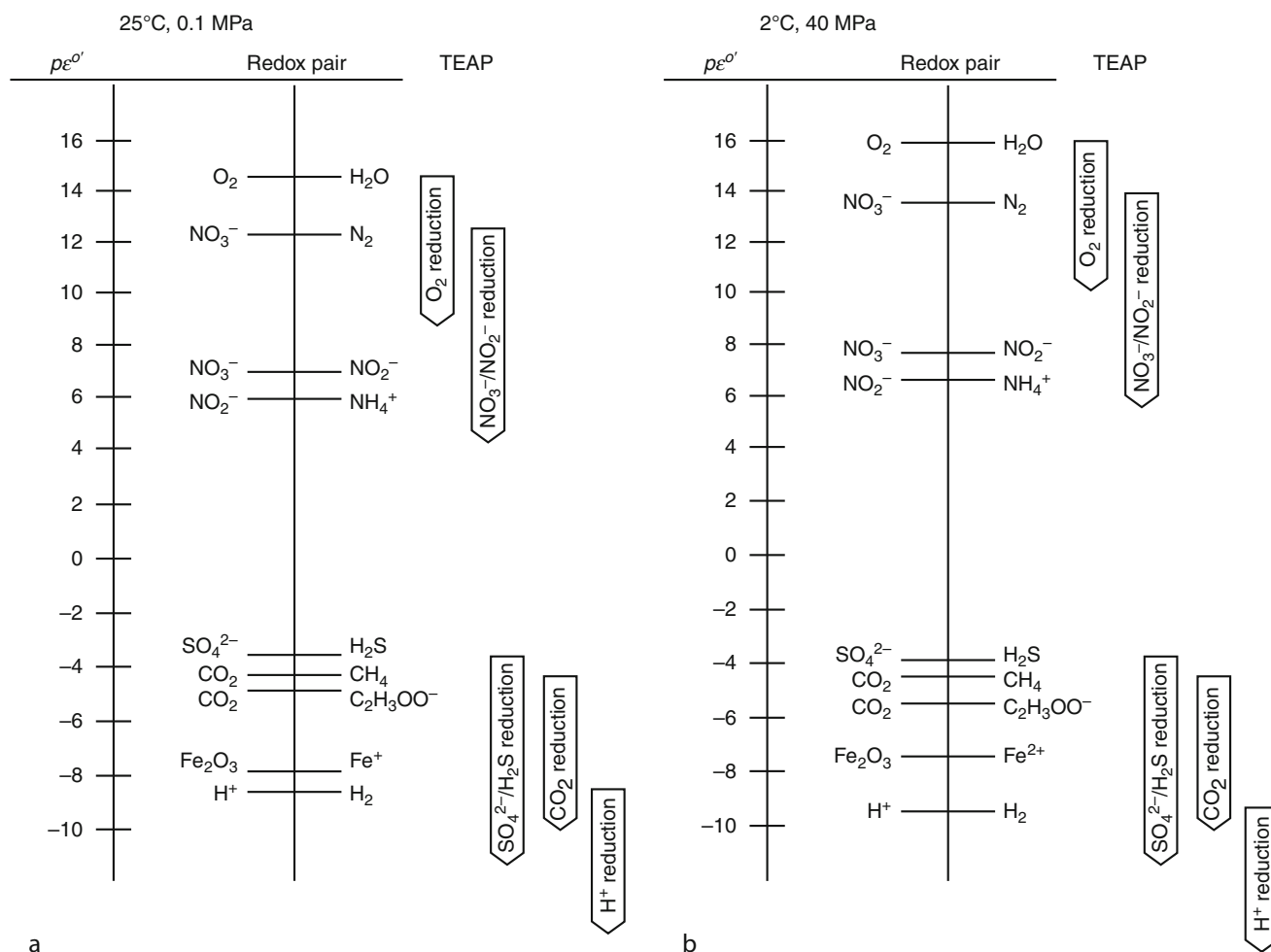
has raised the $p\epsilon^{o'}$ values of the first four reactions commonly taking place at more positive redox potentials and lowered the $p\epsilon^{o'}$ values (electron activity at the biological standard state) for other six reactions that take place at more reducing conditions. Thus, redox reactions prevailing at more oxidative condition (O_2 , NO_3^-/NO_2^- reduction, etc.) yield slightly more energy in the deep-sea low temperature–high pressure conditions, whereas those that dominate in more reducing conditions yield relative less energy, compared to surface environments (25°C , 0.1 MPa).

Lipid biosynthesis and carbon isotope fractionation of piezophilic bacteria

The most abundant lipids detected in piezophilic bacteria are *n*-alkyl, acetogenic lipids (i.e., fatty acids); no polyisoprenoids are detected (Fang, unpublished data). Fatty acids biosynthesized by piezophilic bacteria include C_{12-19}

saturated, monounsaturated, terminal methyl-branched, β -hydroxyl, and cyclopropane fatty acids (Fang et al., 2003; Fang and Bazylinski, 2008). Piezophilic bacteria are unique and distinctive in fatty acid composition in that: (1) they synthesize abundant polyunsaturated fatty acids (PUFA), i.e., eicosapentaenoic acid (EPA, $20:5\omega3$ *cis*-5,8,11,14,17) and docosahexaenoic acid (DHA, $22:6\omega3$ *cis*-4,7,10,13,16,19) and (2) the abundance of unsaturated fatty acids is high and can comprise up to 70% or more of the total fatty acids.

It is generally believed that the carbon isotopic composition of cell components and lipid biomarkers can provide information on modes of microbial metabolism and physiology. The carbon isotope signature is especially useful in deep-sea geomicrobiology for two reasons (Fang and Kato, 2007). First, environmental conditions (temperature and pressure) of the deep sea may exert a greater influence on carbon isotope fractionation in the biosynthesis of fatty acids and therefore the carbon isotopic ratios of individual



Piezophilic Bacteria, Figure 1 Sequence of microbially-mediated reduction reactions based on values of electron activity at biological standard state ($p\epsilon^{o'}$) at 25°C and 1 bar (a) and 2°C and 400 bar (b).

compounds (Fang et al., 2006). Second, the deep-sea piezophilic bacteria possess two simultaneously operating biosynthetic pathways of fatty acids which dictate distinctive carbon isotope signature for the long-chain polyunsaturated fatty acids (Metz et al., 2001).

Recently, Fang et al. (2006) examined carbon isotope fractionation during fatty acid biosynthesis in *Moritella japonica* strain DSK1 and demonstrated that carbon isotope fractionation in biosynthesis of fatty acids of piezophilic bacteria is pressure-dependent. Fatty acids became progressively more depleted in ^{13}C with growth pressure. There was a strong linear correlation between carbon isotopic fractionation and hydrostatic pressure. Overall, piezophilic bacteria fractionate carbon isotopes significantly (14–18‰) more than surface heterotrophic bacteria. Thus, the recycling and re-synthesis of fatty acids by piezophilic bacteria utilizing organic matter originating from primary production will greatly alter the carbon isotope signature of both short-chain bacterial and long-chain planktonic fatty acids in oceanic environments and marine sediments. Further, PUFA had much more negative $\delta^{13}\text{C}$ values than other short-chain saturated and monounsaturated fatty acids. This was attributed to the operation of two different fatty acid biosynthetic systems in piezophilic bacteria: the FAS (fatty acid synthase)- and PKS (polyketide synthases)-based pathways (Fang and Kato, 2007). The FAS-based pathway is one common to surface bacteria which synthesizes typical bacterial fatty acids. The PKS-based pathway is a fundamentally different pathway which involves polyketide synthases (Metz et al., 2001) which catalyze the biosynthesis of long-chain polyunsaturated fatty acids. The PKS pathway appear to be widely distributed in marine bacteria (Wallis et al., 2002) as genes with high homology to the *Shewanella* EPA gene cluster (*Shewanella* sp. SCRC-2738) (Yazawa, 1996) have been found in *Photobacterium profundum* strain SS9 which synthesizes EPA (Allen et al., 1999) and in *Moritella marina* strain MP-1 which produces DHA (Tanaka et al., 1999). Thus, fatty acids (particularly EPA and DHA) and carbon isotope signatures become more informative in deep-sea geomicrobiology.

Conclusions

Piezophilic bacteria are prokaryotes that display optimal growth at pressures greater than 0.1 MPa or that showing a requirement for increased pressure for growth. The deep-sea piezosphere, influenced by hydrostatic pressure, probably represents the largest biotope of the Earth. The evidence obtained thus far suggests that microbial life in the deep sea is diverse and that microbial biomass there can be significant. All of the piezophilic isolates reported thus far are facultatively anaerobic, some demonstrating anaerobic respiration as well as fermentation. It has been shown that the degradation of organic matter by fermentation and anaerobic respiration is the principal energy generation processes in both surface and subsurface marine sediments (Parkes et al., 2005). Thus, marine bacteria

must play an important role in the overall ocean carbon cycle. These microorganisms surely influence the surface of the Earth by changing the chemistry of the ocean and by affecting the rate of organic carbon burial, with consequences for both the marine carbon cycle and global climate (Fang and Bazylinski, 2008).

Bibliography

- Allen, E. E., Facciotti, D., and Bartlett, D. H., 1999. Monounsaturated but not polyunsaturated fatty acids are required for growth of the deep-sea bacterium *Photobacterium profundum* SS9 at high pressure and low temperature. *Applied and Environmental Microbiology*, **65**, 1710–1720.
- DeLong, E. F., Franks, D. G., and Yayanos, A. A., 1997. Evolutionary relationship of cultivated psychrophilic and barophilic deep-sea bacteria. *Applied and Environmental Microbiology*, **63**, 2105–2108.
- Deming, J. W., and Colwell, R. R., 1985. Observation of barophilic microbial activity in samples of sediment and intercepted particulates from the Demerara Abyssal Plain. *Applied and Environmental Microbiology*, **50**, 1002–1006.
- Dietz, A. S., and Yayanos, A. A., 1978. Silica gel media for isolating and studying bacteria under hydrostatic pressure. *Applied and Environmental Microbiology*, **36**, 966–968.
- Eardly, D. F., Carton, M. W., Gallagher, J. M., and Patching, J. W., 2001. Bacterial abundance and activity in deep-sea sediments from the eastern North Atlantic. *Progress in Oceanography*, **50**, 245–259.
- Fang, J., Kato, C., Sato, T., Chan, O., Peeples, T., and Niggemeyer, K., 2003. Phospholipid fatty acid profiles of deep-sea piezophilic bacteria from the deep sea. *Lipids*, **38**, 885–887.
- Fang, J., Uhle, M., Billmark, K., Bartlett, D. H., and Kato, C., 2006. Fractionation of carbon isotopes in biosynthesis of fatty acids by a piezophilic bacterium *Moritella japonica* DSK1. *Geochimica et Cosmochimica Acta*, **70**, 1753–1760.
- Fang, J., and Bazylinski, D. A., 2008. Deep-Sea Geomicrobiology. In Michiels, C., and Bartlett, D. H. (eds.), *High-Pressure Microbiology*. Washington, DC: American Society for Microbiology, pp. 237–264.
- Fang, J., and Kato, C., 2007. FAS or PKS, lipid biosynthesis and stable carbon isotope fractionation in deep-sea piezophilic bacteria. In Méndez-Vilas, A. (ed.), *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, The Formatex Microbiology Book Series. Spain: Formatex Center, pp. 190–200.
- Fang, J., and Kato, C., 2010. Deep-sea piezophilic bacteria: geomicrobiology and biotechnology. In: *Geomicrobiology: Biodiversity and Biotechnology* (S. K. Jain, ed.), pp. 47–77. Blackwell Publishing.
- Jannasch, H. W., and Taylor, C. D., 1984. Deep-sea microbiology. *Annual Review in Microbiology*, **38**, 487–514.
- Kato, C., Sato, T., and Horikoshi, K., 1995. Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. *Biodiversity and Conservation*, **4**, 1–9.
- Metz, J. G., Roessler, P., Facciotti, D., Leverine, C., Dittrich, F., Lassner, M., Valentine, R., Lardizabal, K., Domergue, F., Yamada, A., Yazawa, K., Knauf, V., and Browne, J., 2001. Production of polyunsaturated fatty acids by polyketide synthases in both prokaryotes and eukaryotes. *Science*, **293**, 290–293.
- Nogi, Y., Kato, C., and Horikoshi, K., 2002. *Psychromonas* *kaikoi* sp. nov., isolation of novel piezophilic bacteria from the deepest cold-seep sediments in the Japan Trench. *International Journal of Systematic and Evolutionary Microbiology*, **52**, 1527–1532.
- Parkes, R. J., Webster, G., Cragg, B. A., Weightman, A. J., Newberry, C. J., Ferdelman, T. G., Kallmeyer, J., Jørgensen, B. B.,

- Aiello, I. W., and Fry, J. C., 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geologic time. *Nature*, **436**, 390–394.
- Tanaka, M., Ueno, A., Kawasaki, K., Yumoto, I., Ohgiya, S., Hoshino, T., Ishizaki, K., Okuyama, H., and Morita, N., 1999. Isolation of clustered genes that are notably homologous to the eicosapentaenoic acid biosynthesis gene cluster from the docosahexaenoic acid-producing bacterium *Vibrio marinus* strain MP-1. *Biotechnology Letters*, **21**, 939–945.
- Vezzi, A., et al. 2005. Life at depth: Photobacterium profundum genome sequence and expression analysis. *Science* **307**, 1459–1461.
- Wallis, J. G., Watts, J. L., and Browse, J., 2002. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends in Biological Science*, **27**, 467–473.
- Yayanos, A. A., 1986. Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. *Proceedings of the National Academy of Sciences of the USA*, **83**, 9542–9546.
- Yayanos, A. A., 1998. Empirical and theoretical aspects of life at high pressure in the deep sea. In Horikoshi, K., and Grant, W. D. (eds.), *Extremophiles, Microbial Life in Extreme Environments*. New York: Wiley, pp. 47–92.
- Yayanos, A. A., and DeLong, E. F., 1987. Deep-sea bacterial fitness to environmental temperatures and pressures. In Jannasch, H. W., Marquis, R. E., and Zimmerman, A. M. (eds.), *Current Perspectives in High Pressure Biology*. London: Academic Press, pp. 17–32.
- Yayanos, A. A., Dietz, A. S., and Van Boxtel, R., 1981. Obligately barophilic bacterium from the Mariana Trench. *Proceedings of the National Academy of Sciences of the USA*, **78**, 5212–5215.
- Yazawa, K. 1996. Production of eicosapentaenoic acid from marine bacteria. *Lipids*, **31**, S297–S300.
- ZoBell, C. E., and Johnson, F. H., 1949. The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. *Journal of Bacteriology*, **57**, 179–189.
- ZoBell, C. E., and Morita, R. Y., 1956. Barophilic bacteria in some deep sea sediments. *Journal of Bacteriology*, **73**, 563–568.

Cross-references

Bacteria
 Biogeochemical Cycles
 Biomarkers (Molecular Fossils)
 Deep Biosphere of the Oceanic Deep Sea
 Isotopes (Methods)
 Terrestrial Deep Biosphere

PORE WATERS

Sabine Kasten
 Alfred Wegener Institute for Polar and Marine Research,
 Bremerhaven, Germany

Synonyms

Interstitial waters

Definition

The term *pore water* refers to the water contained in the interstices/pore space of aquatic sediments.

Introduction

Extracting pore waters and analyzing various dissolved constituents is a central approach in marine sediment

geochemistry, biogeochemistry, and geo(micro)biology. The ensuing pore-water concentration-depth profiles are used (a) to characterize the geochemical environment and redox zonation of aquatic sediments (Froelich et al., 1979; Berner, 1981; Postma and Jakobsen, 1996), (b) to infer and identify early diagenetic and biogeochemical processes by compounds which are either consumed or released by the particular reaction (Reeburgh, 1969; Froelich et al., 1979), (c) to calculate diffusive fluxes of dissolved constituents (Hensen et al., 2006; Zabel and Hensen, 2006), and (d) to model reaction and turnover rates of biogeochemical and diagenetic processes – including mineral dissolution and precipitation (Berner, 1980; Van Cappellen and Wang, 1995; Boudreau, 1996). Furthermore, pore-water profiles are applied to quantify the abundance of gas hydrates in marine deposits (Ussler and Paull, 2001; Bohrmann and Torres, 2006) and to study subsurface fluid flow (Saffer and Scretton, 2003).

Pore-water profiles evolve as a consequence of the complex interplay between successive microbial degradation of organic matter by various electron acceptors and a broad spectrum of secondary diagenetic reactions on the one hand and different transport processes (diffusion, advection, bioturbation, and bioirrigation) on the other hand. Due to the close coupling between geochemistry and microbiology, concentration-depth profiles of dissolved chemical species often point to “new” – i.e., to date unknown – biogeochemical processes and metabolic pathways and pave the way for the discovery of novel microorganisms or consortia of microbes which mediate the particular reactions. A well-known example is the process of anaerobic oxidation of methane by sulfate (AOM) which was already postulated to occur on the base of pore-water profiles in the late 1960s (Reeburgh, 1969). Only about 30 years later, new and advanced techniques in molecular biology ultimately were able to prove the existence of microbial consortia of archaea and sulfate-reducing bacteria mediating this process (Boetius et al., 2000; Orphan et al., 2001).

Extraction and analyses of pore waters

The classical ways of extracting pore waters are (a) by squeezers where pressure is applied either by an inert gas (e.g., nitrogen, argon) or with a hydraulic press, or (b) by centrifuging and subsequent decanting (Gieskes et al., 1991; Schlüter, 1990; De Lange et al., 1992; for a review see Schulz, 2006, and references therein). In recent years, a new technique for a rapid and more or less nondestructive collection of pore waters from marine sediments has been developed and established – the so-called rhizon sampling (Seeberg-Elverfeldt et al., 2005; Dickens et al., 2007). Rhizon samplers are thin tubes of hydrophilic porous polymer which have a minimum pore size of 0.1 µm. They are inserted into the sediment and are connected to either vacuum vials, spring-loaded syringes, or operated with peristaltic pumps which allow to

Microbialites, Stromatolites, and Thrombolites
 Nanocrystals, Microbially Induced
 Ores, Microbial Precipitation and Oxidation
 Photosynthesis
 Salinity History of the Earth's Ocean
 Sediment Diagenesis – Biologically Controlled
 Snowball Earth
 Stromatolites

BASALT (GLASS, ENDOLITHS)

Ingunn H. Thorseth
 University of Bergen, Bergen, Norway

Synonyms

Endolithic microorganisms in basaltic glass; Microbial life in glassy basalt

Definition

Basalt endoliths. Microorganisms that colonize and live inside fractures, vesicles, and other cavities in basaltic rocks.

Basalt. The most common volcanic rock formed by eruptions of magma of basaltic composition.

Seafloor basalt. Basalt that is exposed at the seafloor, most commonly in or close to the ocean spreading ridge axes where the crust is young, or at intraplate seamounts.

Subseafloor basalt. Basalt that has been buried by younger lava flows and/or deep-sea sediments, as the crust aged and moved away from the spreading ridges.

Basaltic glass and endolithic microorganisms

Introduction

Basalt is continuously formed during volcanic eruptions along the global mid-ocean ridge system, and constitutes the upper 0.5–1 km thick layer of the ocean crust which covers more than 50% of the Earth's surface. When the basaltic magma is extruded into the cold seawater, the 1–2 cm thick outermost surface layer is cooled so rapidly that its chemical species do not have enough time to be arranged into minerals, but freeze to an amorphous glass rind. These glassy margins become heavily fractured due to cooling-related contraction, and the fracture networks provide an immense surface area for rock-water interactions and colonization by microorganisms. As the basaltic glass dissolves and alters more quickly than the minerals (pyroxene, plagioclase) in the crystalline interior of the lava, the glassy proportion plays an important role in the alteration of the ocean crust and the chemical exchange with the ocean water. The alteration is not only controlled by physiochemical factors but also by microbial processes, implied by the presence of endolithic microorganisms (see *Endoliths*) within the glassy margins. The microbial

activity may, on the other hand, be controlled by the geological environment, which includes a variety of complex fluid temperature and chemistry conditions ranging from seafloor weathering to various hydrothermal systems.

Basaltic glass alteration

Low-temperature alteration of basaltic glass results in the formation of concentrically zoned rims of a yellow-brown, hydrous residual alteration product, frequently called palagonite, along the outer surfaces, and internal vesicles and fractures of the glassy margins of lava flows. The altered glass is amorphous (gel-palagonite) to poorly crystalline with clayish character (fibro-palagonite) (e.g., Stroncik and Schmincke, 2001), and is usually highly enriched in oxidized Fe and Ti, and depleted in Si and alkaline and alkaline earth elements compared to the fresh, parental glass. The term palagonite was used for the first time by von Waltershausen in 1845 for the brown groundmass of a tuff from Palagonia, Sicily, and was then thought to be a new mineral phase. Although palagonite was later shown to be a heterogeneous material, the term has commonly been used in the literature. However, several authors now recommend referring to this material as altered glass. During the last 2 decades, various field and laboratory studies have shown that the alteration rate and the chemical and crystalline characteristics of the altered glass strongly depend on the physiochemical and hydrological conditions (e.g., Crovisier et al., 2003). The understanding of how the endolithic microorganisms in the glassy margins of basaltic lava flows influence and interact with the alteration process is, however, still poor.

Evidence of microbial life in glassy basalt

The first in situ observations of endolithic microorganisms in basaltic glass was reported from a scanning electron microscopy (SEM) study of recent (<1 million years old (Ma)) volcanic glass deposits from Iceland (Thorseth et al., 1992). Microbial biofilms together with irregular alteration fronts at the fresh-altered glass boundary lead to the suggestion that the degradation of the volcanic glass could be biocatalyzed by the development of more aggressive microenvironments in the near vicinity of the microbial cells. This was subsequently supported by experimental alteration studies, where development of corrosion marks in the glass surface only was observed in combination with biofilm formation and not in sterile controls (Thorseth et al., 1995a; Staudigel et al., 1995, 1998). Similar alteration textures resembling microbial cell morphologies observed in glassy margins of 6 Ma ocean basalt, sampled 432 m below the seafloor at the Costa Rica Rift during the Ocean Drilling Program (ODP) Leg 148, were then stained using DNA-specific dyes to confirm the presence of DNA within these structures (Thorseth et al., 1995b; Giovannoni et al., 1996; Furnes et al., 1996). Further fluorescence in situ

hybridization (FISH) were used to document the existence of indigenous Bacteria (see *Bacteria*) and Archaea (see *Archaea*) in this glassy basalt (Torsvik et al., 1998). Additional evidence for microbial activity in oceanic basalt came from the demonstration of carbon and nitrogen enrichments in the alteration textures (Giovannoni et al., 1996; Torsvik et al., 1998), and stable carbon isotope fractionation from disseminated carbonate that is consistent with microbial processes (Torsvik et al., 1998). During the last decade, further morphological, geochemical, biomolecular, and cultivation studies of seafloor and subseafloor basalts of various ages, water depths, depth of burial and temperature regimes, as well as of hydrothermal fluids, have revealed that diverse endolithic microbial communities colonize the upper oceanic crust at the spreading ridges. In addition, detection of comparable alteration textures and geochemical and isotopic biosignatures (see *Biosignatures in Rocks*) in lava margins of ancient oceanic basalt implies that microbial life and associated biodegradation in the ocean crust occurred very early in the Earth's history (Furnes et al., 2004; Staudigel et al., 2006).

Microbial life in seafloor basalt

Several electron microscopy studies of seafloor lavas from the Arctic Mohs and Knipovich Ridges and the Australian Antarctic Discordance (AAD) area of the Southeast Indian Ridge (SEIR) have demonstrated that a diverse and numerous endolithic microbial community of various coccoid, rod-shaped, filamentous, and stalked cells colonize the fracture network in the glassy margins of the basalts soon after its formation and exposure to the cold, oxygenated seawater (Figure 1) (Thorseth et al., 2001, 2003; Kruber et al., 2008a, b). The presence of microbial cells in lavas from different regions of the seafloor suggests that microbial colonization and growth is a general feature of mid-ocean ridge basalts. In very young basalts (0 Ma), the microbial cells are observed in the central fractures attached to the outer surface of alteration rims, within porous zones in the rims, and at the alteration front attached to the fresh glass (Figure 1b and c). The cells act as nucleation sites for precipitation and become encrusted and embedded in the secondary alteration products with time. Alteration rims where porous zones containing numerous hollow, apparently fossilized cells (see *Microbial Biomineralization*), alternate with compact zones lacking distinctive cell structures, indicate that the microbial growth in the fractures is discontinuous (Figure 1b). In older (2.5 Ma) seafloor basalts where the alteration rims are much thicker, no evident cell structures have been observed at the alteration fronts (Figure 1d), but occur in porous zones of the rims closer to the central fracture, and within in the fractures attached to secondary zeolite crystals (Figure 1e). The cells within the rims are much lower in carbon than those in the fractures, indicating that they are fossilized or partially decayed. This implies that

the fluid availability decreases through the alteration rims, away from the fractures (Thorseth et al., 2003).

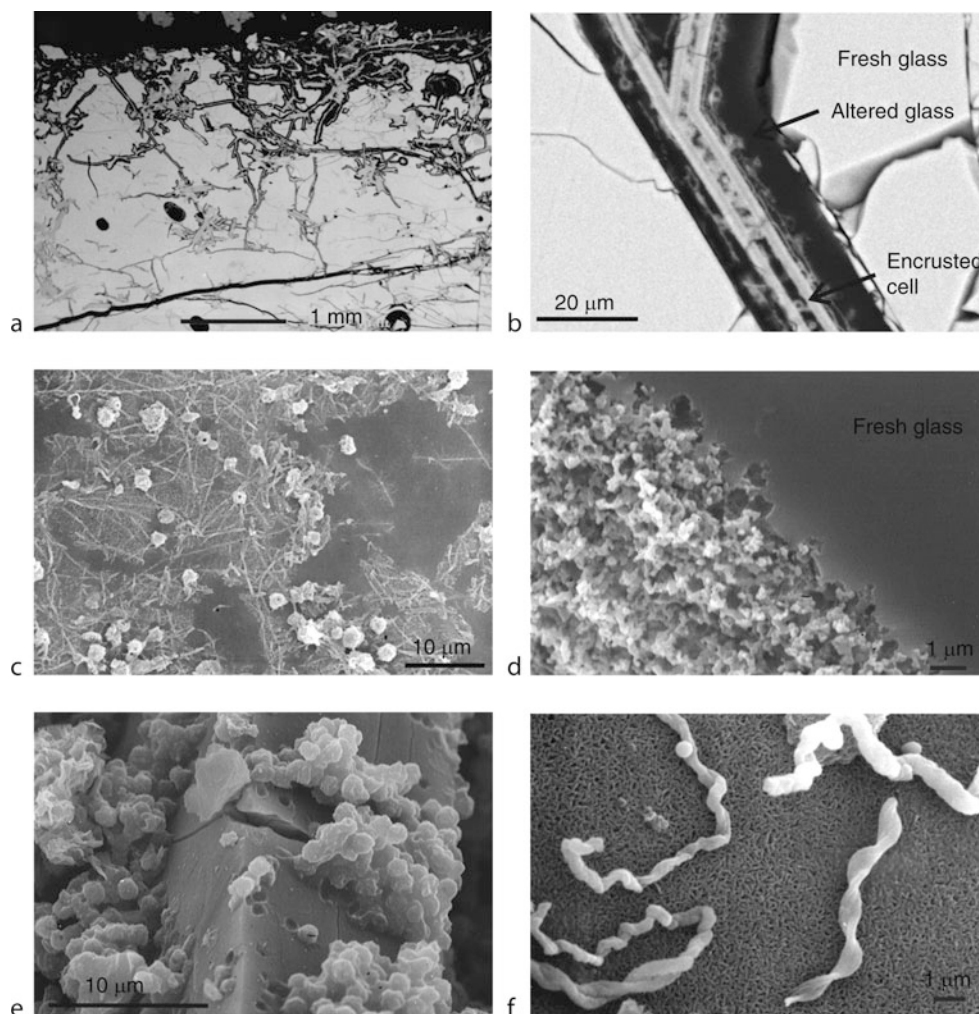
Biomass

The presence of abundant alteration rims with closely packed microbial cell structures indicate high cell numbers in the glassy margins of young seafloor basalt. However, a significant fraction of these structures are fossilized cells that have accumulated through time. Thus, while the number of living cells measured by real-time quantitative PCR and fluorescence microscopy is estimated to be around 10^6 cells/g of glassy seafloor basalt (Einen et al., 2008), calculations based on the organic carbon content in the altered glass indicate a considerable larger endolithic biomass (around 10^9 cells/g of glassy basalt, assuming 10^{-13} g of carbon in one cell and that 1% of the glass is altered (Kruber et al., 2008a)). Even if this calculation is highly tentative, the discrepancy suggests that the biogenic material in the fossilized cells is preserved for some time before it is completely decayed. This is further supported by detection of carbon in individual cell encrustations by microanalytical techniques (Thorseth et al., 2003). Due to the highly porous and fractured character of the upper extrusive basalt layer at spreading axes, it is supposed that a similar biomass may also be present in the subseafloor basalt at depths down to several tens to hundreds of meters.

Phylogenetic and metabolic diversity

The endolithic microbial communities in the Arctic seafloor basalts are shown to be almost exclusively from the domain *Bacteria*, and only small proportions are from the *Archaea* domain (Einen et al., 2008). The most frequently retrieved bacterial DNA sequences from these basalts affiliate with the *Gamma-proteobacteria*, *Alpha-proteobacteria*, *Chloroflexi*, *Firmicutes*, and *Actinobacteria*, while the archaeal sequences were restricted to the marine Group 1 *Crenarchaeota* (Lysnes et al., 2004a). The phylogenetic diversity of basalt endoliths is found to be distinct and different from those observed in the surrounding deep seawater.

In contrast to deep-sea sediments where biogenic debris from the photic zone provides an important energy and carbon source for the inhabiting microbial communities, the basaltic basement is rich in reduced inorganic compounds that together with dissolved inorganic carbon may support growth of lithoautotrophic (see *Chemolithotrophy*) microorganisms (Bach and Edwards, 2003). Mid-ocean ridge basalts typically have iron contents of 7–9 wt%, of which approximately 80% is in the divalent state. As the basaltic glass alters to palagonite, Fe(II) is predominantly oxidized to Fe(III). The alteration of the glass may thus provide both potential energy and electron acceptors for the endolithic microbial communities. Observations of branching, twisted filamentous structures resembling stalks of the neutrophilic, iron-oxidizing bacteria (see *Fe(II)-Oxidizing Prokaryotes*) *Gallionella*



Basalt (Glass, Endoliths), Figure 1 Scanning electron microscopy images of seafloor basalts. (a) Glassy margin of zero-age basalt from the Mohs Ridge with typical network of cracks and fractures where the glass reacts with the seawater and alters to palagonite; (b) close up of fracture with regular alteration rims at both sides, showing alternating solid and porous zones with abundant cell encrustations (hollow spheres); (c) microbial cells attached to the fresh glass at the alteration front in the Arctic seafloor basalt; (d) interface between fresh and altered glass in 2.5 Ma seafloor basalt from the SEIR, where no evident cell structures occur; (e) zeolite crystal in fracture of the 2.5 Ma basaltic glass with clusters of coccoid cells attached to it; (f) filamentous structures resembling stalks of iron-oxidizing bacteria (see *Gallionella*) within fracture of zero-age basaltic glass from the Knipovich Ridge.

ferruginea (see *Gallionella*) and the newly characterized *Mariprofundus ferrooxidans* (Emerson et al., 2007) at the glass-palagonite interface (Figure 1f) indeed indicate that microbial oxidation of Fe(II) from the dissolving glass is associated with the weathering of basalt (Thorseth et al., 2001). Culturing of novel Fe-oxidizing lithoautotrophs from inactive sulfide chimneys (Edwards et al., 2003) furthermore suggests that other, previous unknown Fe-oxidizing species could occur in basalt as well. In addition, preferential accumulation of manganese in some cell encrustations can be explained by the activity of Mn-oxidizing bacteria (Thorseth et al., 2001, 2003; Kruber et al., 2008a, b). Isolation of numbers of diverse heterotrophic Mn(II)-oxidizing bacteria from the surfaces

of recent basalt flows from the Loihi Seamount strongly support this (Templeton et al., 2005). Results from DNA analyses and enrichments have furthermore shown that Fe-reducing bacteria (see *Fe(III)-Reducing Prokaryotes*) affiliating with *Shewanella frigidimarina* (see *Shewanella*) as well as other unknown Fe-reducers also are associated with the weathering of glassy seafloor basalt (Lysnes et al., 2004a). Microbial catalyzed cycling of Fe (and Mn) may thus be important in low-temperature alteration of oceanic basalt. Although seafloor basalts are in direct contact with the ambient, oxygenated seawater, the presence of microaerophilic microorganisms such as *G. ferruginea* and *M. ferrooxidans* demonstrates that redox-gradients are formed in the seawater-filled fractures

during an early stage of alteration and microbial colonization of glassy margins. The detection of Fe-reducers furthermore indicates that anaerobic microenvironments locally develop in the fracture network of the glassy margins. Neither sulfate reducing bacteria (SRB) nor secondary sulfide phases, which are commonly reported from marine sediment, have however, been detected.

Production of methane from hydrogen and carbon dioxide during enrichments indicates in addition that lithotrophic methanogens are present in glassy seafloor basalt, although DNA sequences from basalt and enrichments have not matched DNA sequences from any known methanogens (Lysnes et al., 2004a). The hydrogen source for methanogenesis in the basaltic glass is however, unclear. Degassing from deep magma chambers or production by high-temperature water-rock interactions associated with volcanic eruptions and serpentinization of ultramafic rocks (Holloway and O'Day, 2000; Kelley et al., 2002) are all unlikely processes at the actual sampling sites. Simulations of geochemical reactions between water and ultramafic rocks indicate, however, hydrogen production also at low temperatures (Palandri and Reed, 2004). Hydrogen production during low-temperature alteration of basalt has, however, been a matter of debate (e.g., Andersen et al., 1998). Hydrogen could, on the other hand, be produced by microbial processes.

Microbial impact on the alteration: biomineralization and microbial mediated dissolution

The microbial catalysed oxidation and reduction of iron and manganese, and nucleation and precipitation of alteration products on the microbial cells and their extracellular material, are likely to partially control the mobility of elements from the dissolving glass and reacting seawater. In addition, as the microbial growth and fossilization are main controls on the porosity and texture of the alteration rims, these processes are also expected to affect the fluid exchange rate and thus, the dissolution rate in the fractures. Together with the relatively large endolithic biomass, this suggests that the potential microbial impact on the weathering of seafloor basalt and the chemical exchange with the seawater is significant. Observation of pit marks in the glass beneath the attached microbes

(Figure 2a) furthermore suggests that the dissolution of the glass is to some extent controlled by microbial processes (Thorseth et al., 2001). It is, however, not known if the pitting is actively caused by local enhanced microbial dissolution due to the utilization of energy or nutrients present in the glass, or to the passive accumulations of metabolic waste products and the development of corrosive microenvironments at the interface between the microbial cells and the glass. Results from recent laboratory experiments have shown that organotrophs (Daughney et al., 2004) as well as lithoautotrophs (Edwards et al., 2004) have the potential to increase the dissolution rate and the alteration of basalt at the seafloor.

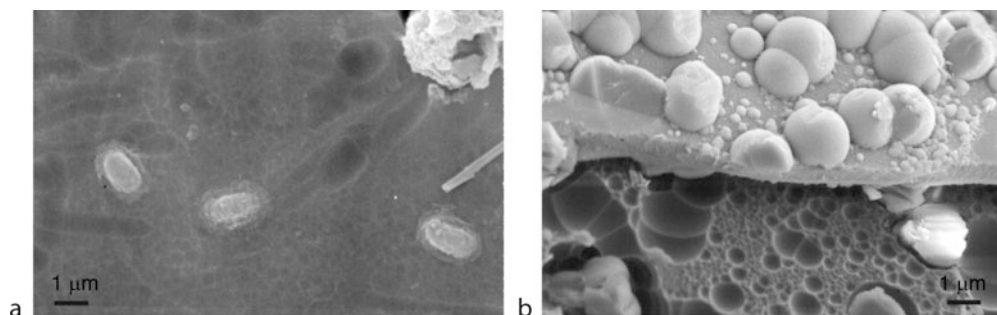
In addition to putative microbial mediated pitting, pit marks of similar form and size to microbes that are filled by hemispherical alteration structures and not by characteristic hollow cell encrustations (Figure 2b) suggest that abiotic processes may produce comparable dissolution textures to microbial processes. Such abiogenic corrosion marks most frequently occur in narrow fractures, which suggest that they are related to dissolution at low water-rock ratios (Kruber et al., 2008a).

Microbial life in subsurface basalt

Drill cores: textural, geochemical, isotope, and biomolecular biosignatures

The first reported evidence of endolithic microbes in basalt from the Costa Rica Rift subsequently stimulated to additional studies of basaltic glass in subsurface rocks from various locations and ages. Most of the studies report of similar textural, geochemical, isotope, and biomolecular evidences for microbial activity in the glass, as in the first report (Furnes and Staudigel, 1999; Fisk et al., 1998, 2003; Furnes et al., 1999, 2001a, b, 2003; Banerjee and Muehlenbachs, 2003; Thorseth et al., 2003; Benzerara et al., 2007). The phylogenetic and metabolic diversity of subseafloor endolithic microbial communities inhabiting oceanic basalts have, however, only been described in the study by Lysnes et al. (2004b), and with more limited data in the study by Fisk et al. (2003).

While abiotic alteration of isotropic basaltic glass is expected to produce regular zoned alteration rims with



Basalt (Glass, Endoliths), Figure 2 (a) Pit marks in the glass with similar form and size as the attached microbial cells; (b) pit marks in the glass that are filled by solid hemispherical alteration structures and not by hollow microbial encrustations.

smooth interfaces between the fresh and altered glasses, the commonly observed irregular alteration fronts have been interpreted to be a result of enhanced dissolution or corrosion mediated by endolithic microbial cells. The most frequently putative biogenic alteration textures reported are (1) irregular patches or zones of individual or coalesced spheres with diameter around 1 μm (also characterized as a granular texture type), and (2) channels or tubular forms 1 μm to few μm wide and up to more than 100 μm in length (Figure 3). After the first reports of DNA-specific staining of altered glass from the Costa Rica Rift, similar textures in samples from other locations have also been shown to contain nucleic acids by fluorescent dyes (Furnes et al., 2001a; Banerjee and Muehlenbachs, 2003; Fisk et al., 2003), as well as elevated amounts of carbon, nitrogen, and phosphorus.

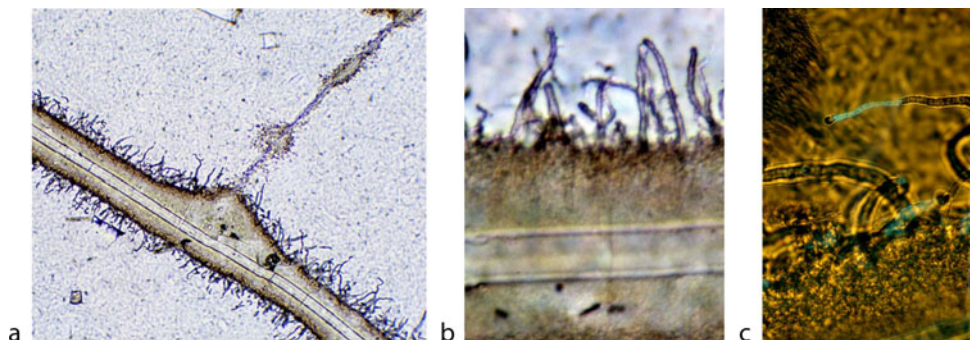
Using alteration textures as discriminating criteria, the extent and importance of biogenic versus abiotic alteration in the oceanic basement has been estimated. Thus, bioalteration has been observed in glasses from 0 to 170 Ma crust at 0–1.5 km depths of burial of lava flows and deep-sea sediments (Fisk et al., 1998; Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006). The degree of this bioalteration is highly variable in the upper 300 m of the crust (20–90%), but tends to decrease with depth. The granular texture is found to dominate at all depths. The tubular texture only represents a small fraction of the total bioalteration, and is more common at deeper levels (Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006).

The stable carbon isotope composition ($\delta^{13}\text{C}$) of disseminated carbonate provides additional evidence for endolithic microbial activity in altered oceanic basalts. Most investigated samples show $\delta^{13}\text{C}$ values that are significantly lower than magmatic carbon (5 to 7), indicating microbial utilization and oxidation of organic carbon which leads to ^{12}C -enriched CO_2 and carbonate minerals (Furnes et al., 2001a). Positive $\delta^{13}\text{C}$ values of some samples from the Atlantic Ocean have, on the other hand, been explained by lithoautotrophic utilization of H_2 and CO_2

and production of methane. During this process ^{12}C -enriched methane is lost, and the remaining carbonate is enriched in ^{13}C . This could result from more extensive serpentinization and abiotic formation of H_2 at the slow-spreading Atlantic ridge compared to other faster spreading ridges.

Despite the evidence for ongoing microbial activity in subseafloor basalt, few direct observations of evident microbial cell structures, which are easily observed in seafloor basalt, have been reported. Possible candidates are hollow and solid filamentous structures detected in basalt tuff recovered from the Ontong Java Plateau during the ODP Leg 192 (Banerjee and Muehlenbachs, 2003), and hollow spherical structures in pillow basalt recovered from the AAD area of the SEIR during ODP Leg 187 (Thorseth et al., 2003). However, these structures were all supposed to represent fossilized cells.

Petrographic inspection of samples drilled from 14 to 28 Ma ocean crust in the AAD area during the ODP Leg 187 indicated mostly low-temperature, oxygenated alteration conditions. Comparison of 2.5 Ma seafloor basalt from the same area with the much older subseafloor basalt indicated that a significant proportion of the glass alteration developed prior to burial. However, diffuse and highly irregular fresh-altered glass interfaces were only observed in the subseafloor samples and thus most likely developed after burial. These diffuse alteration fronts are apparently caused by dissolution and alteration of the glass into minute globules (0.05–0.2 μm in diameter), and no evident microbial cell structures could be observed to be associated. Some hollow, Mn-containing spherical structures that were observed within zeolite-filled fractures in one sample could represent fossilized equivalents of Mn-rich encrusted, apparently active microbes, observed in 2.5 Ma seafloor basalt (Figure 1e). However, the presence of few cell-like structures in the subsurface glassy basalt suggests that most of the numerous encrusted and fossilized cells developed during the surface stage are totally destroyed or masked by alteration and mineralization during burial. The fossilized cells within zeolite-filled



Basalt (Glass, Endoliths), Figure 3 (a) Petrographic thin section of basaltic glass from Costa Rica Rift, showing tubular structures at the alteration front of palagonite rim along a fracture; (b) close up of A; (c) DNA-specific DAPI staining (blue color) of the tubular structures, indicating the presence of DNA-containing cells.

fractures in subseafloor basalt, on the other hand, possibly indicate that microbial activity continued in the fractures after burial, for as long as circulation continued.

Pit marks and development of irregular alteration fronts both with and without the presence of microbes, and ambient fractures where smooth alteration interfaces are combined with numerous microorganisms attached to the fresh glass, as observed in seafloor basalt, together with irregular alteration fronts without detectable cells in subseafloor basalt, imply that great care should be taken before using alteration textures as the only criterion for microbial activity. Clearly, much more detailed knowledge is needed to fully understand the mechanisms that control both biotic and abiotic pit mark formation in basaltic glasses.

Drill cores: genetic and metabolic diversity

Drilling of 14–28 Ma ocean crust during ODP Leg 187 was partly aimed at studying the genetic and functional diversity of the subsurface microbial community (Christie et al., 2001; Pedersen et al., 2004; Lysnes et al., 2004b). Despite the sparse detection of only fossilized cells by SEM (Thorseth et al., 2003), the identification of a microbial community in these samples by biomolecular techniques that differ from that of surface seawater used as drilling fluid, document the presence of living cells in this old, sediment buried ocean floor basalts, and demonstrate that water-rock interactions also support a diverse endolithic microbial community in old ocean crust. This microbial community is probably present in fluid transporting fractures developed during tectonical events after burial. The nondetection of microbial DNA in a significant proportion of subseafloor samples could thus reflect not only a lower biomass in subseafloor basalts, but also a low number of open fractures in the samples analyzed, compared to seafloor lavas where DNA was retrieved from nearly all investigated samples (Lysnes et al., 2004a, b).

A major fraction of the endolithic microbial community in the subseafloor basalt from the ADD area was shown to be from the domain *Bacteria*, similar to the Arctic seafloor basalt. The five phylogenetic classes *Gamma-proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Firmicutes* that were present in the seafloor basalt were also present in the old subseafloor basalt. The main differences observed were the higher frequency of *Gamma-proteobacteria* in subseafloor basalt, the restricted presence of *Alpha-proteobacteria* in seafloor basalt, and *Beta-proteobacteria* in subseafloor basalt. Fewer and more unequal distributions of the phylotypes in subseafloor samples suggest a decrease in biodiversity and biomass with burial and aging of the crust. The results from this study furthermore indicate that microbial communities inhabiting nonhydrothermal subseafloor basalts participate in the cycling of iron and in the production of methane from H_2 and CO_2 . A major part of the microbial populations showed no close relation to previously characterized microorganisms. The correspondence between

the microbial populations in nonhydrothermal seafloor (Lysnes et al., 2004a) and subseafloor basalts (Lysnes et al., 2004b) may reflect the low-temperature and oxidised conditions, as indicated by the secondary alteration phases and their chemical composition, in both environments. The genetic and physiological diversity of microorganisms in the nonhydrothermal oceanic basalt from the AAD area differ from those reported from hydrothermal fluids from hydrothermal active regions of the ocean crust.

Recently, evidence of the presence of also eukaryotic, fungal life in oceanic basalt was reported (Schumann et al., 2004). The filamentous structures, suggested to represent fossil fungi, were observed within carbonate-filled vesicles in 46 Ma subseafloor basalt recovered during the ODP Leg 200 in the Pacific ocean. These findings imply that fungal life may possibly still exist in the subseafloor basaltic environment.

Hydrothermal fluids

Several studies of the microbial diversity in hydrothermal fluids (see *Hydrothermal Environments, Marine*) associated with eruptions in the central volcanic active zone of spreading ridges have been carried out during the last years (Summit and Baross, 1998, 2001; Huber et al., 2002, 2003). These studies have shown that the vent fluids inhabit microorganisms that are different from indigenous seawater microorganisms, suggesting that the vent fluids carry endolithic microbial cells from the basaltic subsurface environment. In the studies by Huber et al. (2002, 2003) dominant subseafloor phylotypes affiliated with the *Alpha*-, *Gamma*-, *Delta*- and *Epsilon-proteobacteria*, *Firmicutes*, novel candidate divisions, Group I *Crenarchaeota*, and Group II *Euryarchaeota* were detected. Most of these phylotypes were reported to be unique, differing from those of surrounding seawater, and affiliated with both low- and high-temperature groups. Considerable overlap with seawater was only found within *Alpha*- and *Gamma-proteobacteria*. To explain the retrieved diversity, Huber et al. (2003) proposed a schematic model of the basaltic subseafloor at the sampling site, based on hydrothermal fluid and seawater mixing that included three different compartments: a deep, anaerobic zone dominated by the hot hydrothermal fluid that contains mostly thermophilic microorganisms; an intermediate zone with both seawater and hydrothermal fluid and the highest variability in microbial community composition, probably including anaerobes, aerobes, and microaerophilic organisms; and an uppermost low-temperature, aerobic zone that is seawater-dominated, possibly containing seawater organisms. Unique subseafloor microorganisms flushed out from this uppermost zone by the vent water could thus be similar to microorganisms detected in nonhydrothermal basalts. Some of the *Gamma*- and *Epsilon-proteobacteria* sequences retrieved from both nonhydrothermal seafloor and subseafloor samples (Lysnes et al., 2004a, b) were phylogenetic affiliated

to sequences retrieved from the $<50^{\circ}\text{C}$ diffuse flow vent fluids associated with an eruptive event on the Juan de Fuca Ridge (Huber et al., 2003).

Investigation of 65°C subsurface fluids from aging (3.5 Ma) ocean crust by Cowen et al. (2003) also demonstrated the presence of a diverse bacterial and archaeal community in hydrothermal active areas of the mid-ocean ridge flanks. In this reduced, hydrothermal environment DNA sequences related to the ammonia-producing *Ammonifex degensii* and sulfate reducers of the *Delta-proteobacteria*, the low G + C Gram-positive genus *Desulfotomaculum*, and *Archaea* (*Archaeoglobus*) were dominant. A later study of 20°C subseafloor fluids from the same area demonstrated that the microbial community in the fluids composed of species indigenous to the subseafloor, as well as species from seawater and sediments (Huber et al., 2006). No sequences retrieved from nonhydrothermal seafloor and subseafloor basalts resembled those obtained from these 65°C subseafloor fluids.

Using available published 16S rDNA sequences from basalt, hydrothermal fluids, basalt enrichments, and data from other relevant environments, Mason et al. (2007) examined the phylogenetic diversity of microbial communities associated with marine basalts. Based on these data, it was suggested that the microorganisms have a cosmopolitan distribution. Most groups were found in both basalts and sediments, but are distinct from microbes in other marine habitats. One group of Marine Group I *Crenarchaeota* seems furthermore to be specifically adapted to basalt.

Biosignatures in ophiolites and greenstone belts

During the last years, terrestrial fragments of ancient ocean crust (ophiolites) and greenstone belts have been investigated for similar biosignatures as those observed within in situ oceanic basalt. For example, the results from the well-preserved ~ 90 Ma Troodos ophiolite in Cyprus revealed comparable traces of microbial life in the glassy lava margins as reported from studies of in situ oceanic crust, except for DNA that could not be detected by specific staining (Furnes et al., 2001c). This, together with geochemical and isotope data indicative of seawater alteration under oxidizing condition, imply that the main fraction of the alteration occurred prior to obduction during an early stage of ocean-floor alteration. Comparable textural, geochemical, and isotopic data obtained from lava margins from the ~ 3.5 billion-year-old Barberton greenstone Belt in South Africa further suggest subaqueous microbial activity during the early history of the Earth (Furnes et al., 2004) (see *Early Earth*). Thus, these biosignatures could be of great relevance in the search for life elsewhere in the universe (see *Astrobiology*).

Conclusions

Endolithic microbial communities have been documented in glassy margins of seafloor and subseafloor basaltic lavas from various locations of the ocean crust. In seafloor

basalt the microbial cells are easily recognized by electron microscopy, and are shown to be closely associated with the alteration of the glass. In subseafloor basalt the microbial activity has mainly been recognized by the use of biosignatures such as characteristic cell-like alteration textures in the glass, elevated concentrations of carbon and nitrogen in these textures, and stable carbon isotope ratios of disseminated carbonate consistent with biological processes. DNA-specific staining indicates the presence of DNA in the cell-like alteration textures. Culturing and biomolecular studies have revealed the existence of diverse, endolithic microbial communities in the glassy margins of oceanic lavas. The results suggest that iron and manganese cycling are important microbial processes in cold, nonhydrothermal regions, while sulfur cycling plays a major role at elevated temperatures in hydrothermal active areas. The endolithic microbial life in oceanic basalt may have a significant impact on the alteration of the basalt and the chemical exchange between the ocean crust and seawater.

Bibliography

- Andersen, T., Chapelle, F. H., and Lovley, D. R., 1998. Evidence against hydrogen-based microbial ecosystems in basalt aquifers. *Science*, **281**, 976–977.
- Bach, W., and Edwards, K. J., 2003. Iron and sulfide oxidation within the Basaltic ocean crust: implications for chemolithoautotrophic microbial biomass production. *Geochimica et Cosmochimica Acta*, **67**, 3871–3887.
- Banerjee, N. R., and Muehlenbachs, K., 2003. Tuff life: bioalteration in volcanoclastic rocks from the Ontong Java Plateau. *Geochemistry, Geophysics, Geosystems*, **4**, doi: 10.1029/2002GC000470.
- Benzerara, K., Menguy, N., Banerjee, N. R., Tylliszczak, T., Brown, G. E., and Guyot, F., 2007. Alteration of submarine basaltic glass from the Ontong Java Plateau: A STEM and TEM study. *Earth and Planetary Science Letters*, **260**, 187–200.
- Christie, D. M., Pedersen, R. B., Miller, D. J., et al., 2001. *Proceedings of the Ocean Drilling Program, Initial Reports*, 187 (Online). Available from World Wide Web: http://www-odp.tamu.edu/publications/187_IR/187ir.htm. doi:10.2973/odp.proc.ir.187.2001.
- Cowen, J. P., Giovannoni, S. J., Kenig, F., Johnson, H. P., Butterfield, D., Rappé, M. S., Hutnak, M., and Lam, P., 2003. Fluids from aging ocean crust that support microbial life. *Science*, **299**, 120–123.
- Crovisier, J. L., Thierry, A., and Dussossoy, J. L., 2003. Nature and role of natural alteration gels formed on the surface of ancient volcanic glasses (natural analogs of waste containment glasses). *Journal of Nuclear Materials*, **321**, 91–109.
- Daughney, C. J., Rioux, J. P., Fortin, D., and Pichler, T., 2004. Laboratory investigation of the role of bacteria in the weathering of basalt near deep sea hydrothermal vents. *Geomicrobiology Journal*, **21**, 21–31.
- Edwards, K. J., Rogers, D. R., Wirsén, C. O., and McCollum, T. M., 2003. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing chemolithoautotrophic α - and γ -proteobacteria from the deep-sea. *Applied and Environmental Microbiology*, **69**, 2906–2913.
- Edwards, K. J., Bach, W., McCollum, T. M., and Rogers, D. R., 2004. Neutrophilic iron-oxidizing bacteria in the ocean: their habitats, diversity, and roles in mineral deposition, rock

- alteration, and biomass production in the deep-sea. *Geomicrobiology Journal*, **21**, 393–404.
- Einen, J., Thorseth, I. H., and Øvreås, L., 2008. Enumeration of *Archaea* and *Bacteria* in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. *FEMS Microbiology Letters*, **282**, 182–187.
- Emerson, D., Rentz, J. A., Lilburn, T. G., Davis, R. E., Aldrich, H., Chan, C., and Moyer, C. L., 2007. A novel lineage of proteobacteria involved in formation of marine Fe-oxidizing microbial mat communities. *PLoS ONE*, **2**, doi: 10.1371/journal.pone.0000667.
- Fisk, M. R., Giovannoni, S. J., and Thorseth, I. H., 1998. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science*, **281**, 978–979.
- Fisk, M. R., Storrer-Lombardi, M. C., Douglas, S., Popa, R., McDonald, G., and Di Meo-Savoie, C., 2003. Evidence of biological activity in Hawaiian subsurface basalts. *Geochemistry, Geophysics, Geosystems*, **4**, doi: 10.1029/2002GC000387.
- Furnes, H., and Staudigel, H., 1999. Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth and Planetary Science Letters*, **166**, 97–103.
- Furnes, H., Thorseth, I. H., Tumyr, O., Torsvik, T., and Fisk, M., 1996. Microbial activity in the alteration of glass from pillow lavas from ODP Hole 896A. In Alt, J. C., Kinoshita, H., Stokking, L. B., and Michael, P. J. (eds.), *Proceedings of the Ocean Drilling Program Scientific Results*, Vol. 148, pp. 191–206.
- Furnes, H., Muehlenbachs, K., Tumyr, O., Torsvik, T., and Thorseth, I. H., 1999. Depth of active bio-alteration in the ocean crust: Costa Rica Rift (Hole 504B). *Terra Nova*, **11**, 228–233.
- Furnes, H., Muehlenbachs, K., Torsvik, T., Thorseth, I. H., and Tumyr, O., 2001a. Microbial fractionation of carbon isotopes in altered basaltic glass from the Atlantic Ocean, Lau Basin and Costa Rica Rift. *Chemical Geology*, **173**, 313–330.
- Furnes, H., Staudigel, H., Thorseth, I. H., Torsvik, T., Muehlenbachs, K., and Tumyr, O., 2001b. Bioalteration of basaltic glass in the oceanic crust. *Geochemistry, Geophysics, Geosystems*, **2**, paper number 2000GC000150.
- Furnes, H., Muehlenbachs, K., Tumyr, O., Torsvik, T., and Xenophontos, C., 2001c. Biogenic alteration of volcanic glass from the Troodos ophiolite, Cyprus. *Journal of the Geological Society, London*, **158**, 75–84.
- Furnes, H., Thorseth, I. H., Torsvik, T., Muehlenbachs, K., Staudigel, H., and Tumyr, O., (2003). Identifying bio-interaction with basaltic glass in oceanic crust and implications for estimating the depth of the oceanic biosphere: a review. In: Smellie, J. L. and Chapman, M. G. (eds.), *Volcano-Ice Interactions on Earth and Mars*, Geological Society, Special Publication, London, **202**, 407–421.
- Furnes, H., Banerjee, N. R., Muehlenbachs, M., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- Giovannoni, S. J., Fisk, M. R., Mullins, T. D., and Furnes, H., 1996. Genetic evidence for endolithic microbial life colonizing basaltic glass-seawater interfaces. *Proceedings of Ocean Drilling Program. Scientific Results*, **148**, 207–214.
- Holloway, J. R., and O'Day, P. A., 2000. Production of CO₂ and H₂ by dike-eruptive events at mid-ocean ridges; implications for abiotic organic synthesis and global geochemical cycling. *International Geology Review*, **42**, 673–683.
- Huber, J. A., Butterfield, D. A., and Baross, J. A., 2002. Temporal changes in archaeal diversity and chemistry in a mid-ocean ridge subseafloor habitat. *Applied and Environmental Microbiology*, **68**, 1585–1594.
- Huber, J. A., Butterfield, D. A., and Baross, J. A., 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiology Ecology*, **43**, 393–409.
- Huber, J. A., Johnson, H. P., Butterfield, D. A., and Baross, J. A., 2006. Microbial life in ridge flank crustal fluids. *Environmental Microbiology*, **8**, 88–99.
- Kelley, D. S., Baross, J. A., and Delaney, J. R., 2002. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annual Review of Earth and Planetary Sciences*, **30**, 385–491.
- Kruber, C., Thorseth, I. H., and Pedersen, R. B., 2008a. Seafloor alteration of basaltic glass: textures, geochemistry and endolithic microorganisms. *Geochemistry, Geophysics, Geosystems*, **9**, 12, doi: 10.1029/2008GC002119.
- Kruber, C., Thorseth, I. H., and Pedersen, R. B., 2008b. Fossilised endolithic microorganisms in glassy seafloor basalt: a TEM study. (submitted to *Earth and Planetary Science Letters*).
- Lysnes, K., Thorseth, I. H., Steinsbu, B. O., Øvreås, L., Torsvik, T., and Pedersen, R. B., 2004a. Microbial community diversity in seafloor basalt from the arctic spreading ridges. *FEMS Microbial Ecology*, **50**, 213–230.
- Lysnes, K., Torsvik, T., Thorseth, I. H., and Pedersen, R. B., 2004b. Microbial populations in ocean floor basalt: results from ODP Leg 187. In Pedersen, R. B., Christie, D. M., and Miller, D. J. (eds.), *Proceedings of the Ocean Drilling Program Scientific Results*, **187**, 1–27, doi:10.2973/odp.proc.sr.187.203.2004.
- Mason, O. U., Stingl, U., Wilhelm, L. J., Moeseneder, M. M., Di Meo-Savoie, C. A., Fisk, M. R., and Giovannoni, S. J., 2007. The phylogeny of endolithic microbes associated with marine basalts. *Environmental Microbiology*, **9**, 2539–2550.
- Palandri, J. L., and Reed, M. H., 2004. Geochemical models of metasomatism in ultramafic systems: serpentinization, rodingitization, and sea floor carbonate chimney precipitation. *Geochimica et Cosmochimica Acta*, **68**, 1115–1133.
- Pedersen, R. B., Christie, D. M., and Miller, D. J., (eds.), 2004. *Proceedings of the Ocean Drilling Program Scientific Results*, **187**, College Station, TX (Ocean Drilling Program). doi:10.2973/odp.proc.sr.187.
- Schumann, G., Manz, W., Reitner, J., and Lustrino, M., 2004. Ancient fungal life in North Pacific Eocene oceanic crust. *Geomicrobiology Journal*, **21**, 241–246.
- Staudigel, H., Chastain, R. A., Yayanos, A., and Bourcier, W., 1995. Biologically mediated dissolution of glass. *Chemical Geology*, **126**, 147–154.
- Staudigel, H., Yayanos, A., Chastain, R. A., Davies, G., Verdurmen, E. A. T., Sciffman, P., Bourcier, W., and De Baar, H., 1998. Biologically mediated dissolution of volcanic glass in seawater. *Earth and Planetary Science Letters*, **164**, 233–244.
- Staudigel, H., Furnes, H., Banerjee, N. R., Dilek, Y., and Muehlenbachs, K., 2006. Microbes and volcanoes: a tale from the oceans, ophiolites, and greenstone belts. *GSA Today*, **16**, 4–10.
- Stronck, N. A., and Schmincke, H. U., 2001. Evolution of palagonite: crystallization, chemical changes, and element budget. *Geochemistry, Geophysics, Geosystems*, **2**, Paper number 2000GC000102.
- Summit, M., and Baross, J. A., 1998. Thermophilic subseafloor microorganisms from the 1996 North Gorda eruption. *Deep-Sea Research. II*, **45**, 2751–2766.
- Summit, M., and Baross, J. A., 2001. A novel microbial habitat in the mid-ocean ridge subseafloor. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2158–2163.
- Templeton, A., Staudigel, H., and Tebo, B., 2005. Diverse Mn(II)-oxidizing bacteria isolated from submarine basalts at Loihi seamount. *Geomicrobiology Journal*, **22**, 127–139.
- Thorseth, I. H., Furnes, H., and Heldal, M., 1992. The importance of microbiological activity in the alteration of natural basaltic glass. *Geochimica et Cosmochimica Acta*, **56**, 845–850.

- Thorseth, I. H., Furnes, H., and Tumyr, O., 1995a. Textural and chemical effects of bacterial activity on basaltic glass: an experimental approach. *Chemical Geology*, **119**, 139–160.
- Thorseth, I. H., Torsvik, T., Furnes, H., and Muehlenbachs, K., 1995b. Microbes play an important role in the alteration of oceanic crust. *Chemical Geology*, **126**, 137–146.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daac, F. L., Pedersen, R. B., and the Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Thorseth, I. H., Pedersen, R. B., and Christie, D. M., 2003. Microbial alteration of 0–30 Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic discordance. *Earth and Planetary Science Letters*, **215**, 237–247.
- Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I. H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters*, **162**, 165–176.
- von Waltershausen, W. S., 1845. Über die submarinen vulkanischen Ausbrüche in der Tertiär-Formation des Val di Noto im Vergleiche mit verwandten Erscheinungen am Ätna. *Göttinger Studien*, **1**, 371–431.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Biological Volcanic Rock Weathering
 Biosignatures in Rocks
 Deep Biosphere of the Ocean Deep Sea
 Early Earth
 Endoliths
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Fluorescence In Situ Hybridisation (FISH)
 Gallionella
 Hydrogen
 Hydrothermal Environments, Marine
 Isotopes and Geobiology
 Microbial Biomineralization
 Origin of Life
 Shewanella
 Sulfate-Reducing Bacteria

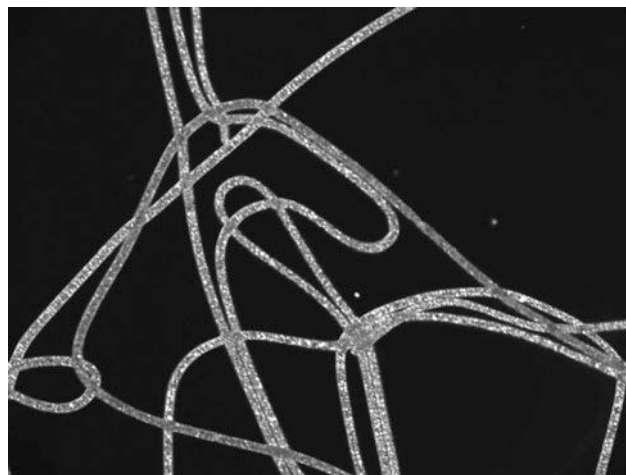
BEGGIATO

Heide N. Schulz-Vogt
 Max Planck Institute for Marine Microbiology,
 Bremen, Germany

Definition

Beggiatoa is a colorless, sulfur-storing bacterium belonging to the Gammaproteobacteria, filamentous, motile, and not attached.

Bacteria of the genus *Beggiatoa* live by the oxidation of sulfide with oxygen or nitrate. They are benthic



Beggiatoa, Figure 1 *Beggiatoa* filaments of 6 μm diameter seen under the light microscope with dark field illumination. The sulfur globules reflect the light and give the filaments their shining white appearance.

organisms, which occur in almost all freshwater and marine environments where sulfide is present, e.g., wastewater, areas of coastal upwelling, salt marshes, hydrothermal vents, cold seeps, methane hydrates, etc. In contrast to most bacteria, *Beggiatoa* can be recognized by its typical morphology. It is a filamentous bacterium, which is motile by gliding and stores elemental sulfur inside, giving it a shining white appearance (Figure 1). The filaments can be several millimeters to centimeters long and are formed by long rows of cells with diameters between 1 and 200 μm . The larger species of *Beggiatoa* (>5–10 μm in diameter) have a central vacuole, which they use for storing nitrate in high concentrations (ca 10–300 mM).

Beggiatoa filaments typically occur as mats on top of the sediment or within the upper few centimeters. They actively glide to the overlapping zone of oxygen, supplied from the open water, and sulfide evolving in the sediment. In addition to oxygen, the larger species can also use internally stored nitrate for the oxidation of sulfide and thereby increase their rates of sulfide oxidation or survive periods of anoxia. Sulfide is first oxidized to sulfur, which forms intracellular sulfur globules, and then further on to sulfate. If sulfide concentrations are high, the cells are filled with many sulfur globules, which are disappearing in the absence of sulfide. Apart from sulfur and nitrate, *Beggiatoa* filaments were also observed to store polyphosphate and poly-beta-hydroxybutyrate (PHB).

Bibliography

- Teske, A., and Nelson, D. C., 2006. The genera *Beggiatoa* and *Thioploca*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, pp. 784–810.

- Thorseth, I. H., Furnes, H., and Tumyr, O., 1995a. Textural and chemical effects of bacterial activity on basaltic glass: an experimental approach. *Chemical Geology*, **119**, 139–160.
- Thorseth, I. H., Torsvik, T., Furnes, H., and Muehlenbachs, K., 1995b. Microbes play an important role in the alteration of oceanic crust. *Chemical Geology*, **126**, 137–146.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daac, F. L., Pedersen, R. B., and the Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Thorseth, I. H., Pedersen, R. B., and Christie, D. M., 2003. Microbial alteration of 0–30 Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic discordance. *Earth and Planetary Science Letters*, **215**, 237–247.
- Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I. H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters*, **162**, 165–176.
- von Waltershausen, W. S., 1845. Über die submarinen vulkanischen Ausbrüche in der Tertiär-Formation des Val di Noto im Vergleiche mit verwandten Erscheinungen am Ätna. *Göttinger Studien*, **1**, 371–431.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Biological Volcanic Rock Weathering
 Biosignatures in Rocks
 Deep Biosphere of the Ocean Deep Sea
 Early Earth
 Endoliths
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Fluorescence In Situ Hybridisation (FISH)
 Gallionella
 Hydrogen
 Hydrothermal Environments, Marine
 Isotopes and Geobiology
 Microbial Biomineralization
 Origin of Life
 Shewanella
 Sulfate-Reducing Bacteria

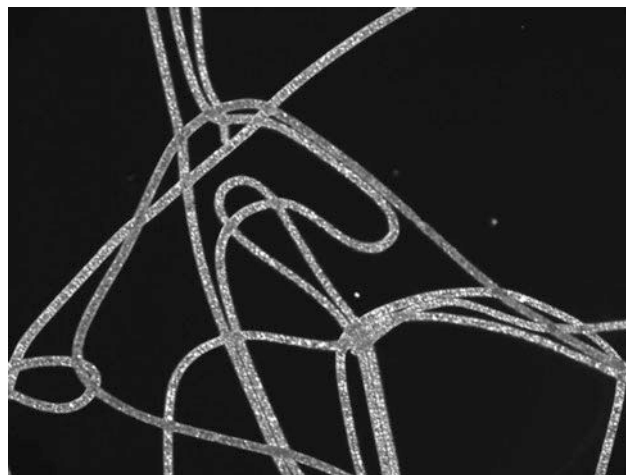
BEGGIATO

Heide N. Schulz-Vogt
 Max Planck Institute for Marine Microbiology,
 Bremen, Germany

Definition

Beggiatoa is a colorless, sulfur-storing bacterium belonging to the Gammaproteobacteria, filamentous, motile, and not attached.

Bacteria of the genus *Beggiatoa* live by the oxidation of sulfide with oxygen or nitrate. They are benthic



Beggiatoa, Figure 1 *Beggiatoa* filaments of 6 μm diameter seen under the light microscope with dark field illumination. The sulfur globules reflect the light and give the filaments their shining white appearance.

organisms, which occur in almost all freshwater and marine environments where sulfide is present, e.g., wastewater, areas of coastal upwelling, salt marshes, hydrothermal vents, cold seeps, methane hydrates, etc. In contrast to most bacteria, *Beggiatoa* can be recognized by its typical morphology. It is a filamentous bacterium, which is motile by gliding and stores elemental sulfur inside, giving it a shining white appearance (Figure 1). The filaments can be several millimeters to centimeters long and are formed by long rows of cells with diameters between 1 and 200 μm . The larger species of *Beggiatoa* (>5–10 μm in diameter) have a central vacuole, which they use for storing nitrate in high concentrations (ca 10–300 mM).

Beggiatoa filaments typically occur as mats on top of the sediment or within the upper few centimeters. They actively glide to the overlapping zone of oxygen, supplied from the open water, and sulfide evolving in the sediment. In addition to oxygen, the larger species can also use internally stored nitrate for the oxidation of sulfide and thereby increase their rates of sulfide oxidation or survive periods of anoxia. Sulfide is first oxidized to sulfur, which forms intracellular sulfur globules, and then further on to sulfate. If sulfide concentrations are high, the cells are filled with many sulfur globules, which are disappearing in the absence of sulfide. Apart from sulfur and nitrate, *Beggiatoa* filaments were also observed to store polyphosphate and poly-beta-hydroxybutyrate (PHB).

Bibliography

- Teske, A., and Nelson, D. C., 2006. The genera *Beggiatoa* and *Thioploca*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, pp. 784–810.

Cross-references

Bacteria
Sulfur Cycle
Thiomargarita
Thiotrophic Bacteria

BIODETERIORATION (OF STONE)

Christina Beimforde
University of Göttingen, Göttingen, Germany

Definition

Biodeterioration: Undesirable alteration of the properties of materials caused by vital activities of organisms.

Introduction

Materials are exposed to weathering processes caused by physical, chemical, and biological factors, leading to an irreversible loss of our cultural heritage. The reduction of decay by conservation strategies is of particular importance to protect historical monuments or significant cultural artifacts.

Generally, the velocity of decay depends on the chemical nature and physical structure of materials as well as external factors associated to the locations where the objects are exposed. In many studies it was demonstrated that the process of weathering consists of various mechanisms acting together in coassociation with more or less intensity, making it impossible to separate individual aspects such as physical, chemical, or biological factors from each other (Koestler et al., 1994; Ehrlich, 2002). In many cases microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial consortia, rather the microbial activity, is also affected by the surrounding chemical and physical factors.

Solar irradiation, wind, and variations in temperature and humidity predominate the physical effects. Wind and humidity, of course, lead to the erosion of particles from stone surfaces. Variations in temperature, due to diurnal and seasonal changes, cause tensile stresses that can break up the structure of the material, resulting in a loss of persistence. Humidity, in combination with frost and salt, can intensify this effect. The crystallization of water or saline solutions is connected with an increase in volume that leads to “frost shattering” or “saline shattering” (Steiger and Dannecker, 1995).

Chemical weathering may be caused by rain water which dissolves carbon dioxide originating from the ambient air or from respiration of present microorganisms. Generated carbon acid reacts with calcium carbonate, thereby producing calcium hydrogen carbonate with a higher solubility than calcium carbonate (Bell, 1993).

According to Krumbein and Schönborn (1987), biological damages of building material can be divided into

different categories: (1) Anthropogenically induced damages due to atmospheric pollution as a result of the industrialization. These processes lead to an increasing level of inorganic (SO_2 , NO_x) and organic (aliphatic and aromatic hydrocarbons) compounds in the air. This implicates acid rain that has a corrosive effect on stone buildings or other exposed materials (Pitzurra et al., 2003; Winkler, 1987). (2) Damages caused by higher plants and animals. The growth of higher plants, for example, leads to the physical destruction of materials. Moreover, excrements of birds contain ammoniac and nitrite, which can be utilized by chemolithotrophic bacteria. (3) Microorganisms such as bacteria, algae, fungi, and lichens can cause a degradation of materials. These damaging effects will be specified below.

Furthermore, biological colonization and the intensity of associated biodeterioration processes are strongly influenced by water availability. This is determined by specific material parameters such as porosity and permeability, as well as by environmental conditions.

Microbial degradation processes

How do microbial associations interact within these complex weathering processes? A considerable amount of studies have been done to figure out the role of microbiological agents in weathering processes (e.g., Urzi and Krumbein, 1994; Griffin et al., 1991; Warscheid and Braams, 2000). Generally, microorganisms play an important role within the regulation of the global cycles of materials, contributing to the function of the ecosystems. However, microorganisms also play a crucial role concerning the stability of materials. The interactions between microbial associations and materials can be divided into three different categories (Weber, 1993, cf. Figure 1): (1) The material can be decomposed enzymatically and used as a nutrient source by microbial associations. (2) Microorganisms can excrete metabolic compounds such as inorganic and organic acids that interact with mineral compounds of the material, thus causing dissolution and discoloration. (3) The material can be damaged by physical (mechanical) forces due to the growth of microorganisms.

For the most part, microorganisms conglomerate to biofilms of different natures such as crusts, fluffs, or slime, giving them the ability to colonize nearly all biotic and abiotic surfaces (Costeron et al., 1987). Biofilms can exhibit a highly spatial structured composition (Stoodley et al., 2002), thereby consisting of different prokaryotic and/or eukaryotic populations, extracellular polymeric substances (EPS), accumulated polyvalent cations, as well as solvated and dissolved organic and inorganic particles. The formation of biofilms provides considerable advantages including mechanical stability, the facility of symbiotic and syntrophic interactions, the building of a diffusion barrier and an increased resistance against toxic environmental conditions, biocides, aridity, or pH variabilities (Wolfaardt et al., 1999). However, the presence of biofilms can lead to biophysical determined damages of

oxygen ligands. Stoichiometry and temperature dependence of the metal–bacteria adsorption reactions can also be extracted using this approach (Gorman-Lewis et al., 2006).

Summary

Microbial–metal adsorption includes the electrostatic and chemical association of metals with the organic functional groups of microbial cell surfaces. This can be considered a passive process driven by the electronegativity of the microbial cell wall/EPS and the Gibbs free energy of the adsorption reactions. These adsorption reactions seem to be dominated by binding of cations to the oxygen containing moieties on the cell surface. Future advances in elucidating the mechanisms of metal adsorption to microbes will come from combining novel analytical techniques, such as colorimetry and spectroscopy, with bulk partitioning studies and site-specific adsorption models.

Bibliography

- Aislabie, J., and Loutit, M. W., 1986. Accumulation of Cr(III) by bacteria isolated from polluted sediment. *Marine Environmental Research*, **20**, 221–232.
- Beveridge, T. J., 1984. Mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In Reddy, C. A., and Klug, M. J. (eds.), *Current Perspectives in Microbial Ecology*. Washington, DC: American Society for Microbiology, pp. 601–607.
- Beveridge, T. J., and Koval, S. F., 1981. Binding of metals to cell envelopes of *Escherichia coli* K-12. *Applied and Environmental Microbiology*, **42**, 325–335.
- Beveridge, T. J., and Murray, R. G. E., 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *Journal of Bacteriology*, **127**, 1502–1518.
- Boyanov, M. L., Kelly, S. D., Kemner, K. M., Bunker, B. A., Fein, J. B., and Fowle, D. A., 2003. Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: a pH dependent X-ray absorption fine structure spectroscopy study. *Geochimica et Cosmochimica Acta*, **67**, 3299–3311.
- Carstensen, E. L., and Marquis, R. E., 1968. Passive electrical properties of microorganisms. III. Conductivity of isolated bacterial cell walls. *Biophysics Journal*, **8**, 536–548.
- Fein, J. B., 2000. Quantifying the effects of bacteria on adsorption reactions in water-rock systems. *Chemical Geology*, **169**, 265–280.
- Fowle, D. A., and Fein, J. B., 2000. Experimental measurements of the reversibility of metal-bacteria adsorption reactions. *Chemical Geology*, **168**, 27–36.
- Geesey, G. G., and Jang, L., 1989. Interactions between metal ions and capsular polymers. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: John Wiley, pp. 325–357.
- Gorman-Lewis, D., Fein, J. B., and Jensen, M. P., 2006. Enthalpies and entropies of proton and cadmium adsorption onto *Bacillus subtilis* bacterial cells from calorimetric measurements. *Geochimica et Cosmochimica Acta*, **70**, 4862–4873.
- Harden, V. P., and Harris, J. O., 1953. The isoelectric point of bacterial cells. *Journal of Bacteriology*, **65**, 198–202.
- Kelly, S. D., Boyanov, M. I., Fein, J. B., Fowle, D. A., Yee, N., and Kemner, K. M., 2002. X-ray absorption fine structure determination of pH-dependent U-bacterial cell wall interactions. *Geochimica et Cosmochimica Acta*, **66**, 3855–3871.
- Markai, S., Andrés, Y., Montavon, G., and Grambow, B., 2003. Study of the interaction between europium (III) and *Bacillus subtilis*: fixation sites, biosorption modelling and reversibility. *Journal of Interface and Colloid Science*, **243**, 73–80.
- Panak, P., Raff, J., Selenska-Pobell, S., Geipel, G., Bernhard, G., and Nitsche, H., 2000. *Radiochimica Acta*, **88**, 71–76.
- Rudd, T., Sterritt, R. M., and Lester, J. N., 1983. Mass balance of heavy metal uptake by encapsulated cultures of *Klebsiella aerogenes*. *Microbial Ecology*, **9**, 261–272.
- Texier, A. C., Andrés, Y., Illemassene, M., and Le Cloirec, P., 2000. Characterization of lanthanide ions binding sites in the cell wall of *Pseudomonas aeruginosa*. *Environmental Science and Technology*, **34**, 610–615.
- Warren, L. A., and Haack, E. A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. *Earth-Science Reviews*, **54**, 261–320.
- Williams, R. J. P., 1981. Physico-chemical aspects of inorganic element transfer through membranes. *Philosophical Transactions of the Royal Society of London*, **B294**, 57–74.

Cross-references

Carbonates
Clay Authigenesis, Bacterial
Dolomite, Microbial
Metalloenzymes
Microbial Biomineralization
Microbial Surface Reactivity
Nanocrystals, Microbially Induced
Organomineralization

MICROBIOCORROSION

Aline Tribollet¹, Stjepko Golubic², Gudrun Radtke³,
Joachim Reitner⁴
¹Institut de Recherche pour le Développement, Nouméa,
Nouvelle-Calédonie, France
²Boston University, Boston, MA, USA
³Hessisches Landesamt für Umwelt und Geologie,
Wiesbaden, Germany
⁴University of Göttingen, Göttingen, Germany

Definition

Destruction of rocks and minerals by biological activities has been termed bioerosion (Neumann, 1966). It includes mechanical as well as chemical effects, that is, bioabrasion and biocorrosion (Schneider, 1976; Golubic and Schneider, 1979). However, both the processes often co-occur; they are functionally interconnected and mutually supportive. Biocorrosion can result from the activity of macro- or microorganisms and, thus, is called macrobiocorrosion and microbiocorrosion. Microbiocorrosion can also be closely associated with microbial rock formation and consolidation in stromatolitic structures (Reid et al., 2000; Macintyre et al., 2000; Garcia-Pichel et al., 2004; Dupraz and Visscher, 2005). In fact, the oldest known fossils of microboring organisms were located in lithified horizons of silicified stromatolites (Zhang and Golubic, 1987).

Bibliography

- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–338.
- Garcia-Pichel, F., Al-Horani, F., Ludwig, R., Farmer, J., and Wade, B., 2004. Balance between calcification and bioerosion in modern stromatolites. *Geobiology*, **2**, 49–57.
- Golubic, S., and Schneider, J., 1979. Carbonate dissolution. In Trudinger, P. A., and Swaine, D. J. (eds.), *Biogeochemical Cycling of Mineral-Forming Elements*. Amsterdam: Elsevier, pp. 107–129.
- Macintyre, I. G., Prufert-Bebout, L., and Reid, R. P., 2000. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology*, **47**, 915–921.
- Neumann, A. C., 1966. Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge *Cliona lampa*. *Limnology and Oceanography*, **11**, 92–108.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C. P., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Schneider, J., 1976. Biological and inorganic factors in the destruction of limestone coasts. *Contributions to Sedimentology*, **6**, 1–112.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early Proterozoic stromatolites, Hebei, China. *Acta Micropaleont Sinica*, **4**, 1–12.

MICROSENSORS FOR SEDIMENTS, MICROBIAL MATS, AND BIOFILMS

Dirk de Beer

Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

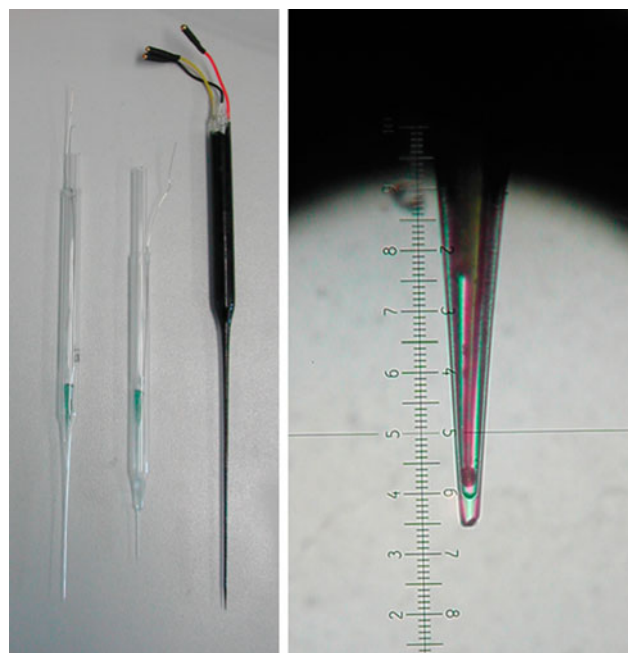
Amperometry; Diagenesis; Diffusion-reaction; Micro-electrode; Microenvironment; Potentiometry

Definition

Microsensors are needle-shaped sensors ([Figure 1](#)) that can be inserted in biologically active matrices, such as sediments, to measure directly concentrations of certain compounds.

Introduction

In sediments, microbial mats, and biofilms steep gradients of substrates and products develop, due to high metabolic activities and mass transfer limitations (see Chapters [Biofilms](#); [Microbial Mats](#); [Sediment Diagenesis – Biologically Controlled](#)). Stratifications of microenvironments develop that determine the existence and activity of microbial consortia. For example, in active biofilms oxygen can penetrate less than 50 μm (de Beer et al., 1994c; de Beer et al., 1993), in sediments oxygen penetrates typically less than 2 mm (de Beer, 2001; Jørgensen and Revsbech, 1985; Meyers et al., 1987; Revsbech,



Microsensors for Sediments, Microbial Mats, and Biofilms, Figure 1 Left panel, microsensors for oxygen (left sensor), for pH (middle), and for H_2S (right sensor). The right panel zooms further in on the tip of an oxygen sensor, showing the gold-plated electrode behind a silicon membrane, the ticks on the ruler are 1 μm .

1983; Sorensen et al., 1981; Sweerts and de Beer, 1989). Below the oxic zone anaerobic microbial processes can occur, such as denitrification and sulfate reduction (see Chapter [Sulfur Cycle](#)), that determine the element cycling (the coupled degradative and chemolithotrophic processes) in the system as a whole. Stratifications on such a small scale cannot be studied by more classical techniques such as pore-water extraction and chemical analyses. Extraction is impossible on such small scales, as it is highly destructive for the system and concentrations will have changed during the extraction procedure. To study such stratified systems we need microsensors that are minimally invasive and directly measure the concentrations without disturbing the system in which we measure. Moreover, as the system is not disturbed by microsensor analyses, we can perform sequences of measurements and, for example, study how the system responds to environmental changes. Microsensor measurements have enormously enhanced the insight in the vertical distribution of microbial processes in sediments. Microsensors were originally developed for physiological studies in animal and plant tissues, and even for intracellular measurements (Ammann, 1986; Hinke, 1969; Thomas, 1978), but soon applied in environmental studies (Bungay et al., 1969). They became an established technique for ecological studies by the developing work of Revsbech, who made them more robust and invented a large diversity of sensors (Kühl and Revsbech, 2000; Revsbech and Jørgensen, 1986).

Cross-references

Bacteria
Sulfur Cycle
Thiomargarita
Thiotrophic Bacteria

BIODETERIORATION (OF STONE)

Christina Beimforde
University of Göttingen, Göttingen, Germany

Definition

Biodeterioration: Undesirable alteration of the properties of materials caused by vital activities of organisms.

Introduction

Materials are exposed to weathering processes caused by physical, chemical, and biological factors, leading to an irreversible loss of our cultural heritage. The reduction of decay by conservation strategies is of particular importance to protect historical monuments or significant cultural artifacts.

Generally, the velocity of decay depends on the chemical nature and physical structure of materials as well as external factors associated to the locations where the objects are exposed. In many studies it was demonstrated that the process of weathering consists of various mechanisms acting together in coassociation with more or less intensity, making it impossible to separate individual aspects such as physical, chemical, or biological factors from each other (Koestler et al., 1994; Ehrlich, 2002). In many cases microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial consortia, rather the microbial activity, is also affected by the surrounding chemical and physical factors.

Solar irradiation, wind, and variations in temperature and humidity predominate the physical effects. Wind and humidity, of course, lead to the erosion of particles from stone surfaces. Variations in temperature, due to diurnal and seasonal changes, cause tensile stresses that can break up the structure of the material, resulting in a loss of persistence. Humidity, in combination with frost and salt, can intensify this effect. The crystallization of water or saline solutions is connected with an increase in volume that leads to “frost shattering” or “saline shattering” (Steiger and Dannecker, 1995).

Chemical weathering may be caused by rain water which dissolves carbon dioxide originating from the ambient air or from respiration of present microorganisms. Generated carbon acid reacts with calcium carbonate, thereby producing calcium hydrogen carbonate with a higher solubility than calcium carbonate (Bell, 1993).

According to Krumbein and Schönborn (1987), biological damages of building material can be divided into

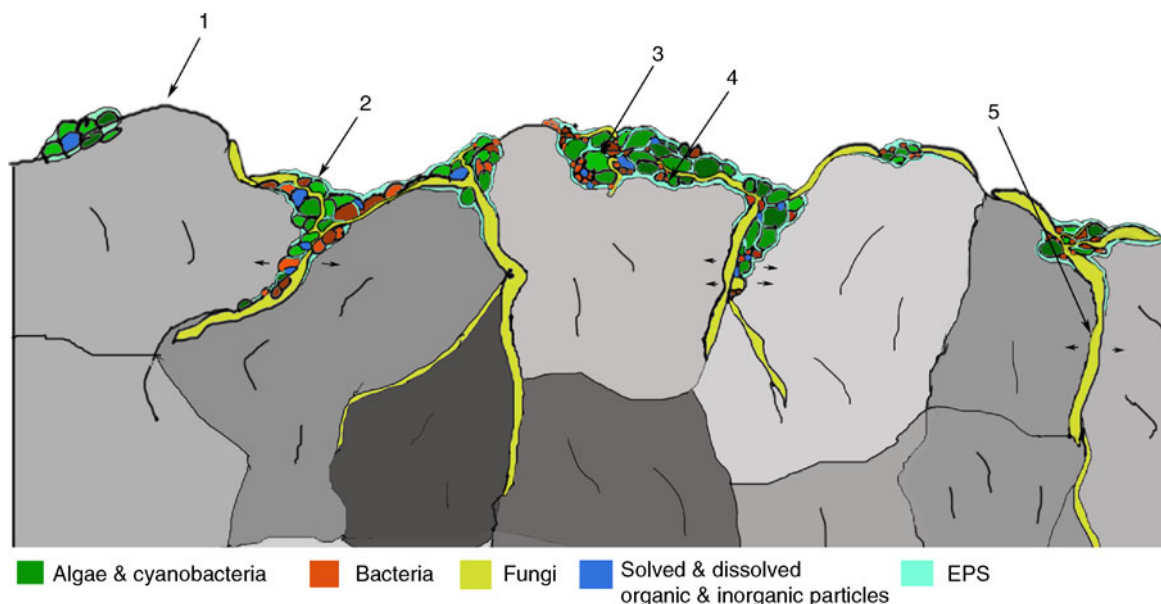
different categories: (1) Anthropogenically induced damages due to atmospheric pollution as a result of the industrialization. These processes lead to an increasing level of inorganic (SO_2 , NO_x) and organic (aliphatic and aromatic hydrocarbons) compounds in the air. This implicates acid rain that has a corrosive effect on stone buildings or other exposed materials (Pitzurra et al., 2003; Winkler, 1987). (2) Damages caused by higher plants and animals. The growth of higher plants, for example, leads to the physical destruction of materials. Moreover, excrements of birds contain ammoniac and nitrite, which can be utilized by chemolithotrophic bacteria. (3) Microorganisms such as bacteria, algae, fungi, and lichens can cause a degradation of materials. These damaging effects will be specified below.

Furthermore, biological colonization and the intensity of associated biodeterioration processes are strongly influenced by water availability. This is determined by specific material parameters such as porosity and permeability, as well as by environmental conditions.

Microbial degradation processes

How do microbial associations interact within these complex weathering processes? A considerable amount of studies have been done to figure out the role of microbiological agents in weathering processes (e.g., Urzi and Krumbein, 1994; Griffin et al., 1991; Warscheid and Braams, 2000). Generally, microorganisms play an important role within the regulation of the global cycles of materials, contributing to the function of the ecosystems. However, microorganisms also play a crucial role concerning the stability of materials. The interactions between microbial associations and materials can be divided into three different categories (Weber, 1993, cf. Figure 1): (1) The material can be decomposed enzymatically and used as a nutrient source by microbial associations. (2) Microorganisms can excrete metabolic compounds such as inorganic and organic acids that interact with mineral compounds of the material, thus causing dissolution and discoloration. (3) The material can be damaged by physical (mechanical) forces due to the growth of microorganisms.

For the most part, microorganisms conglomerate to biofilms of different natures such as crusts, fluffs, or slime, giving them the ability to colonize nearly all biotic and abiotic surfaces (Costeron et al., 1987). Biofilms can exhibit a highly spatial structured composition (Stoodley et al., 2002), thereby consisting of different prokaryotic and/or eukaryotic populations, extracellular polymeric substances (EPS), accumulated polyvalent cations, as well as solvated and dissolved organic and inorganic particles. The formation of biofilms provides considerable advantages including mechanical stability, the facility of symbiotic and syntrophic interactions, the building of a diffusion barrier and an increased resistance against toxic environmental conditions, biocides, aridity, or pH variabilities (Wolfaardt et al., 1999). However, the presence of biofilms can lead to biophysical determined damages of



Biodeterioration (of Stone), Figure 1 Interactions between materials, environmental conditions, and subaerial biofilms include (1) atmosphere substrate interactions (2) interactions between atmosphere and biofilms (3) interactions of metabolic compounds and substrate; enzymatic degradation (4) physicochemical interactions between biofilm and substrate (5) physical forces due to microbial growth.

surface structures of stone (Gorbushina and Krumbein, 2000; Gorbushina, 2007). Variations in temperature and humidity induce shrinking and swelling cycles of the film, leading to an alteration of hydrophobic–hydrophilic properties of rock surfaces. As a consequence, hydrophobic properties of stone surfaces can turn into hydrophilic.

Microorganisms involved in biodeterioration processes

The ecology of microbial associations colonizing rocks is highly complex and involves different groups of microorganisms with different physiological properties. These ecosystems can develop in various ways, depending on environmental conditions and physicochemical properties of the material. Therefore, only a small range of examples can be discussed in this framework, representing the complexity of the interactions between microorganisms and their substrate.

Phototrophic microorganisms

Phototrophic microorganisms such as algae, cyanobacteria and lichens, but also mosses and even higher plants cover stone surfaces, thus changing the face of new buildings or cultural heritage. In many cases they form biofilms or crusts to protect themselves from unfavorable environmental factors. These crusts or films can appear in a deep or light green color under humid conditions or in black tint under dry environmental conditions (Urzi and Krumbein, 1994; Krumbein and Warscheid, 1992). Under particular

climatic conditions (e.g., tropical environments) these biofilms provide a protective effect, isolating the stone surface from aggressive environments and regulating humidity and temperature (Krumbein and Warscheid, 1992). But the formed biopatina can also cause damages by physical and chemical processes (Krumbein and Warscheid, 1992; De los Rios et al., 2009). Associated biodeterioration processes can be induced by the excretion of corrosive acids (Arino et al., 1997), the uptake of substrate compounds such as sulfur and calcium and accumulation into their cells (Orgenta Calvo et al., 1994), and by the alteration of stone forming minerals (Ortega-Morales et al., 2000; Crispim and Gaylarde, 2004). Finally, the penetration of hyphae and roots causes enlargement of pores and loosening of stone particles from the material (Ascaso et al., 1998). The formation of biofilms intensifies this loss of structural consistency (Orgenta-Calvo et al., 1991).

Due to the capability of fixing atmospheric nitrogen, phototrophic organisms are often the primary settlers of habitats especially in combination with fungi as lichens. In rural or strongly industrialized areas where the air is enriched with nitrogen from fertilizers or industrialization, their growth can be enhanced (Cadot-Leroux, 1996; Cicek et al., 2008). Some phototrophic microorganisms (epilithicphototrophs) may penetrate several millimeters into the stone pore system (Golubic et al., 1981). Additionally, some of these microorganisms can colonize extreme locations, whereby a special pigmentation protects them against strong light irradiation (Krumbein and Jens, 1981).

Chemolithotrophic and chemoorganotrophic microorganisms

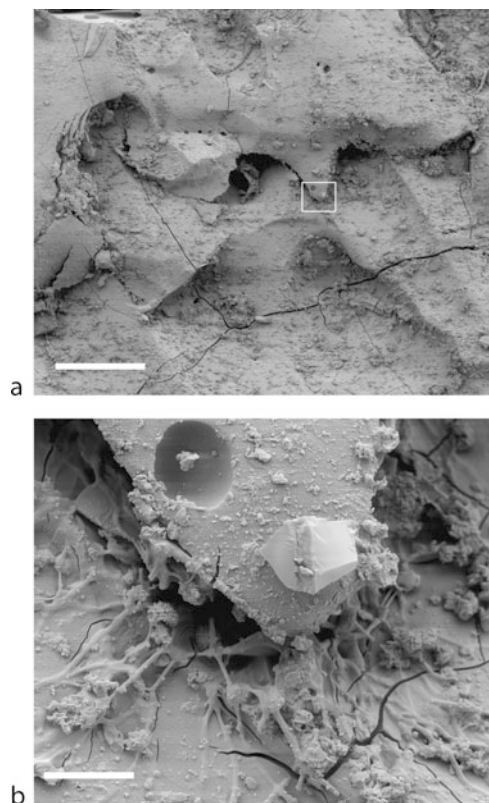
Even the natural biomass accumulated by photosynthetic microorganisms – especially in combination with anthropogenic pollution – can serve as nutrient source for the establishment of heterotrophic microorganisms (Warscheid et al., 1991; Zanardini et al., 2000).

Besides, even without a primary settlement of phototrophic microorganisms an establishment of heterotrophic organisms is possible (Krasilnikov, 1949). A sufficient amount of inorganic compounds (e.g., sulfur, ammonium, sulfide) enables the appearance of chemolithoautotrophic bacteria (Kaufmann, 1960; Pochon and Jaton, 1967). The formation of nitrous acid (HNO_2 by *Nitrosomas*) or nitric acid (HNO_3 by *Nitrobacter*) and sulfuric acid (H_2SO_4 , *Thiobacillus*) results in the solution of mineral rock compounds. Acidic metabolic compounds resulting from the oxidation of sulfur, for example, interact with calcium carbonate of the stone structure. This reaction leads to the formation of calcium sulfate, which is more soluble than calcium carbonate.

The most numerous microorganisms in natural building stone are chemoorganotrophic bacteria. Results of Warscheid et al. (1993) indicate that they colonize natural stone within weeks. They can excrete acid compounds but, generally, their destructive activity has been evaluated as low (Lewis et al., 1988). Nevertheless, they are extensively involved in the formation of biofilms, thus causing physicochemical properties of the mineral structure described above. Materials can contain or be associated with organic compounds such as dust, natural resin, wax, or atmospheric aerosols that can act as nutrient source for chemoorganotrophic microorganisms (Warscheid, 1990). As an effect of organic pollution, chemoorganotrophic microorganisms can be the first settlers on buildings in highly industrialized locations (Krumbein, 1966). Many chemoorganotrophic bacteria are able to use low degradable compounds, offering nutrients for other microorganisms of the microbial consortia (Warscheid et al., 1991; Zanardini et al., 2000).

Fungi

The colonization of stone surfaces or other materials by fungi causes damages, especially by physical (mechanical) forces (cf. Figure 2). The formation of penetration hyphae enables some fungi to enter actively into materials, despreding within the material and affecting the so-called biopitting structures on rock surfaces (Sterflinger and Krumbein, 1997). Furthermore, the production of aggressive acidic metabolites leads to biocorrosion (Kurakov et al., 1999). In comparison to bacteria, fungi can establish rapidly, especially in environments with low humidity due to their widespread system of hyphae (Weber, 1993). Their damaging effects also include the discoloration of surfaces due to the building and excretion of melanins (Gorbishina et al., 1993). The synthesis of special pigments such as melanin, carotenoids, and mycosporines provides



Biodeterioration (of Stone), Figure 2 (a) Scanning electron microscopic demonstrate colonization of amber surface by recent microorganisms. Fungal hyphae cover the amber surface and accumulate in cracks leading to loss of material persistence by physical stresses; scale bar 100 μm . (b) closeup, scale bar 10 μm . (Reitner and Beimforde, 2008).

protection against UV radiation and enables some species (e.g., black fungi) to colonize even at locations with extreme UV radiation (Chertov et al., 2004).

Measures and preventive treatments

In general, a microbial colonization is primarily dependent on the availability of water and nutrients and specific material parameters such as porosity, permeability, architectural conditions (considering buildings), and environmental factors. Natural selective processes can lead to the formation of populations that reveal a perfect adaption between organisms, substrate, and environment. A previous individual and comprehensive analysis of all factors and relationships is essential before preventive treatments or cleaning of the material is applied. A mechanical cleaning of historical buildings from dust or biological films should integrate a chemical analysis to characterize the surface dirt. Otherwise, the cleaning procedure can fail to have a long-lasting effect and even accelerate the degradation processes or alteration within the composition of microbial associations affecting changes of microbial activity (Dornieden et al., 2000).

Water cleaning can help remove soluble salts and biological infections, but in the long term it can lead to a wider dispersion of the microbial infection because of increased humidity. Furthermore, consolidation treatments can be applied, but their effectiveness is strongly connected to the physical structure of the stones. The application on stone surfaces with insufficient consistence and absorbent capacity can intensify physical damages (Sattler, 1992; Wendler, 1992; Zoghalmi and Gómez-Gras, 2004). Other preventive agents impregnate surface structures and are often based on high polymer polysaccharide, silane, or siloxane. Various studies have shown that the application of consolidants or water repellents induce an increasing activity or colonization of microorganisms using these restoration agents as nutrient source (Krumbein et al., 1993; Petersen et al., 1991, cf. Figure 3). Studies of Urzi and

De Leo (2007) and De Leo and Urzi (2003) have shown that water repellents alone do not stop microbial colonization, but in combination with biocides, microbial growth can be prevented.

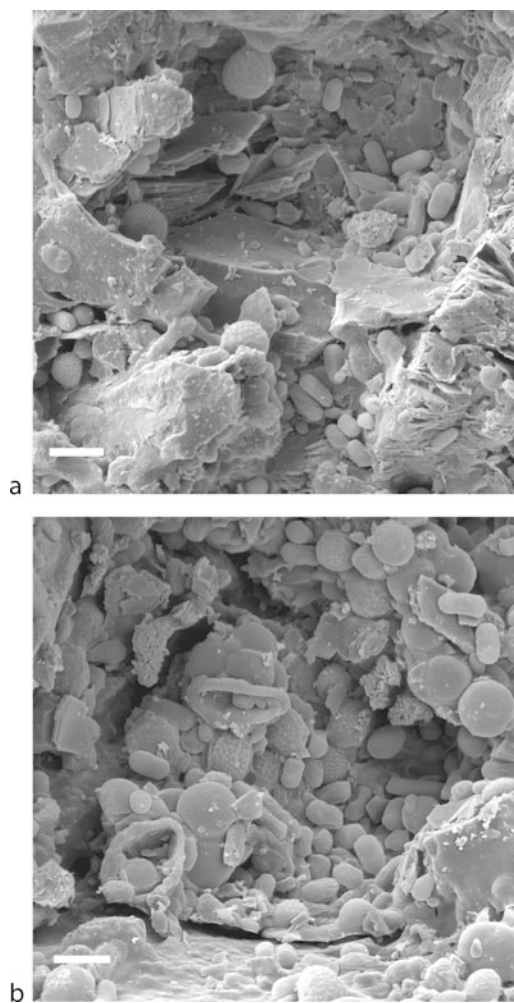
Finally, it is important to mention that the treatment with biocides should only be applied in instances where biodeterioration processes cannot be controlled and chemical applications are unavoidable. An inappropriate treatment with biocides or other preventive arrangements can lead to an increasing selective microbial colonization that may accelerate the deterioration.

Summary

The process of weathering consists of physical, chemical and biological factors acting together with more or less intensity, leading to an irreversible loss of our cultural heritage. Microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial community or rather the microbial activity is also affected by the surrounding chemical and physical factors. Interactions between microbial associations and materials contain the enzymatic decomposition of materials, excretion of metabolic compounds such as inorganic or organic acids, and damages by physical (mechanical) forces due to the growth of microorganisms.

Bibliography

- Arino, X., Gomez-Bolea, A., and Saiz-Jimenez, C., 1997. Lichens on ancient mortars. *International Biodeterioration and Biodegradation*, **40**, 217–224.
- Ascaso, C., Wierzbos, J., and Castello, R., 1998. Study of the biogenic weathering of calcareous litharenite stones caused by lichen and endolithic microorganisms. *International Biodeterioration and Biodegradation*, **42**, 29–38.
- Beimforde, C., 2006. Einfluss von Restaurierungsmitteln auf gesteinsbesiedelnde mikrobielle Vergesellschaftungen: mikroskopischer und molekularbiologischer Vergleich. Thesis, Oldenburg.
- Beimforde, C., and Schmidt, A. R., 2010. Microbes in resinous habitats: a compilation from modern and fossil resins. In Reitner, J., Queric, N.-V., and Arp, G. (eds.), *Advances in Stromatolite Geobiology. Lecture Notes in Earth Sciences* (in press).
- Bell, F. G., 1993. Durability of carbonate rock as building stone with comments on its preservation. *Environmental Geology*, **21**, 187–200.
- Cadot-Leroux, L., 1996. Rural nitrogenous pollution and deterioration of granitic monuments: results of a statistical study. In Riederer, J. (ed.), *Proceedings of the Eighth International Congress on Deterioration and Conservation of Stone*, Rathgen-Forschungslabor: Berlin, Vol. 1, pp. 301–309.
- Chertov, O., Gorbushina, A. A., and Deventer, B., 2004. A model for microcolonial fungi growth on rock surfaces. *Ecological Modelling*, **177**, 415–426.
- Cicek, A., Aslan, A., Yazc, K., and Savas Koparal, A., 2008. Effects of environmental conditions on historical buildings: lichens and NO_x gases. *Environmental Monitoring Assessment*, Published online.
- Costeron, J. W., Cheng, K.-J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., and Marrie, T. J., 1987. Bacterial biofilms in nature and disease. *Annual Reviews in Microbiology*, **41**, 435–464.



Biodeterioration (of Stone), Figure 3 Sandstone treated with (a) consolidation agents and (b) water repellents. A high microbial colonizations was observed 3 month after treatment and outdoor exposure, scale bar 10 μ m (photograph by A. A. Gorbushina and C. Beimforde, Beimforde, 2006).

- Crispim, C. A., and Gaylarde, C. C., 2004. Cyanobacteria and biodegradation of cultural heritage: a review. *Microbiological Ecology*, **49**, 1–9.
- De Leo, F., and Urzi, C., 2003. Fungal colonisation on treated and untreated stone surfaces. In Saiz Jimenez, C. (ed.), *Molecular Biology and Cultural Heritage*. The Netherlands: Swets & Zeitlinger, pp. 213–217.
- De los Rios, A., Cámara, B., García Del Cura, Á., Víctor, M. A. G., Rico, V. J., Galván, V., and Ascaso, C., 2009. Deteriorating effects of lichen and microbial colonization of carbonate building rocks in the Romanesque churches of Segovia (Spain). *Science of the Total Environment*, **407**, 1123–1134.
- Dornieden, Th., Gorbushina, A. A., and Krumbein, W. E., 2000. Biodecay of cultural heritage as a space/time-related ecological situation - an evaluation of a series of studies. *International Biodeterioration and Biodegradation*, **46**, 261–270.
- Ehrlich, H. L., 2002. *Geomicrobiology*, 4th edn. New York: Marcel Dekker.
- Golubic, S., Friedman, E., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**, 475–478.
- Gorbushina, A. A., 2007. Life on the Rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Gorbushina, A. A., and Krumbein, W. E., 2000. Subaerial microbial mats and their effects on soil and rock. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 161–170.
- Gorbushina, A. A., Krumbein, W. E., Hamman, C. H., Panina, L., Soukharjevsky, S., and Wollenzien, U., 1993. Role of black fungi in color change and biodeterioration of antique marbles. *Geomicrobiology Journal*, **11**, 205–220.
- Griffin, P. S., Indictor, N., and Koestler, R. J., 1991. The biodeterioration of stone: a review of deterioration mechanisms. *Conservation Case International Biodeterioration*, **28**, 187–207.
- Kaufmann, J., 1960. Corrosion et protection des pierres calcaires des monuments. *Corrosion Anticorrosion*, **8**, 87–95.
- Koestler, R. J., Brimblecomb, P., Camuo, D., Ginell, W., Graedel, T., Leavengood, P., Petushkova, J., Steiger, M., Urzi, C., Verges-Belmin, V., Warscheid, Th., 1994. Group report: How do external environmental factors accelerate change? In Krumbein, W. K., Brimblecombe, P., Cosgrove, D. E., and Staniforth, S. (eds.), *Durability and Change*. Chichester: Wiley, pp. 149–163.
- Krasilnikov, N. A., 1949. The role of microorganisms in the weathering of rocks. I. Microora of the surface layer of rocks. *Microbiologiya*, **18**, 318–323.
- Krumbein, W. E., 1966. Zur Frage der Gesteinsverwitterung (über geochemische und mikrobiologische Bereiche der exogenen Dynamik). Dissertation, Würzburg.
- Krumbein, W. E., and Jens, K., 1981. Biogenic rock varnishes of the Negev desert (Israel) an ecological study of iron and manganese transformation by cyanobacteria and fungi. *Oecologia*, **50**, 25–38.
- Krumbein, W. E., and Schönborn-Krumbein, C. E., 1987. Biogene Bauschäden: Anamnese, Diagnose und Therapie in Bautenschutz und Denkmalpflege. *Bautenschutz-Bausanierung*, **10**, 14–23.
- Krumbein, W. E., and Warscheid, T., 1992. Patina – ein schillernder Begriff. Biologisch beschleunigte Alterung mineralischer Werkstoffe und Baudenkmäler. *Die Geowissenschaften*, **10**, 274–276.
- Krumbein, W. E., Braams, J., and Grote, G., 1993. Anti-Graffiti-Beschichtungen – Haltbarkeit und biologische Wechselwirkungen. *Bautenschutz-Bausanierung*, **16**, 17–21.
- Kurakov, A. V., Somova, N. G., and Ivanovskii, R. N., 1999. Micromycetes populating limestone and red brick surfaces of the Novodevichii Convent Masonry. *Microbiology*, **68**, 232–241.
- Lewis, F. J., May, E., and Bravery, A. F., 1988. Metabolic activities of bacteria isolated from building stone and their relationship to stone decay. In Houghton, D. R., Smith, R. N., and Eggins, H. O. W. (eds.), *Biodeterioration*. London, New York: Elsevier Applied Science, Vol. 7, pp. 107–112.
- Ortega-Calvo, J. J., Arino, X., Stal, L. J., and Saiz-Jimenez, C., 1994. Cyanobacterial sulfate accumulation from black crusts of a historic building. *Geomicrobiology Journal*, **12**, 15–22.
- Ortega-Calvo, J. J., Hernandez-Marine, M., and Saiz-Jimenez, C., 1991. Biodeterioration of building materials by Cyanobacteria and Algae. *International Biodeterioration*, **28**, 165–186.
- Ortega-Morales, O., Guezennec, J., Hernández-Duque, G., Gaylarde, C. C., and Gaylarde, P. M., 2000. Phototrophic biofilms on ancient Mayan buildings in Yucatan, Mexico. *Current Microbiology*, **40**, 81–85.
- Petersen, K., Janssen, D. D., Schostak, V., Bode-Warscheid, K., and Krumbein, W. E., 1991. On the influence of restoration activities on microbial biodeterioration of mural paintings. In Baer, N. S., Sabbioni, C., and Sors, A. I. (eds.), *Proceedings of the European Symposium, Bologna, Italy*, Luxembourg: Butterworth-Heinemann Ltd.
- Pitzurra, L., Moroni, B., Nocentini, A., Sbaraglia, G., Poli, G., and Bistoni, F., 2003. Microbial growth and air pollution in carbonate rock weathering. *International Biodeterioration and Biodegradation*, **52**, 63–68.
- Pochon, J., and Jaton, C., 1967. The role of microbiological agencies in the deterioration of stone. *Chemistry and Industry*, **9**, 1587–1589.
- Reitner, J., and Beimforde, C., 2008. Mikroben und Bernstein – Einblicke in eine wenig verstandene geo-biologische Beziehung. *Jahrbuch der Akademie der Wissenschaften zu Göttingen*. Berlin: Walter de Gruyter GmbH & Co. KG, pp. 188–198.
- Sattler, L., 1992. Wirksamkeitskontrollen an klassischen Methoden der Steinkonservierung. *Die Geowissenschaften*, **10**, 88–89.
- Steiger, M., and Dannecker, W., 1995. Hygroskopische Eigenschaften und Kristallisationsverhalten von Salzgemischen. In *Jahresberichte Steinzerfall – Steinkonservierung, Band 5-1993*. Berlin: Ernst und Sohn Verlag, pp. 115–127.
- Sterflinger, K., and Krumbein, W. E., 1997. Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones. *Geomicrobiology Journal*, **14**, 219–230.
- Stoodley, P., Sauer, K., Davies, D. G., and Costerton, J. W., 2002. Biofilms as complex differentiated communities. *Annual Reviews in Microbiology*, **56**, 187–209.
- Urzi, C., and De Leo, F., 2007. Evaluation of the efficiency of water-repellent and biocide compounds against microbial colonization of mortars. *International Biodeterioration and Biodegradation*, **60**, 25–34.
- Urzi, C., and Krumbein, W. E., 1994. Microbiological impacts on the cultural heritage. In Krumbein W. E., Brimblecombe, P., Cosgrove, D. E., and Staniforth, S. (eds.), *Durability and Change*. Chichester: Wiley, pp. 107–135.
- Warscheid, Th., 1990. Untersuchungen zur Biodeterioration von Sandsteinen unter besonderer Berücksichtigung der chemoorganotrophen Bakterien. Dissertation, Oldenburg.
- Warscheid, Th., and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, **46**, 343–368.
- Warscheid, Th., Oelting, M., and Krumbein, W. E., 1991. Physico-chemical aspects of biodeterioration processes on rocks with special regard to organic pollution. *International Biodeterioration*, **28**, 37–48.
- Warscheid, Th., Becker, Th., Braams, J., Brüggerhoff, S., Gehrmann, C., Krumbein, W. E., and Petersen, K., 1993. Studies on the temporal development of microbial infection of different types of sedimentary rocks and its effect on the alteration of the physico-chemical properties in building materials. In Thiel,

- M.-J. (ed.), *Conservation of Stone and Other Materials*. London: E & FN Spon, Vol. 1, pp. 303–310.
- Weber, H., 1993. *Allgemeine Mykologie*. Jena: Gustav Fischer Verlag.
- Wendler, E., 1992. Modifizierung von Steinschutzmitteln. *Die Geowissenschaften*, **10**, 290–292.
- Winkler, E. M., 1987. Weathering and weathering rates of natural stone. *Environmental Geology and Water Science*, **9**, 85–92.
- Wolfaardt, G. M., Lawrence, J. R., and Korber, D. R., 1999. Function of EPS. In Wingender, J., Neu, T. R., and Flemming, H. C. (eds.), *Microbial Extracellular Substances (Characterization, Structure and Function)*. Berlin, Heidelberg: Springer, pp. 171–200.
- Zanardini, E., Abbruscato, P., Ghedini, N., Realini, M., and Sorlini, C., 2000. Influence of atmospheric pollutants on the biodeterioration of stone. *International Biodeterioration and Biodegradation*, **45**, 35–42.
- Zoghalmi, K., and Gómez-Gras, D., 2004. Determination of the distribution of consolidants and interpretation of mercury porosimetry data in a sandstone porous network using LSCM. *Microscopy Research and Technique*, **65**, 270–275.

Cross-references

Amber
 Bioerosion
 Biological Volcanic Rock Weathering
 Bioprotection
 Endoliths
 Fungi and Lichens
 Geomycology
 Microbial Biomineralization
 Microbial Degradation
 Microbiocorrosion

BIOEROSION

Aline Tribollet¹, Gudrun Radtke², Stjepko Golubic³

¹Institut de Recherche pour le Développement, Nouméa, Nouvelle-Calédonie, France

²Hessisches Landesamt für Umwelt und Geologie, Wiesbaden, Germany

³Boston University, Boston, MA, USA

Definition

Bioerosion is defined as erosion, i.e., removal and transport of materials by the action of organisms. Bioabrasion refers to removal by mechanical means: scraping, rasping, drilling. Biocorrosion refers to removal by chemical means: etching, dissolving, boring. Bioerosion is often carried out by a combination of bioerosional tools. Bioerosion by microorganisms is referred to as microbioerosion as opposed to macrobioerosion performed by animals and plants. External bioerosion is carried out by organisms that live outside the substrate but remove it in the process of seeking food (grazing). Internal bioerosion is carried out by organisms that excavate the substrate for shelter and live inside as endoliths. They penetrate the substrate actively as euendoliths, or

occupy existing cavities as cryptoendoliths or fissures in the rocks as chasmoendoliths.

Concept of bioerosion

The concept of erosion is usually discussed in geology, particularly in geomorphology, sedimentology, and soil sciences, in reference to degradation and transport of rocks and sediments by environmental forces such as wind, water, or ice. The participation of organisms in these processes and their long-term geological impact are often ignored. The role of organisms in sediment stabilization, organization, and buildup is becoming increasingly recognized as biosedimentation, and so is bioerosion, i.e., the participation of organisms in destruction of rocks and sediments.

Similar to weathering and erosion by environmental forces, the destruction of hard substrates by organisms, i.e., biological erosion or bioerosion (Neumann, 1966) includes two mechanisms: (a) biological corrosion, which refers to destruction of substrates by chemical means introduced by organisms, thus promoting dissolution of solid substrates, and (b) biological abrasion, which refers to mechanical destruction and removal of substrate by organisms (Golubic and Schneider, 1979). Sediment particles are frequently produced during biological abrasion and are redeposited (Neumann, 1966; Schneider and Torunski, 1983).

Bioerosion affects various substrates, most commonly the relatively soluble ones such as carbonates, phosphates, and sulfates. Bioerosion of siliceous rocks occurs at much lower rates and is rarely conspicuous. The objects of bioerosion include solid rocks, sediment particles, and mineralized skeletons of various live and dead organisms. Bioerosion takes place in a wide variety of aquatic and terrestrial environments. Its effects are especially noticeable in coastal carbonate rocks, coral reef structures, and molluscan calcareous skeletons, but are less apparent in skeletons of live corals (Le Campion-Alsumard et al., 1995a), encrusting coralline algae (Pantazidou et al., 2000; Tribollet and Payri, 2001; Ghirardelli, 2002), foraminifera (Golubic et al., 1984), calcareous ooid sand particles (Al-Thukair and Golubic, 1991), and fish teeth (Underwood et al., 1999).

Many researchers describe penetration into hard substrate as boring activity, in contrast to burrowing, which describes digging into loose substrate such as sand and mud; however, some use the term burrowing for penetrating any substrate. Others suggested that activities of bioeroding organisms be distinguished on the basis of their objectives (e.g., food vs. shelter). Many bioeroding organisms are fully or partially embedded in the rocky substrate, occupying an endolithic habitat, while others erode the substrate from outside. Accordingly, they have been distinguished as internal versus external bioeroders. Many investigators view bioerosion as behavior (Boucot, 1990) and relate bioeroding organisms to the traces they leave in modern and ancient hard substrates. Researchers

B

BACTERIA

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms

Eubacteria (term abandoned)

Definition

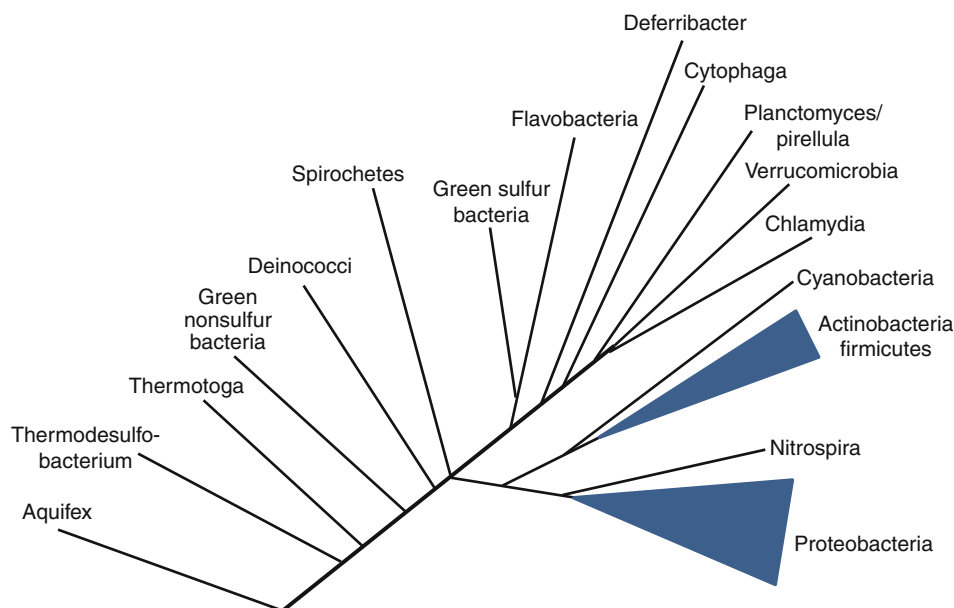
Prokaryotes that constitute, besides Archaea and Eukarya, a domain of life. According to (old Greek) bakterion “small rod.”

History

During the nineteenth century, several fundamental discoveries defined the beginning of modern microbiology (cf. Schlegel, 1999). Louis Pasteur (1822–1895) found that growth of bacteria in nutrient broths is not due to spontaneous generation and that fermentation is caused by the growth of microorganisms. Ferdinand Cohn (1828–1898) could state that bacteria must belong to a phylogenetic group separated from other unicellular plants or animals, due to their size, shape, the mode of cell division, and their metabolic properties. Robert Koch (1842–1910) developed the concept of infectious diseases. The final proof that bacteria must be distinct from plants and animals could be shown not until the distinct compartmentation of bacterial cells was noticed by electron microscopy, in the 1960s. Especially, the absence of a nuclear membrane was a clear indicator for some fundamental differences in cellular processes. Hence, the term “prokaryote” for bacteria (including Archaea) was introduced. The separation of *Archaea* from Bacteria (as phylogenetic groups) was achieved by Woese and Fox (1977).

Besides the missing nuclear membrane as a common cytological feature, Bacteria and Archaea are structurally and physiologically distinct. The Bacteria are the phylogenetically most diverse domain, with a rapidly growing number of phyla. Currently, more than 80 phyla could be identified (Pace, 2009). About two-third of these phyla are just represented by rDNA sequences from environmental samples. Twenty-five phyla are represented by known species. Among them, twelve phyla contain the majority of currently known and well-characterized species. The well-known representatives of phyla, however, stand for an incomplete and subjective view on the bacterial domain. Human pathogenic species are, for example, overrepresented, because of their importance for human medicine. The significance of several groups relevant for environmental processes, such as Planctomycetes or the *Roseobacter* clade has just been addressed recently. General knowledge on the physiology of these groups is still limited. Most in-depth analyses on microbial physiology and molecular biology has been performed with selected species from a small number of phyla, such as *Escherichia coli*, *Salmonella*, and *Pseudomonas* species (Proteobacteria) or *Bacillus* (Firmicutes). Some organisms like *Ralstonia* (an aerobic hydrogen oxidizing Proteobacterium) or *Rhodobacter* (a photosynthetic bacterium) are model organisms for environmentally relevant processes.

The phylogenetic tree (Figure 1), based upon 16S-rDNA data shows an overview of these major, well-characterized lineages (Cole et al., 2009; Madigan et al., 2009). In-depth analysis based upon whole genomes may allow, in future, a more accurate positioning of genes and organisms in phylogenetic trees (Ciccarelli et al., 2006; Wu and Eisen, 2008). Though the major phyla have been confirmed, some repositioning may be necessary. Some reports imply that Firmicutes are descendants of



Bacteria, Figure 1 Phylogenetic tree of Bacteria, derived from 16S rDNA sequences. (Redrawn after Madigan et al., 2009.)

the stem group, other than expected from rDNA analysis (Ciccarelli et al., 2006).

In the following chapter, a short survey on the hitherto well-investigated groups will illustrate the metabolic diversity of the organisms. Terms denoting defined phyla are capitalized.

Diversity in phylogenetic lineages

Important metabolic key processes are unique to bacteria and have just been overtaken by endosymbiosis to eukaryotes, such as chlorophyll-dependent photosynthesis and mitochondrial respiration. Thus, metabolism in every eukaryote is based upon bacterial metabolism. Eukaryotes, however, received just a small range of the highly various metabolic features from bacteria. Virtually, every reduced organic compound and numerous reduced inorganic molecules can be oxidized in a bacterial metabolism that may be either respiratory, that is, generating a membrane potential, driven by an electron flow toward an oxidized acceptor (oxygen, nitrate, and others) or fermentative (generation of ATP independent of a membrane potential). These metabolic features are unsuitable to discriminate between large groups because they are mostly not specific for only one phylum (Tables 1–4; Schlegel and Jannasch, 2006; Zinder and Dworkin, 2006). Some strategies and bacterial life-styles are, however, suitable to discriminate between certain subgroups within a phylum. In the following, some key features that may be confined to the respective groups are specified. The groups are assigned to phyla according to the “classical” phylogenetic scheme, obtained by

16S-rDNA sequence analysis (Cole et al., 2008; cf. Madigan et al., 2009).

Ancient phyla

Several representatives of the ancient bacterial groups clustering deep in the phylogenetic tree are hyperthermophilic, some of them are chemolithotrophs. The hyperthermophilic genus *Aquifex* is an obligately hydrogen oxidizing, microaerophilic, chemolithotrophic (see *Chemolithotrophy*) bacterium (Huber and Eder, 2006). The fixation of carbon dioxide does not depend on the Calvin cycle (ribulose biphosphate carboxylase as key enzyme), like in further derived phyla and green plants, but, instead, on a reversed citrate cycle. The organisms are not capable to grow on organic molecules, that is, they are obligate chemolithotrophs utilizing molecular hydrogen or reduced sulfur compounds as electron donors, at optimum growth temperatures of 85°C. Thus, the environmental conditions for the ecological niche of *Aquifex* are equivalent to the reducing conditions of the earth’s early atmosphere. The related genera *Hydrogenobacter* and *Thermocrinis* share most of *Aquifex*’ properties, but are aerobic and also utilize organic compounds.

Several important cellular features that are found in most bacterial phyla are already present in this ancient lineage: the cytoplasmic membrane is surrounded by another membrane of lipid bilayer type (outer membrane). Thus, the membranes enclose an additional compartment, the periplasmic space. The periplasmic space contains the rigid murein (peptidoglycan) sacculus. Another feature found in all, including the most ancient bacterial phyla is the bacterial flagellum (Figure 2a). Though analogous

Bacteria, Table 1 Groups of chemolithotrophic bacteria (After Schlegel and Jannasch, 2006, with modifications)

Typical species	Lineage	Metabolic process	Electron donor	Electron acceptor	Product
<i>Aquifex</i>	Aquificales	Hydrogen oxidation	H ₂	O ₂	H ₂ O
		Sulfur oxidation	S ₂ O ₃ ²⁻ , S ₀	O ₂	SO ₄ ²⁻
<i>Ralstonia eutropha</i>	Beta-Proteobacteria	Hydrogen oxidation	H ₂	O ₂	H ₂ O
<i>Pseudomonas carboxydovorans</i>	Gamma-Proteobacteria	CO oxidation	CO	O ₂	CO ₂
<i>Nitrosomonas europaea</i>	Beta-Proteobacteria	Ammonium oxidation	NH ₄ ⁺	O ₂	NO ₂ ⁻
<i>Nitobacter</i>	Alpha-Proteobacteria	Nitrite oxidation	CO ₂	O ₂	NO ₃ ⁻
<i>Thiobacillus thiooxidans</i>	Beta-Proteobacteria	Sulfur oxidation	H ₂ S, S ₀	O ₂	SO ₄ ²⁻
<i>Thiobacillus ferrooxidans</i>	Beta-Proteobacteria	Iron oxidation	Fe ²⁺	O ₂	Fe ³⁺
<i>Beggiatoa alba</i>	Gamma-Proteobacteria	Sulfur oxidation	H ₂ S, S ₀	O ₂ , NO ₃ ⁻	SO ₄ ²⁻ , N ₂
<i>Acetobacterium woodii</i>	Firmicutes	Acetogenesis	H ₂	CO ₂	Acetate

Bacteria, Table 2 Groups of phototrophic bacteria (After Schlegel and Jannasch, 2006, with modifications)

Bacterial group	Lineage	Key genus	Chemoorganotrophic growth	Electron donors for photosynthesis
Purple non-sulfur bacteria	Alpha-/beta-Proteobacteria	<i>Rhodospirillum</i>	+	H ₂ , organic, (S ²⁻)
Purple sulfur bacteria	Gamma-Proteobacteria	<i>Chromatium</i>	—	S ²⁻ , S ⁰ , S ₂ O ₃ ²⁻ , H ₂
Green sulfur bacteria	Chlorobi	<i>Chlorobium</i>	—	S ²⁻ , S ⁰ , S ₂ O ₃ ²⁻ , H ₂
<i>Chloroflexus</i> group	Chloroflexi	<i>Chloroflexus</i>	+	Organic (S ²⁻)
Heliobacteriaceae	Firmicutes	<i>Heliobacterium</i>	—	Organic
Aerobic phototrophic Proteobacteria	Alpha-Proteobacteria	<i>Roseobacter</i>	—	Organic
Cyanobacteria	Cyanobacteria	<i>Synechococcus</i> <i>Oscillatoria</i>	—	H ₂ O H ₂ O (S ²⁻)

Bacteria, Table 3 Bacterial groups and their electron acceptors for respiratory processes (After Schlegel and Jannasch, 2006, with modifications)

Physiological group	Typical species	Lineage	Respiratory process	Electron acceptor	Reduction products
Denitrifiers	<i>Pseudomonas denitrificans</i>	Gamma-Proteobacteria	Aerobic respiration nitrite respiration	O ₂ , NO ₃ ⁻	N ₂ , N ₂ O, NO ₂ ⁻
Sulfate reducers	<i>Desulfovibrio vulgaris</i>	Delta-Proteobacteria	Sulfate respiration	SO ₄ ²⁻	S ²⁻
Sulfur reducers	<i>Desulfuromonas acetoxidans</i>	Delta-Proteobacteria	Sulfur respiration	S ⁰	S ²⁻
Acetogenic bacteria	<i>Acetobacterium woodii</i>	Firmicutes	Carbonate respiration	CO ₂	acetate
Succinogenic bacteria	<i>Wolinella succinogenes</i>	Epsilon-Proteobacteria	Fumarate respiration	fumarate	succinate
Iron reducer	<i>Pseudomonas GS-15</i>	Gamma-Proteobacteria	Iron respiration	Fe ³⁺	Fe ²⁺

motility structures are also found in Archaea (and in Eukarya), they are phylogenetically completely unrelated. For other types of appendages (bacterial pili, Figure 2a), homologues in Archaea could be described (Szabo et al., 2007).

Thermotoga and related genera also comprise chemoorganotrophic hyperthermophilic representatives. Comparative genomics revealed that more than 20% of the *Thermotoga* genome shares strong homologies with genes from various hyperthermophilic archaeal species, accounting for an extensive inter-domain horizontal gene transfer (Nelson et al., 1999).

Chloroflexi and Chlorobi

(Bacterio)chlorophyll-dependent photosynthesis (see *Photosynthesis*) is unique for bacterial organisms (Table 2; Overmann and Garcia-Pichel, 2006). Ancestors of green nonsulfur bacteria (Chloroflexi) were very likely the first photosynthetic organisms of the bacterial domain (Oyaizu et al., 1987). The key genus *Chloroflexus* is a thermophilic representative of the group. Besides photoautotrophic growth, *Chloroflexus* is also able to use organic compounds. Carbon dioxide fixation is still Rubisco independent and is carried out by transfer of hydrogen carbonate to activated acetate and propionate

Bacteria, Table 4 Groups of fermentative bacteria

Fermentative process	Typical species	Lineage	Preferred substrates	Fermentation products (minor products)
Ethanol fermentation	<i>Zymomonas mobilis</i>	Alpha-Proteobacteria	Glucose	Ethanol (CO ₂)
Lactate fermentation – homofermentative – heterofermentative	<i>Lactobacillus casei</i> <i>Leuconostoc mesenteroides</i>	Firmicutes	Glucose, lactose Glucose, lactose	Lactate Lactate (ethanol, CO ₂)
Butyrate fermentation	<i>Clostridium butyricum</i> <i>Clostridium acetobutylicum</i> <i>Clostridium klyuveri</i>	Firmicutes Firmicutes Firmicutes	Glucose Glucose Ethanol+acetate	Butyrate (acetate, H ₂ , CO ₂) Butyrate, butanol (acetone, 2-propanol)
Homoacetate fermentation	<i>Clostridium acetivum</i>	Firmicutes	Fructose	Acetate
Propionate and succinate fermentation	<i>Propionibacterium pentosaceum</i> <i>Veillonella alcalescens</i> <i>Bacteroides ruminicola</i>	Actinobacteria Firmicutes	Sugars, Lactate Lactate Sugars	Propionate (succinate) Propionate (acetate, H ₂ , CO ₂) Propionate
Mixed acid and butanediol fermentation	<i>Escherichia coli</i> <i>Enterobacter aerogenes</i>	Gamma-Proteobacteria	Glucose, lactose Glucose	Lactate, ethanol acetate (formate, succinate, H ₂ , CO ₂) 2,3 butanediol, ethanol (formate, H ₂ , CO ₂)
Amino acid fermentation	<i>Clostridium tetanomorphum</i> <i>Clostridium sticklandii</i> <i>Clostridium oroticum</i>	Firmicutes Firmicutes Firmicutes	Glutamate Lysine Orotate	Butyrate (acetate, CO ₂ , NH ₃) Butyrate (acetate, NH ₃) Acetate (CO ₂ , NH ₃)

(acetyl-coenzyme A, propionyl-coenzyme A) in the hydroxypropionate pathway. Green sulfur bacteria (Chlorobi) represent a separate lineage of anoxygenic photosynthetic bacteria. Similar to photosynthetic Proteobacteria, the organisms use reduced sulfur compounds (H₂S, S₀) as well as organic compounds as electron donors. The organisms use a reverse citric acid cycle for carbon dioxide fixation. A remarkable structural feature, the chlorosome, consisting of densely stacked self-assembled antenna bacteriochlorophylls, is a simple but very effective light harvesting complex. This arrangement transfers light energy (excitons) effectively to the reaction centre in the cytoplasmic membrane. The organisms are, hence, adapted to very low light conditions. Chlorosome genes were transferred, by horizontal gene transfer, to some phototrophic lineages, including some genera of the *Chloroflexus* group (cf. Hanada and Pierson, 2006).

Spirochetes

Spirochetes are rather structurally than metabolically remarkable (Charon et al., 1992). The spirochete endoflagellum is of the standard bacterial type, but is located in the periplasmic space. In all other bacterial groups, the flagellum (if present) traverses all layers of the envelope. The endoflagellum is helically wound around the flexible protoplast and gives the cell its unique, helically coiled appearance. The movement of the flagellum drives the cell body in a drill-like manner. By this way, movement of the cell in highly viscous media like tissue is facilitated. In fact, numerous symbiotic and

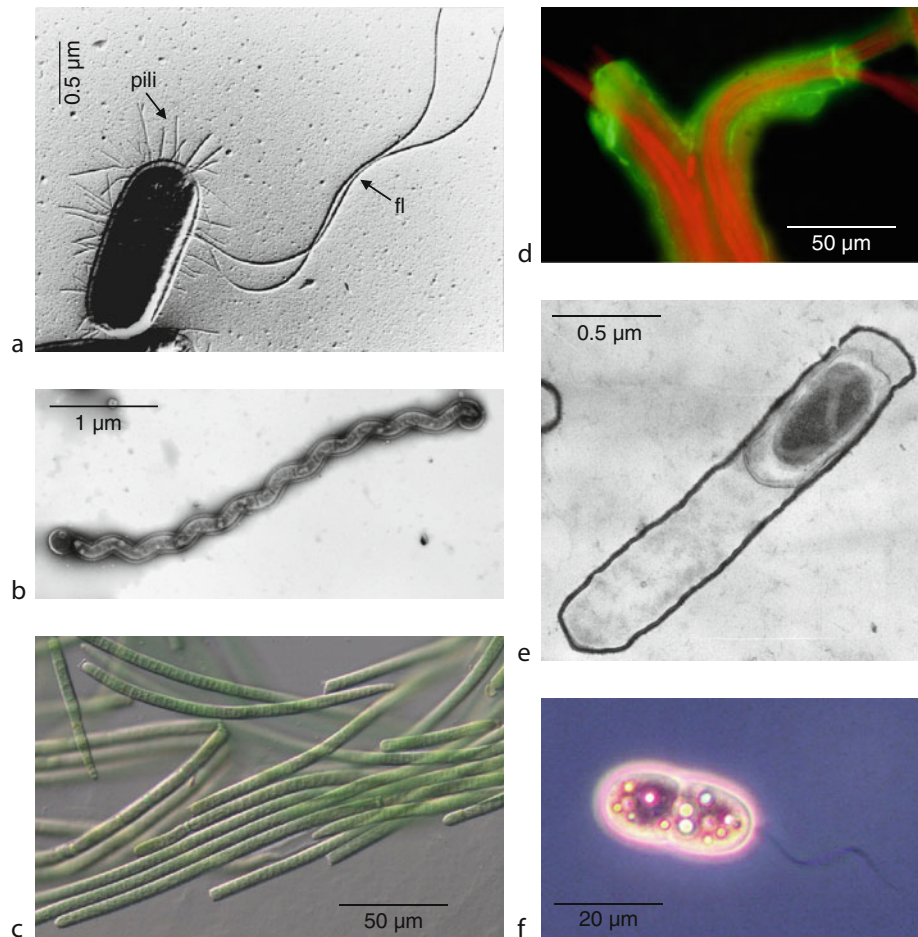
pathogenic organisms belong to this group (*Teponema*, *Borrelia*, *Leptospira*).

Deinococcus/Thermus

Another separate lineage of extremophilic bacteria is represented by the key genera *Deinococcus* and *Thermus*. The organisms mainly oxidize carbohydrates and proteins. *Thermus* species are thermophilic and grow between 50°C and 80°C. The remarkable members of the *Deinococcus* group are adapted to desiccation and ionizing or ultraviolet radiation. The composition and thickness of the cell wall, DNA compaction, and DNA repair systems are special protective features (Da Costa et al., 2006).

Flavobacteria/Cytophaga

The Flavobacteria/Cytophaga phylum comprises diverse, mostly heterotrophic, anaerobic or aerobic bacteria (Bernardet and Nakagawa, 2006). Within this group, the members of the genus *Bacteroides* represent the dominant bacteria in the human large intestine. The second key genus *Flavobacterium* is mostly found in freshwater and marine habitats. The Acidobacteria with the genera *Acidobacterium*, *Geothrix*, and *Halophaga* are heterotrophic soil inhabitants. It is known from phylogenetic analysis of environmental DNA that uncultured members of the genera are widespread in other terrestrial and aquatic habitats and may thus play an important ecological role. Several members of *Cytophaga* and related genera are important degraders of cellulose and other polysaccharides. Cells move by gliding, a surface motility mechanism that is also observable in other bacterial lineages.



Bacteria, Figure 2 Some cytological features of bacteria. (a) The bacterial flagellum (fl) is a unique motility structure of the bacterial kingdom. The short spikelike appendages (pili) are also found in Archaea (electron micrograph of a metal shadowed *Escherichia coli* cell). (b) The corkscrew-like *Spirochete* morphology (here, *Spirochaeta thermophila*) is determined by the flagellum ("axial filament") wound around the elongate cell body. The axial filament propels the whole cell in a drill-like manner. (c) Aquatic organisms tend to form long filaments (filamentous cyanobacterium *Phormidium* sp.). Light micrograph, differential interference contrast. (d) Several filaments of the cyanobacterium *Microcoleus*, frequently found in terrestrial habitats, are surrounded by a polysaccharide sheath (green). The filaments (red) glide out of the tubelike sheath. Sheaths are found in various bacterial groups. Fluorescence light micrograph. (e) *Bacillus* mother cell with endospore. Electron micrograph of an ultrathin section. (f) Purple sulfur bacterium (*Chromatium*) with intracellular sulfur granula. Light micrograph, phase contrast.

The gliding motility of *Cytophaga* and relatives, however, is based on a unique, not yet understood, cell envelope-associated mechanism.

Planctomyces/Verrucomicrobia

The *Planctomyces/Verrucomicrobia* lineages comprise morphologically uncommon groups of uncertain phylogeny. Planctomycetes develop protein stalks as attachment structures and divide by budding (instead of binary fission, the common way of bacterial and archaeal cell division). Peptidoglycan is missing. Planctomycetes and the related phylum of Verrucomicrobia also show a highly compartmentalized cytoplasm (Lee et al., 2009). Endocytosis-like processes, a typical feature of eukaryotes, could also be observed (Lonhienne et al., 2010).

In *Gemmata* spp. the nucleoid is surrounded by an intracytoplasmic unit membrane. The anammoxosome in *Brocadia anammoxidans* is a structurally similar compartment, but appears to be involved in the compartmentation of anaerobic oxidation of ammonia, which is a unique feature of this group. The genera *Verrucomicrobium* and *Prostheco bacter* possess lobe-like cellular extensions. The organisms are mostly found in freshwater habitats. The lobed appendages increase the cell surface and facilitate the uptake of substrate molecules in an oligotrophic life-style.

Cyanobacteria

Cyanobacteria and prochlorophytes differ from all other phototrophic bacteria in fundamental ways (Overmann

and Garcia-Pichel, 2006). The oxygenic photosynthesis, conducted by cyanobacteria, requires photosystems acting over a wide redox span. To split the water molecules, the reduction potential of chlorophyll in photosystem II must be more positive than that of the O_2/H_2O couple. Excited electrons are transferred, via several redox carriers, to a second photosystem (PS I), capable of NAD(P)H regeneration. The reducing power is particularly needed for carbon dioxide fixation (for fixation of 1 mol CO_2 , 2 mol NADPH is required), carried out by the Calvin cycle. A unique feature for Cyanobacteria are phycobilins as accessory (light harvesting) pigments, an adaptation to low light conditions. Like plastids, cyanobacteria contain intracytoplasmic thylakoid membranes as additional compartments for the membrane-bound photosystems.

Cyanobacteria are the morphologically most diverse group of bacteria, with some organizational levels analogous to eukaryotic algae (Figure 2c; Waterbury, 2006). Many species are filamentous. They are present in marine and freshwater, as well as in terrestrial habitats (Figure 2c, d). Some live in symbiotic relationship to higher plants (owing to the cyanobacterial nitrogen fixing activity). Several filamentous species carry out nitrogen fixation in specialized cells (heterocysts), lacking the oxygen evolving photosystem II.

Since cyanobacteria are responsible for oxygenation of the early atmosphere, the group has left the possibly deepest impact ever on earth's ecosystems. They are still of global importance for carbon cycling. Marine species are responsible for an estimated 20–40% of carbon dioxide fixation in oceans (e.g., Campbell and Vault, 1993). Since recent Cyanobacteria and the endosymbiotic plastids of green plants have common ancestors, most of the photosynthetic processes are basically due to the activity of Cyanobacteria and their ancestors.

Firmicutes/Actinobacteria

Next related to Cyanobacteria are the Firmicutes/Actinobacteria phyla (Stackebrandt and Schumann, 2006; Madigan et al., 2009). A common feature of all species within these groups is the lack of an outer membrane. Most members of the groups are chemoheterotroph and exhibit a thick peptidoglycan layer, resulting in a positive reaction of the Gram stain. Thus, the organisms are also referred to as “Gram-positive.” The thick cell wall may be also considered as an adaptation to the high osmotic stresses in terrestrial ecosystems (i.e., soil). The genera *Bacillus* and *Clostridium*, but especially the filamentous Actinobacteria (the genus *Streptomyces*, in particular) are important groups in soil habitats. Interestingly, also a small group of photosynthetic genera belongs to the Firmicutes. *Heliobacterium* and related genera are anaerobic, photoheterotrophic soil bacteria, with a minimal photosynthetic apparatus and incapable of carbon dioxide fixation (Asao and Madigan, 2010). *Frankia* genera are important nitrogen-fixing symbionts in alder roots. The production of different antibiotic agents

is another important feature, especially in Actinobacteria and *Bacillus*. Another unique feature within this lineage is the formation of endospores (Figure 2e).

Key genera of endospore formers are *Bacillus* and *Clostridium*. Whereas bacilli are aerobic respiratory organisms, clostridia lack a respiratory chain and are obligate anaerobic, fermenting organisms. They are important for anaerobic degradation of organic compounds, mostly producing organic acids (Table 4). Several clostridia as well as related acetogenic bacteria (e.g., *Acetobacterium*, *Acetogenium*) grow anaerobically either by using carbon dioxide as the terminal electron acceptor in anaerobic respiration with hydrogen as substrate (i.e., chemolithoautotrophically), or by fermenting carbohydrates. In either case, the acetyl-CoA pathway is used to reduce carbon dioxide to acetate. In the course of carbon dioxide reduction to acetate, a sodium ion gradient (instead of a proton gradient) across the membrane is generated that drives ATP synthesis.

Lactic acid bacteria obtain energy solely by fermentative processes. They typically have limited biosynthetic abilities, and need external sources of amino acids, vitamins, purines, and pyrimidines. This life-style is an adaptation to commensalism with other organisms: lactic acid bacteria mainly live on (decaying) plant leaves and fruit, the skin surface and the intestinal mucosa of mammals. Due to their ability to produce high amounts of lactic acid from carbohydrates (including lactose), their habitats are difficult to colonize by competing microorganisms. This also excludes pathogens from mucosal tissue. Another beneficial effect is the conservation of food (especially dairy products) by lactic acid fermentation.

Interestingly, some members of the Gram-positive lineage lost their peptidoglycan layer. These cell wall-less bacteria (key genera *Mycoplasma*, *Spiroplasma*) live as parasites on animal and plant tissue. The lack of a cell wall facilitates the flow of substrates from the host to the bacterium.

Proteobacteria

The most diverse bacterial lineage, the Proteobacteria, is divided into five subdivisions (alpha, beta, gamma, delta, and epsilon). Within most subdivisions, all metabolic key processes, ranging from chemo- and photoautotrophy to chemoheterotrophy, respiration, and fermentation (Tables 1–4), are common (Kersters et al., 2006; Madigan et al., 2009). Here, just the ecologically most important life-styles are pointed out. At least in three subdivisions, photoautotrophs are present (Overmann and Garcia-Pichel, 2006). All of them carry out anoxygenic photosynthesis with one photosystem; different types of intracytoplasmic membranes (lamellar, spheroidal) can be found (Tucker et al., 2010). Electron donors are reduced sulfur compounds (such as H_2S) in purple sulfur bacteria (Figure 2f) and mostly organic compounds (H_2S also in low concentrations) in purple non-sulfur bacteria. Purple non-sulfur bacteria are photoheterotrophic

organisms that utilize external carbon sources, if available. Most of them also fix carbon dioxide by the Calvin cycle.

Chemolithoautotrophy occurs in all proteobacterial subdivisions. Interestingly, ammonia and nitrite oxidation is mainly confined to the Proteobacteria. In four subdivisions (and one additional, recently discovered lineage, related to Proteobacteria) obligate chemolithotrophic nitrifying bacteria, oxidizing either ammonia to nitrite or nitrite to nitrate are present. Like phototrophs, also nitrifying bacteria contain internal membranes. Oxidation of sulfur and iron is represented by several groups in four proteobacterial subdivisions. Sulfur-oxidizing genera have been found in four proteobacterial subdivisions. Some genera are acidiphilic (e.g., *Acidithiobacillus*, *Thiomonas*). Among sulfur oxidizing chemolithotrophs (thiotrophic bacteria), exceptionally large morphotypes were described (*Beggiatoa*, *Thioploca*, *Thiomargarita*). Several sulfur oxidizers, especially from the gamma-subgroup, live in symbiosis with marine mollusks (Dubilier et al., 2008). Oxidation of hydrogen, already mentioned for some bacterial stem lineages, is also widespread in Proteobacteria. All hydrogen oxidizers are facultative chemolithotrophs, that is, they are also able to grow on organic compounds (Kelly and Wood, 2006).

Up to now, the aerobic methanotrophy is mainly confined to Proteobacteria, though recently, a methanotrophic verrucomicrobial bacterium could be found (Chistoserdova et al., 2009). Methylotrophic bacteria oxidize a variety of C₁ compounds (methanol, methylamine, formate). All methanotrophs possess methane monooxygenase as key enzyme for hydroxylation of the chemically inert methane to methanol. Some methanotrophic bacteria are also symbionts in marine animals.

Several genera are well-known model organisms especially for medical microbiology, such as pseudomonads and enterobacteria. Both groups are chemoorganotrophic, respiratory bacteria, enteric bacteria are facultative anaerobes. In global cycles, however, these organisms are largely insignificant. Especially enteric bacteria (within the gamma-subdivision), as commensals and pathogens of diverse higher organisms are well adapted to their hosts.

Sulfate and sulfur reduction is not only restricted to Proteobacteria. *Archaeoglobus*, as an example, is an archaeon, *Thermodesulfobacterium* is a deeply branching genus close to the root of the bacterial phylogenetic tree. Most of the sulfate- and sulfur-reducers are, however, proteobacteria that belong to the delta-subdivision. They are obligate anaerobes using mainly hydrogen, fatty and other organic acids or alcohols as energy sources. Sulfate or elemental sulfur serve as the terminal electron acceptors in an anaerobic respiration with hydrogen sulfide as product. The organic compounds are either oxidized to acetate or to carbon dioxide. These organisms are geochemically important, and are widespread in anaerobic sulfate-rich environments (Barton and Fauque, 2009).

Nitrogen fixation is a feature of multiple bacterial and archaeal genera, only a few groups are important symbiotic organisms. The alpha-proteobacterial Rhizobia

conduct specific symbiosis with legume plants by formation of root nodules.

Several proteobacterial groups exhibit differentiated life cycles: filamentous beta-Proteobacteria like *Spaerotilus* form bundles of sheathed filaments in eutrophic freshwater habitats. Under unfavorable conditions, flagellated swarmer cells are liberated from the open tip of a sheath. Sheaths of *Leptothrix* are involved in the precipitation of Fe³⁺ and oxidation of Mn⁴⁺. In iron-rich waters, mass developments of iron oxide-covered sheaths form large flocculent layers, which are also involved in the formation of bog iron deposits (Crerar et al., 1979). Prosthecae bacteria are primarily found among the alpha-proteobacterial subgroup. Members of this group have many different types of extensions (prosthecae) that contain cytoplasm and are bounded by the cell wall. Similar extensions are formed by the genera *Hyphomicrobium* and *Rhodomicrobium*. Daughter cells arise as growing buds from the hyphal tips. In *Caulobacter*, a stalk attaches the cell to a surface, whereas at the distal cell pole, a flagellated swarmer cell is formed. After division, the swarmer attaches to a new place and will form a new stalk at the formerly flagellated pole (Angert, 2005). The myxobacteria have a complex life cycle, which culminates in the production of fruiting bodies containing resting structures called myxospores. The formation of these structures requires the cooperation and coordinated differentiation of large numbers of cells. Aggregates of cells form through a chemotactic response (Zusman et al., 2007).

Summary

The brief overview points out that bacteria are taxonomically as well as metabolically more diverse than Archaea. Though the phylogenetic relationships among the bacterial phyla is still a matter of debate, several lines of evolutionary developments can be stated.

Chlorophyll-dependent photosynthesis must have been developed early in bacterial evolution, though some lineages may also have acquired genes of the photosynthetic apparatus by horizontal gene transfer. Fixation of carbon dioxide is not conducted via the Calvin cycle in stem groups, but a common feature in Cyanobacteria and Proteobacteria.

Except from Firmicutes/Actinobacteria and Planctomycetes, bacterial cell envelopes consist of two double-layer membranes. It is a matter of debate, if the Firmicutes are a lineage closest to the root of the bacterial phylogenetic tree or if the membrane got lost as a secondary event. The volume enclosed by both membranes, the periplasmic space, is, especially in Proteobacteria, of significance for cellular transport, DNA transfer, and cellular motility. Intracytoplasmic membranes are widespread in Proteobacteria as well as Cyanobacteria, but have not yet been detected in the basal lineages or in Archaea.

Several major phyla contain symbiotic and pathogenic organisms, especially Firmicutes and Proteobacteria

(as far as we know, no Archaeon is pathogenic in a strict sense). Especially within the bacterial “crown group,” complex host–symbiont/pathogen interaction has been developed. Mitochondria have very likely an alpha-proteobacterial ancestor (Gray et al., 1999).

Though biofilms, as an elementary type of multicellular organization, are already found in Archaea, some true multicellular stages in bacteria exhibit elaborate cellular differentiations and complex life cycles. In aquatic habitats, the organisms sometimes switch from planktonic to sessile stages. Several groups of bacteria exhibit diverse adaptations to terrestrial habitats: Firmicutes develop extremely durable and heat-resistant endospores, streptomycetes grow and develop like zygomycete or ascomycete fungal mycelia with branched hyphae and conidia-like exospores. A similar convergence with life cycles to a eukaryote is carried out by myxobacteria. Their life cycle has some striking analogies to cellular slime molds and represents the most complex pattern of bacterial differentiation known so far.

In brief, the development of photosynthesis, the colonization of terrestrial (soil habitats), and the interaction with eukaryotic hosts, are besides the diverse metabolic capabilities, the major achievements of bacteria.

Bibliography

- Asao, M., and Madigan, M. T., 2010. Taxonomy, phylogeny and ecology of the heliobacteria. *Photosynthesis Research*, **104**, 103–111.
- Barton, L., and Fauque, G. D., 2009. Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. *Advances in Applied Microbiology*, **68**, 41–98.
- Bernadet, J.-F., and Nakagawa, Y., 2006. An introduction to the family *Flavobacteriaceae*. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 455–480.
- Campbell, L., and Vaulot, D., 1993. Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). *Deep-Sea Research Part I: Oceanographic Research Papers*, **40**, 2043–2060.
- Charon, N. W., Greenberg, E. P., Koopman, M. B., and Limberger, R. J., 1992. Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella. *Microbiological Research*, **143**, 597–603.
- Chistoserdova, L., Kalyuzhnaya, M. G., and Lidstrom, M. E., 2009. The Expanding World of Methylotrophic Metabolism. *Annual Review of Microbiology*, **63**, 477–499.
- Ciccarelli, F. D., Doerks, T., von Mering, C., Creevey, C. J., Snel, B., and Bork, P., 2006. Toward Automatic Reconstruction of a highly resolved tree of life. *Science*, **311**, 1283–1287.
- Cole, J. R., Wang, Q., Cardenas, E., Fish, J., Chai, B., Farris, R. J., Kulam-Syed-Mohideen, A. S., McGarrell, D. A., Marsh, T., Garrity, G. M., and Tiedje, J. M., 2008. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. *Nucleic Acids Research*, **37**, D141–D145.
- Crerar, D. A., Knox, G. W., and Means, J. L., 1979. Biogeochemistry of bog iron in the New Jersey Pine Barrens. *Chemical Geology*, **24**, 111–135.
- Da Costa, M. S., Rainey, F. A., and Nobre, M. F., 2006. The genus *Thermus* and relatives. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 797–812.
- Dubilier, N., Bergin, C., and Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosymbiosis. *Nature Reviews Microbiology*, **6**, 725–740.
- Gray, M. W., Burger, G., and Lang, B. F., 1999. Mitochondrial evolution. *Science*, **283**, 1476–1481.
- Hanada, S., and Pierson, B. K., 2006. The family Chloroflexaceae. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 815–842.
- Huber, R., and Eder, W., 2006. Aquificales. In Balows, et al. (eds.), *The Prokaryotes* 7. Heidelberg: Springer, pp. 925–938.
- Kelly, D. P., and Wood, A. P., 2006. The chemolithotrophic Bacteria. In Balows, et al. (eds.), *The Prokaryotes* 2. Heidelberg: Springer, pp. 441–456.
- Kerstens, K., de Vos, P., Gillis, M., Swings, J., Vandamme, P., and Stackebrandt, E., 2006. Introduction to Proteobacteria. In Balows, et al. (eds.), *The Prokaryotes* 5. Heidelberg: Springer, pp. 3–37.
- Lee, K. C., Webb, R. I., Janssen, P. H., Sangwan, P., Romeo, T., Staley, J. T., and Fuerst, J. A., 2009. Phylum Verrucomicrobia representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. *BMC Microbiology*, **9**, 5.
- Lonhienne, T. G., Sagulenko, E., Webb, R. I., Lee, K. C., Franke, J., Devos, D. P., Nouwens, A., Carroll, B. J., and Fuerst, J. A., 2010. Endocytosis-like protein uptake in the bacterium *Gemmata obscuriglobus*. *Proceedings of the National Academy of Sciences USA*, **107**, 12883–12888.
- Madigan, M. T., Dunlap, P. V., and Clark, D. P., 2009. *Brock Biology of Microorganisms*, 12th edn. San Francisco: Pearson/Benjamin Cummings.
- Nelson, K. E., Clayton, R. A., Gill, S. R. et al., 1999. Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. *Nature*, **399**, 323–329.
- Overmann, J., and Garcia-Pichel, F., 2006. The phototrophic way of life. In Balows, et al. (eds.), *The Prokaryotes* 1. Heidelberg: Springer, pp. 82–85.
- Oyaizu, H., Debrunner-Vossbrinck, B., Mandelco, L., Studier, J. A., and Woese, C. R., 1987. The green non-sulfur bacteria: a deep branching in the eubacterial line of descent. *Systematic and Applied Microbiology*, **9**, 47–53.
- Pace, N. R., 2009. Mapping the tree of life. *Microbiology and Molecular Biology Reviews*, **73**, 565–576.
- Schlegel, H. G., 1999. *Geschichte der Mikrobiologie (Acta Historica Leopoldina 28)*. Halle (Saale): Deutsche Akademie der Naturforscher Leopoldina.
- Schlegel, H. G., and Jannasch, H. W., 2006. Prokaryotes and their habitats. In Balows, et al. (eds.), *The Prokaryotes* 1. Heidelberg: Springer, pp. 137–184.
- Stackebrandt, E., and Schumann, P., 2006. Introduction to the taxonomy of Actinobacteria. In Balows, et al. (eds.), *The Prokaryotes* 3. Heidelberg: Springer, pp. 297–321.
- Szabó, Z., Stahl, A. O., Albers, S. V., Kissinger, J. C., Driessen, A. J., and Pohlschröder, M., 2007. Identification of diverse archaeal proteins with class III signal peptides cleaved by distinct archaeal prepilin peptidases. *J Bacteriol*, **189**, 772–778.
- Tucker, J. D., Siebert, C. A., Escalante, M., Adams, P. G., Olsen, J. D., Otto, C., Stokes, D. L., and Hunter, C. N., 2010. Membrane invagination in *Rhodobacter sphaeroides* is initiated at curved regions of the cytoplasmic membrane, then forms both budded and fully detached spherical vesicles. *Molecular Microbiology*, **76**, 833–847.
- Waterbury, J. B., 2006. The Cyanobacteria – isolation, purification, and identification. In Balows, et al. (eds.), *The Prokaryotes*. Heidelberg: Springer, pp. 1053–1073.
- Woese, C. R., and Fox, G. E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Science USA*, **74**, 5088–5090.
- Wu, M., and Eisen, J. A., 2008. A simple, fast, and accurate method of phylogenomic inference. *Genome Biology*, **9**, R151.

- Zinder, S. H., and Dworkin, M., 2006. Morphological and physiological diversity. In Balows, et al. (eds.), *The Prokaryotes 1*. Heidelberg: Springer, pp. 185–220.
- Zusman, D. R., Scott, A. E., Yang, Z., and Kirby, J. R., 2007. Chemosensory pathways, motility and development in *Myxococcus xanthus*. *Nature Reviews of Microbiology*, **5**, 862–872.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Bacterioplankton](#)
[Cyanobacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fluorescence In Situ Hybridisation \(FISH\)](#)
[Gallionella](#)
[Halobacteria – Halophiles](#)
[Histology](#)
[Hydrogen](#)
[Leptothrix](#)
[Metallogenium](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Communities, Structure, and Function](#)
[Nan\(n\)obacteria](#)
[Piezophilic Bacteria](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

BACTERIOPLANKTON

Thomas Pommier
 CNRS-Université Lyon, Villeurbanne, France

Synonyms

Aquatic bacteria

Definition

Bacterial component of the plankton that drifts in the water column of both seawater and freshwater ecosystems.

Introduction

The name bacterioplankton comes from the association of the Greek word *πλαγκτός* “*planktós*,” meaning “wanderer” or “drifter,” and bacterium, a word coined in the nineteenth century by Christian Gottfried Ehrenberg (Thurman, 1997). In contrast to land, microbes drive the ecology of the aquatic environments both as producers and consumers of fixed carbon. Their colossal biomass vastly outreaches the ones of all other members of the oceanic and freshwaters biota. Considering its size, the Ocean encompasses more bacteria than the count of known stars (estimated to 10^{21}) in the Universe. Though its role in aquatic ecosystems was for long eluded, the bacterioplankton carries out the largest fraction of the biological activity and occupies a range of ecological niches.

Enumeration and description

Since the beginning of microbiology, counting the number of bacteria that occur in various aquatic environments has been an exciting subject of investigation. Because of the size and the mobility of the bacterioplankton, early bacteriologists had difficulties to simply enumerate them with a conventional microscope. With the development of appropriate techniques (e.g., size selection on membrane filters and DNA/RNA specific fluorochromes stainings), as well as advances in microscopy, bacterioplanktonic cells could be observed and counted (Hobbie et al., 1977). This method indicated that the bacterioplankton were on an average $\sim 10^6$ cells per milliliter of water. Recent developments in flow cytometry have confirmed and sharpened these counts (Amann et al., 1990). In the water column, the bacterioplankton is also exposed to grazing activity by larger protists and viral infections, but irrespective to variations in primary productivity, these two causes of mortality seem to maintain a relatively constant abundance.

Though it is not always mentioned in the current literature, a clear distinction should be made between the fraction of the bacterioplankton that is attached to drifting particles of organic matters (also called “marine snow” in the marine environment) and the fraction that is free-living in the water column. A common trait for free-living bacterioplankton is their small size, or small cell volume, ranging from 0.1 to $0.22 \mu\text{m}^3$, which is about ten times lower than that of the most attached bacterioplankton in aquatic habitats or in soils (Fenchel et al., 1998) and the most cultured cells.

Bacterioplankton biological activity

Based on both direct observations and indirect measurements of bacterial growth rates in whole seawater (e.g., frequency of dividing cells (Hagström et al., 1979), radioactive thymidine or leucine incorporation (Fuhrman and Azam, 1982; Chin-Leo and Kirchman, 1988)) as well as when bacterial grazers (protozoans) were removed (Vacelet, 1972), generation times (i.e., the time required to double population) of most bacterioplanktons range from 0.049 to 0.080 h^{-1} .

Because a significant proportion of the bacterioplankton (99%) of the total counted cells fail to grow on nutrient-rich agar plates, it has been suggested that most counted cells were either starving cells, dormant (Stevenson and Schmidt, 1998) or even non-viable cells (Kjelleberg et al., 1987). Different in situ measurements of amino acids or glucose uptake by microautoradiography (Hoppe, 1976) indicate that from 5% to >60% of the cells are metabolically active in different aquatic environments (Tabor and Neihof, 1984; Fuhrman and Ferguson, 1986; Smith and del Giorgio, 2003). Whether or not most bacteria in aquatic environment are dormant remains controversial, most scientists will agree however that the bacterial community, as a whole and over

long period of time, plays a paramount role in global biochemical processes.

The blue-green algae, or *Cyanobacteria*, are the “plants” of the bacterioplankton and carry out photosynthesis, which supports the entire ocean food web. In the open ocean, these phototrophic bacteria are responsible for about 80% of the total oceanic primary production, which represents about half of the planet’s primary production (ranging from 35 to 65 Gt of carbon per year) (del Giorgio and Duarte, 2002). Moreover, a new type of phototrophy has recently been discovered among marine bacterioplankton that carry bacteriorhodopsin molecules, which scavenge light to create an energetic gradient of protons (Beja et al., 2001). Since ~13% of the bacterioplankton cells in the photic zone may carry out proteorhodopsin-based phototrophy (Sabeji et al., 2005), this metabolism surely has a global significance and may be the foremost future investigation field in aquatic microbiology.

However in numbers, marine bacterioplankton are considered mostly heterotrophs, or more precisely saprotrophs, and obtain energy by consuming dissolved organic matter (DOM) released by larger aquatic animals (Smith et al., 1992) or by phytoplankton exudation (Larsson and Hagström, 1979). This activity, known as the “microbial loop” (Azam et al., 1983), is crucial to the aquatic trophic foodwebs because essential inorganic nutrients are recycled through bacterial metabolism into the water column.

Heterotrophic and phototrophic bacterioplankton are continuously grazed by protozoans (i.e., mostly flagellates and ciliates), which are later consumed by larger zooplankton, which are subsequently consumed by fishes. Phages (i.e., viruses infecting bacteria) also affect DOM turnover by lysing cells, which releases organic matter available to surrounding bacterioplankton cells (Proctor and Fuhrman, 1990).

A large fraction of the carbon flow in the aquatic ecosystem is processed by heterotrophic bacterioplankton that respire particulate and dissolved organic carbon (DOC) (del Giorgio and Duarte, 2002). Especially with the rapid increase in global atmospheric CO₂ due to modern human activities, this bacterial process is recognized as a key component in global climate regulation (Falkowski et al., 2000).

Bacterioplankton diversity and global distribution

While growth on agar plates, microscopic observations were intensively used to delineate several types of bacteria, their capacity of differentiation showed quickly to be too restrictive and new methods were required to distinguish between two species with similar cell and colony morphologies. Moreover, a general concern grew among microbiologists when they realized that many bacteria counted under the microscope could not be cultivated on agar plate or in liquid media. For instance, marine microbiologists estimated that only 1% of the cells counted

under the microscope formed colonies on agar plates, and named this issue the “Great plate count anomaly” (Jannasch and Jones, 1959; Kogure et al., 1979).

In the early 1980s, a breakthrough in bacterial taxonomy was achieved by Woese et al., who utilized ribosomal ribonucleic acids (rRNA) to catalogue related bacterial species (Fox et al., 1980; Woese et al., 1985, 1987). Applying this approach to aquatic environmental samples often recovers members from about 18 bacterial clades, mostly represented within the α -*Proteobacteria* and γ -*Proteobacteria* phyla. Moreover, members from the *Cyanobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctobacteria*, *Fibrobacter*, *Chloroflexi* and β -*Proteobacteria* are often retrieved from environmental rRNA genes clone libraries. Interestingly, though some members of the global bacterioplankton community (e.g., the so-called SAR11 clade) are recurrently found in environmental samples, and therefore show a ubiquitous distribution, endemism of rRNA genes is the most common trait of the bacterioplankton, and their diversity follows a latitudinal gradient as observed in the other macro-organisms (Pommier et al., 2007).

The extensive use of the SSU rRNA as phylogenetic marker has actually reached the level where variations in the bacterioplankton composition can be linked to observed changes both in natural (Acinas et al., 1999; Cottrell and Kirchman, 2000; Hollibaugh et al., 2002) and reconstructed environments (i.e., meso- or microcosms) (Riemann et al., 2000). The current view of marine bacterioplankton phylogeny is however being questioned together with the relevance of the SSU rRNA gene to delineate bacterial species.

Conclusions

Though it was for long considered a mass of dormant cells, bacterioplankton has proven to be a key actor in aquatic ecosystems throughout the world. As for many ecosystems, the bacterioplankton drives the whole biological activity of the water foodweb and occupies a huge variety of niches throughout the water column. Further research aims to show the importance of local bacterioplanktonic processes involved in global biogeochemistry.

Summary

The bacterial component of the plankton that drifts in the water column of all aquatic ecosystems is named *Bacterioplankton*. Though it was for long considered solely dormant, this part of the biota is now recognized as a determinant actor on the global recycling of nutrients and at the basis of all aquatic foodwebs. With a million cells per millimeter of water, they represent the most successful organisms, as well as the most diverse biome on our planet. Modern approaches combining molecular biology to epi-fluorescence microscopy have sharpened our knowledge on the abundances, the diversity and the ecology of the bacterioplankton.

Bibliography

- Acinas, S. G., Anton, J., and Rodriguez-Valera, F., 1999. Diversity of free-living and attached bacteria in offshore western mediterranean waters as depicted by analysis of genes encoding 16S rRNA. *Applied and Environmental Microbiology*, **65**, 514–522.
- Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., and Stahl, D. A., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology*, **56**, 1919–1925.
- Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A., and Thingstad, F., 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*, **10**, 257–263.
- Beja, O., Spudich, E. N., Spudich, J. L., Leclerc, M., and DeLong, E. F., 2001. Proteorhodopsin phototrophy in the ocean. *Nature*, **411**, 786–789.
- Chin-Leo, G., and Kirchman, D. L., 1988. Estimating bacterial production in marine waters from the simultaneous incorporation of thymidine and leucine. *Applied and Environmental Microbiology*, **54**, 1934–1939.
- Cottrell, M. T., and Kirchman, D. L., 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence in situ hybridization. *Applied and Environmental Microbiology*, **66**, 5116–5122.
- del Giorgio, P. A., and Duarte, C. M., 2002. Respiration in the open ocean. *Nature*, **420**, 379–384.
- Eilers, H., Pernthaler, J., Peplies, J., Glockner, F. O., Gerdt, G., and Amann, R., 2001. Isolation of novel pelagic bacteria from the German bight and their seasonal contributions to surface picoplankton. *Applied and Environmental Microbiology*, **67**, 5134–5142.
- Falkowski, P., Scholes, R. J., Boyle, E., Canadell, J., Canfield, D., Elser, J., Gruber, N., Hibbard, K., Hogberg, P., Linder, S., Mackenzie, F. T., Moore, B. III, Pedersen, T., Rosenthal, Y., Seitzinger, S., Smetacek, V., and Steffen, W., 2000. The global carbon cycle: a test of our knowledge of earth as a system. *Science*, **290**, 291–296.
- Fenchel, T., King, G. M., and Blackburn, T. H., 1998. *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*. London: Academic.
- Fox, G. E., Stackebrandt, E., Hespell, R. B., Gibson, J., Maniloff, J., et al., 1980. The phylogeny of prokaryotes. *Science*, **209**, 457–463.
- Fuhrman, J. A., and Azam, F., 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Marine Biology*, **66**, 109–120.
- Fuhrman, J. A., Ferguson, R. I., 1986. Nanomolar concentrations and rapid turnover of dissolved free amino acids in seawater: agreement between chemical and microbiological measurements. *Marine Ecology Progress Series*, **33**, 237–242.
- Giovannoni, S. J., Rappé, M. S., Vergin, K. L., and Adair, N. L., 1996. 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 7979–7984.
- González, J. M., and Moran, M. A., 1997. Numerical dominance of a group of marine bacteria in the alpha-subclass of the class proteobacteria in coastal seawater. *Applied and Environmental Microbiology*, **63**, 4237–4242.
- Gordon, D. A., and Giovannoni, S. J., 1996. Detection of stratified microbial populations related to *Chlorobium* and *Fibrobacter* species in the Atlantic and Pacific Oceans. *Applied and Environmental Microbiology*, **62**, 1171–1177.
- Hagström, Å., Larsson, U., Hörstedt, P., and Normark, S., 1979. Frequency of dividing cells, a new approach to the determination of bacterial growth rates in aquatic environments. *Applied and Environmental Microbiology*, **37**, 805–812.
- Hobbie, J. E., Daley, R. J., and Jasper, S., 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology*, **33**, 1225–1228.
- Hollihaugh, J. T., Bano, N., and Ducklow, H. W., 2002. Widespread distribution in polar oceans of a 16S rRNA gene sequence with affinity to *Nitrospira*-like ammonia-oxidizing bacteria. *Applied and Environmental Microbiology*, **68**, 1478–1484.
- Hoppe, H. G., 1976. Determination and properties of actively metabolizing heterotrophic bacteria in the sea, investigated by means of micro-autoradiography. *Marine Biology*, **36**, 291–302.
- Jannasch, H. W., and Jones, G. E., 1959. Bacterial populations in sea water as determined by different methods of enumeration. *Limnology and Oceanography*, **4**, 128–139.
- Kjelleberg, S., Hermansson, M., Mården, P., and Jones, G. W., 1987. The transient phase between growth and nongrowth of heterotrophic bacteria, with emphasis on the marine environment. *Annual Review of Microbiology*, **41**, 25–49.
- Kogure, K., Simidu, U., and Taga, N., 1979. A tentative direct microscopic method for counting living marine bacteria. *Canadian Journal of Microbiology*, **25**, 415–420.
- Larsson, U., and Hagström, Å., 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. *Marine Biology*, **52**, 199–206.
- Morris, R. M., Rappé, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, **420**, 806–810.
- Pernthaler, A., Pernthaler, J., and Amann, R. I., 2002. Fluorescent in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, **68**, 3094–3101.
- Pinhassi, J., and Hagström, Å., 2000. Seasonal succession in marine bacterioplankton. *Aquatic Microbial Ecology*, **21**, 245–256.
- Pinhassi, J., Winding, A., Binnerup, S., Zweifel, U. L., Riemann, B., and Hagström, Å., 2003. Spatial variability in bacterioplankton community composition at the Skagerrak-Kattegat Front. *Marine Ecology Progress Series*, **255**, 1–13.
- Pommier, T., Canbäck, B., Riemann, L., Boström, H. K., Lundberg, P., Tunlid, A., and Hagström, Å., 2007. Global patterns of diversity and community structure in marine bacterioplankton. *Molecular Ecology*, **16**, 867–880.
- Proctor, L. M., and Fuhrman, J. A., 1990. Viral mortality of marine bacteria and cyanobacteria. *Nature*, **343**, 60–62.
- Rappé, M. S., Connon, S. A., Vergin, K. L., and Giovannoni, S. J., 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature*, **418**, 630–633.
- Riemann, L., Steward, G. F., and Azam, F., 2000. Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Applied and Environmental Microbiology*, **66**, 578–587.
- Sabehi, G., Loy, A., Jung, K. H., Partha, R., Spudich, J. L., Isaacson, T., Hirschberg, J., Wagner, M., and Beja, O., 2005. New insights into metabolic properties of marine bacteria encoding proteorhodopsins. *PLOS Biology*, **3**, 1409–1417.
- Salomon, P. S., Janson, S., and Granéli, E., 2003. Molecular identification of bacteria associated with filaments of *Nodularia spumigena* and their effect on the cyanobacterial growth. *Harmful Algae*, **2**, 261–272.
- Smith, E. M., and del Giorgio, P. A., 2003. Low fractions of active bacteria in natural aquatic communities? *Aquatic Microbial Ecology*, **31**, 203–208.
- Smith, D. C., Simon, M., Alldredge, A. L., and Azam, F., 1992. Intense hydrolytic enzyme activity on marine aggregates and implications for rapid particle dissolution. *Nature*, **359**, 139–142.

- Stevenson, B. S., and Schmidt, T. M., 1998. Growth rate-dependent accumulation of RNA from plasmid-borne rRNA operons in *Escherichia coli*. *Journal of Bacteriology*, **180**, 1970–1972.
- Suzuki, M. T., Preston, C. M., Chavez, F. P., and Delong, E. F., 2001. Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey bay. *Aquatic Microbial Ecology*, **24**, 117–127.
- Tabor, P. S., and Neihof, R. A., 1984. Direct determination of activities for microorganisms of Chesapeake bay populations. *Applied and Environmental Microbiology*, **48**, 1012–1019.
- Thurman, H. V., 1997. *Introductory Oceanography*. New Jersey, USA: Prentice Hall College. ISBN 0132620723.
- Vacelet, E., 1972. Generation time measured in minutes for marine bacteria. *Comptes rendus hebdomadaires des séances de l'Académie des sciences. Série D: Sciences naturelles*, **274**, 2083–2085.
- Woese, C. R., 1987. Bacterial evolution. *Microbiological Reviews*, **51**, 221–271.
- Woese, C. R., Stackebrandt, E., Macke, T. J., and Fox, G. E., 1985. A phylogenetic definition of the major eubacterial taxa. *Systematic and Applied Microbiology*, **6**, 143–151.

Cross-references

Algae (Eukaryotic)
 Bacteria
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cyanobacteria
 Fluorescence In Situ Hybridisation (FISH)
 Metals, Acquisition by Marine Bacteria
 Piezophilic Bacteria
 Protozoa (Heterotroph, Eukaryotic)

BANDED IRON FORMATIONS

Nicole R. Posth¹, Kurt O. Konhauser², Andreas Kappler¹
¹University of Tübingen, Tübingen, Germany
²University of Alberta, Edmonton, Alberta, Canada

Synonyms

BIFs

Definition

Sedimentary deposits of alternating iron-rich (~20–40% Fe) and iron-poor, siliceous (~40–50% SiO₂) mineral layers that primarily precipitated throughout much of the late Archean (2.7–2.5 Ga) and Paleoproterozoic (2.5–1.8 Ga), but then remerged in the Neoproterozoic (~0.8 Ga).

BIF characterization, mineralogy, and distribution

BIFs are globally distributed, with some of the larger formations being those of the Hamersley range in Australia and the Transvaal Supergroup in South Africa (Figures 1 and 2). Other major BIFs include Krivoy Rog Supergroup, Ukraine (2.2 Ga); Labrador Trough, Canada; Lake Superior Region, USA; Gunflint and Biwabik, North America

(2.2–2.0 Ga), Carajás Formation (2.6 Ga) and Urucum Region, Brazil (0.8 Ga).

The mineralogy of the least metamorphosed BIFs consists of chert, magnetite, hematite, carbonates (siderite and dolomite–ankerite), greenalite, stilpnomelane, and riebeckite (Klein, 2005): the presence of both ferric and ferrous minerals gives BIF an average oxidation state of Fe^{2.4+} (Klein and Beukes, 1992). It is generally agreed that none of the minerals in BIF are primary, but that instead, the minerals reflect both diagenetic and metamorphic overprinting. For instance, the primary iron minerals were most likely ferric hydroxide (Fe(OH)₃), greenalite ((Fe)₃Si₂O₅(OH)₄), and siderite (FeCO₃) (Klein, 2005).

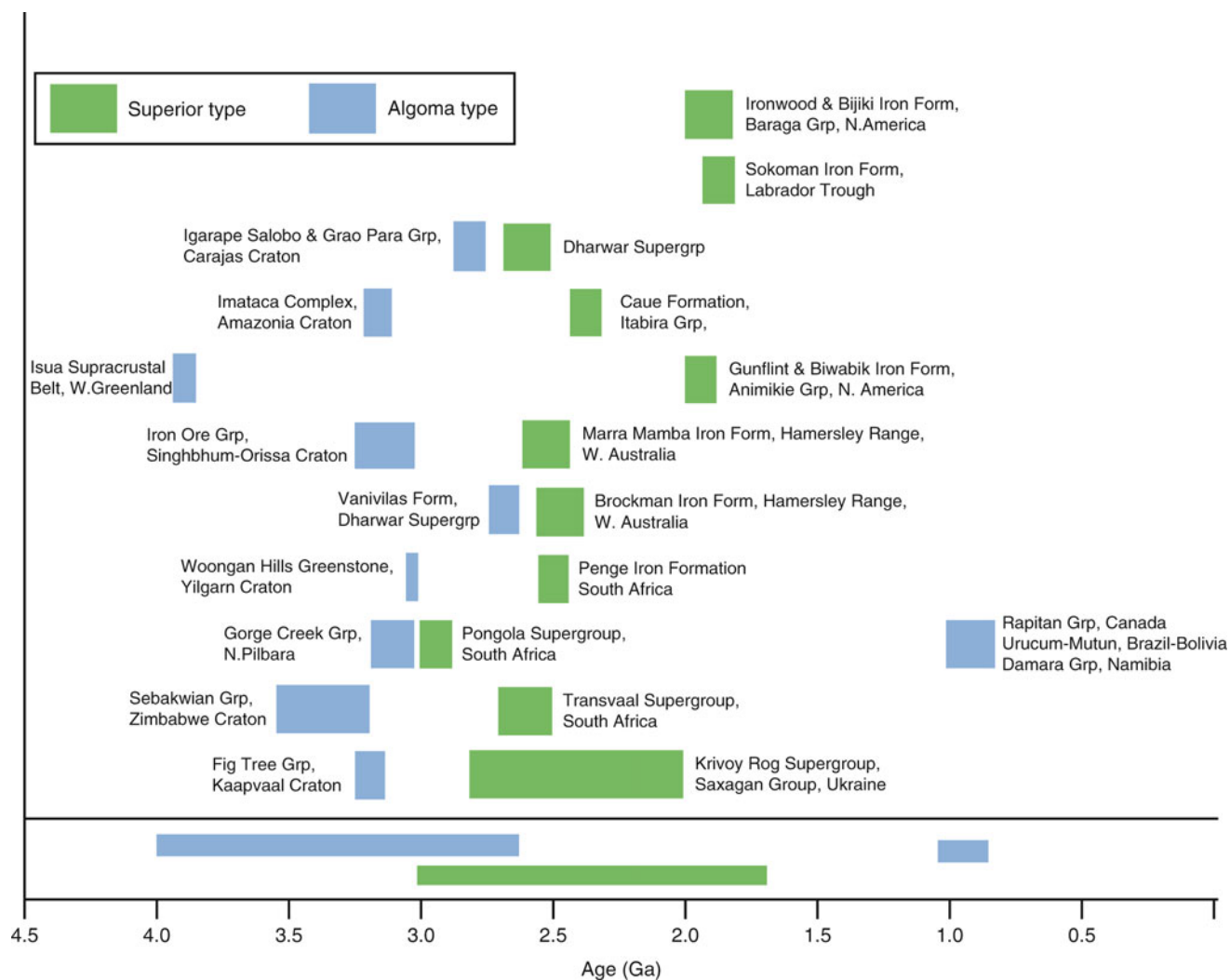
Based on their depositional environment, BIFs have been categorized as Algoma or Superior type. Algoma type BIFs are generally small in lateral extent and display a volcanic association (Gross, 1965, 1980; Huston and Logan, 2004), while Superior type BIFs are found in shelf environments, are vast, and are without a dominant volcanic influence. The “Superior” type BIFs, including those in the Hamersley Group, Western Australia and the Transvaal Supergroup, South Africa, are hundreds of meters thick, over 10⁵ km² in areal extent, and contain >10¹³ t of iron (Trendall, 2002). They are characteristically laminated, with alternating Fe-rich and Si-rich layers (Figures 2 and 3). Banding can be observed on a wide range of scales, from coarse macrobands (meters in thickness) to mesobands (centimeter-thick units) to millimeter and sub-millimeter layers (Trendall and Blockely, 1970). Among the latter is the wide variety of varve-like repetitive laminae, known as microbands.

BIFs have also been categorized according to their Fe oxide composition, as hematite or magnetite BIFs (Figure 3) (James, 1954). The former refers to BIFs facies consisting principally or entirely of interbedded chert and (primary) hematite, which are in some places oolitic (e.g., the Gunflint range). The latter describes Fe oxide layers of magnetite that may grade imperceptibly into silicate rock in some areas, be mixed with carbonate, or sprinkled with hematite or chert lenses (James, 1954).

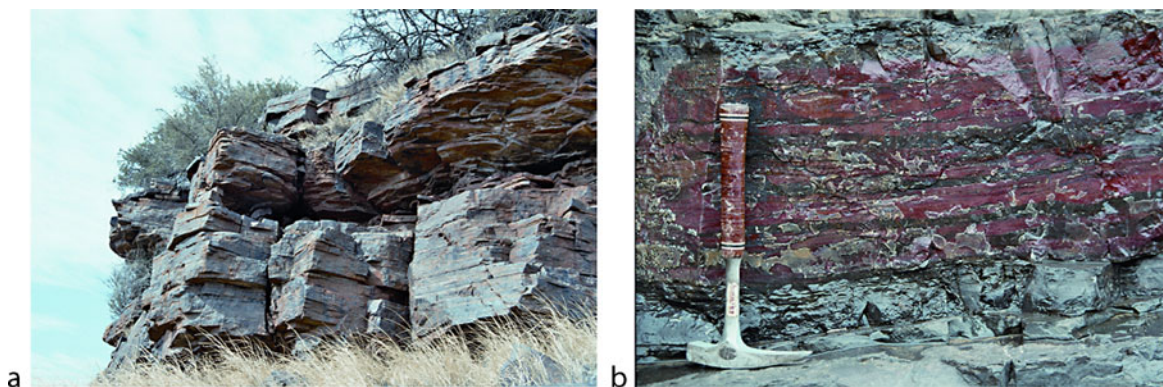
A third categorization of BIF is based on the granular versus nongranular nature of the silica layers. Granules, seen in the 2.0 Gyr Gunflint BIF are approximately 0.5 mm in diameter, irregular, and can be within a chert matrix. They can have a greenalite or magnetite core. These granules, however, do not show the concentric layering seen in hematitic oolites. Non-granular BIFs are typically thin-bedded or laminated and may chiefly be composed of minnesotaite with magnetite and carbonate in some layers (James, 1954).

Banded iron formations and the study of the early Earth

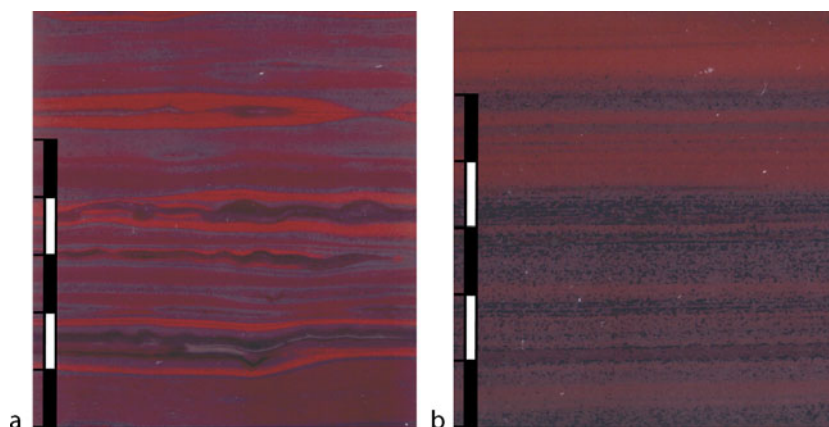
The height of BIF deposition was 2.7–2.4 Ga (Trendall, 2002; Klein, 2005), a timeframe which overlaps with the currently estimated age for the rise of oxygen on the Earth ~2.5 Ga (Anbar et al., 2007). As it is agreed that the



Banded Iron Formations, Figure 1 Appearance of BIFs in geological time and marked as Algoma and Superior types.



Banded Iron Formations, Figure 2 BIF outcrop, Gamoha Hill, South Africa (a), and White Mfolozi Gorge, Kwazulu Natal, South Africa, Jasper and Magnetite, 3.0 billion-years-old (b). Pictures by Nicole Posth.



Banded Iron Formations, Figure 3 Thin sections of banded iron formation, Mamatwan Manganese Mine, Hotazel, northern Cape Province, South Africa. Samples were taken from a depth of 83.4 m (a) and 87.5 m (b).

source of iron for these deposits was Fe(II), the Fe(III) minerals in BIF indicate that an oxidizing mechanism was present. Once oxygen began its rise due to the advent of cyanobacteria, it is plausible that an oxic mechanism formed BIF, perhaps even in pockets or oxygen oases. In the case of BIF dated prior to 2.7 Ga, however, an anoxic oxidizing mechanism is required to explain the formation of these vast deposits. The latest BIFs (0.8 Ga), which seem to represent a revival later in the geologic record, may be archives of major climate changes related to the Snowball Earth (Kirschvink, 1992; Kopp et al., 2005). Due to the intricacies of the depositional mechanisms, BIFs have been studied for decades regarding their potential as archives of the early Earth environment, but may also prove to be a detailed record of the early Earth biosphere.

BIF deposition

Depositional setting

Early studies of BIF mineral association (James, 1954), as well as comparative BIFs studies (Trendall, 1968; Beukes, 1973; Gole and Klein, 1981; Simonson, 1985; to name a few) postulated that these sedimentary deposits likely formed on the margins of cratons in semi-restricted, marine basins with minimal wave action (for a comprehensive review, Klein, 2005). They also laid a foundation for both theoretical and experimental geochemical modeling based on atmospheric (oxygen and carbon dioxide) and oceanic (iron and silica) concentrations, which are largely linked to mineralogical, petrologic, and isotopic analyses and have been invoked to describe the BIF deposition mechanism (Drever, 1974; Cairns-Smith, 1978; Han, 1978; Braterman et al., 1983; Baur et al., 1985; Francois, 1986; Garrels, 1987; Morris, 1993).

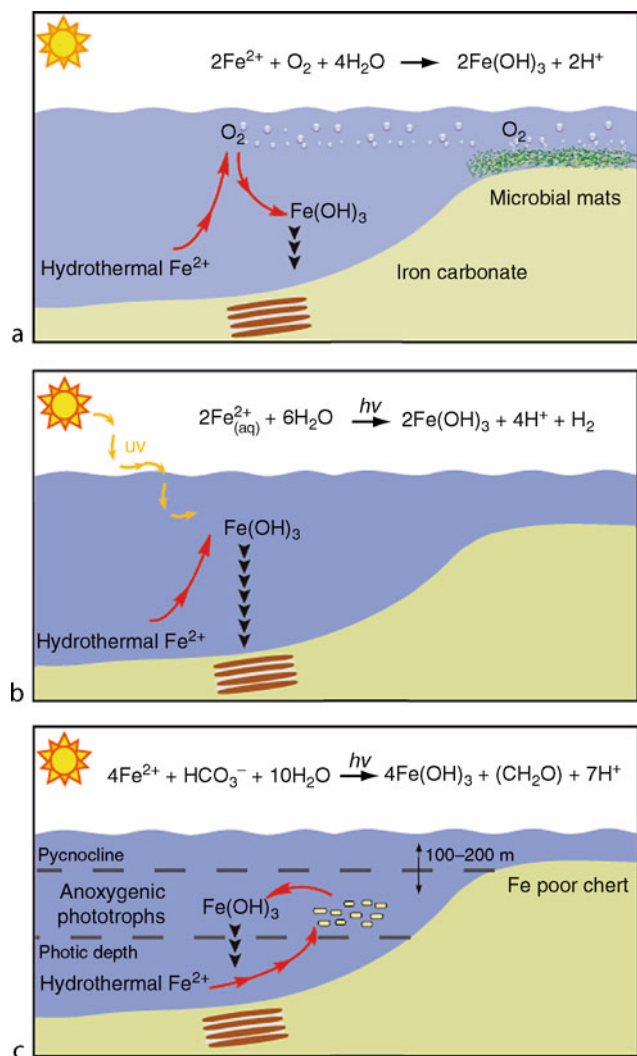
There is ample evidence that the dominant source of Fe (II) into the Archean ocean was hydrothermal (e.g., Jacobsen and Pimentel-Klose, 1988; Bau and Möller,

1993), but it has also recently been suggested that a significant fraction of the Fe in Archean seawater came from the continents (Alexander et al., 2008). The proximity of the Fe(II) source to the site of deposition, however, is still unclear. The estimated Fe(II) concentration for these basins ranges from 0.02 to 0.5 mM (Holland, 1973; Morris, 1993). This Fe(II) may have been delivered from the deep ocean to the outer continental shelf by upwelling currents from a mid-ocean ridge system (e.g., Holland, 1973). Accordingly, BIFs would sediment from below the wave base (free of wave- and storm-induced currents) onto partially submerged platforms of the continental shelves. Alternatively, Fe(II) could have been directly supplied from plumes in a seamount type system (Isley, 1995), which would curtail the difficulties introduced by the high upwelling rates required in the continental shelf model. After deposition, these sediments would undergo diagenesis, with some BIF sediment possibly even being redistributed to deeper waters via turbidity currents (Krapež et al., 2003).

There is significant evidence that Archean oceans also contained elevated concentrations of dissolved silica, possibly even to saturation with respect to amorphous silica (~2.20 mM; Siever, 1992; Maliva et al., 2005). Under such silica-rich conditions, the direct precipitation of amorphous silica could have taken place directly from the water column onto the sea basin floor (Krapež et al., 2003). Moreover, silica could have reacted with dissolved Fe(II) and precipitated as ferrous or ferric-silicates (Konhauser et al., 2007).

Theories of Precambrian BIF deposition

BIF mineralogy dictates that some form of Fe(II) oxidation was necessary for their formation, yet which mechanism(s) dominated is still highly uncertain (Figure 4). While dissolved Fe(II) readily oxidizes in the presence of oxygen at circumneutral pH, to the rise of oxygen on Earth occurred approximately 2.5 Ga (Kopp et al., 2005; Anbar et al., 2007). An alternative, O₂-independent



Banded Iron Formations, Figure 4 Models of banded iron formation deposition: The traditional model of BIF deposition involves the production of oxygen by cyanobacteria, which is released into the water column to chemically react with hydrothermal dissolved Fe(II) (a). The two proposed mechanisms of deposition in an anoxic ocean water column are the abiotic Fe(II) photooxidation by UV light, which has recently been discounted (Konhauser et al., 2007) (b), and direct microbial Fe(II) oxidation via anoxygenic Fe(II)-oxidizing phototrophy (c).

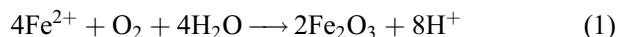
mechanism is therefore necessary to explain the deposition of the earliest BIF.

Both abiotic and biotic mechanisms of BIF deposition have been put forth. The potential role of microorganisms in BIF was first proposed 4 decades ago (Cloud, 1968) and in part addressed the difficulties in explaining these deposits based on geochemical processes alone (Brown et al., 1995). Yet, BIFs contain very little or no organic carbon (0.5% [w/w], Beukes and Klein, 1992) which makes it difficult to constrain the responsible microbial process

simply based on geochemical and petrographic studies. Any fossil information about the presence of life in the Archean has therefore been garnered from contemporaneous formations. For this reason, isotopic, biomarker, and ecophysiological studies with modern analogues have been implemented in order to supplement the geochemical BIF data and test the plausibility of the depositional models and the role of microorganisms.

Oxygenic photosynthesis model

The traditional model of BIF deposition was first postulated by Cloud (1968). Herein, Fe(II) is abiotically oxidized by photosynthetically produced O_2 , allowing for the indirect biogenic precipitation of ferric hydroxide as shown in Equation 1 (Figure 4a). Under an anoxic atmosphere, this O_2 could have been confined to local "oxygen oases" associated with cyanobacterial blooms in coastal settings (Cloud, 1965, 1973). Cloud (1965, 1973) further proposed that such primitive O_2 -producing photosynthetic bacteria, which lacked suitably advanced oxygen-mediating enzymes, required ferrous iron to detoxify oxygen. If so, these microorganisms would have flourished specifically when Fe(II) and nutrients were made episodically available. Once oxygen was present, aerobic Fe(II) oxidizers could also have contributed to biogenic Fe minerals precipitation (Holm, 1989).



The oldest evidence to support the presence of cyanobacteria during the time of BIF deposition came from the 3.45 billion-year-old Apex cherts, Warrawoona, Australia (Schopf, 1993). These findings, however, have since been challenged by Brasier et al. (2002) as being secondary artefacts formed by Fischer–Tropsch type reactions associated with seafloor hydrothermal systems. In fact, Buick (1988) maintained that the chert matrix of the "oldest microfossils" is a secondary hydrothermal deposit of younger age that cross-cuts the primary bedding. The use of kerogen as a marker of past life in these formations has also been challenged by experiments demonstrating the abiotic pathways of kerogen synthesis; simple organic hydrocarbons of abiological origins (e.g., formaldehyde) readily condense onto silica–carbonate inorganic filaments, and subsequently polymerize under gentle heating to yield kerogenous products (García-Ruiz et al., 2003).

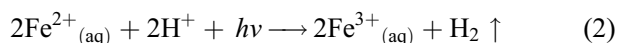
In light of these studies, stronger evidence for the presence of cyanobacteria comes from the stromatolitic assemblages of the 2.7 Gyr Tumbiana Formation, Western Australia. The presence of oxygenic photosynthesis in these formations is based on their likely habitat in sulfate-deficient evaporative lakes (Buick, 1992). This is further supported by the earliest undisputed fossil assemblages of the 2.6 Gyr Campbell Group, South Africa, that include cyanobacterial genera which helped form stromatolitic reefs in shallow subtidal to intertidal settings (Altermann and Schopf, 1995).

Biomarkers, organic compounds derived from more complex precursors which still resemble the original biological molecule after a long burial history, may be used to identify a specific group of organisms in ancient rock. For example, 2 α -methylhopanes are derivatives of prominent lipids in cyanobacteria (methylbacteriohopanepolyols). Abundant amounts were extracted from bitumens of the 2.6 Gyr Marra Mamba Iron Formation and the 2.5 Gyr Mt. McRae Shale of the Hamersley Group, Western Australia (Brocks et al., 1999; Summons et al., 1999). As these compounds were first solely found in cyanobacteria, their presence was first interpreted as diagnostic of oxygenic photosynthesis, however, they have recently been detected in anoxygenic phototrophs (Rashby et al., 2007), which weakens the use of this biomarker as conclusive proof of oxygenic photosynthesis in ancient rock.

Specific steranes are more indicative of this metabolism. These are 28- to 30-carbon isomers and are unique alteration products of sterols known from extant eukaryotic cell membranes. The only prokaryotes known to synthesize sterols have biosynthetic pathways leading to different structural isomers. Importantly, O₂ is required for the biosynthesis of sterols, hence, their extraction from Archean rock suggests that at least some dissolved oxygen (~ 0.002 ml O₂ L⁻¹) was present at the time of their production (Runnegar, 1991). To date, there is no experimental evidence to support the alternative theory that steranes may have been produced via an anaerobic pathway utilizing hydrogen peroxide or organic peroxides (Fischer and Pearson, 2007), which make them a specific biomarker of oxygenic photosynthesis. They are found, for example, in the 2.7 billion-years-old shales of the Jeerinah Formation, Hamersley Group (Brocks et al., 1999). Their presence in these shales is particularly intriguing as other redox-sensitive proxies suggest that oxygen arose much later in earth history (Rye and Holland, 1998; Rasmussen and Buick, 1999; Farquhar et al., 2000). Only the presence of localized oxygen oases can explain these conflicting results.

UV photooxidation model

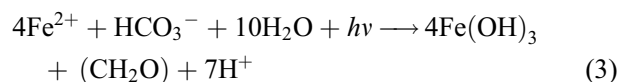
Prior to the rise of atmospheric oxygen and the development of a protective ozone layer, the Earth's surface was also subjected to high levels of ultraviolet radiation. This led to a theory of BIF deposition in which UV radiation drives an abiotic mechanism of Fe²⁺ oxidation and Fe(III) mineral precipitation. Specifically, under conditions of anoxia and high dissolved Fe(II) concentrations, dissolved ferrous iron species, such as Fe²⁺ or Fe(OH)⁺, can absorb radiation in the 200–400 nm range. Dissolved ferric iron is formed (Equation 2), which hydrolyzes to form ferric hydroxide at circumneutral pH (Cairns-Smith, 1978; Braterman et al., 1983) (Figure 4b).



These experiments, however, were recently carried out in complex solution simulating disequilibrium Precambrian ocean water chemistry in which Fe(II)-rich hydrothermal waters react with ambient Si-saturated seawater also containing high HCO₃⁻ concentrations (Konhauser et al., 2007). It was shown that the oxidation effects of either UVA or UVC were negligible in fluids with high dissolved Fe(II), Si, and HCO₃⁻, compared to the precipitation of ferrous-iron-silicates and ferrous carbonate. This suggests that under such conditions Fe²⁺ would have been largely deposited as Fe(II) minerals instead of being oxidized by UV radiation. The fact that Fe(III) minerals are present in BIF therefore suggests that in the absence of O₂ the alternative anoxic depositional mechanism, Fe(II) oxidation by anoxygenic phototrophs, drove Fe oxide deposition.

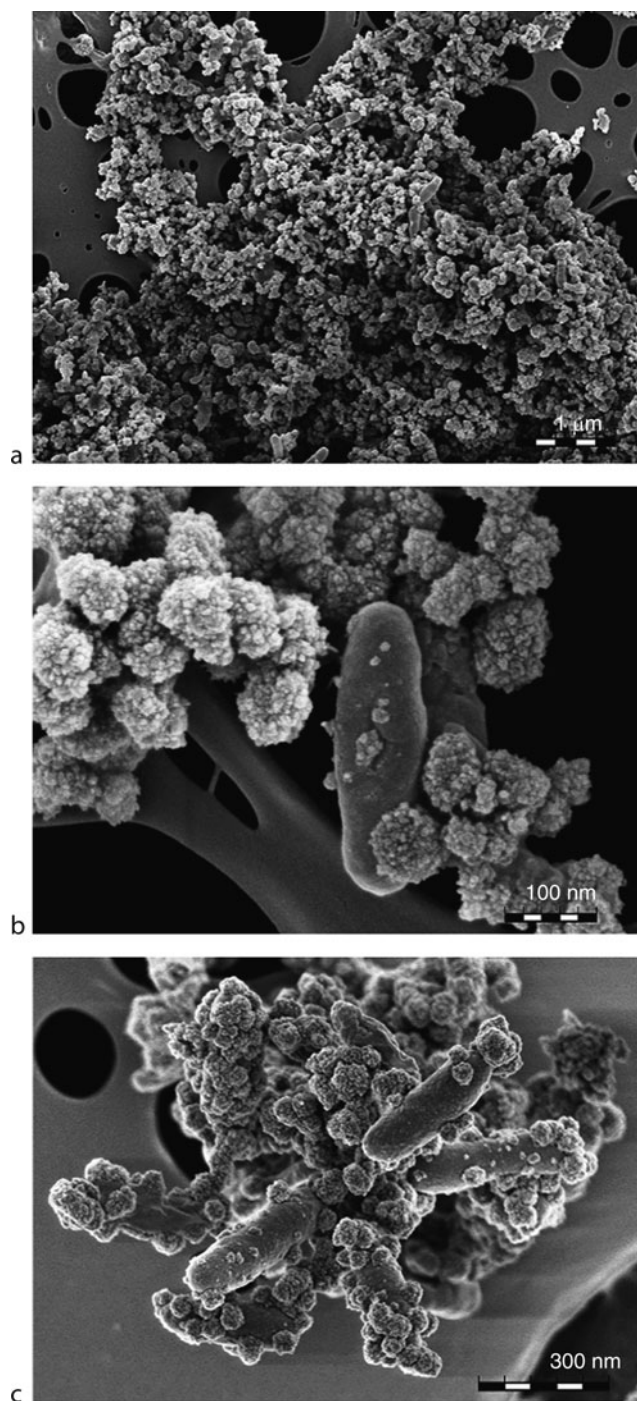
Anoxygenic Fe(II)-oxidizing phototroph model

Anoxygenic photosynthesis was first suggested as a BIF deposition mechanism by Garrels et al. (1973) and Hartman (1984). They proposed that light, not O₂, may have coupled the carbon and iron cycles via photosynthesis that used Fe(II) rather than H₂O as an electron donor and produced Fe(III) rather than O₂ (Figure 2c). It was only 2 decades later that the plausibility of this theory was validated by the discovery of a modern Fe(II)-oxidizing phototroph (Widdel et al., 1993). Seven extant strains of anoxygenic Fe(II)-oxidizing phototrophs are now known to use light energy to catalyze the oxidation of Fe(II) and the corresponding reduction of CO₂, which yields biomass and ferric hydroxide as shown in Equation 3.



In the context of BIFs, anoxygenic Fe(II)-oxidizing photosynthesis (photoferrotrophy) is the only direct biological process that can precipitate Fe(III) minerals in the absence of oxygen.

While no direct evidence exists for the presence of these strains on the Archean Earth, various lines of evidence support the plausibility of anoxygenic phototrophs in BIF deposition. Ecophysiological studies performed with modern anoxygenic Fe(II)-oxidizing phototrophs test the plausibility of this deposition mechanism. It was shown that these strains inhabit a wide range of growth environments; being globally distributed and present in fresh and marine waters (Ehrenreich and Widdel, 1994; Heising et al., 1999; Straub et al., 1999; Croal et al., 2004; Jiao et al., 2005). These organisms do not have a unique form, all being rod-shaped (Figure 5), yet morphological variety is apparent, one example being the presence of vacuoles in one strain, *Thiodictyon* sp. strain F4 (Croal et al., 2004). These strains also utilize a variety of substrates, such as acetate, FeS and H₂, which suggests that they are versatile enough to be an integral group in both



Banded Iron Formations, Figure 5 Anoxic Fe(II)-oxidizing phototrophs "*Rhodobacter ferrooxidans*" sp. strain SW2 (a, b) and *Chlorobium ferrooxidans* sp. strain KoFox (c) form aggregates with the Fe(III) hydroxides produced by their metabolism. Many cells are not encrusted by, but only associated with, the Fe(III) minerals that may have been the primary minerals in BIFs.

ancient and modern environments. Modeling of the photo-synthetic Fe(II) oxidation rates determined in these studies suggest that such microorganisms could have accounted for all of the Fe(III) initially deposited in primary BIF sediment (Kappler et al., 2005). Importantly, while the Fe(II) oxidation rate of these strains is dependent on light intensity, anoxygenic phototrophs can oxidize Fe(II) in light regimes befitting the photic zone of ocean water (of a few hundred meters depth) (Kappler et al., 2005). Also, Fe(II) oxidation is not hindered by high concentrations of dissolved silica (tested at 2 mM Archean ocean concentration) (Siever, 1992; Konhauser et al., 2007).

While modern studies show that cyanobacteria are indeed important in stromatolite building through the trapping and binding of carbonate grains in their filaments and extracellular polymers, several anaerobic pathways, such as sulfate reduction (e.g., Visscher et al., 1998; Paerl et al., 2001), are also integral in carbonate cementation and localized alkalinity generation. Indeed, sequence analysis of small subunit rRNA genes amplified with PCR from genomic DNA showed that anoxygenic phototrophs represent a considerable fraction of biomass in the modern stromatolite communities of Hamelin Pool, Shark Bay, Australia (Papineau et al., 2005). Recently, Bosak et al. (2007) experimentally demonstrated that the anoxygenic phototroph, *Rhodospseudomonas palustris*, stimulates the precipitation of calcite in saturated solutions and builds stromatolite-like structures.

While 2 α -methylhopanes were first considered diagnostic of oxygenic photosynthesis, they may have a structural function and are not related to cell metabolism. Indeed, several facultative and obligate anaerobes possess the genes for hopanoid biosynthesis, and *Geobacter sulfurreducens* produces a wide variety of complex hopanoids structurally related to 2 α -methylhopane under strictly anoxic conditions in pure culture (Härtner et al., 2005; Fischer and Pearson, 2007). Moreover, it was recently demonstrated that an anoxygenic Fe(II)-oxidizing phototroph, *Rhodospseudomonas palustris*, generates substantial quantities of 2-methylhopanoids in the absence of oxygen (Rashby et al., 2007), which renders their presence in ancient rocks alone as an unsatisfactory marker for a specific metabolism.

Pigment biomarkers may offer further evidence of a microbial role in BIF. Okenane, the fossil hydrocarbon biomarker of the carotenoid pigment precursor, okenone, was recently found in the 1.64 billion-year-old Barney Creek Formation in northern Australia, a marine, sub-wavebase base succession. This pigment is exclusively found in purple sulfur bacteria and in recent sediments under euxinic conditions (Brocks, 2005).

Isotopic evidence and the BIF depositional mechanism

The Fe isotope composition of iron minerals in BIFs is markedly different from the homogenous values seen in

igneous rocks and many modern marine sediments ($\delta^{56}\text{Fe}$ $0.00 \pm 0.05\text{‰}$). Specifically, the hematite, magnetite, Fe-carbonate, and pyrite Fe isotope compositions reflect the various processes contributing to BIF formation; equilibrium fractionation of minerals, a variation in the fluid isotope composition from which the minerals precipitated, as well as microbial processes (Johnson et al., 2003; Johnson and Beard, 2005).

In an effort to elucidate the role of microorganisms in the formation of BIF, the Fe isotope fractionation carried out by Fe metabolizing microorganisms was analyzed in controlled laboratory studies of both pure and enrichment cultures. These show that ferric hydroxides are enriched in the heavy isotope relative to Fe(II) for anoxygenic Fe(II)-oxidizing phototrophy ($1.5 \pm 0.2\text{‰}$) (Croal et al., 2004), microbially catalyzed aerobic Fe(II) oxidation at low pH (Balci et al., 2006), aerobic chemical oxidation (Bullen et al., 2001), and UV Fe(II) oxidation (Straton et al., 2006), suggesting that all major processes produce similar Fe isotope fractionations.

The isotopic fractionations reported from laboratory experiments correlate well in part with the values recovered from Archean to early Proterozoic banded rocks of the Transvaal Supergroup, South Africa. $^{56}\text{Fe}/^{54}\text{Fe}$ values in hematite are as high as $+0.75$ to $+1.0\text{‰}$ (Johnson et al., 2003) when compared to the Fe from hydrothermal vents ($\pm 0\text{‰}$). As UV photooxidation was recently ruled out as a significant BIF deposition mechanism (Konhauser et al., 2007), these positive to near-zero $\delta^{56}\text{Fe}$ values are even consistent with (although not proof of) phototrophic Fe(II) oxidation in the early Archean oceans. However, the similarity in values from anoxygenic phototrophs with those of Fe(III) oxides formed by chemical oxidation and microbially catalyzed aerobic Fe(II) oxidation make it difficult to distinguish the key biotic and abiotic processes (Bullen et al., 2001; Balci et al., 2006). Iron isotope fractionation during oxidation itself is obviously independent of the oxidation mechanism (chemical or biological) because of the very rapid isotopic exchange between aqueous Fe^{2+} and Fe^{3+} , allowing isotopic equilibrium to be attained between reduced and oxidized aqueous species. Moreover, in these ancient formations interpretation of the isotopic signature is complicated by the complexity of the depositional environments, the diagenetic processes which have altered the Fe mineralogy, including the infiltration of external fluids during diagenesis and/or low temperature metamorphism which may have erased the primary isotope record (Hoefs, 1997; Johnson et al., 2003). Indeed, the large variation of $\delta^{56}\text{Fe}$ (-2.5 to $+1.0\text{‰}$ relative to the bulk Earth) in late Archean and early Paleoproterozoic BIFs from the Transvaal Supergroup, South Africa incorporates the entire range of values measured on the Earth.

The carbon isotopic record of BIFs is constrained by the low occurrence of organic carbon (0.5% [w/w], Beukes and Klein, 1992) in these structures. Carbon isotope signatures observed in contemporaneous formations may offer information about organisms which were present at the

time of deposition, but whose record was erased, for example, by postdeposition diagenetic processes. In such ancient formations, negative carbon isotope signatures are often interpreted as an indicator of life. Organisms transform inorganic carbon (e.g., CO_2 or HCO_3^-) via autotrophic pathways into organic carbon, preferentially incorporating the lighter isotope, ^{12}C , into the organic phase and producing residual CO_2 enriched in the heavier isotope, ^{13}C . Cyanobacteria, for example, display a $\delta^{13}\text{C}$ range of -4 to -35‰ (Schidlowski, 2000). For example, early photosynthesis has been linked to mat-forming communities in microfossiliferous units within Archean formations from Western Australia and South Africa (e.g., Altermann and Kazmierczak, 2003; Tice and Lowe, 2004) by carbon isotopic values ranging from -20‰ to -35‰ , as well as the $-31.0\text{‰} \pm 4.7$ values from organic carbon in early Proterozoic cherts (Strauss et al., 1992).

Key to this approach is the consideration of overlapping degrees of fractionation produced by various carbon fixation pathways (reductive acetyl-CoA, reductive citric acid, Calvin cycle, and hydroxypropionate). This makes it impossible to determine whether these putative biological residues were remnants of chemolithoautotrophs, evidence for early photosynthesis, or abiological C-isotopic fractionations, such as those associated with Fischer-Tropsch type processes ($\Delta^{13}\text{C}$ between -50‰ and -100‰).

Theories of iron and silica mineral layering

One of the characteristic features of BIF are the alternating Fe-rich (hematite, magnetite, and siderite) and the silicate/carbonate (chert, jasper, dolomite, and ankerite) layers that form bands varying in thickness from the microscale (μm in thickness) to meter-thick units (Trendall, 1968; Klein and Beukes, 1992; Klein, 2005). Some deposits, such as in the Dales Gorge Member, Hamersley Group, Western Australia, show laterally contiguous layers up to a 100 km in distance. This suggests that some unifying trigger of iron and silicate mineral precipitation exists over large areas with some regularity, most likely one which largely subdues the precipitation of one mineral while stimulating that of the other. Furthermore, given the range of band thickness from the micro- to macro-scale, as well as the complex mineralogy, it is most likely that layering in BIF is determined by a multilayered mechanism; one producing primary layering and a second mechanism acting post deposition via diagenesis.

Following this observation, many models attribute the primary genesis of micro and mesobands to seasonal stratification, or to yearly or decadal climatic cycles. Episodic hydrothermal pulsation and upwelling of Fe(II)-rich waters into depositional basins already saturated with dissolved silica have been invoked to explain these bands; silicification is driven by seasonal evaporation of basin waters (Holland, 1973; Garrels, 1987; Jacobsen and Pimentel-Klose, 1988; Siever, 1992). This mechanism, however, does not explain the laterally contiguous fine-scale banding, nor does it resolve why the iron and silica

do not co-precipitate but rather form distinct mesobands (Trendall, 1968; Garrels, 1987; Morris, 1993). Furthermore, in light of increasing evidence for an active microbial component in the Fe cycle (Johnson et al., 2003), the potential importance of microorganisms in BIF deposition models has been suggested. Indeed, a mechanism of alternating Fe and silicate mineral layer deposition with a microbial driver triggered by temperature has been recently presented (Posth et al., 2008).

Diagenesis in BIFs: oxidation and the reduction of Fe(III) minerals

The average oxidation state of $\text{Fe}^{2.4+}$ can be described by the simultaneous deposition of Fe(II) and Fe(III). Yet, this mixed oxidation state can also be explained by partial biotic or abiotic diagenetic re-reduction of precipitated Fe(III) to Fe(II). Experimental studies testing such diagenetic mineral transformations in the presence and in the absence of biomass have not yet been reported. Nonetheless, the presence of magnetite as (1) disseminated grains within but obscuring sedimentary laminae, (2) laminated beds that clearly truncate sedimentary layering, (3) layer-discordant veins, and (4) cleavage fills, suggests such a secondary origin (Ewers and Morris, 1981; Krapež et al., 2003).

The diagenetic or microbial modification of Fe(III) after sedimentation to produce siderite (FeCO_3) and magnetite (Fe_3O_4) requires a reductant. Even if a surface water oxic zone was generated by cyanobacterial activity (Summons et al., 1999), deep waters remained anoxic (e.g., Canfield, 1998). The dominant anaerobic respiratory processes would have oxidized fermentation products in the bottom waters and/or shallow sediments (Rothman et al., 2003). The scarcity of O_2 limited both nitrate and sulfate. This is supported by both negligible sulfur isotopic fractionations between late Archean and early Paleoproterozoic sulfide and sulfate minerals (Strauss, 2003), as well as the absence of pyrite in BIF, except in association with interlayered shaley units (Ewers and Morris, 1981). The significance of manganese pathways is also refuted by the low relative concentrations of Mn(II) released in hydrothermal effluent, as well as the lack of phototrophic or nitrate-independent Mn(II) oxidizing bacteria in modern environments. In contrast, abundant ferric hydroxide deposited as BIF may point to the importance of a microbial process which coupled the oxidation of organic carbon, dead planktonic bacteria, to the reduction of ferric iron producing such reduced iron mineral phases.

Evidence of BIF diagenesis via ancient microbial Fe(III) reduction comes from Fe isotopic ratios in Fe-bearing minerals from Archean sedimentary rocks that closely mimic those observed during modern dissimilatory Fe(III) reduction. For instance, analyses of carbon- and magnetite-rich rocks, from the 2.9 Gyr Rietkuil Formation, Witwatersrand Supergroup, South Africa, revealed $\delta^{56}\text{Fe}$ values as low as -2.3‰ (Yamaguchi et al., 2005). These negative fractionations are very similar to what might be expected from multiple stages of Fe(III)

reduction (Johnson et al., 2004), where each single-step bacterial Fe(III) reduction leads to isotopically light ($\delta^{56}\text{Fe} < 1.2\text{‰}$) aqueous Fe(II) relative to the initial ferric hydroxide substrate (Icopini et al., 2004). The importance of Fe(III) reduction as a means to explain the iron fractionations has, however, been challenged by Rouxel et al. (2005). They suggested that highly variable, but negative values in pyrite from black shales (0.5‰ to -3.5‰) between 2.8 and 2.3 Ga more likely reflect the initial deposition of Fe oxides (e.g., BIFs) which preferentially removed isotopically heavy ^{56}Fe , driving the ocean waters to the negative $\delta^{56}\text{Fe}$ values recorded in pyrite. This interpretation is consistent with the notion that partial biological and abiological processes oxidized the dissolved Fe(II) brought into the shallow waters, but is problematic in light of recent studies showing significant isotopic variations for minerals within close proximity and thus, time of deposition (Johnson et al., 2008). This variation is perhaps most easily explained if the Fe isotopes reflect diagenetic pathways and not the bulk seawater (Johnson et al., 2008).

BIF diagenesis and the Archean marine Fe cycle

BIFs have recently been studied to develop a model of the Archean marine Fe cycle. In doing so, Konhauser et al. (2005) made two assumptions. First, they assumed that the bulk of the Fe(II) component in Fe-rich BIF-type macrobands formed diagenetically through biological Fe(III) reduction, i.e., the magnetite is not primary. Based on a predicted rate of Fe(III) deposition annually (1 mm year^{-1}), they then quantified the electrons that were needed to generate the amount of magnetite as reported in BIF (one third of Fe minerals; Morris, 1993). Second, they quantified the amount of photosynthetic Fe(II)-oxidizer biomass that may have been generated in the photic zone of the water column (based on Kappler et al., 2005), in order to estimate the amount of Fe recycled prior to burial. The results demonstrated that under ideal growth conditions, as much as 70% of the biologically formed Fe(III) could have been recycled back into the water column via fermentation and organic carbon oxidation coupled to microbial Fe(III) reduction. It was also suggested that some of the biomass may have been ultimately consumed via methanogenesis, i.e., coupling the oxidation of acetate or H_2 to methane formation. The hypothesis is to some extent corroborated with the analyses of kerogens (extracted from rocks 2.8 and 2.6 Gyr) with highly negative $\delta^{13}\text{C}$ signatures (between -40‰ and -60‰) that possibly formed as the result of methanogenic ^{12}C -rich gas production, the incorporation of the methane into the biomass of methanotrophic bacteria and inevitably the preservation of ^{12}C -enriched organic matter (Hayes, 1983).

Summary

Banded iron formations (BIFs) have been studied for decades in regard to their importance as an economically

viable source of iron ore, but their significance also extends to their potential as archives of the early Earth environment.

In spite of this effort, the mechanism of their deposition and in particular whether microorganisms drove the precipitation of BIF minerals, is still uncertain. Increasing evidence of a predominantly anoxic Earth until ~2.5–2.4 Ga forces the investigation of O₂-independent mechanisms for BIF deposition. For this reason, recent studies have explored the long-standing proposition that Archean BIFs may have been formed, and diagenetically modified, by anaerobic microbial metabolisms.

Bibliography

- Alexander, B. W., Bau, M., Andersson, P., and Dulski, P., 2008. Continentally-derived solutes in shallow Archean seawater: rare earth element and Nd isotope evidence in iron formation from the 2.9 Ga Pongola Supergroup, South Africa. *Geochimica et Cosmochimica Acta*, **72**, 378–394.
- Altermann, W., and Kazmierczak, J., 2003. Archean microfossils: a reappraisal of early life on Earth. *Research in Microbiology*, **154**, 611–617.
- Altermann, W., and Schopf, J. W., 1995. Microfossils from the Neoproterozoic Campbell Group, Griqualand West Sequence of the Transvaal Supergroup, and their paleoenvironmental and evolutionary implications. *Precambrian Research*, **75**, 65–90.
- Anbar, A. D., Duan, Y., Lyons, T. W., Arnold, G. L., Kendall, B., Creaser, R. A., Kaufman, A. J., Gordon, G. W., Scott, C., Garvin, J., and Buick, R., 2007. A whiff of oxygen before the Great Oxidation event? *Science*, **317**, 1903–1906.
- Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W., 2006. Iron isotope fractionation during microbially stimulated Fe(II) oxidation and Fe(III) precipitation. *Geochimica et Cosmochimica Acta*, **70**, 622–639.
- Barns, S. M., and Nierzwicki-Bauer, S. A., 1997. Microbial diversity in ocean, surface and subsurface environments. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals*. Washington, DC: Mineralogical Society of America, pp. 35–79.
- Bau, M., and Möller, P., 1993. Rare earth element systematics of the chemically precipitated component in Early Precambrian iron-formations and the evolution of the terrestrial atmosphere-hydrosphere-lithosphere system. *Geochimica et Cosmochimica Acta*, **57**, 2239–2249.
- Baur, M. E., Hayes, J. M., Studley, S. A., and Walter, M. R., 1985. Millimeter-scale variations of stable isotope abundances in carbonates from banded iron formations in the Hamersley Group of Western Australia. *Economic Geology*, **80**, 270–282.
- Beukes, N. J., 1973. Precambrian iron-formations of Southern Africa. *Economic Geology*, **68**, 960–1004.
- Beukes, N., 2004. Early options in photosynthesis. *Nature*, **431**, 522–523.
- Beukes, N. J., and Klein, C., 1992. Models for iron-formation deposition. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: University of Cambridge.
- Bosak, T., Greene, S. E., and Newman, D. K., 2007. A likely role for anoxygenic photosynthetic microbes in the formation of ancient stromatolites. *Geobiology*, **5**(2), 119–126.
- Brasier, M. D., Green, O. R., Jephcoat, A. P., Klepepe, A. K., Van Kranendonk, M. J., Lindsay, J. F., Steele, A., and Grassineau, N. V., 2002. Questioning the evidence for Earth's oldest fossils. *Nature*, **416**, 76–81.
- Braterman, P. S., Cairns-Smith, A. G., and Sloper, R. W., 1983. Photo-oxidation of hydrated Fe²⁺ – significance for banded iron formations. *Nature*, **303**, 163–164.
- Brocks, J. J., 2005. Biomarker evidence for green and purple sulphur bacteria in a stratified Palaeoproterozoic sea. *Nature*, **437**, 866–870.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1036.
- Brown, A. D., Gross, G. A., and Sawicki, J. A., 1995. A review of the microbial geochemistry of banded iron formations. *The Canadian Mineralogist*, **33**, 1321–1333.
- Buick, R., 1988. Carbonaceous filaments from North Pole, West Australia: are they fossil bacteria in Archean stromatolites? A reply. *Precambrian Research*, **39**, 311–317.
- Buick, R., 1992. The antiquity of oxygenic photosynthesis: evidence for stromatolites in sulphate-deficient Archean lakes. *Science*, **255**, 74–77.
- Bullen, T. D., White, A. F., Childs, C. W., Vivit, D. V., and Schulz, M. J., 2001. Demonstration of significant abiotic iron isotope fractionation in nature. *Geology*, **29**(8), 699–702.
- Cairns-Smith, A. G., 1978. Precambrian solution photochemistry, inverse segregation, and banded iron formations. *Nature*, **276**, 807–808.
- Campbell, A. C., Palmer, M. R., Klinkhammer, G. P., Bowers, T. S., Edmond, J. M., Lawrence, J. R., Casey, J. F., Thompson, G., Humphris, S., Rona, P., and Karson, J. A., 1988. Chemistry of hot springs on the mid-Atlantic ridge. *Nature*, **335**, 514–519.
- Canfield, D. E., 1998. A new model for Proterozoic ocean chemistry. *Nature*, **396**, 450–453.
- Cloud, P., 1965. Significance of the Gunflint (Precambrian) microflora. *Science*, **148**, 27–35.
- Cloud, P., 1968. Atmospheric and hydrospheric evolution on the primitive Earth. *Science*, **160**, 729–736.
- Cloud, P., 1972. A working model of the primitive earth. *American Journal of Science*, **272**, 537–548.
- Cloud, P., 1973. Paleocological significance of the banded iron-formation. *Economic Geology*, **68**, 1135–1143.
- Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K., 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, **68**, 1227–1242.
- Drever, J. I., 1974. Geochemical model for the origin of Precambrian banded iron formations. *Geological Society of America Bulletin*, **85**, 1099–1106.
- Ehrenreich, A., and Widdel, F., 1994. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Applied and Environmental Microbiology*, **60**(12), 4517–4526.
- Ewers, W. E., and Morris, R. C., 1981. Studies of the Dales Gorge member of the Brockman iron formation, Western Australia. *Economic Geology*, **76**, 1929–1953.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulfur cycle. *Science*, **289**, 756–758.
- Fischer, W. W., and Pearson, A., 2007. Hypotheses for the origin and early evolution of triterpenoid cyclases. *Geobiology*, **5**, 19–34.
- Francois, L. M., 1986. Extensive deposition of banded iron formations was possible without photosynthesis. *Nature*, **320**, 352–354.
- García-Ruiz, J. M., Hyde, S. T., Camerup, A. M., Christy, A. G., Van Kranendonk, M. J., and Welham, N. J., 2003. Self-assembled silica-carbonate structures and detection of ancient microfossils. *Science*, **302**(4), 1194–1197.

- Garrels, R. M., 1987. A model for the deposition of the microbanded Precambrian iron formations. *American Journal of Science*, **287**, 81–105.
- Garrels, R. M., Perry, E. A., and MacKenzie, F. T., 1973. Genesis of Precambrian iron-formations and the development of atmospheric oxygen. *Economic Geology*, **68**, 1173–1179.
- Gole, M. J., and Klein, C., 1981. Banded iron formations through much of Precambrian time. *Journal of Geology*, **89**, 169–183.
- Gross, G. A., 1965. Geology of iron deposits in Canada, Vol. 1. General geology and evaluation of iron deposits. *Geological Survey of Canada Economic Report*, **22**(1), 181 p.
- Gross, G. A., 1980. A classification of iron formations based on depositional environments. *Canadian Mineralogist*, **18**, 215–222.
- Han, T. M., 1978. Microstructures of magnetite as guides to its origin in some Precambrian iron-formations. *Fortschritte der Mineralogie*, **56**(1), 105–142.
- Hartman, H., 1984. The evolution of photosynthesis and microbial mats: a speculation on banded iron formations. In Cohen, Y., Castenholz, R. W., and Halvorson, H. O. (eds.), *Microbial Mats: Stromatolites*. New York: Alan Liss, pp. 451–453.
- Härtner, T., Straub, K. L., and Kannenberg, E., 2005. Occurrence of hopanoid lipids in anaerobic geobacter species. *FEMS Microbiology Letters*, **243**, 59–64.
- Hayes, J. M., 1983. Geochemical evidence bearing on the origin of aerobiosis, a speculative hypothesis. In Schopf, J. W. (ed.), *Earth's Earliest Biosphere, Its Origin and Evolution*. Princeton, NJ: Princeton University Press, pp. 291–301.
- Heising, S., Richter, L., Ludwig, W., and Schink, B., 1999. *Chlorobium ferrooxidans* sp. nov., a phototrophic green sulfur bacterium that oxidizes ferrous iron in coculture with a *Geospirillum* sp. strain. *Arch Microbiology*, **172**, 116–124.
- Hoefs, J., 1997. *Stable Isotope Geochemistry*, 4th edn. Heidelberg: Springer.
- Holland, H. D., 1973. The oceans: a possible source of iron in iron-formations. *Economic Geologist*, **68**, 1169–1172.
- Holm, N. G., 1989. The $^{13}\text{C}/^{12}\text{C}$ ratios of siderite and organic matter of a modern metalliferous hydrothermal sediment and their implications for banded iron formations. *Chemical Geology*, **77**, 41–45.
- Huston, D. L., and Logan, G. A., 2004. Barite, BIFs and bugs: evidence for the evolution of the Earth's early hydrosphere. *Earth and Planetary Science Letters*, **694**, 1–15.
- Icopini, G. A., Anbar, A. D., Ruebush, S. R., Tien, M., and Brantley, S. L., 2004. Iron isotope fractionation during microbial reduction of iron. *Geology*, **32**(3), 205–208.
- Isley, A. E., 1995. Hydrothermal plumes and the delivery of iron to banded iron formation. *Journal of Geology*, **103**, 169–185.
- Jacobsen, S. B., and Pimentel-Klose, M. R., 1988. A Nd isotopic study of the hamersley and michipicoten banded iron formations: the source of REE and Fe in Archean oceans. *Earth and Planetary Science Letters*, **87**, 29–44.
- James, H. L., 1954. Sedimentary facies of iron-formation. *Economic Geology*, **49**(3), 236–294.
- Jiao, Y., Kappler, A., Croal, L., and Newman, D. K., 2005. Isolation and characterization of a genetically tractable photoautotrophic Fe(II)-oxidizing bacterium, *Rhodopseudomonas palustris* strain TIE-1. *Applied and Environmental Microbiology*, **71**(8), 1–10.
- Johnson, C. M., and Beard, B. L., 2005. Biogeochemical cycling of iron isotopes. *Science*, **309**, 1025–1027.
- Johnson, C. M., Beard, B. L., Beukes, N. J., Klein, C., and O'Leary, J. M., 2003. Ancient geochemical cycling in the Earth as inferred from Fe isotope studies of banded iron formations from the Transvaal Craton. *Contributions to Mineralogy and Petrology*, **144**, 523–547.
- Johnson, C. M., Beard, B. L., Klein, C., Beukes, N. J., and Roden, E. E., 2008. Iron isotopes constrain biologic and abiologic processes in banded iron formation genesis. *Geochimica et Cosmochimica Acta*, **72**, 151–169.
- Johnson, C. M., Beard, B. L., Roden, E. E., Newman, D. K., and Nealon, K. H., 2004. Isotopic constraints on biogeochemical cycling of Fe. *Reviews in Mineralogy and Geochemistry*, **55**, 359–408.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, **33**, 865–868.
- Kirschvink, J. L., 1992. Late proterozoic low-latitude global glaciation: The snowball Earth. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: Cambridge University Press, pp. 51–52.
- Klein, C., 2005. Some Precambrian banded iron formations (BIFs) from around the world: their age, geologic setting, mineralogy, metamorphism, geochemistry, and origin. *The American Mineralogist*, **90**, 1473–1499.
- Klein, C., and Beukes, N. J., 1992. Time distribution, stratigraphy, and sedimentologic setting, and geochemistry of Precambrian iron-formations. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge: University of Cambridge, pp. 139–146.
- Konhauser, K., Hamade, T., Raiswell, R., Morris, R. C., Ferris, F. G., Southam, G., and Canfield, D. E., 2002. Could bacteria have formed the Precambrian banded iron formations? *Geology*, **30**, 1079–1082.
- Konhauser, K., Newman, D. K., and Kappler, A., 2005. The potential significance of microbial Fe(III) reduction during deposition of Precambrian banded iron formations. *Geobiology*, **3**, 167–177.
- Konhauser, K. O., Amskold, L., Lalonde, S. V., Posth, N. R., Kappler, A., and Anbar, A., 2007. Decoupling photochemical Fe(II) oxidation from shallow-water deposition. *Earth and Planetary Science Letters*, **258**, 87–100.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A., and Nash, C. Z., 2005. The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Science*, **102**(32), 11131–11136.
- Krapež, B., Barley, M. E., and Pickard, A. L., 2003. Hydrothermal and resedimented origins of the precursor sediments to banded iron formation: sedimentological evidence from the Early Paleoproterozoic Brockman supersequence of Western Australia. *Sedimentology*, **50**, 979–1011.
- Lovley, D. R., Kashefi, K., Vargas, M., Tor, J. M., and Blunt-Harris, E. L., 2000. Reduction of humic substances and Fe(III) by hyperthermophilic microorganisms. *Chemical Geology*, **169**, 289–298.
- Maliva, R. G., Knoll, A. H., and Simonson, B. M., 2005. Secular change in the Precambrian silica cycle: insights from chert petrology. *Geological Society of America Bulletin*, **117**, 835–845.
- Morris, R. C., 1993. Genetic modelling for banded iron-formation of the Hamersley Group, Pilbara Craton, Western Australia. *Precambrian Research*, **60**, 243–286.
- Morris, R. C., and Horwitz, R. C., 1983. The origin of the iron-formation-rich Hamersley Group of Western Australia – deposition on a platform. *Precambrian Research*, **21**, 273–297.
- Olson, J. M., and Blankenship, R. E., 2004. Thinking about the evolution of photosynthesis. *Photosynthesis Research*, **80**, 373–386.
- Paerl, H. W., Steppe, T. F., and Reid, R. P., 2001. Bacterially mediated precipitation in marine stromatolites. *Environmental Microbiology*, **3**, 123–130.
- Papineau, D., Walker, J. J., Mojzsis, S. J., and Pace, N. R., 2005. Composition and structure of microbial communities from

- stromatolites of Hamelin Pool in Shark Bay, Western Australia. *Applied and Environmental Microbiology*, **71**(8), 4822–4832.
- Pasteris, J. D., and Wopenka, B., 2003. Necessary, but not sufficient Raman identification of disordered carbon as a signature of ancient life. *Astrobiology*, **3**, 727–738.
- Perry, E. C., Tan, F. C., and Morey, G. B., 1973. Geology and stable isotope geochemistry of the Biwabik iron formation, Northern Minnesota. *Economic Geology*, **68**, 1110–1125.
- Posth, N. R., Hegler, F., Konhauser, K. O. and Kappler, A., 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. *Nature Geoscience*, **1**(10), 703–708.
- Posth, N. R., Konhauser, K. O., and Kappler, A., 2008. Microbiological processes in BIF deposition. In Glenn, C., and Jarvis, I. (eds.), *Authigenic Minerals: Sedimentology, Geochemistry, Origins, Distribution and Applications*. J. Sedimentology IAS Special Publication Series, in press.
- Rashby, S. E., Sessions, A. L., Summons, R. E., and Newman, D. K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of National Academy of Sciences of the United States of America*, **104**(38), 15099–15104.
- Rasmussen, B., and Buick, R., 1999. Redox state of the Archean atmosphere: evidence from detrital heavy metals in ca. 3250–2750 Ma sandstones from the Pilbara Craton, Australia. *Geology*, **27**, 115–118.
- Rothman, D. H., Hayes, J. M., and Summons, R. E., 2003. Dynamics of the Neoproterozoic carbon cycle. *Proceedings of National Academy of Sciences of the United States of America*, **100**, 8124–8129.
- Rouxel, O. J., Bekker, A., and Edwards, K. J., 2005. Iron isotope constraints on the Archaean and Paleoproterozoic. *Science*, **307**, 1088–1091.
- Runnegar, B., 1991. Precambrian oxygen levels estimated from the biochemistry and physiology of early eukaryotes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **71**, 97–111.
- Rye, R., and Holland, H. D., 1998. Paleosols and the evolution of atmospheric oxygen: a critical review. *American Journal of Science*, **298**, 621–672.
- Schidlowski, M., 2000. Carbon isotopes and microbial sediments. In Riding, R., and Awramik, S.M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 84–95.
- Schopf, J. W., 1993. Microfossils of the Early Archean Apex Chert: new evidence of the antiquity of life. *Science*, **260**, 640–646.
- Schopf, J. W., and Packer, B. M., 1987. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Science*, **237**, 70–73.
- Schopf, J. W., Kudryavtsev, A. B., Agresti, D. G., Wdowiak, T. J., and Czaja, A. D., 2002. Laser-Raman imagery of Earth's earliest fossils. *Nature*, **416**, 73–76.
- Siever, R., 1992. The silica cycle in the Precambrian. *Geochimica et Cosmochimica Acta*, **56**, 3265–3272.
- Simonson, B. M., 1985. Sedimentological constraints on the origins of Precambrian iron-formations. *Geological Society of America Bulletin*, **96**, 244–252.
- Straton, S., Amskold, L., Anbar, A., and Konhauser, K. O., 2006. Iron isotope fractionation during Fe(II) photo-oxidation. *Astrobiology Science Conference 4*, Washington, DC.
- Straub, K. L., Rainey, F. R., and Widdel, F., 1999. *Rhodovulum iodolum* sp. nov. and *Rhodovulum robiginosum* sp. nov., two new marine phototrophic ferrous-iron-oxidizing purple bacteria. *International Journal of Systematic Bacteriology*, **49**, 729–735.
- Strauss, H., 2003. Sulphur isotopes and the early Archean sulphur cycle. *Precambrian Research*, **126**, 349–361.
- Strauss, H., Des Marais, D. J., Hayes, J. M., and Summons, R. E., 1992. Concentrations of organic carbon and maturities and elemental compositions of kerogens. In Schopf, J.W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 95–99.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Tice, M. M., and Lowe, D. R., 2004. Photosynthetic microbial mats in the 3,416 Myr old ocean. *Nature*, **431**, 549–552.
- Trendall, A. F., 1968. Three great basins of Precambrian banded iron formation deposition: a systematic comparison. *Geological Society of America Bulletin*, **79**, 1527–1544.
- Trendall, A. F., 2002. The significance of iron-formation in the Precambrian stratigraphic record. *International Association of Sedimentologists. Special Publication*, **33**, 33–66.
- Trendall, A. F., and Blockely, J. G., 1970. The iron formations of the Precambrian Hamersley Group, Western Australia; with special reference to the associated crocidolite. *Western Australia Geological Survey Bulletin*, **119**, 336.
- Van Kranendonk, M. J., 2006. Volcanic degassing, hydrothermal circulation and the flourishing of early life on Earth: a review of the evidence from c. 3490–3240 Ma rocks of the Pilbara Supergroup, Pilbara Craton, Western Australia. *Earth Science Reviews*, **74**, 197–240.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovely, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.
- Visscher, P. T., Reid, R. P., Bebout, B. M., Hoefft, S. E., Macintyre, I. G., and Thompson, J. A., 1998. Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): the role of sulfur cycling. *American Mineralogist*, **83**, 1482–1493.
- Walker, J. C. G., 1984. Suboxic diagenesis in banded iron formations. *Nature*, **309**, 340–342.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature*, **362**, 834–836.
- Wright, D. T., and Altermann, W., 2000. Microfacies development in Late Archaean Stromatolites and oolites of the Campbellrand Subgroup, South Africa. In Insalco, E., Skelton, P. W., and Palmer, T. J. (eds.), *Carbonate Platform Systems: Components and Interactions*. London: Geological Society, Special Publication, **178**, 51–70.
- Yamaguchi, K. E., Johnson, C. M., Beard, B. L., and Ohmoto, H., 2005. Biogeochemical cycling of iron in the Archean Paleoproterozoic Earth: constraints from iron isotope variations in sedimentary rocks from the Kaapvaal and Pilbara Cratons. *Chemical Geology*, **218**, 135–169.

Cross-references

[Algae \(Eukaryotic\)](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biosignatures in Rocks](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Geobacter](#)
[Hydrothermal Environments, Marine](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

Microbialites, Stromatolites, and Thrombolites
 Nanocrystals, Microbially Induced
 Ores, Microbial Precipitation and Oxidation
 Photosynthesis
 Salinity History of the Earth's Ocean
 Sediment Diagenesis – Biologically Controlled
 Snowball Earth
 Stromatolites

BASALT (GLASS, ENDOLITHS)

Ingunn H. Thorseth
 University of Bergen, Bergen, Norway

Synonyms

Endolithic microorganisms in basaltic glass; Microbial life in glassy basalt

Definition

Basalt endoliths. Microorganisms that colonize and live inside fractures, vesicles, and other cavities in basaltic rocks.

Basalt. The most common volcanic rock formed by eruptions of magma of basaltic composition.

Seafloor basalt. Basalt that is exposed at the seafloor, most commonly in or close to the ocean spreading ridge axes where the crust is young, or at intraplate seamounts.

Subseafloor basalt. Basalt that has been buried by younger lava flows and/or deep-sea sediments, as the crust aged and moved away from the spreading ridges.

Basaltic glass and endolithic microorganisms

Introduction

Basalt is continuously formed during volcanic eruptions along the global mid-ocean ridge system, and constitutes the upper 0.5–1 km thick layer of the ocean crust which covers more than 50% of the Earth's surface. When the basaltic magma is extruded into the cold seawater, the 1–2 cm thick outermost surface layer is cooled so rapidly that its chemical species do not have enough time to be arranged into minerals, but freeze to an amorphous glass rind. These glassy margins become heavily fractured due to cooling-related contraction, and the fracture networks provide an immense surface area for rock-water interactions and colonization by microorganisms. As the basaltic glass dissolves and alters more quickly than the minerals (pyroxene, plagioclase) in the crystalline interior of the lava, the glassy proportion plays an important role in the alteration of the ocean crust and the chemical exchange with the ocean water. The alteration is not only controlled by physiochemical factors but also by microbial processes, implied by the presence of endolithic microorganisms (see *Endoliths*) within the glassy margins. The microbial

activity may, on the other hand, be controlled by the geological environment, which includes a variety of complex fluid temperature and chemistry conditions ranging from seafloor weathering to various hydrothermal systems.

Basaltic glass alteration

Low-temperature alteration of basaltic glass results in the formation of concentrically zoned rims of a yellow-brown, hydrous residual alteration product, frequently called palagonite, along the outer surfaces, and internal vesicles and fractures of the glassy margins of lava flows. The altered glass is amorphous (gel-palagonite) to poorly crystalline with clayish character (fibro-palagonite) (e.g., Stroncik and Schmincke, 2001), and is usually highly enriched in oxidized Fe and Ti, and depleted in Si and alkaline and alkaline earth elements compared to the fresh, parental glass. The term palagonite was used for the first time by von Waltershausen in 1845 for the brown groundmass of a tuff from Palagonia, Sicily, and was then thought to be a new mineral phase. Although palagonite was later shown to be a heterogeneous material, the term has commonly been used in the literature. However, several authors now recommend referring to this material as altered glass. During the last 2 decades, various field and laboratory studies have shown that the alteration rate and the chemical and crystalline characteristics of the altered glass strongly depend on the physiochemical and hydrological conditions (e.g., Crovisier et al., 2003). The understanding of how the endolithic microorganisms in the glassy margins of basaltic lava flows influence and interact with the alteration process is, however, still poor.

Evidence of microbial life in glassy basalt

The first in situ observations of endolithic microorganisms in basaltic glass was reported from a scanning electron microscopy (SEM) study of recent (<1 million years old (Ma)) volcanic glass deposits from Iceland (Thorseth et al., 1992). Microbial biofilms together with irregular alteration fronts at the fresh-altered glass boundary lead to the suggestion that the degradation of the volcanic glass could be biocatalyzed by the development of more aggressive microenvironments in the near vicinity of the microbial cells. This was subsequently supported by experimental alteration studies, where development of corrosion marks in the glass surface only was observed in combination with biofilm formation and not in sterile controls (Thorseth et al., 1995a; Staudigel et al., 1995, 1998). Similar alteration textures resembling microbial cell morphologies observed in glassy margins of 6 Ma ocean basalt, sampled 432 m below the seafloor at the Costa Rica Rift during the Ocean Drilling Program (ODP) Leg 148, were then stained using DNA-specific dyes to confirm the presence of DNA within these structures (Thorseth et al., 1995b; Giovannoni et al., 1996; Furnes et al., 1996). Further fluorescence in situ

hybridization (FISH) were used to document the existence of indigenous Bacteria (see *Bacteria*) and Archaea (see *Archaea*) in this glassy basalt (Torsvik et al., 1998). Additional evidence for microbial activity in oceanic basalt came from the demonstration of carbon and nitrogen enrichments in the alteration textures (Giovannoni et al., 1996; Torsvik et al., 1998), and stable carbon isotope fractionation from disseminated carbonate that is consistent with microbial processes (Torsvik et al., 1998). During the last decade, further morphological, geochemical, biomolecular, and cultivation studies of seafloor and subseafloor basalts of various ages, water depths, depth of burial and temperature regimes, as well as of hydrothermal fluids, have revealed that diverse endolithic microbial communities colonize the upper oceanic crust at the spreading ridges. In addition, detection of comparable alteration textures and geochemical and isotopic biosignatures (see *Biosignatures in Rocks*) in lava margins of ancient oceanic basalt implies that microbial life and associated biodegradation in the ocean crust occurred very early in the Earth's history (Furnes et al., 2004; Staudigel et al., 2006).

Microbial life in seafloor basalt

Several electron microscopy studies of seafloor lavas from the Arctic Mohs and Knipovich Ridges and the Australian Antarctic Discordance (AAD) area of the Southeast Indian Ridge (SEIR) have demonstrated that a diverse and numerous endolithic microbial community of various coccoid, rod-shaped, filamentous, and stalked cells colonize the fracture network in the glassy margins of the basalts soon after its formation and exposure to the cold, oxygenated seawater (Figure 1) (Thorseth et al., 2001, 2003; Kruber et al., 2008a, b). The presence of microbial cells in lavas from different regions of the seafloor suggests that microbial colonization and growth is a general feature of mid-ocean ridge basalts. In very young basalts (0 Ma), the microbial cells are observed in the central fractures attached to the outer surface of alteration rims, within porous zones in the rims, and at the alteration front attached to the fresh glass (Figure 1b and c). The cells act as nucleation sites for precipitation and become encrusted and embedded in the secondary alteration products with time. Alteration rims where porous zones containing numerous hollow, apparently fossilized cells (see *Microbial Biomineralization*), alternate with compact zones lacking distinctive cell structures, indicate that the microbial growth in the fractures is discontinuous (Figure 1b). In older (2.5 Ma) seafloor basalts where the alteration rims are much thicker, no evident cell structures have been observed at the alteration fronts (Figure 1d), but occur in porous zones of the rims closer to the central fracture, and within in the fractures attached to secondary zeolite crystals (Figure 1e). The cells within the rims are much lower in carbon than those in the fractures, indicating that they are fossilized or partially decayed. This implies that

the fluid availability decreases through the alteration rims, away from the fractures (Thorseth et al., 2003).

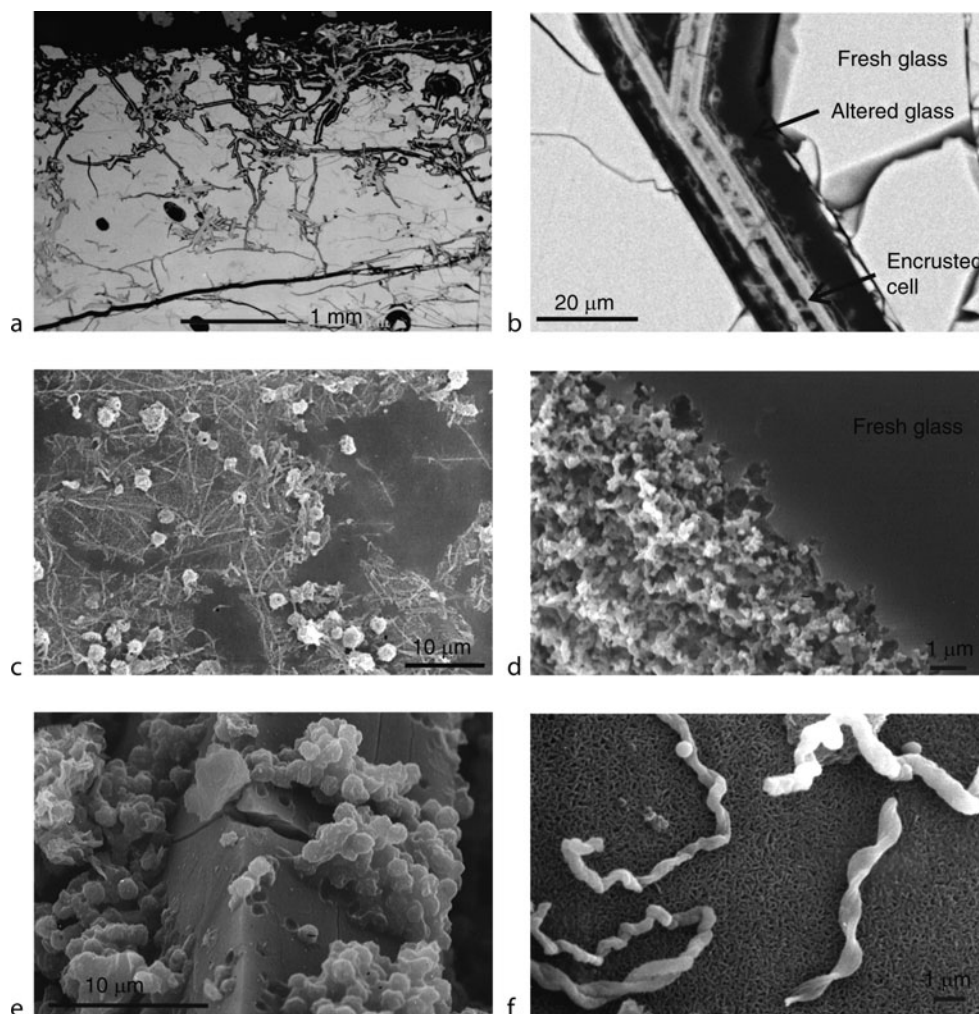
Biomass

The presence of abundant alteration rims with closely packed microbial cell structures indicate high cell numbers in the glassy margins of young seafloor basalt. However, a significant fraction of these structures are fossilized cells that have accumulated through time. Thus, while the number of living cells measured by real-time quantitative PCR and fluorescence microscopy is estimated to be around 10^6 cells/g of glassy seafloor basalt (Einen et al., 2008), calculations based on the organic carbon content in the altered glass indicate a considerable larger endolithic biomass (around 10^9 cells/g of glassy basalt, assuming 10^{-13} g of carbon in one cell and that 1% of the glass is altered (Kruber et al., 2008a)). Even if this calculation is highly tentative, the discrepancy suggests that the biogenic material in the fossilized cells is preserved for some time before it is completely decayed. This is further supported by detection of carbon in individual cell encrustations by microanalytical techniques (Thorseth et al., 2003). Due to the highly porous and fractured character of the upper extrusive basalt layer at spreading axes, it is supposed that a similar biomass may also be present in the subseafloor basalt at depths down to several tens to hundreds of meters.

Phylogenetic and metabolic diversity

The endolithic microbial communities in the Arctic seafloor basalts are shown to be almost exclusively from the domain *Bacteria*, and only small proportions are from the *Archaea* domain (Einen et al., 2008). The most frequently retrieved bacterial DNA sequences from these basalts affiliate with the *Gamma-proteobacteria*, *Alpha-proteobacteria*, *Chloroflexi*, *Firmicutes*, and *Actinobacteria*, while the archaeal sequences were restricted to the marine Group 1 *Crenarchaeota* (Lysnes et al., 2004a). The phylogenetic diversity of basalt endoliths is found to be distinct and different from those observed in the surrounding deep seawater.

In contrast to deep-sea sediments where biogenic debris from the photic zone provides an important energy and carbon source for the inhabiting microbial communities, the basaltic basement is rich in reduced inorganic compounds that together with dissolved inorganic carbon may support growth of lithoautotrophic (see *Chemolithotrophy*) microorganisms (Bach and Edwards, 2003). Mid-ocean ridge basalts typically have iron contents of 7–9 wt%, of which approximately 80% is in the divalent state. As the basaltic glass alters to palagonite, Fe(II) is predominantly oxidized to Fe(III). The alteration of the glass may thus provide both potential energy and electron acceptors for the endolithic microbial communities. Observations of branching, twisted filamentous structures resembling stalks of the neutrophilic, iron-oxidizing bacteria (see *Fe(II)-Oxidizing Prokaryotes*) *Gallionella*



Basalt (Glass, Endoliths), Figure 1 Scanning electron microscopy images of seafloor basalts. (a) Glassy margin of zero-age basalt from the Mohs Ridge with typical network of cracks and fractures where the glass reacts with the seawater and alters to palagonite; (b) close up of fracture with regular alteration rims at both sides, showing alternating solid and porous zones with abundant cell encrustations (hollow spheres); (c) microbial cells attached to the fresh glass at the alteration front in the Arctic seafloor basalt; (d) interface between fresh and altered glass in 2.5 Ma seafloor basalt from the SEIR, where no evident cell structures occur; (e) zeolite crystal in fracture of the 2.5 Ma basaltic glass with clusters of coccoid cells attached to it; (f) filamentous structures resembling stalks of iron-oxidizing bacteria (see *Gallionella*) within fracture of zero-age basaltic glass from the Knipovich Ridge.

ferruginea (see *Gallionella*) and the newly characterized *Mariprofundus ferrooxidans* (Emerson et al., 2007) at the glass-palagonite interface (Figure 1f) indeed indicate that microbial oxidation of Fe(II) from the dissolving glass is associated with the weathering of basalt (Thorseth et al., 2001). Culturing of novel Fe-oxidizing lithoautotrophs from inactive sulfide chimneys (Edwards et al., 2003) furthermore suggests that other, previous unknown Fe-oxidizing species could occur in basalt as well. In addition, preferential accumulation of manganese in some cell encrustations can be explained by the activity of Mn-oxidizing bacteria (Thorseth et al., 2001, 2003; Kruber et al., 2008a, b). Isolation of numbers of diverse heterotrophic Mn(II)-oxidizing bacteria from the surfaces

of recent basalt flows from the Loihi Seamount strongly support this (Templeton et al., 2005). Results from DNA analyses and enrichments have furthermore shown that Fe-reducing bacteria (see *Fe(III)-Reducing Prokaryotes*) affiliating with *Shewanella frigidimarina* (see *Shewanella*) as well as other unknown Fe-reducers also are associated with the weathering of glassy seafloor basalt (Lysnes et al., 2004a). Microbial catalyzed cycling of Fe (and Mn) may thus be important in low-temperature alteration of oceanic basalt. Although seafloor basalts are in direct contact with the ambient, oxygenated seawater, the presence of microaerophilic microorganisms such as *G. ferruginea* and *M. ferrooxidans* demonstrates that redox-gradients are formed in the seawater-filled fractures

during an early stage of alteration and microbial colonization of glassy margins. The detection of Fe-reducers furthermore indicates that anaerobic microenvironments locally develop in the fracture network of the glassy margins. Neither sulfate reducing bacteria (SRB) nor secondary sulfide phases, which are commonly reported from marine sediment, have however, been detected.

Production of methane from hydrogen and carbon dioxide during enrichments indicates in addition that lithotrophic methanogens are present in glassy seafloor basalt, although DNA sequences from basalt and enrichments have not matched DNA sequences from any known methanogens (Lysnes et al., 2004a). The hydrogen source for methanogenesis in the basaltic glass is however, unclear. Degassing from deep magma chambers or production by high-temperature water-rock interactions associated with volcanic eruptions and serpentinization of ultramafic rocks (Holloway and O'Day, 2000; Kelley et al., 2002) are all unlikely processes at the actual sampling sites. Simulations of geochemical reactions between water and ultramafic rocks indicate, however, hydrogen production also at low temperatures (Palandri and Reed, 2004). Hydrogen production during low-temperature alteration of basalt has, however, been a matter of debate (e.g., Andersen et al., 1998). Hydrogen could, on the other hand, be produced by microbial processes.

Microbial impact on the alteration: biomineralization and microbial mediated dissolution

The microbial catalysed oxidation and reduction of iron and manganese, and nucleation and precipitation of alteration products on the microbial cells and their extracellular material, are likely to partially control the mobility of elements from the dissolving glass and reacting seawater. In addition, as the microbial growth and fossilization are main controls on the porosity and texture of the alteration rims, these processes are also expected to affect the fluid exchange rate and thus, the dissolution rate in the fractures. Together with the relatively large endolithic biomass, this suggests that the potential microbial impact on the weathering of seafloor basalt and the chemical exchange with the seawater is significant. Observation of pit marks in the glass beneath the attached microbes

(Figure 2a) furthermore suggests that the dissolution of the glass is to some extent controlled by microbial processes (Thorseth et al., 2001). It is, however, not known if the pitting is actively caused by local enhanced microbial dissolution due to the utilization of energy or nutrients present in the glass, or to the passive accumulations of metabolic waste products and the development of corrosive microenvironments at the interface between the microbial cells and the glass. Results from recent laboratory experiments have shown that organotrophs (Daughney et al., 2004) as well as lithoautotrophs (Edwards et al., 2004) have the potential to increase the dissolution rate and the alteration of basalt at the seafloor.

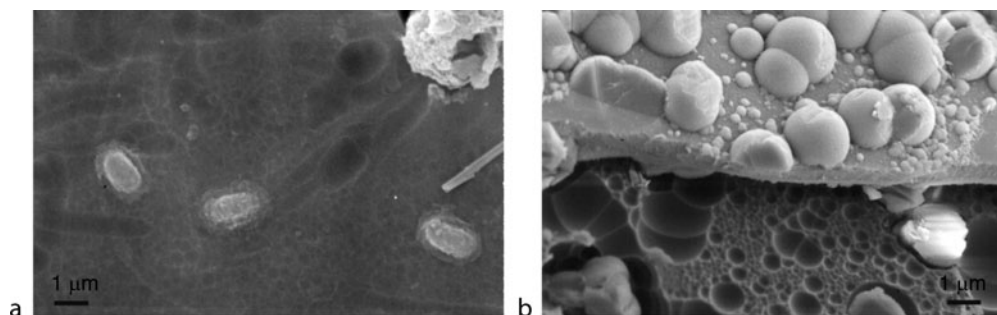
In addition to putative microbial mediated pitting, pit marks of similar form and size to microbes that are filled by hemispherical alteration structures and not by characteristic hollow cell encrustations (Figure 2b) suggest that abiotic processes may produce comparable dissolution textures to microbial processes. Such abiogenic corrosion marks most frequently occur in narrow fractures, which suggest that they are related to dissolution at low water-rock ratios (Kruber et al., 2008a).

Microbial life in subsurface basalt

Drill cores: textural, geochemical, isotope, and biomolecular biosignatures

The first reported evidence of endolithic microbes in basalt from the Costa Rica Rift subsequently stimulated to additional studies of basaltic glass in subsurface rocks from various locations and ages. Most of the studies report of similar textural, geochemical, isotope, and biomolecular evidences for microbial activity in the glass, as in the first report (Furnes and Staudigel, 1999; Fisk et al., 1998, 2003; Furnes et al., 1999, 2001a, b, 2003; Banerjee and Muehlenbachs, 2003; Thorseth et al., 2003; Benzerara et al., 2007). The phylogenetic and metabolic diversity of subsurface endolithic microbial communities inhabiting oceanic basalts have, however, only been described in the study by Lysnes et al. (2004b), and with more limited data in the study by Fisk et al. (2003).

While abiotic alteration of isotropic basaltic glass is expected to produce regular zoned alteration rims with



Basalt (Glass, Endoliths), Figure 2 (a) Pit marks in the glass with similar form and size as the attached microbial cells; (b) pit marks in the glass that are filled by solid hemispherical alteration structures and not by hollow microbial encrustations.

smooth interfaces between the fresh and altered glasses, the commonly observed irregular alteration fronts have been interpreted to be a result of enhanced dissolution or corrosion mediated by endolithic microbial cells. The most frequently putative biogenic alteration textures reported are (1) irregular patches or zones of individual or coalesced spheres with diameter around 1 μm (also characterized as a granular texture type), and (2) channels or tubular forms 1 μm to few μm wide and up to more than 100 μm in length (Figure 3). After the first reports of DNA-specific staining of altered glass from the Costa Rica Rift, similar textures in samples from other locations have also been shown to contain nucleic acids by fluorescent dyes (Furnes et al., 2001a; Banerjee and Muehlenbachs, 2003; Fisk et al., 2003), as well as elevated amounts of carbon, nitrogen, and phosphorus.

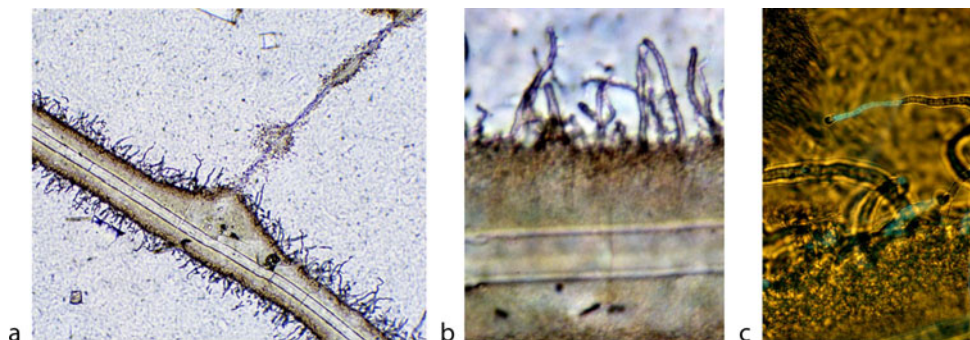
Using alteration textures as discriminating criteria, the extent and importance of biogenic versus abiotic alteration in the oceanic basement has been estimated. Thus, bioalteration has been observed in glasses from 0 to 170 Ma crust at 0–1.5 km depths of burial of lava flows and deep-sea sediments (Fisk et al., 1998; Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006). The degree of this bioalteration is highly variable in the upper 300 m of the crust (20–90%), but tends to decrease with depth. The granular texture is found to dominate at all depths. The tubular texture only represents a small fraction of the total bioalteration, and is more common at deeper levels (Furnes and Staudigel, 1999; Furnes et al., 2001b; Staudigel et al., 2006).

The stable carbon isotope composition ($\delta^{13}\text{C}$) of disseminated carbonate provides additional evidence for endolithic microbial activity in altered oceanic basalts. Most investigated samples show $\delta^{13}\text{C}$ values that are significantly lower than magmatic carbon (5 to 7), indicating microbial utilization and oxidation of organic carbon which leads to ^{12}C -enriched CO_2 and carbonate minerals (Furnes et al., 2001a). Positive $\delta^{13}\text{C}$ values of some samples from the Atlantic Ocean have, on the other hand, been explained by lithoautotrophic utilization of H_2 and CO_2

and production of methane. During this process ^{12}C -enriched methane is lost, and the remaining carbonate is enriched in ^{13}C . This could result from more extensive serpentinization and abiotic formation of H_2 at the slow-spreading Atlantic ridge compared to other faster spreading ridges.

Despite the evidence for ongoing microbial activity in subseafloor basalt, few direct observations of evident microbial cell structures, which are easily observed in seafloor basalt, have been reported. Possible candidates are hollow and solid filamentous structures detected in basalt tuff recovered from the Ontong Java Plateau during the ODP Leg 192 (Banerjee and Muehlenbachs, 2003), and hollow spherical structures in pillow basalt recovered from the AAD area of the SEIR during ODP Leg 187 (Thorseth et al., 2003). However, these structures were all supposed to represent fossilized cells.

Petrographic inspection of samples drilled from 14 to 28 Ma ocean crust in the AAD area during the ODP Leg 187 indicated mostly low-temperature, oxygenated alteration conditions. Comparison of 2.5 Ma seafloor basalt from the same area with the much older subseafloor basalt indicated that a significant proportion of the glass alteration developed prior to burial. However, diffuse and highly irregular fresh-altered glass interfaces were only observed in the subseafloor samples and thus most likely developed after burial. These diffuse alteration fronts are apparently caused by dissolution and alteration of the glass into minute globules (0.05–0.2 μm in diameter), and no evident microbial cell structures could be observed to be associated. Some hollow, Mn-containing spherical structures that were observed within zeolite-filled fractures in one sample could represent fossilized equivalents of Mn-rich encrusted, apparently active microbes, observed in 2.5 Ma seafloor basalt (Figure 1e). However, the presence of few cell-like structures in the subsurface glassy basalt suggests that most of the numerous encrusted and fossilized cells developed during the surface stage are totally destroyed or masked by alteration and mineralization during burial. The fossilized cells within zeolite-filled



Basalt (Glass, Endoliths), Figure 3 (a) Petrographic thin section of basaltic glass from Costa Rica Rift, showing tubular structures at the alteration front of palagonite rim along a fracture; (b) close up of A; (c) DNA-specific DAPI staining (blue color) of the tubular structures, indicating the presence of DNA-containing cells.

fractures in subseafloor basalt, on the other hand, possibly indicate that microbial activity continued in the fractures after burial, for as long as circulation continued.

Pit marks and development of irregular alteration fronts both with and without the presence of microbes, and ambient fractures where smooth alteration interfaces are combined with numerous microorganisms attached to the fresh glass, as observed in seafloor basalt, together with irregular alteration fronts without detectable cells in subseafloor basalt, imply that great care should be taken before using alteration textures as the only criterion for microbial activity. Clearly, much more detailed knowledge is needed to fully understand the mechanisms that control both biotic and abiotic pit mark formation in basaltic glasses.

Drill cores: genetic and metabolic diversity

Drilling of 14–28 Ma ocean crust during ODP Leg 187 was partly aimed at studying the genetic and functional diversity of the subsurface microbial community (Christie et al., 2001; Pedersen et al., 2004; Lysnes et al., 2004b). Despite the sparse detection of only fossilized cells by SEM (Thorseth et al., 2003), the identification of a microbial community in these samples by biomolecular techniques that differ from that of surface seawater used as drilling fluid, document the presence of living cells in this old, sediment buried ocean floor basalts, and demonstrate that water-rock interactions also support a diverse endolithic microbial community in old ocean crust. This microbial community is probably present in fluid transporting fractures developed during tectonical events after burial. The nondetection of microbial DNA in a significant proportion of subseafloor samples could thus reflect not only a lower biomass in subseafloor basalts, but also a low number of open fractures in the samples analyzed, compared to seafloor lavas where DNA was retrieved from nearly all investigated samples (Lysnes et al., 2004a, b).

A major fraction of the endolithic microbial community in the subseafloor basalt from the ADD area was shown to be from the domain *Bacteria*, similar to the Arctic seafloor basalt. The five phylogenetic classes *Gamma-proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, and *Firmicutes* that were present in the seafloor basalt were also present in the old subseafloor basalt. The main differences observed were the higher frequency of *Gamma-proteobacteria* in subseafloor basalt, the restricted presence of *Alpha-proteobacteria* in seafloor basalt, and *Beta-proteobacteria* in subseafloor basalt. Fewer and more unequal distributions of the phylotypes in subseafloor samples suggest a decrease in biodiversity and biomass with burial and aging of the crust. The results from this study furthermore indicate that microbial communities inhabiting nonhydrothermal subseafloor basalts participate in the cycling of iron and in the production of methane from H_2 and CO_2 . A major part of the microbial populations showed no close relation to previously characterized microorganisms. The correspondence between

the microbial populations in nonhydrothermal seafloor (Lysnes et al., 2004a) and subseafloor basalts (Lysnes et al., 2004b) may reflect the low-temperature and oxidised conditions, as indicated by the secondary alteration phases and their chemical composition, in both environments. The genetic and physiological diversity of microorganisms in the nonhydrothermal oceanic basalt from the AAD area differ from those reported from hydrothermal fluids from hydrothermal active regions of the ocean crust.

Recently, evidence of the presence of also eukaryotic, fungal life in oceanic basalt was reported (Schumann et al., 2004). The filamentous structures, suggested to represent fossil fungi, were observed within carbonate-filled vesicles in 46 Ma subseafloor basalt recovered during the ODP Leg 200 in the Pacific ocean. These findings imply that fungal life may possibly still exist in the subseafloor basaltic environment.

Hydrothermal fluids

Several studies of the microbial diversity in hydrothermal fluids (see *Hydrothermal Environments, Marine*) associated with eruptions in the central volcanic active zone of spreading ridges have been carried out during the last years (Summit and Baross, 1998, 2001; Huber et al., 2002, 2003). These studies have shown that the vent fluids inhabit microorganisms that are different from indigenous seawater microorganisms, suggesting that the vent fluids carry endolithic microbial cells from the basaltic subsurface environment. In the studies by Huber et al. (2002, 2003) dominant subseafloor phylotypes affiliated with the *Alpha*-, *Gamma*-, *Delta*- and *Epsilon-proteobacteria*, *Firmicutes*, novel candidate divisions, Group I *Crenarchaeota*, and Group II *Euryarchaeota* were detected. Most of these phylotypes were reported to be unique, differing from those of surrounding seawater, and affiliated with both low- and high-temperature groups. Considerable overlap with seawater was only found within *Alpha*- and *Gamma-proteobacteria*. To explain the retrieved diversity, Huber et al. (2003) proposed a schematic model of the basaltic subseafloor at the sampling site, based on hydrothermal fluid and seawater mixing that included three different compartments: a deep, anaerobic zone dominated by the hot hydrothermal fluid that contains mostly thermophilic microorganisms; an intermediate zone with both seawater and hydrothermal fluid and the highest variability in microbial community composition, probably including anaerobes, aerobes, and microaerophilic organisms; and an uppermost low-temperature, aerobic zone that is seawater-dominated, possibly containing seawater organisms. Unique subseafloor microorganisms flushed out from this uppermost zone by the vent water could thus be similar to microorganisms detected in nonhydrothermal basalts. Some of the *Gamma*- and *Epsilon-proteobacteria* sequences retrieved from both nonhydrothermal seafloor and subseafloor samples (Lysnes et al., 2004a, b) were phylogenetic affiliated

to sequences retrieved from the <50°C diffuse flow vent fluids associated with an eruptive event on the Juan de Fuca Ridge (Huber et al., 2003).

Investigation of 65°C subsurface fluids from aging (3.5 Ma) ocean crust by Cowen et al. (2003) also demonstrated the presence of a diverse bacterial and archaeal community in hydrothermal active areas of the mid-ocean ridge flanks. In this reduced, hydrothermal environment DNA sequences related to the ammonia-producing *Ammonifex degensii* and sulfate reducers of the *Delta-proteobacteria*, the low G + C Gram-positive genus *Desulfotomaculum*, and *Archaea* (*Archaeoglobus*) were dominant. A later study of 20°C subseafloor fluids from the same area demonstrated that the microbial community in the fluids composed of species indigenous to the subseafloor, as well as species from seawater and sediments (Huber et al., 2006). No sequences retrieved from nonhydrothermal seafloor and subseafloor basalts resembled those obtained from these 65°C subseafloor fluids.

Using available published 16S rDNA sequences from basalt, hydrothermal fluids, basalt enrichments, and data from other relevant environments, Mason et al. (2007) examined the phylogenetic diversity of microbial communities associated with marine basalts. Based on these data, it was suggested that the microorganisms have a cosmopolitan distribution. Most groups were found in both basalts and sediments, but are distinct from microbes in other marine habitats. One group of Marine Group I *Crenarchaeota* seems furthermore to be specifically adapted to basalt.

Biosignatures in ophiolites and greenstone belts

During the last years, terrestrial fragments of ancient ocean crust (ophiolites) and greenstone belts have been investigated for similar biosignatures as those observed within in situ oceanic basalt. For example, the results from the well-preserved ~90 Ma Troodos ophiolite in Cyprus revealed comparable traces of microbial life in the glassy lava margins as reported from studies of in situ oceanic crust, except for DNA that could not be detected by specific staining (Furnes et al., 2001c). This, together with geochemical and isotope data indicative of seawater alteration under oxidizing condition, imply that the main fraction of the alteration occurred prior to obduction during an early stage of ocean-floor alteration. Comparable textural, geochemical, and isotopic data obtained from lava margins from the ~3.5 billion-year-old Barberton greenstone Belt in South Africa further suggest subaqueous microbial activity during the early history of the Earth (Furnes et al., 2004) (see *Early Earth*). Thus, these biosignatures could be of great relevance in the search for life elsewhere in the universe (see *Astrobiology*).

Conclusions

Endolithic microbial communities have been documented in glassy margins of seafloor and subseafloor basaltic lavas from various locations of the ocean crust. In seafloor

basalt the microbial cells are easily recognized by electron microscopy, and are shown to be closely associated with the alteration of the glass. In subseafloor basalt the microbial activity has mainly been recognized by the use of biosignatures such as characteristic cell-like alteration textures in the glass, elevated concentrations of carbon and nitrogen in these textures, and stable carbon isotope ratios of disseminated carbonate consistent with biological processes. DNA-specific staining indicates the presence of DNA in the cell-like alteration textures. Culturing and biomolecular studies have revealed the existence of diverse, endolithic microbial communities in the glassy margins of oceanic lavas. The results suggest that iron and manganese cycling are important microbial processes in cold, nonhydrothermal regions, while sulfur cycling plays a major role at elevated temperatures in hydrothermal active areas. The endolithic microbial life in oceanic basalt may have a significant impact on the alteration of the basalt and the chemical exchange between the ocean crust and seawater.

Bibliography

- Andersen, T., Chapelle, F. H., and Lovley, D. R., 1998. Evidence against hydrogen-based microbial ecosystems in basalt aquifers. *Science*, **281**, 976–977.
- Bach, W., and Edwards, K. J., 2003. Iron and sulfide oxidation within the Basaltic ocean crust: implications for chemolithoautotrophic microbial biomass production. *Geochimica et Cosmochimica Acta*, **67**, 3871–3887.
- Banerjee, N. R., and Muehlenbachs, K., 2003. Tuff life: bioalteration in volcanoclastic rocks from the Ontong Java Plateau. *Geochemistry, Geophysics, Geosystems*, **4**, doi: 10.1029/2002GC000470.
- Benzerara, K., Menguy, N., Banerjee, N. R., Tylliszczak, T., Brown, G. E., and Guyot, F., 2007. Alteration of submarine basaltic glass from the Ontong Java Plateau: A STEM and TEM study. *Earth and Planetary Science Letters*, **260**, 187–200.
- Christie, D. M., Pedersen, R. B., Miller, D. J., et al., 2001. *Proceedings of the Ocean Drilling Program, Initial Reports*, 187 (Online). Available from World Wide Web: http://www-odp.tamu.edu/publications/187_IR/187ir.htm. doi:10.2973/odp.proc.ir.187.2001.
- Cowen, J. P., Giovannoni, S. J., Kenig, F., Johnson, H. P., Butterfield, D., Rappé, M. S., Hutnak, M., and Lam, P., 2003. Fluids from aging ocean crust that support microbial life. *Science*, **299**, 120–123.
- Crovisier, J. L., Thierry, A., and Dussossoy, J. L., 2003. Nature and role of natural alteration gels formed on the surface of ancient volcanic glasses (natural analogs of waste containment glasses). *Journal of Nuclear Materials*, **321**, 91–109.
- Daughney, C. J., Rioux, J. P., Fortin, D., and Pichler, T., 2004. Laboratory investigation of the role of bacteria in the weathering of basalt near deep sea hydrothermal vents. *Geomicrobiology Journal*, **21**, 21–31.
- Edwards, K. J., Rogers, D. R., Wirsén, C. O., and McCollum, T. M., 2003. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing chemolithoautotrophic α - and γ -proteobacteria from the deep-sea. *Applied and Environmental Microbiology*, **69**, 2906–2913.
- Edwards, K. J., Bach, W., McCollum, T. M., and Rogers, D. R., 2004. Neutrophilic iron-oxidizing bacteria in the ocean: their habitats, diversity, and roles in mineral deposition, rock

- alteration, and biomass production in the deep-sea. *Geomicrobiology Journal*, **21**, 393–404.
- Einen, J., Thorseth, I. H., and Øvreås, L., 2008. Enumeration of *Archaea* and *Bacteria* in seafloor basalt using real-time quantitative PCR and fluorescence microscopy. *FEMS Microbiology Letters*, **282**, 182–187.
- Emerson, D., Rentz, J. A., Lilburn, T. G., Davis, R. E., Aldrich, H., Chan, C., and Moyer, C. L., 2007. A novel lineage of proteobacteria involved in formation of marine Fe-oxidizing microbial mat communities. *PLoS ONE*, **2**, doi: 10.1371/journal.pone.0000667.
- Fisk, M. R., Giovannoni, S. J., and Thorseth, I. H., 1998. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science*, **281**, 978–979.
- Fisk, M. R., Storrer-Lombardi, M. C., Douglas, S., Popa, R., McDonald, G., and Di Meo-Savoie, C., 2003. Evidence of biological activity in Hawaiian subsurface basalts. *Geochemistry, Geophysics, Geosystems*, **4**, doi: 10.1029/2002GC000387.
- Furnes, H., and Staudigel, H., 1999. Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth and Planetary Science Letters*, **166**, 97–103.
- Furnes, H., Thorseth, I. H., Tumyr, O., Torsvik, T., and Fisk, M., 1996. Microbial activity in the alteration of glass from pillow lavas from ODP Hole 896A. In Alt, J. C., Kinoshita, H., Stokking, L. B., and Michael, P. J. (eds.), *Proceedings of the Ocean Drilling Program Scientific Results*, Vol. 148, pp. 191–206.
- Furnes, H., Muehlenbachs, K., Tumyr, O., Torsvik, T., and Thorseth, I. H., 1999. Depth of active bio-alteration in the ocean crust: Costa Rica Rift (Hole 504B). *Terra Nova*, **11**, 228–233.
- Furnes, H., Muehlenbachs, K., Torsvik, T., Thorseth, I. H., and Tumyr, O., 2001a. Microbial fractionation of carbon isotopes in altered basaltic glass from the Atlantic Ocean, Lau Basin and Costa Rica Rift. *Chemical Geology*, **173**, 313–330.
- Furnes, H., Staudigel, H., Thorseth, I. H., Torsvik, T., Muehlenbachs, K., and Tumyr, O., 2001b. Bioalteration of basaltic glass in the oceanic crust. *Geochemistry, Geophysics, Geosystems*, **2**, paper number 2000GC000150.
- Furnes, H., Muehlenbachs, K., Tumyr, O., Torsvik, T., and Xenophontos, C., 2001c. Biogenic alteration of volcanic glass from the Troodos ophiolite, Cyprus. *Journal of the Geological Society, London*, **158**, 75–84.
- Furnes, H., Thorseth, I. H., Torsvik, T., Muehlenbachs, K., Staudigel, H., and Tumyr, O., (2003). Identifying bio-interaction with basaltic glass in oceanic crust and implications for estimating the depth of the oceanic biosphere: a review. In: Smellie, J. L. and Chapman, M. G. (eds.), *Volcano-Ice Interactions on Earth and Mars*, Geological Society, Special Publication, London, **202**, 407–421.
- Furnes, H., Banerjee, N. R., Muehlenbachs, M., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- Giovannoni, S. J., Fisk, M. R., Mullins, T. D., and Furnes, H., 1996. Genetic evidence for endolithic microbial life colonizing basaltic glass-seawater interfaces. *Proceedings of Ocean Drilling Program. Scientific Results*, **148**, 207–214.
- Holloway, J. R., and O'Day, P. A., 2000. Production of CO₂ and H₂ by diking-eruptive events at mid-ocean ridges; implications for abiotic organic synthesis and global geochemical cycling. *International Geology Review*, **42**, 673–683.
- Huber, J. A., Butterfield, D. A., and Baross, J. A., 2002. Temporal changes in archaeal diversity and chemistry in a mid-ocean ridge subseafloor habitat. *Applied and Environmental Microbiology*, **68**, 1585–1594.
- Huber, J. A., Butterfield, D. A., and Baross, J. A., 2003. Bacterial diversity in a subseafloor habitat following a deep-sea volcanic eruption. *FEMS Microbiology Ecology*, **43**, 393–409.
- Huber, J. A., Johnson, H. P., Butterfield, D. A., and Baross, J. A., 2006. Microbial life in ridge flank crustal fluids. *Environmental Microbiology*, **8**, 88–99.
- Kelley, D. S., Baross, J. A., and Delaney, J. R., 2002. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annual Review of Earth and Planetary Sciences*, **30**, 385–491.
- Kruber, C., Thorseth, I. H., and Pedersen, R. B., 2008a. Seafloor alteration of basaltic glass: textures, geochemistry and endolithic microorganisms. *Geochemistry, Geophysics, Geosystems*, **9**, 12, doi: 10.1029/2008GC002119.
- Kruber, C., Thorseth, I. H., and Pedersen, R. B., 2008b. Fossilised endolithic microorganisms in glassy seafloor basalt: a TEM study. (submitted to *Earth and Planetary Science Letters*).
- Lysnes, K., Thorseth, I. H., Steinsbu, B. O., Øvreås, L., Torsvik, T., and Pedersen, R. B., 2004a. Microbial community diversity in seafloor basalt from the arctic spreading ridges. *FEMS Microbial Ecology*, **50**, 213–230.
- Lysnes, K., Torsvik, T., Thorseth, I. H., and Pedersen, R. B., 2004b. Microbial populations in ocean floor basalt: results from ODP Leg 187. In Pedersen, R. B., Christie, D. M., and Miller, D. J. (eds.), *Proceedings of the Ocean Drilling Program Scientific Results*, **187**, 1–27, doi:10.2973/odp.proc.sr.187.203.2004.
- Mason, O. U., Stingl, U., Wilhelm, L. J., Moeseneder, M. M., Di Meo-Savoie, C. A., Fisk, M. R., and Giovannoni, S. J., 2007. The phylogeny of endolithic microbes associated with marine basalts. *Environmental Microbiology*, **9**, 2539–2550.
- Palandri, J. L., and Reed, M. H., 2004. Geochemical models of metasomatism in ultramafic systems: serpentinization, rodingitization, and sea floor carbonate chimney precipitation. *Geochimica et Cosmochimica Acta*, **68**, 1115–1133.
- Pedersen, R. B., Christie, D. M., and Miller, D. J., (eds.), 2004. *Proceedings of the Ocean Drilling Program Scientific Results*, **187**, College Station, TX (Ocean Drilling Program). doi:10.2973/odp.proc.sr.187.
- Schumann, G., Manz, W., Reitner, J., and Lustrino, M., 2004. Ancient fungal life in North Pacific Eocene oceanic crust. *Geomicrobiology Journal*, **21**, 241–246.
- Staudigel, H., Chastain, R. A., Yayanos, A., and Bourcier, W., 1995. Biologically mediated dissolution of glass. *Chemical Geology*, **126**, 147–154.
- Staudigel, H., Yayanos, A., Chastain, R. A., Davies, G., Verdurmen, E. A. T., Sciffman, P., Bourcier, W., and De Baar, H., 1998. Biologically mediated dissolution of volcanic glass in seawater. *Earth and Planetary Science Letters*, **164**, 233–244.
- Staudigel, H., Furnes, H., Banerjee, N. R., Dilek, Y., and Muehlenbachs, K., 2006. Microbes and volcanoes: a tale from the oceans, ophiolites, and greenstone belts. *GSA Today*, **16**, 4–10.
- Stronck, N. A., and Schmincke, H. U., 2001. Evolution of palagonite: crystallization, chemical changes, and element budget. *Geochemistry, Geophysics, Geosystems*, **2**, Paper number 2000GC000102.
- Summit, M., and Baross, J. A., 1998. Thermophilic subseafloor microorganisms from the 1996 North Gorda eruption. *Deep-Sea Research. II*, **45**, 2751–2766.
- Summit, M., and Baross, J. A., 2001. A novel microbial habitat in the mid-ocean ridge subseafloor. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2158–2163.
- Templeton, A., Staudigel, H., and Tebo, B., 2005. Diverse Mn(II)-oxidizing bacteria isolated from submarine basalts at Loihi seamount. *Geomicrobiology Journal*, **22**, 127–139.
- Thorseth, I. H., Furnes, H., and Heldal, M., 1992. The importance of microbiological activity in the alteration of natural basaltic glass. *Geochimica et Cosmochimica Acta*, **56**, 845–850.

- Thorseth, I. H., Furnes, H., and Tumyr, O., 1995a. Textural and chemical effects of bacterial activity on basaltic glass: an experimental approach. *Chemical Geology*, **119**, 139–160.
- Thorseth, I. H., Torsvik, T., Furnes, H., and Muehlenbachs, K., 1995b. Microbes play an important role in the alteration of oceanic crust. *Chemical Geology*, **126**, 137–146.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daac, F. L., Pedersen, R. B., and the Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Thorseth, I. H., Pedersen, R. B., and Christie, D. M., 2003. Microbial alteration of 0–30 Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic discordance. *Earth and Planetary Science Letters*, **215**, 237–247.
- Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I. H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters*, **162**, 165–176.
- von Waltershausen, W. S., 1845. Über die submarinen vulkanischen Ausbrüche in der Tertiär-Formation des Val di Noto im Vergleiche mit verwandten Erscheinungen am Ätna. *Göttinger Studien*, **1**, 371–431.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Biological Volcanic Rock Weathering
 Biosignatures in Rocks
 Deep Biosphere of the Ocean Deep Sea
 Early Earth
 Endoliths
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Fluorescence In Situ Hybridisation (FISH)
 Gallionella
 Hydrogen
 Hydrothermal Environments, Marine
 Isotopes and Geobiology
 Microbial Biomineralization
 Origin of Life
 Shewanella
 Sulfate-Reducing Bacteria

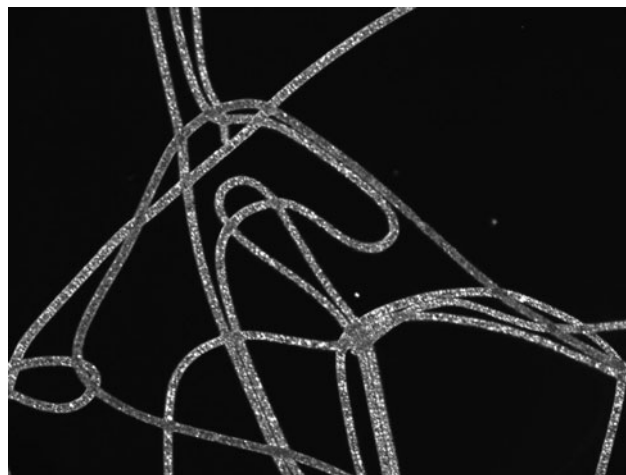
BEGGIATO

Heide N. Schulz-Vogt
 Max Planck Institute for Marine Microbiology,
 Bremen, Germany

Definition

Beggiatoa is a colorless, sulfur-storing bacterium belonging to the Gammaproteobacteria, filamentous, motile, and not attached.

Bacteria of the genus *Beggiatoa* live by the oxidation of sulfide with oxygen or nitrate. They are benthic



Beggiatoa, Figure 1 *Beggiatoa* filaments of 6 μm diameter seen under the light microscope with dark field illumination. The sulfur globules reflect the light and give the filaments their shining white appearance.

organisms, which occur in almost all freshwater and marine environments where sulfide is present, e.g., wastewater, areas of coastal upwelling, salt marshes, hydrothermal vents, cold seeps, methane hydrates, etc. In contrast to most bacteria, *Beggiatoa* can be recognized by its typical morphology. It is a filamentous bacterium, which is motile by gliding and stores elemental sulfur inside, giving it a shining white appearance (Figure 1). The filaments can be several millimeters to centimeters long and are formed by long rows of cells with diameters between 1 and 200 μm . The larger species of *Beggiatoa* (>5–10 μm in diameter) have a central vacuole, which they use for storing nitrate in high concentrations (ca 10–300 mM).

Beggiatoa filaments typically occur as mats on top of the sediment or within the upper few centimeters. They actively glide to the overlapping zone of oxygen, supplied from the open water, and sulfide evolving in the sediment. In addition to oxygen, the larger species can also use internally stored nitrate for the oxidation of sulfide and thereby increase their rates of sulfide oxidation or survive periods of anoxia. Sulfide is first oxidized to sulfur, which forms intracellular sulfur globules, and then further on to sulfate. If sulfide concentrations are high, the cells are filled with many sulfur globules, which are disappearing in the absence of sulfide. Apart from sulfur and nitrate, *Beggiatoa* filaments were also observed to store polyphosphate and poly-beta-hydroxybutyrate (PHB).

Bibliography

- Teske, A., and Nelson, D. C., 2006. The genera *Beggiatoa* and *Thioploca*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, pp. 784–810.

Cross-references

Bacteria
Sulfur Cycle
Thiomargarita
Thiotrophic Bacteria

BIODETERIORATION (OF STONE)

Christina Beimforde
University of Göttingen, Göttingen, Germany

Definition

Biodeterioration: Undesirable alteration of the properties of materials caused by vital activities of organisms.

Introduction

Materials are exposed to weathering processes caused by physical, chemical, and biological factors, leading to an irreversible loss of our cultural heritage. The reduction of decay by conservation strategies is of particular importance to protect historical monuments or significant cultural artifacts.

Generally, the velocity of decay depends on the chemical nature and physical structure of materials as well as external factors associated to the locations where the objects are exposed. In many studies it was demonstrated that the process of weathering consists of various mechanisms acting together in coassociation with more or less intensity, making it impossible to separate individual aspects such as physical, chemical, or biological factors from each other (Koestler et al., 1994; Ehrlich, 2002). In many cases microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial consortia, rather the microbial activity, is also affected by the surrounding chemical and physical factors.

Solar irradiation, wind, and variations in temperature and humidity predominate the physical effects. Wind and humidity, of course, lead to the erosion of particles from stone surfaces. Variations in temperature, due to diurnal and seasonal changes, cause tensile stresses that can break up the structure of the material, resulting in a loss of persistence. Humidity, in combination with frost and salt, can intensify this effect. The crystallization of water or saline solutions is connected with an increase in volume that leads to “frost shattering” or “saline shattering” (Steiger and Dannecker, 1995).

Chemical weathering may be caused by rain water which dissolves carbon dioxide originating from the ambient air or from respiration of present microorganisms. Generated carbon acid reacts with calcium carbonate, thereby producing calcium hydrogen carbonate with a higher solubility than calcium carbonate (Bell, 1993).

According to Krumbein and Schönborn (1987), biological damages of building material can be divided into

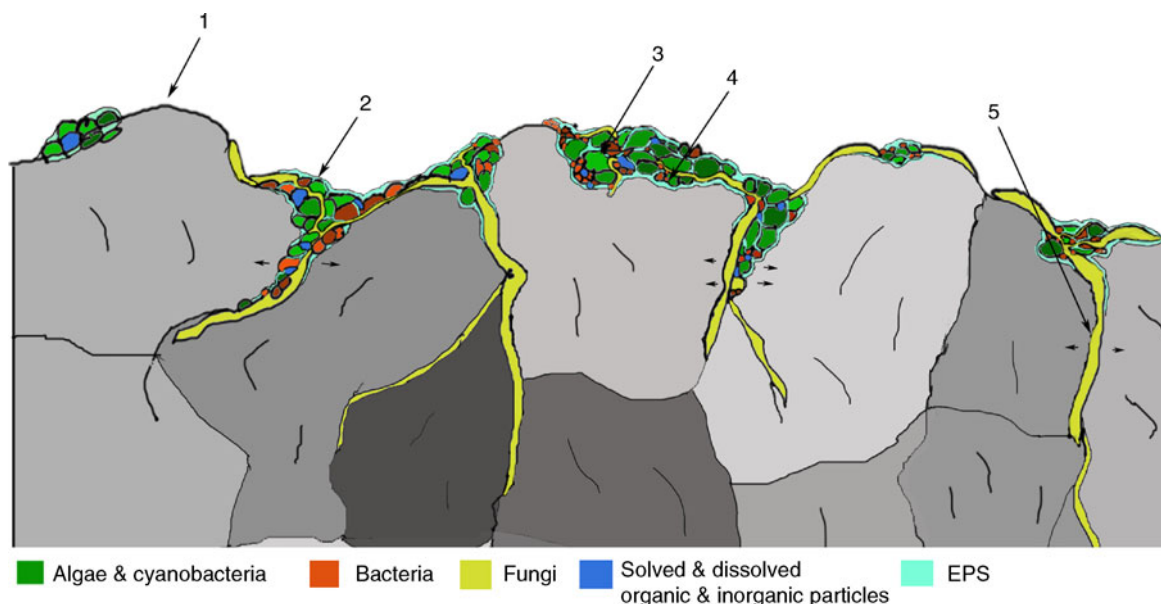
different categories: (1) Anthropogenically induced damages due to atmospheric pollution as a result of the industrialization. These processes lead to an increasing level of inorganic (SO_2 , NO_x) and organic (aliphatic and aromatic hydrocarbons) compounds in the air. This implicates acid rain that has a corrosive effect on stone buildings or other exposed materials (Pitzurra et al., 2003; Winkler, 1987). (2) Damages caused by higher plants and animals. The growth of higher plants, for example, leads to the physical destruction of materials. Moreover, excrements of birds contain ammoniac and nitrite, which can be utilized by chemolithotrophic bacteria. (3) Microorganisms such as bacteria, algae, fungi, and lichens can cause a degradation of materials. These damaging effects will be specified below.

Furthermore, biological colonization and the intensity of associated biodeterioration processes are strongly influenced by water availability. This is determined by specific material parameters such as porosity and permeability, as well as by environmental conditions.

Microbial degradation processes

How do microbial associations interact within these complex weathering processes? A considerable amount of studies have been done to figure out the role of microbiological agents in weathering processes (e.g., Urzi and Krumbein, 1994; Griffin et al., 1991; Warscheid and Braams, 2000). Generally, microorganisms play an important role within the regulation of the global cycles of materials, contributing to the function of the ecosystems. However, microorganisms also play a crucial role concerning the stability of materials. The interactions between microbial associations and materials can be divided into three different categories (Weber, 1993, cf. Figure 1): (1) The material can be decomposed enzymatically and used as a nutrient source by microbial associations. (2) Microorganisms can excrete metabolic compounds such as inorganic and organic acids that interact with mineral compounds of the material, thus causing dissolution and discoloration. (3) The material can be damaged by physical (mechanical) forces due to the growth of microorganisms.

For the most part, microorganisms conglomerate to biofilms of different natures such as crusts, fluffs, or slime, giving them the ability to colonize nearly all biotic and abiotic surfaces (Costeron et al., 1987). Biofilms can exhibit a highly spatial structured composition (Stoodley et al., 2002), thereby consisting of different prokaryotic and/or eukaryotic populations, extracellular polymeric substances (EPS), accumulated polyvalent cations, as well as solvated and dissolved organic and inorganic particles. The formation of biofilms provides considerable advantages including mechanical stability, the facility of symbiotic and syntrophic interactions, the building of a diffusion barrier and an increased resistance against toxic environmental conditions, biocides, aridity, or pH variabilities (Wolfaardt et al., 1999). However, the presence of biofilms can lead to biophysical determined damages of



Biodeterioration (of Stone), Figure 1 Interactions between materials, environmental conditions, and subaerial biofilms include (1) atmosphere substrate interactions (2) interactions between atmosphere and biofilms (3) interactions of metabolic compounds and substrate; enzymatic degradation (4) physicochemical interactions between biofilm and substrate (5) physical forces due to microbial growth.

surface structures of stone (Gorbushina and Krumbein, 2000; Gorbushina, 2007). Variations in temperature and humidity induce shrinking and swelling cycles of the film, leading to an alteration of hydrophobic–hydrophilic properties of rock surfaces. As a consequence, hydrophobic properties of stone surfaces can turn into hydrophilic.

Microorganisms involved in biodeterioration processes

The ecology of microbial associations colonizing rocks is highly complex and involves different groups of microorganisms with different physiological properties. These ecosystems can develop in various ways, depending on environmental conditions and physicochemical properties of the material. Therefore, only a small range of examples can be discussed in this framework, representing the complexity of the interactions between microorganisms and their substrate.

Phototrophic microorganisms

Phototrophic microorganisms such as algae, cyanobacteria and lichens, but also mosses and even higher plants cover stone surfaces, thus changing the face of new buildings or cultural heritage. In many cases they form biofilms or crusts to protect themselves from unfavorable environmental factors. These crusts or films can appear in a deep or light green color under humid conditions or in black tint under dry environmental conditions (Urzi and Krumbein, 1994; Krumbein and Warscheid, 1992). Under particular

climatic conditions (e.g., tropical environments) these biofilms provide a protective effect, isolating the stone surface from aggressive environments and regulating humidity and temperature (Krumbein and Warscheid, 1992). But the formed biopatina can also cause damages by physical and chemical processes (Krumbein and Warscheid, 1992; De los Rios et al., 2009). Associated biodeterioration processes can be induced by the excretion of corrosive acids (Arino et al., 1997), the uptake of substrate compounds such as sulfur and calcium and accumulation into their cells (Orgenta Calvo et al., 1994), and by the alteration of stone forming minerals (Ortega-Morales et al., 2000; Crispim and Gaylarde, 2004). Finally, the penetration of hyphae and roots causes enlargement of pores and loosening of stone particles from the material (Ascaso et al., 1998). The formation of biofilms intensifies this loss of structural consistency (Orgenta-Calvo et al., 1991).

Due to the capability of fixing atmospheric nitrogen, phototrophic organisms are often the primary settlers of habitats especially in combination with fungi as lichens. In rural or strongly industrialized areas where the air is enriched with nitrogen from fertilizers or industrialization, their growth can be enhanced (Cadot-Leroux, 1996; Cicek et al., 2008). Some phototrophic microorganisms (epilithicphototrophs) may penetrate several millimeters into the stone pore system (Golubic et al., 1981). Additionally, some of these microorganisms can colonize extreme locations, whereby a special pigmentation protects them against strong light irradiation (Krumbein and Jens, 1981).

Chemolithotrophic and chemoorganotrophic microorganisms

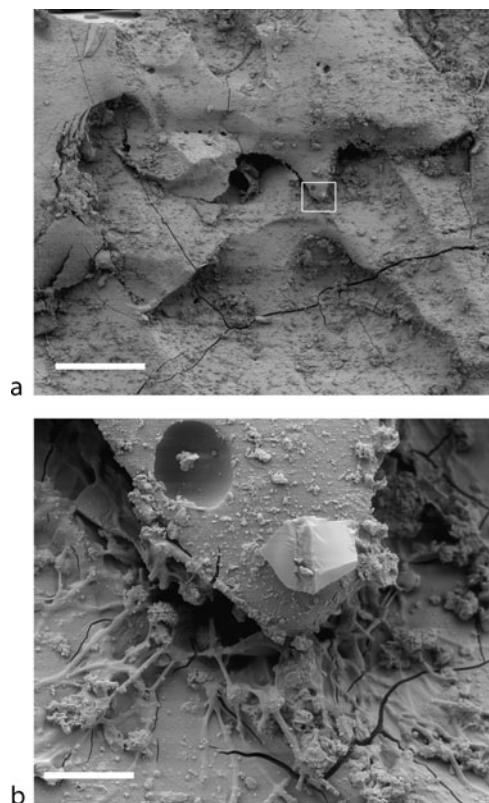
Even the natural biomass accumulated by photosynthetic microorganisms – especially in combination with anthropogenic pollution – can serve as nutrient source for the establishment of heterotrophic microorganisms (Warscheid et al., 1991; Zanardini et al., 2000).

Besides, even without a primary settlement of phototrophic microorganisms an establishment of heterotrophic organisms is possible (Krasilnikov, 1949). A sufficient amount of inorganic compounds (e.g., sulfur, ammonium, sulfide) enables the appearance of chemolithoautotrophic bacteria (Kaufmann, 1960; Pochon and Jaton, 1967). The formation of nitrous acid (HNO_2 by *Nitrosomas*) or nitric acid (HNO_3 by *Nitrobacter*) and sulfuric acid (H_2SO_4 , *Thiobacillus*) results in the solution of mineral rock compounds. Acidic metabolic compounds resulting from the oxidation of sulfur, for example, interact with calcium carbonate of the stone structure. This reaction leads to the formation of calcium sulfate, which is more soluble than calcium carbonate.

The most numerous microorganisms in natural building stone are chemoorganotrophic bacteria. Results of Warscheid et al. (1993) indicate that they colonize natural stone within weeks. They can excrete acid compounds but, generally, their destructive activity has been evaluated as low (Lewis et al., 1988). Nevertheless, they are extensively involved in the formation of biofilms, thus causing physicochemical properties of the mineral structure described above. Materials can contain or be associated with organic compounds such as dust, natural resin, wax, or atmospheric aerosols that can act as nutrient source for chemoorganotrophic microorganisms (Warscheid, 1990). As an effect of organic pollution, chemoorganotrophic microorganisms can be the first settlers on buildings in highly industrialized locations (Krumbein, 1966). Many chemoorganotrophic bacteria are able to use low degradable compounds, offering nutrients for other microorganisms of the microbial consortia (Warscheid et al., 1991; Zanardini et al., 2000).

Fungi

The colonization of stone surfaces or other materials by fungi causes damages, especially by physical (mechanical) forces (cf. Figure 2). The formation of penetration hyphae enables some fungi to enter actively into materials, despreding within the material and affecting the so-called biopitting structures on rock surfaces (Sterflinger and Krumbein, 1997). Furthermore, the production of aggressive acidic metabolites leads to biocorrosion (Kurakov et al., 1999). In comparison to bacteria, fungi can establish rapidly, especially in environments with low humidity due to their widespread system of hyphae (Weber, 1993). Their damaging effects also include the discoloration of surfaces due to the building and excretion of melanins (Gorbishina et al., 1993). The synthesis of special pigments such as melanin, carotenoids, and mycosporines provides



Biodeterioration (of Stone), Figure 2 (a) Scanning electron microscopic demonstrate colonization of amber surface by recent microorganisms. Fungal hyphae cover the amber surface and accumulate in cracks leading to loss of material persistence by physical stresses; scale bar 100 μm . (b) closeup, scale bar 10 μm . (Reitner and Beimforde, 2008).

protection against UV radiation and enables some species (e.g., black fungi) to colonize even at locations with extreme UV radiation (Chertov et al., 2004).

Measures and preventive treatments

In general, a microbial colonization is primarily dependent on the availability of water and nutrients and specific material parameters such as porosity, permeability, architectural conditions (considering buildings), and environmental factors. Natural selective processes can lead to the formation of populations that reveal a perfect adaption between organisms, substrate, and environment. A previous individual and comprehensive analysis of all factors and relationships is essential before preventive treatments or cleaning of the material is applied. A mechanical cleaning of historical buildings from dust or biological films should integrate a chemical analysis to characterize the surface dirt. Otherwise, the cleaning procedure can fail to have a long-lasting effect and even accelerate the degradation processes or alteration within the composition of microbial associations affecting changes of microbial activity (Dornieden et al., 2000).

Water cleaning can help remove soluble salts and biological infections, but in the long term it can lead to a wider dispersion of the microbial infection because of increased humidity. Furthermore, consolidation treatments can be applied, but their effectiveness is strongly connected to the physical structure of the stones. The application on stone surfaces with insufficient consistence and absorbent capacity can intensify physical damages (Sattler, 1992; Wendler, 1992; Zoghalmi and Gómez-Gras, 2004). Other preventive agents impregnate surface structures and are often based on high polymer polysaccharide, silane, or siloxane. Various studies have shown that the application of consolidants or water repellents induce an increasing activity or colonization of microorganisms using these restoration agents as nutrient source (Krumbein et al., 1993; Petersen et al., 1991, cf. Figure 3). Studies of Urzi and

De Leo (2007) and De Leo and Urzi (2003) have shown that water repellents alone do not stop microbial colonization, but in combination with biocides, microbial growth can be prevented.

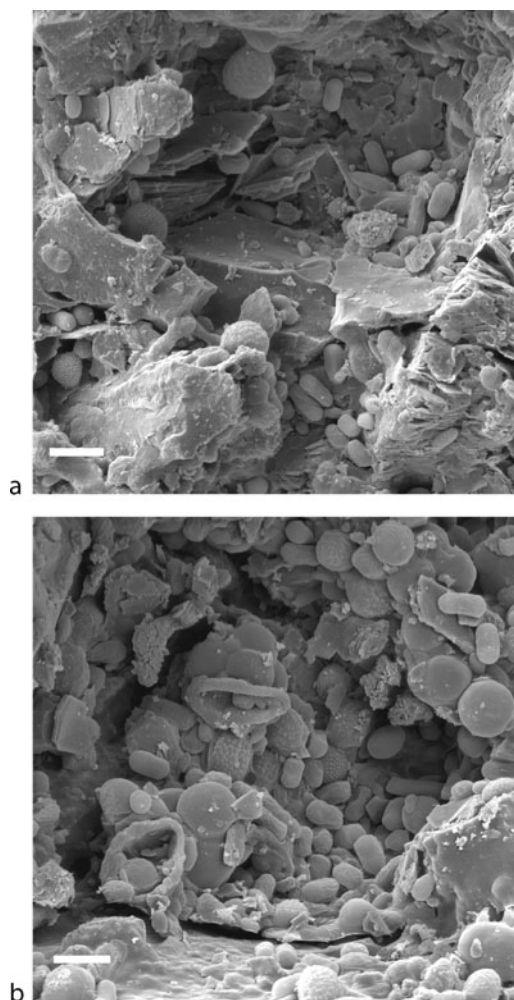
Finally, it is important to mention that the treatment with biocides should only be applied in instances where biodeterioration processes cannot be controlled and chemical applications are unavoidable. An inappropriate treatment with biocides or other preventive arrangements can lead to an increasing selective microbial colonization that may accelerate the deterioration.

Summary

The process of weathering consists of physical, chemical and biological factors acting together with more or less intensity, leading to an irreversible loss of our cultural heritage. Microbial activities can accelerate or trigger physicochemical processes. But the composition of microbial community or rather the microbial activity is also affected by the surrounding chemical and physical factors. Interactions between microbial associations and materials contain the enzymatic decomposition of materials, excretion of metabolic compounds such as inorganic or organic acids, and damages by physical (mechanical) forces due to the growth of microorganisms.

Bibliography

- Arino, X., Gomez-Bolea, A., and Saiz-Jimenez, C., 1997. Lichens on ancient mortars. *International Biodeterioration and Biodegradation*, **40**, 217–224.
- Ascaso, C., Wierchos, J., and Castello, R., 1998. Study of the biogenic weathering of calcereous litharenite stones caused by lichen and endolithic microorganisms. *International Biodeterioration and Biodegradation*, **42**, 29–38.
- Beimforde, C., 2006. Einfluss von Restaurierungsmitteln auf gesteinsbesiedelnde mikrobielle Vergesellschaftungen: mikroskopischer und molekularbiologischer Vergleich. Thesis, Oldenburg.
- Beimforde, C., and Schmidt, A. R., 2010. Microbes in resinous habitats: a compilation from modern and fossil resins. In Reitner, J., Queric, N.-V., and Arp, G. (eds.), *Advances in Stromatolite Geobiology. Lecture Notes in Earth Sciences* (in press).
- Bell, F. G., 1993. Durability of carbonate rock as building stone with comments on its preservation. *Environmental Geology*, **21**, 187–200.
- Cadot-Leroux, L., 1996. Rural nitrogenous pollution and deterioration of granitic monuments: results of a statistical study. In Riederer, J. (ed.), *Proceedings of the Eighth International Congress on Deterioration and Conservation of Stone*, Rathgen-Forschungslabor: Berlin, Vol. 1, pp. 301–309.
- Chertov, O., Gorbushina, A. A., and Deventer, B., 2004. A model for microcolonial fungi growth on rock surfaces. *Ecological Modelling*, **177**, 415–426.
- Cicek, A., Aslan, A., Yazc, K., and Savas Koparal, A., 2008. Effects of environmental conditions on historical buildings: lichens and NO_x gases. *Environmental Monitoring Assessment*, Published online.
- Costeron, J. W., Cheng, K.-J., Geesey, G. G., Ladd, T. I., Nickel, J. C., Dasgupta, M., and Marrie, T. J., 1987. Bacterial biofilms in nature and disease. *Annual Reviews in Microbiology*, **41**, 435–464.



Biodeterioration (of Stone), Figure 3 Sandstone treated with (a) consolidation agents and (b) water repellents. A high microbial colonizations was observed 3 month after treatment and outdoor exposure, scale bar 10 μ m (photograph by A. A. Gorbushina and C. Beimforde, Beimforde, 2006).

- Crispim, C. A., and Gaylarde, C. C., 2004. Cyanobacteria and biodegradation of cultural heritage: a review. *Microbiological Ecology*, **49**, 1–9.
- De Leo, F., and Urzi, C., 2003. Fungal colonisation on treated and untreated stone surfaces. In Saiz Jimenez, C. (ed.), *Molecular Biology and Cultural Heritage*. The Netherlands: Swets & Zeitlinger, pp. 213–217.
- De los Rios, A., Cámara, B., García Del Cura, Á., Víctor, M. A. G., Rico, V. J., Galván, V., and Ascaso, C., 2009. Deteriorating effects of lichen and microbial colonization of carbonate building rocks in the Romanesque churches of Segovia (Spain). *Science of the Total Environment*, **407**, 1123–1134.
- Dornieden, Th., Gorbushina, A. A., and Krumbein, W. E., 2000. Biodecay of cultural heritage as a space/time-related ecological situation - an evaluation of a series of studies. *International Biodeterioration and Biodegradation*, **46**, 261–270.
- Ehrlich, H. L., 2002. *Geomicrobiology*, 4th edn. New York: Marcel Dekker.
- Golubic, S., Friedman, E., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**, 475–478.
- Gorbushina, A. A., 2007. Life on the Rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Gorbushina, A. A., and Krumbein, W. E., 2000. Subaerial microbial mats and their effects on soil and rock. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 161–170.
- Gorbushina, A. A., Krumbein, W. E., Hamman, C. H., Panina, L., Soukharjevsky, S., and Wollenzien, U., 1993. Role of black fungi in color change and biodeterioration of antique marbles. *Geomicrobiology Journal*, **11**, 205–220.
- Griffin, P. S., Indictor, N., and Koestler, R. J., 1991. The biodeterioration of stone: a review of deterioration mechanisms. *Conservation Case International Biodeterioration*, **28**, 187–207.
- Kaufmann, J., 1960. Corrosion et protection des pierres calcaires des monuments. *Corrosion Anticorrosion*, **8**, 87–95.
- Koestler, R. J., Brimblecomb, P., Camuo, D., Ginell, W., Graedel, T., Leavengood, P., Petushkova, J., Steiger, M., Urzi, C., Verges-Belmin, V., Warscheid, Th., 1994. Group report: How do external environmental factors accelerate change? In Krumbein, W. K., Brimblecombe, P., Cosgrove, D. E., and Staniforth, S. (eds.), *Durability and Change*. Chichester: Wiley, pp. 149–163.
- Krasilnikov, N. A., 1949. The role of microorganisms in the weathering of rocks. I. Microora of the surface layer of rocks. *Microbiologiya*, **18**, 318–323.
- Krumbein, W. E., 1966. Zur Frage der Gesteinsverwitterung (über geochemische und mikrobiologische Bereiche der exogenen Dynamik). Dissertation, Würzburg.
- Krumbein, W. E., and Jens, K., 1981. Biogenic rock varnishes of the Negev desert (Israel) an ecological study of iron and manganese transformation by cyanobacteria and fungi. *Oecologia*, **50**, 25–38.
- Krumbein, W. E., and Schönborn-Krumbein, C. E., 1987. Biogene Bauschäden: Anamnese, Diagnose und Therapie in Bautenschutz und Denkmalpflege. *Bautenschutz-Bausanierung*, **10**, 14–23.
- Krumbein, W. E., and Warscheid, T., 1992. Patina – ein schillernder Begriff. Biologisch beschleunigte Alterung mineralischer Werkstoffe und Baudenkmäler. *Die Geowissenschaften*, **10**, 274–276.
- Krumbein, W. E., Braams, J., and Grote, G., 1993. Anti-Graffiti-Beschichtungen – Haltbarkeit und biologische Wechselwirkungen. *Bautenschutz-Bausanierung*, **16**, 17–21.
- Kurakov, A. V., Somova, N. G., and Ivanovskii, R. N., 1999. Micromycetes populating limestone and red brick surfaces of the Novodevichii Convent Masonry. *Microbiology*, **68**, 232–241.
- Lewis, F. J., May, E., and Bravery, A. F., 1988. Metabolic activities of bacteria isolated from building stone and their relationship to stone decay. In Houghton, D. R., Smith, R. N., and Eggins, H. O. W. (eds.), *Biodeterioration*. London, New York: Elsevier Applied Science, Vol. 7, pp. 107–112.
- Ortega-Calvo, J. J., Arino, X., Stal, L. J., and Saiz-Jimenez, C., 1994. Cyanobacterial sulfate accumulation from black crusts of a historic building. *Geomicrobiology Journal*, **12**, 15–22.
- Ortega-Calvo, J. J., Hernandez-Marine, M., and Saiz-Jimenez, C., 1991. Biodeterioration of building materials by Cyanobacteria and Algae. *International Biodeterioration*, **28**, 165–186.
- Ortega-Morales, O., Guezennec, J., Hernández-Duque, G., Gaylarde, C. C., and Gaylarde, P. M., 2000. Phototrophic biofilms on ancient Mayan buildings in Yucatan, Mexico. *Current Microbiology*, **40**, 81–85.
- Petersen, K., Janssen, D. D., Schostak, V., Bode-Warscheid, K., and Krumbein, W. E., 1991. On the influence of restoration activities on microbial biodeterioration of mural paintings. In Baer, N. S., Sabbioni, C., and Sors, A. I. (eds.), *Proceedings of the European Symposium, Bologna, Italy*, Luxembourg: Butterworth-Heinemann Ltd.
- Pitzurra, L., Moroni, B., Nocentini, A., Sbaraglia, G., Poli, G., and Bistoni, F., 2003. Microbial growth and air pollution in carbonate rock weathering. *International Biodeterioration and Biodegradation*, **52**, 63–68.
- Pochon, J., and Jaton, C., 1967. The role of microbiological agencies in the deterioration of stone. *Chemistry and Industry*, **9**, 1587–1589.
- Reitner, J., and Beimforde, C., 2008. Mikroben und Bernstein – Einblicke in eine wenig verstandene geo-biologische Beziehung. *Jahrbuch der Akademie der Wissenschaften zu Göttingen*. Berlin: Walter de Gruyter GmbH & Co. KG, pp. 188–198.
- Sattler, L., 1992. Wirksamkeitskontrollen an klassischen Methoden der Steinkonservierung. *Die Geowissenschaften*, **10**, 88–89.
- Steiger, M., and Dannecker, W., 1995. Hygroskopische Eigenschaften und Kristallisationsverhalten von Salzgemischen. In *Jahresberichte Steinzerfall – Steinkonservierung, Band 5-1993*. Berlin: Ernst und Sohn Verlag, pp. 115–127.
- Sterflinger, K., and Krumbein, W. E., 1997. Dematiaceous fungi as a major agent for biopitting on Mediterranean marbles and limestones. *Geomicrobiology Journal*, **14**, 219–230.
- Stoodley, P., Sauer, K., Davies, D. G., and Costerton, J. W., 2002. Biofilms as complex differentiated communities. *Annual Reviews in Microbiology*, **56**, 187–209.
- Urzi, C., and De Leo, F., 2007. Evaluation of the efficiency of water-repellent and biocide compounds against microbial colonization of mortars. *International Biodeterioration and Biodegradation*, **60**, 25–34.
- Urzi, C., and Krumbein, W. E., 1994. Microbiological impacts on the cultural heritage. In Krumbein W. E., Brimblecombe, P., Cosgrove, D. E., and Staniforth, S. (eds.), *Durability and Change*. Chichester: Wiley, pp. 107–135.
- Warscheid, Th., 1990. Untersuchungen zur Biodeterioration von Sandsteinen unter besonderer Berücksichtigung der chemoorganotrophen Bakterien. Dissertation, Oldenburg.
- Warscheid, Th., and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, **46**, 343–368.
- Warscheid, Th., Oelting, M., and Krumbein, W. E., 1991. Physico-chemical aspects of biodeterioration processes on rocks with special regard to organic pollution. *International Biodeterioration*, **28**, 37–48.
- Warscheid, Th., Becker, Th., Braams, J., Brüggerhoff, S., Gehrmann, C., Krumbein, W. E., and Petersen, K., 1993. Studies on the temporal development of microbial infection of different types of sedimentary rocks and its effect on the alteration of the physico-chemical properties in building materials. In Thiel,

- M.-J. (ed.), *Conservation of Stone and Other Materials*. London: E & FN Spon, Vol. 1, pp. 303–310.
- Weber, H., 1993. *Allgemeine Mykologie*. Jena: Gustav Fischer Verlag.
- Wendler, E., 1992. Modifizierung von Steinschutzmitteln. *Die Geowissenschaften*, **10**, 290–292.
- Winkler, E. M., 1987. Weathering and weathering rates of natural stone. *Environmental Geology and Water Science*, **9**, 85–92.
- Wolfaardt, G. M., Lawrence, J. R., and Korber, D. R., 1999. Function of EPS. In Wingender, J., Neu, T. R., and Flemming, H. C. (eds.), *Microbial Extracellular Substances (Characterization, Structure and Function)*. Berlin, Heidelberg: Springer, pp. 171–200.
- Zanardini, E., Abbruscato, P., Ghedini, N., Realini, M., and Sorlini, C., 2000. Influence of atmospheric pollutants on the biodeterioration of stone. *International Biodeterioration and Biodegradation*, **45**, 35–42.
- Zoghalmi, K., and Gómez-Gras, D., 2004. Determination of the distribution of consolidants and interpretation of mercury porosimetry data in a sandstone porous network using LSCM. *Microscopy Research and Technique*, **65**, 270–275.

Cross-references

Amber
 Bioerosion
 Biological Volcanic Rock Weathering
 Bioprotection
 Endoliths
 Fungi and Lichens
 Geomycology
 Microbial Biomineralization
 Microbial Degradation
 Microbiocorrosion

BIOEROSION

Aline Tribollet¹, Gudrun Radtke², Stjepko Golubic³

¹Institut de Recherche pour le Développement, Nouméa, Nouvelle-Calédonie, France

²Hessisches Landesamt für Umwelt und Geologie, Wiesbaden, Germany

³Boston University, Boston, MA, USA

Definition

Bioerosion is defined as erosion, i.e., removal and transport of materials by the action of organisms. Bioabrasion refers to removal by mechanical means: scraping, rasping, drilling. Biocorrosion refers to removal by chemical means: etching, dissolving, boring. Bioerosion is often carried out by a combination of bioerosional tools. Bioerosion by microorganisms is referred to as microbioerosion as opposed to macrobioerosion performed by animals and plants. External bioerosion is carried out by organisms that live outside the substrate but remove it in the process of seeking food (grazing). Internal bioerosion is carried out by organisms that excavate the substrate for shelter and live inside as endoliths. They penetrate the substrate actively as euendoliths, or

occupy existing cavities as cryptoendoliths or fissures in the rocks as chasmoendoliths.

Concept of bioerosion

The concept of erosion is usually discussed in geology, particularly in geomorphology, sedimentology, and soil sciences, in reference to degradation and transport of rocks and sediments by environmental forces such as wind, water, or ice. The participation of organisms in these processes and their long-term geological impact are often ignored. The role of organisms in sediment stabilization, organization, and buildup is becoming increasingly recognized as biosedimentation, and so is bioerosion, i.e., the participation of organisms in destruction of rocks and sediments.

Similar to weathering and erosion by environmental forces, the destruction of hard substrates by organisms, i.e., biological erosion or bioerosion (Neumann, 1966) includes two mechanisms: (a) biological corrosion, which refers to destruction of substrates by chemical means introduced by organisms, thus promoting dissolution of solid substrates, and (b) biological abrasion, which refers to mechanical destruction and removal of substrate by organisms (Golubic and Schneider, 1979). Sediment particles are frequently produced during biological abrasion and are redeposited (Neumann, 1966; Schneider and Torunski, 1983).

Bioerosion affects various substrates, most commonly the relatively soluble ones such as carbonates, phosphates, and sulfates. Bioerosion of siliceous rocks occurs at much lower rates and is rarely conspicuous. The objects of bioerosion include solid rocks, sediment particles, and mineralized skeletons of various live and dead organisms. Bioerosion takes place in a wide variety of aquatic and terrestrial environments. Its effects are especially noticeable in coastal carbonate rocks, coral reef structures, and molluscan calcareous skeletons, but are less apparent in skeletons of live corals (Le Campion-Alsumard et al., 1995a), encrusting coralline algae (Pantazidou et al., 2000; Tribollet and Payri, 2001; Ghirardelli, 2002), foraminifera (Golubic et al., 1984), calcareous ooid sand particles (Al-Thukair and Golubic, 1991), and fish teeth (Underwood et al., 1999).

Many researchers describe penetration into hard substrate as boring activity, in contrast to burrowing, which describes digging into loose substrate such as sand and mud; however, some use the term burrowing for penetrating any substrate. Others suggested that activities of bioeroding organisms be distinguished on the basis of their objectives (e.g., food vs. shelter). Many bioeroding organisms are fully or partially embedded in the rocky substrate, occupying an endolithic habitat, while others erode the substrate from outside. Accordingly, they have been distinguished as internal versus external bioeroders. Many investigators view bioerosion as behavior (Boucot, 1990) and relate bioeroding organisms to the traces they leave in modern and ancient hard substrates. Researchers

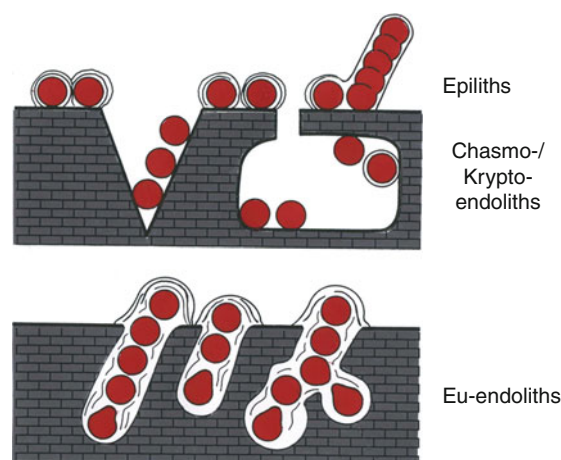
employ a variety of experimental strategies and preparation techniques, including x-ray visualization (Bromley, 1970; Sammarco and Risk, 1990), replication (casting) of borings in polymerizing resins (Golubic et al., 1970), and digital imaging (Tapanila, 2008).

The research of geological history of bioerosion relies on traces left by bioeroding organisms in ancient sediments, so that the study of trace fossils constitutes a separate discipline with its own methods and rules of nomenclature. According to the rules of the International Code of Zoological Nomenclature (ICZN), which governs trace fossil investigations, the traces have to be named separate from the organisms that make them (Bromley, 1990; Bertling et al., 2006). These studies are published in special journals (e.g., *Ichnos*, *Facies*), and as proceedings of symposia and compendia (Carriker et al., 1969; Crimes and Harper, 1970; Frey, 1975; Bromley, 1990; Miller, 2007). The geological roles of bioeroding organisms are periodically reviewed regarding: (a) their diversity and evolution, (b) behavior, (c) recognition and classification of their traces and imprints in the substrate, (d) their impact on sedimentary systems, and (e) other environmental consequences of their activities (Hutchings, 1986; Radtke et al., 1997; Perry and Hepburn, 2008; Tribollet, 2008b). Bibliographies on past and present bioerosion are also displayed on line (see Cross-references below).

Agents of bioerosion

Agents of bioerosion cover a wide range of sizes. Organisms that actively penetrate hard substrates are for practical reasons treated separately as microborers (size <100 µm) and macroborers (size >100 µm). In addition to macro- and microboring organisms, which predominantly act as biocorroding agents (internal bioeroders), bioerosion is strongly affected by bioabrasion by various grazing invertebrate and vertebrate animals (external bioeroders). Predators able to penetrate shells and attack the animal inside constitute a special group of bioeroders that includes predatory snails and cephalopods (called “predators” in this chapter). Bioeroding organisms are metabolically diverse; they employ different feeding strategies and thus affect substrates differently. Some organisms penetrate or perforate hard substrates in search of food, such as fungi and predatory snails. Others penetrate the substrate and use it as a habitat while depending on external energy sources. This is the case with boring phototrophs, such as cyanobacteria, algae, and lichens as well as with filter-feeding invertebrates.

Organisms that occupy the interior of rocky substrates are called endoliths, as opposed to epiliths that adhere to external rock surfaces. At the microscopic scale, hard substrates provide different habitats, which are occupied by different endolithic microorganisms (Figure 1). Cracks and fissures in rocks are occupied by chasmoendoliths, the existing pore spaces between grains in sandstones or structural pores in skeletons are occupied by



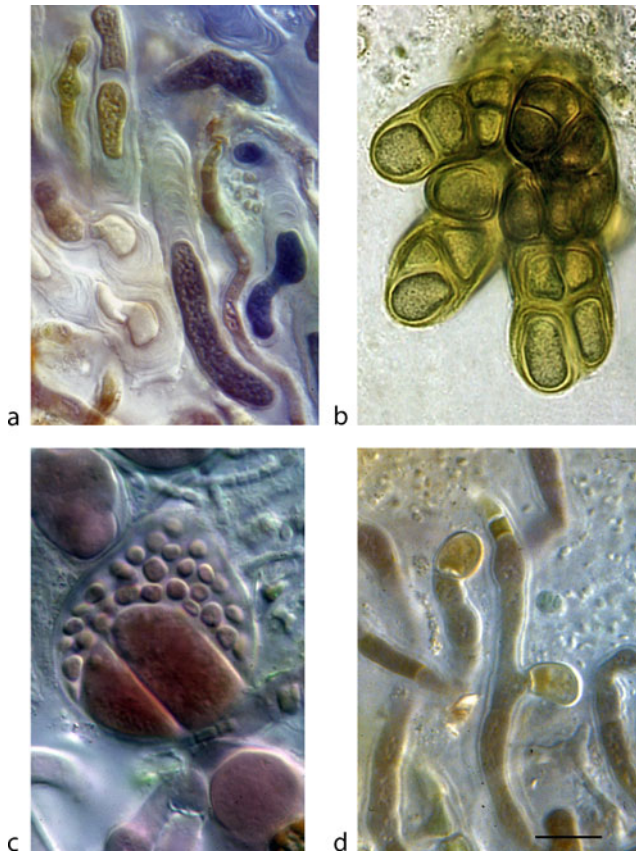
Bioerosion, Figure 1 Microorganisms of a lithobiontic niche: Epiliths occupy the surface of the rock by forming a biofilm, mat, or turf, while endoliths occupy the interior of the rock: chasmoendoliths line the walls of cracks and fissures, cryptoendoliths fill preexisting textural spaces and pores within the rock, and euendoliths penetrate the carbonate substrate and live in the pores of their own making. (Modified from Golubic et al., 1981.)

cryptoendoliths; only euendoliths or microborers actively dissolve substrates and occupy spaces of their own making (Golubic et al., 1981).

The euendoliths are equipped with special chemical or mechanical means of bioerosion, although other inhabitants of the rock interiors may also affect the substrate by modifying the chemistry of the surrounding interstitial waters. Euendoliths, both micro- and macroborers, produce boreholes and tunnels in the substrate that comply closely to the outlines of their bodies (Pohowsky, 1978; Radtke and Golubic, 2005). At a finer scale, they leave etch marks that comply with and reflect crystalline composition of the rock (Golubic et al., 1975). Both, micro- and macroborings preserve well in the fossil record and provide valuable information about past environments (Vogel et al., 1987; Glaub et al., 2007; Bromley, 2004).

Microborers comprise phototrophs such as cyanobacteria and algae (mostly chlorophytes and rhodophytes) and organotrophs (heterotrophs) including fungi, foraminifera, and other, mostly unidentified prokaryotic and eukaryotic light-independent organisms.

Euendolithic cyanobacteria (Figure 2) include members of all orders, Chroococcales (coccoid forms) (Figure 2a–c), Oscillatoriales (non-heterocystous filamentous forms), Nostocales, and Stigonematales (unbranched and branched heterocystous filamentous forms) (Figure 2d). Euendolithic algae include septate and siphonal chlorophytes (Figure 3) and endolithic “Conchocelis” stages in the life cycle of some rhodophytes (Bangiales). Euendolithic fungi include lower (Chytrids) and higher fungi (Ascomycetes, Basidiomycetes), but many are known only by the borings they



Bioerosion, Figure 2 Common euendolithic cyanobacteria. (a) *Solentia achromatica* Ercegovic, common in coastal rock pools, Adriatic Sea, Croatia; (b) *Hyella balani* Lehmann, extracted from a shell, lower intertidal zone, Woods Hole, MA; (c) *Cyanosaccus piriformis* Golubic et Lukas, Atlantic Ocean off the Florida coast; (d) *Mastigocoleus testarum* Lagerheim, a branched, heterocystous, cyanobacterium; note the terminal nitrogen-fixing heterocysts with pigmentation different from that of normal carbon-fixing trichome cells, extracted from a shell, coast at La Parguera, Puerto Rico. Scale bar in d is 10 μ m long valid for all pictures a–d.

produce (Figure 4). Euendolithic and partially endolithic mode of life is known for benthic foraminifera, including fossil forms, and their boring traces are common. However, little is known about the biology or taxonomic identity of euendolithic foraminifera (Bromley et al., 2007).

The mechanisms of carbonate dissolution in the process of penetration into rocky substrate by microbial euendoliths are poorly understood. The observed variations in boring behavior indicate the existence of different mechanisms, which may include calcium pumps (Garcia-Pichel, 2006), respiratory carbonic acid (Tribollet, 2008b), or enzymes such as carbonic anhydrase. Microborers are mainly agents of biocorrosion.

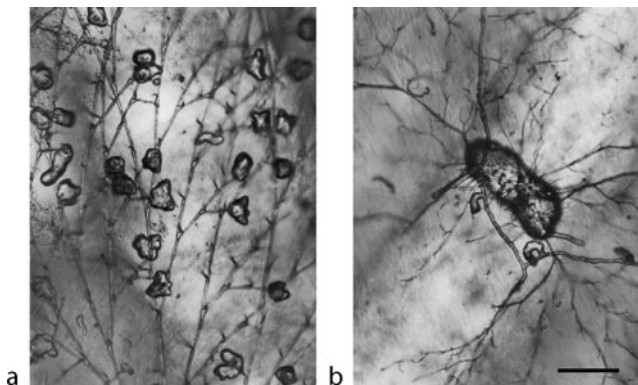
Macrobiorers include specialized sponges (Figure 5), such as clionids (Rützler, 2002), various groups of worms including polychaetes (Wielgus et al., 2006), phoronids (Plewes, 1994) and sipunculids (Gherardi and Bosence, 2001), and other invertebrates including ctenostome bryozoans (Pohowsky, 1978; Todd, 2000), acrothoracic cirripedia (barnacles) (Kolbasov, 2000), and bivalves, such as lithophagid mussels (Figure 6) (Scott and Risk, 1988; Kleemann, 1996).

Boring sponges employ the enzyme carbonic anhydrase for biocorrosion combined with mechanical cleavage of cell-size particles, which are expelled into the environment (Pomponi, 1980). The research on boring sponges was recently reviewed by Schönberg (2008). Polychaetes and pholadid mussels use a combination of chemical and mechanical means of penetration into rocks and shells. For example, polychaetes involve setae for mechanical removal of chemically loosened substrate, whereas the pholadid mussels use rotation movement of their shells (Hutchings, 1986).

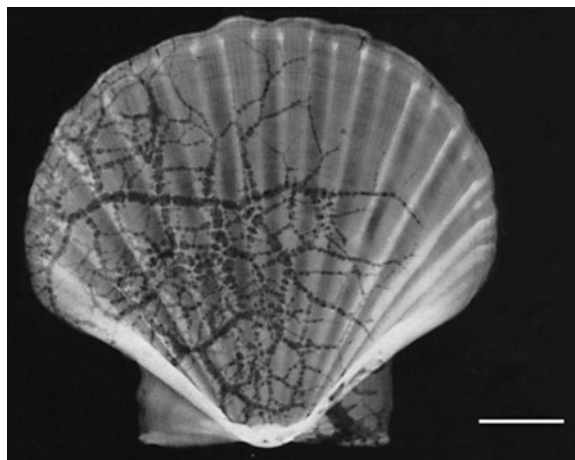
Grazers are the principal agents of biological abrasion (external bioerosion), and comprise gastropods (Schneider and Torunski, 1983; Radtke et al., 1996), polyplacophores (Rasmussen and Frankenberg, 1990), echinoderms (Carreiro-Silva and McClanahan, 2001), and fish (Bruggemann et al., 1994), especially parrotfish on coral reefs (Hoey and Bellwood, 2008). Grazers are



Bioerosion, Figure 3 Resin replica of the complex borings *Ichneoreticulina elegans* produced by the siphonal chlorophyte *Ostreobium quekettii* boring into experimentally exposed calcite spar. Note the prolific tree-like branching patterns with occasional terminal swellings. Scale bar is 100 μ m long. (Courtesy of T. Le Campion-Alsumard and S. Golubic.)



Bioerosion, Figure 4 Euendolithic fungi inside transparent shell fragments, Adriatic Sea, 50 m depth. (a) Forked branches of a fungus identified as *Phytophthora* sp. forms terminal sporangial swellings described as *Saccomorpha terminalis*. (b) A lower fungus with sporangial swellings, from which hyphae radiate outward. Scale bar is 50 μ m long.



Bioerosion, Figure 5 The boring sponge *Cliona vestifica* invading the shell of *Pecten maximus*. X-ray image. Scale bar is 1 cm long. (Modified from Bromley, 1970.)

not endolithic and they do not perforate shells. Instead, they remove layers of the mineral substrate with their specialized hard mouth parts (e.g., “Aristotle’s lantern” of sea urchins, magnetite-enforced radulas of snails and beak-like jaws of parrotfish) while feeding on epilithic algal turf and also removing external and internal microbial biofilms (Golubic and Schneider, 2003).

Predators that successfully attack invertebrates protected by shells include snails of the families Naticidae, Muricidae, and five other families (Walker, 2007), as well as foraminifera (Cedhagen, 1994). Predatory snails use a combination of mechanical and chemical means of making a perforation in the shell of their prey and killing the prey (Carriker, 1969). Euendolithic fungi, which grow inside coral skeletons, from where they regularly attack



Bioerosion, Figure 6 Pholadid bivalves penetrating the coral skeleton of *Porites*. Note that the hole is commensurate with the size and shape of the mussel. Scale bar is 1 cm long.

coral polyps, can also be considered predatory. Fungi may compromise the coral’s ability to overcome environmental stress conditions or to resist diseases inflicted by other causative agents (Le Campion-Alsumard et al., 1995b; Benthis et al., 2000).

Diversity of bioeroding organisms

The ability to exploit resources by eroding rocks and sediment particles evolved in various prokaryotic and eukaryotic groups of organisms, often enhanced by their mutual interactions. Microbial euendoliths contribute significantly to the overall benthic primary production and support a variety of grazing invertebrates and vertebrates. Their continuing activity, however, depends on grazing, which maintains optimal light access to rocky surfaces.

Microbial euendoliths or microborers are ubiquitous. They are distributed globally: in marine (Tribollet, 2008b), freshwater (Schneider and Le Campion-Alsumard, 1999; Anagnostidis and Pantazidou, 1988), and terrestrial environments (lichens) (Golubic and Schneider, 2003). However, they are most diversified in marine environments, where they occupy a variety of ecological niches that range in distribution from the supratidal coastal spray zone to the abyssal depths. Macroborers in carbonate substrates are predominantly marine. There are a number of predatory snails in terrestrial and freshwater habitat, but shell perforation is known only for marine species.

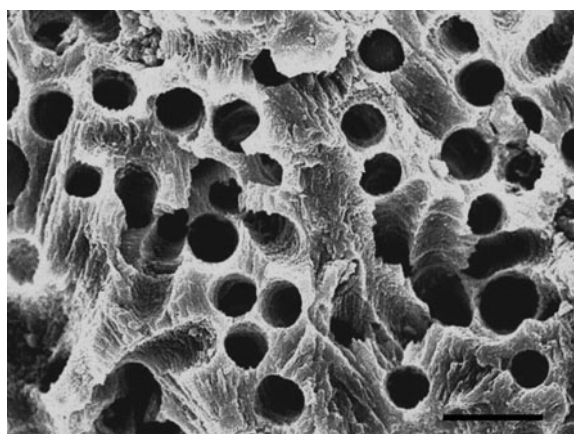
Bioeroding organisms are also highly diversified and specialized, so that different environmental settings, different latitudes and regions are settled by different species and genera. Their regional distribution based on taxonomic distinctions and evolutionary history is beyond the scope of the present article. Instead, we will concentrate on environmental conditions and constraints that determine distribution of particular assemblages of euendoliths and their traces, so that they can be used as environmental and, by extension, as paleoenvironmental

and paleobathymetric indicators (Golubic et al., 1975; Vogel et al., 1987; Glaub et al., 2001, 2007). Further we will present case histories that illustrate synergistic interactions among bioeroding organisms that resulted from their coevolution.

Depth distribution of microbial euendoliths

Light is a critical factor for depth distribution of phototrophic euendolithic organisms, whereas the organotrophs are light-independent and depend on organic food supply. Light is absorbed selectively by the water column as well by the organisms and their pigment systems. The combination of both defines the euphotic and disphotic environments, and triggers adaptations to different light intensities and spectral qualities, as well as responses to quantitative and qualitative shading, resulting in microbial arrangement in strata. Other factors, such as water supply and chemistry, including redox potential and nutrient availability pose further limitations, challenges, and opportunities to which the organisms respond.

The intertidal ranges of carbonate coasts are densely populated by assemblages of endolithic cyanobacteria (Le Campion-Alsumard, 1979). More than half a million euendoliths commonly occupy a square centimeter of limestone rock surface (Figure 7) (Schneider and Le Campion-Alsumard, 1999). Intertidal barnacles and mussels are also subject to cyanobacterial borings whenever the protective periostracum layer of their shells is damaged. Extensive boring by euendolithic cyanobacteria has been shown to inflict lethal damage on mussels by destroying their shells (Kaehler and McQuaid, 1999). An assemblage of intertidal cyanobacteria appears to have cosmopolitan distribution with characteristic morphotypes of *Hyella balani* (Figure 2b), *H. caespitosa*, *Plectonema terebrans*, *Mastigocoleus testarum* (Figure 2d), and *Kyrtuthrix dalmatica* (Figure 8), which



Bioerosion, Figure 7 Cylindrical boreholes produced by filamentous cyanobacteria oriented perpendicular to the surface of dead coral skeleton. Coral rubble in the intertidal range. Scale bar is 10 μ m long. (Compare scale bars in Figures 6 and 7).

are common on Mediterranean coasts (Le Campion-Alsumard, 1979; Le Campion-Alsumard and Golubic, 1985) and also on the coast of South Africa (Kaehler and McQuaid, 1999). Similar assemblages of euendolithic organisms and their traces have been found at different latitudes including northern seas (Wisshak et al., 2005), but their highest diversity and the highest rates of bioerosion have been recorded in lower latitudes (Wisshak, 2006; Tribollet and Golubic, 2005).

Special assemblages of euendolithic cyanobacteria occupy subtidal carbonates. In shoaling sands (ooids), mollusk shells, and shell fragments in subtropical regions of the Bahamas, and the Arabian Gulf, assemblages are dominated by a number of different cyanobacteria of the genus *Hyella* (Al-Thukair and Golubic, 1991; Al-Thukair et al., 1994) that produce boring traces of the ichnogenus *Fascichnus* (Figure 9) (Radtke and Golubic, 2005). Relatively stable carbonate substrates such as bivalve shells, coral skeletons, and carbonate hard grounds in illuminated marine waters are dominated by septate euendolithic chlorophytes, *Phaeophila* and *Eugomontia*, which produce traces of the ichnogenus *Rhopalia* (Radtke, 1991; Golubic and Radtke, 2008) and by the siphonal chlorophyte, *Ostreobium quekettii*, which produces trace *Ichnoreticulina* (Figure 3) (Radtke and Golubic, 2005). Similar assemblages of euendoliths and their traces have been studied in profiles across coral reefs in Mexico (Günther, 1990) and Jamaica (Perry, 1998).

Ostreobium reaches considerable depths in clear oligotrophic tropical seas. This endolith dominates carbonate substrates between 10 and 150 m and is still present in the rocks at 300 m (Vogel et al., 2000). The maximum



Bioerosion, Figure 8 Dense populations of the cyanobacterium *Kyrtuthrix dalmatica* Ercegovic presented in situ. Double embedded preparation renders the rock transparent (lower part of the picture). The line of the rock surface is indicated by arrows. Intertidal zone on limestone cliffs at Marseille, France. Scale bar is 50 μ m long. (Courtesy of T. Le Campion-Alsumard and J. Schneider.)

depth for phototrophic euendoliths is reached by the cyanobacterium *Plectonema* (*Leptolyngbya*) *terebrans*, which was identified alive at 370 m depth (Lukas, 1978). Conchocelis stages of bangialean rhodophytes penetrate carbonate substrates to a depth of 78 m in the North Sea (Clokie et al., 1981). The actual depth of occurrence of all phototrophic euendolithic organisms depends on light penetration in the waters which can be affected by environmental factors such as terrigenous inputs of nutrients and sediments, and eutrophication in general. Depth zones have, therefore been defined by Glaub et al. (2001) in relative terms as: “shallow euphotic zone,” divided in three stages to cover the supratidal, intertidal, and shallow subtidal ranges, “deep euphotic zone,” “disphotic zone” (defined by light penetration between 1% and 0.001% of the incoming illumination at the surface), and “aphotic zone” beneath that.

Euendolithic fungi penetrate carbonate substrates that contain organic matter. They permeate carbonate substrates with their thin, branched hyphae (Figure 4) (Mao-Che et al., 1996). Fungi also parasitize on euendolithic algae (Bentis et al., 2000; Priess et al., 2000; Golubic et al., 2005) and form lichens with cyanobacteria (Le Campion-Alsumard and Golubic, 1985) and some chlorophytes (Golubic and Schneider, 2003). Euendolithic organotrophs such as bacteria and fungi may occur at any depth, including abyssal ranges (Golubic et al., 1984; Golubic et al., 2005). Their traces have been described in deep-water coral reefs (Wisshak, 2006).

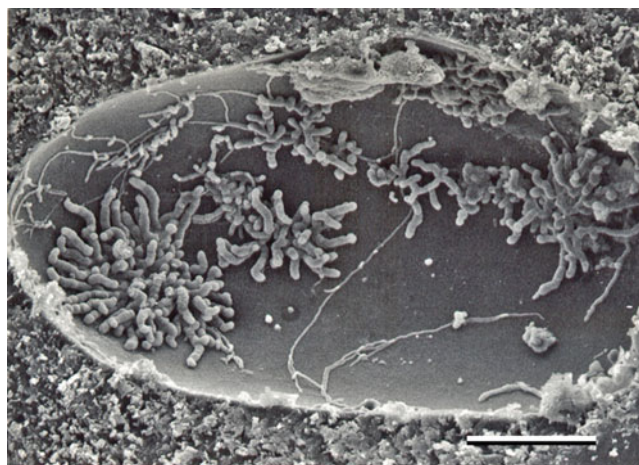
Phototrophic euendoliths in corals

Distribution of phototrophic euendoliths deep into the disphotic zone of the oceans is paralleled by the depth of their penetration into carbonate substrates. *Plectonema*

terebrans and *Ostreobium quekettii* penetrate over 1 mm deep into live and dead coral skeletons (Tribollet, 2008a). These euendolithic phototrophs can survive and grow at light intensities as low as 0.04% of the surface illumination and are, therefore, considered oligophotic (Fork and Larkum, 1989; Shashar and Stambler, 1992; Schlichter et al., 1997). The ability to harvest energy by extending the window of photosynthetically useful radiation is facilitated in cyanobacteria by the auxiliary phycobilin pigments, including optional chromatic adaptation in some taxa. Different strategies are employed by *Ostreobium*. This green alga can increase the proportion of Chlorophyll-b (Schlichter et al., 1997), or use light from the long-wave portion of the solar spectrum (Koehne et al., 1999; Wilhelm and Jakob, 2006).

The ecological niche that euendolithic algae occupy in the carbonate skeletons of live calcifying organisms such as growing corals requires a positive phototropic growth orientation and a rate of growth and carbonate penetration that keeps up with the host's accretion rates. This condition is fulfilled by *Plectonema terebrans* and *Ostreobium quekettii* (Figure 10), and less frequently by Conchocelis stages in the life cycle of bangialean rhodophytes. *Ostreobium quekettii* is omnipresent in live corals (Lukas, 1973, 1974) as euendolith and as cryptoendolith (Schroeder, 1972) (Figure 11).

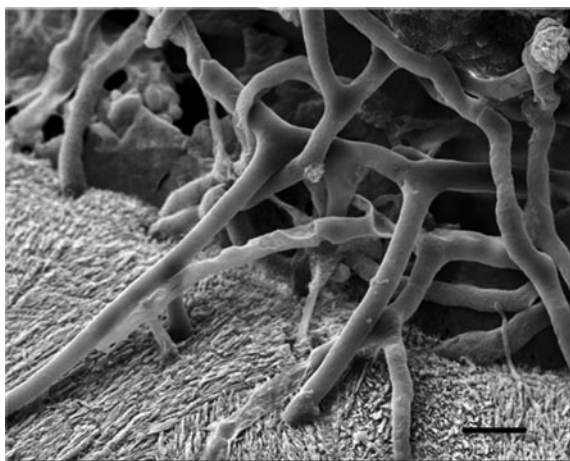
The distribution and abundance of *Ostreobium* in live corals and its possible interactions with the coral host has been the subject of several studies, leading to the



Bioerosion, Figure 9 Resin replicas of cyanobacterial borings in an ooid sand grain, Joultar's Key, Andros Island, Bahamas. The boring patterns are ichnospecies *Fascichnus frutex* produced by colonies of the coccoid cyanobacterium *Hyella gigas*. Scale bar is 50 μ m long.



Bioerosion, Figure 10 Two microboring organisms sharing a coral skeleton: The yellow-green side (left) is permeated by the chlorophyte *Ostreobium quekettii*, while the other side is red from the cyanobacterium *Plectonema* (*Leptolyngbya*) *terebrans*. The coral was growing in deep waters of a fjord on the coast of Chile. (Courtesy of Vreni Häusermann and Günter Försterra.)



Bioerosion, Figure 11 Network of resin-cast tunnels bored through the coral skeleton of *Porites lobata* from Kaneohe Bay (Hawaii) by *Ostreobium quekettii*, partially exposed by dissolving the skeleton with diluted HCl. Scale bar is 10 μ m long. (Courtesy of A. Tribollet.)

suggestion of a symbiotic relationship. This proposal was supported by documenting an exchange of nutrients and metabolic products between the two partners living in close proximity (Shashar and Stambler, 1992; Schlichter et al., 1997). Chemistry of pore waters in corals was reviewed briefly by Risk and Muller (1983). Such an ectosymbiotic relationship between euendoliths and live coral polyps may help the survival and recovery of the latter following the stress of coral bleaching events (Fine et al., 2006). *Ostreobium* responds to coral bleaching first by photoinhibition, followed by rapid accommodation to higher light intensities. This response leads to an increase in growth, identified as a bloom of *Ostreobium* (Fine et al., 2006). Uneven density of *Ostreobium* filaments and their pigmentation in coral skeletons is expressed as green bands (Lukas, 1973), which were interpreted as periodic blooms of the alga (Highsmith, 1981). Le Campion-Alsumard et al. (1995a) showed that euendolithic algae in coral skeletons tend to increase in density beneath the polyp layer whenever coral growth slows down (Figure 12). It is, therefore, conceivable that the green bands studied by Lukas and illustrated by Le Campion-Alsumard et al. (1995a, Fig. 7) represent a record of past coral bleaching events.

Organotrophic euendoliths in corals

Regular inhabitants of coral skeletons other than phototrophs are euendolithic fungi. Their penetration depends on the distribution of organic matter in the bored substrate, including that which was contributed by euendolithic phototrophs. It has also been documented that fungi attack euendolithic algae in coral skeleton as well as the live coral polyps (Priess et al., 2000; Benthis et al., 2000). To protect itself, the coral responds by initiating deposition of a dense carbonate layer over the fungal

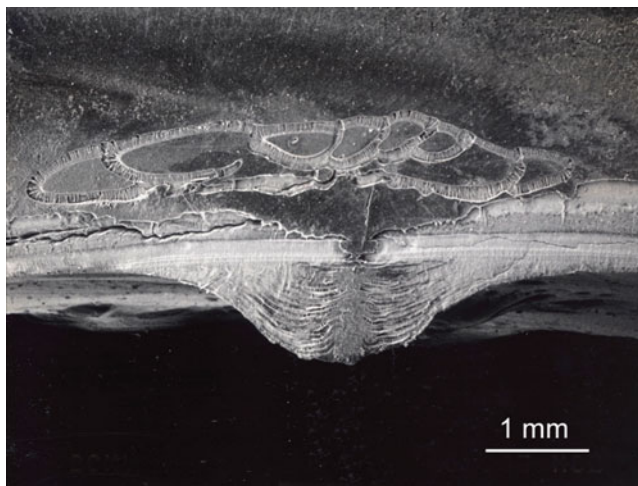


Bioerosion, Figure 12 A head of a hermatypic coral *Porites lutea* cut open to reveal perforations by a green zone of phototrophic microborer *Ostreobium quekettii*, underneath the polyps, and deeper inside the skeleton a macroborer, *Cliona* sp., which undermines the coral base. Lee Stocking Island, Bahamas.

borehole. As the fungus continues to penetrate, such deposits tend to accumulate, building conical structures reminiscent of deposition of pearls around foreign bodies by oysters (Le Campion-Alsumard et al., 1995b). The reported interrelations between fungi and corals (Priess et al., 2000; Benthis et al., 2000) have been recently complemented experimentally by demonstrating stimulation of cultured coral skeletogenic cells by the basidiomycete fungus *Cryptococcus*, independently isolated from a coral skeleton (Domart-Coulon et al., 2004). Several strains of ascomycete fungi were isolated earlier from corals in conjunction with the study of their parasitic role (Kendrick et al., 1982).

Boring foraminifera

Carbonate penetration by benthic foraminifera is a widespread but little known activity. Some euendolithic foraminifera construct their tests inside their boreholes (Cherchi and Schroeder, 1991), but more often, only boring traces of soft-bodied parts remain inside carbonate substrate, leaving only spine-like traces of their pseudopodia. Foraminifera include taxa that are strictly organotrophic as well as those that contain phototrophic endosymbionts (*Symbiodinium*, zooxanthellae). The latter group is expected to be limited in distribution to illuminated parts of the ocean, but their relation to microboring activity is not known. Boring traces of foraminifera overlap in size the ranges usually assigned to micro- or macroendoliths. Most of them are small, possibly temporary anchoring devices. Boring foraminifera include also large forms (Rosalinidae) that parasitize on mollusks by perforating their shells (Smyth, 1988; Cedhagen, 1994). The mollusk responds to the attack in a way similar to coral's defense from predatory fungi by producing layers



Bioerosion, Figure 13 Predatory foraminifer *Rosalina* sp. attacking a mussel. The protist has perforated the shell as well as layer by layer of the nacreous pearl-like cone that the mollusk has produced in defense. Polished and lightly etched petrographic section. SEM of the MCZ, Harvard University.

of nacre over the area of penetration (Figure 13) (reviewed by Beuck et al., 2008).

The naming of boring foraminifera became a subject of controversy when the organism described as *Globodendrina*, on the basis of the interpretation of its fossil trace (Plewes et al., 1993), was subsequently formally described as the trace *Semidendrina* (Bromley et al., 2007).

Distribution of macroborers

Marine macroborers are mostly filter-feeders, and thus need to maintain contact with the substrate surface in order to access food from the surrounding water column. Accordingly, these internal bioeroders tend to thrive in eutrophic coastal waters (Risk et al., 1995), and are less abundant in the offshore oligotrophic settings (Tribollet and Golubic, 2005), with particularly strong offshore decline recorded for the abundance of boring bivalves (Sammarco and Risk, 1990).

Depth distribution of macroboring organisms has not been assessed as vigorously as for microboring organisms. The boring sponges seem to show an increase in abundance with depth within the range between 10 and 30 m (Zundelevich et al., 2007). Most work on macroboring organisms concentrates on shallow marine habitats and reefs (Mariani et al., 2000). Macroboring traces, however, have been also observed in deep-sea corals and shells (Freiwald et al., 1997; Wisshak, 2006).

Depth of penetration of macroborers into substrates varies, depending on the organism and its size. Small polychaetes, larvae, and juveniles do not penetrate deep inside substrates, and the depth of penetration and volume of the substrate excavated increase with age of the macroborer (Figure 6). Some macroborers are nearly

microscopic in size. The zooetia of boring bryozoa, for example, are connected by stolones that bore tunnels close in dimensions to those of endolithic algae and to borings of microbial heterotrophs described as *Orthogonum* (Radtke, 1991). Larger euendoliths include cirriped crustaceans and various boring worms, such as polychaetes, phoronids, and sipunculids. These borers remove relatively small amounts of substrate due to their small size when their population density is low. In higher concentrations, they are known to effectively damage mollusk shells (Bergman et al., 1982).

Interaction between macroendoliths and their coral hosts has produced distinctive patterns and taphonomic alterations (Scoffin and Bradshaw, 2000), analogous to distinction between live and dead corals shown for microbial euendoliths. The exceptionally pervasive macroborers are clionids (Mariani et al., 2000), which produce extensive branched and interconnected systems of galleries within carbonate substrates (Figure 5) while communicating with the surface by numerous small openings; other boring sponges are partially endolithic with bodies that overgrow the substrate as well as penetrate inside (Rützler, 2002). Clionid sponges are known to attack the substrate at the cellular level by carving out silt-to sand-sized chips that are then expelled into the environment contributing to formation of fine-grained sediments (Pomponi, 1980). However, the chemical dissolution that accompanies this process removes more than three times the amount of carbonate than is removed by the mechanical chip removal (Zundelevich et al., 2007). Traces of sponge borings are known by the name of *Entobia* (Bromley and D'Alessandro, 1989), which are characterized by species-specific boring patterns. Like microborers and sponges, other macroborers, such as bryozoans, cirripeds, and bivalves excavate spaces that comply closely with the outlines of their bodies (e.g., Hutchings, 1986; Scott and Risk, 1988). All these macroboring organisms preserve well in the fossil record and provide useful information for paleoecological research.

Bioerosion over geological time

Commonly asked questions about the euendolithic mode of life are: Why do they bore? When and how did this habit evolve, and which selective pressures supported that habit. An early hypothesis that microbial euendoliths evolved seeking shelter to escape the grazing metazoans when those evolved around 540 million years ago, was rejected since Proterozoic euendoliths were discovered in much older strata (Campbell, 1982). Entire assemblages of fossil euendolithic cyanobacteria have subsequently been discovered in Neoproterozoic silicified ooids (Knoll et al., 1986). The oldest microbial euendoliths were found to bore ancient stromatolites in over 1,500 Myrs. old rocks (Zhang and Golubic, 1987).

The observation that many euendoliths enter the substrate via a small opening, from which they expand laterally, i.e., parallel to the surface, suggests that they

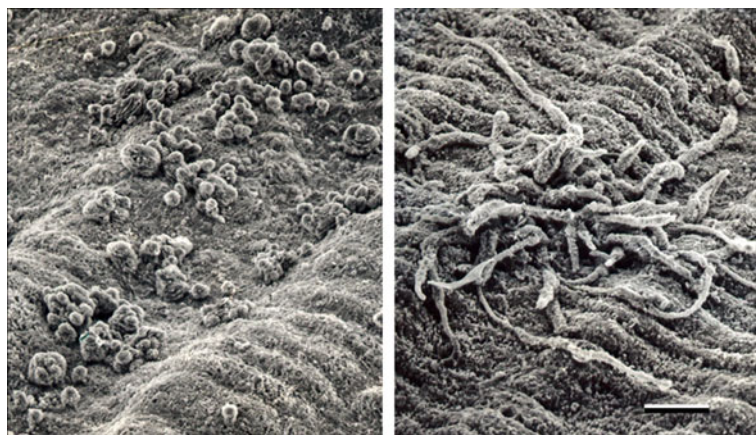
successfully avoid competition with epiliths for settlement ground. The phototrophic euendoliths evolved efficient light-harvesting systems to operate at low light levels exploiting different spectral ranges. This adaptation is important because the light available to euendoliths changes qualitatively and quantitatively as it is filtered by the water column, mineral substrate, coral endosymbionts, and/or epilithic turf and crusts (Fork and Larkum, 1989; Schlichter et al., 1997; Koehne et al., 1999).

The evolution of invertebrates with mineralized skeletons in the course of the early Phanerozoic provided microbial euendoliths with new substrates. Euendoliths have occupied these substrates successfully, leaving a wealth of microboring traces throughout the Phanerozoic (Glaub and Vogel, 2004; Glaub et al., 2007). Organically preserved euendolithic rhodophytes were found occupying crinoid ossicles in rocks of Upper Silurian age (Kazmierczak and Golubic, 1976). They were identified and described as *Palaeoconchocelis starmachii* (Campbell, 1980). Many microborings remained preserved as natural casts due to differential solubility of borehole fill versus bored matrix (e.g., Harris et al., 1979); however, surprisingly high number of boreholes remained empty, so that they have been successfully cast by polymerizing resins and observed by scanning electron microscopy (Golubic et al., 1970). This finding illustrates the role of microborers in contributing to the microporosity of rocks, which serve as petroleum reservoirs (Cantrell and Hagerty, 1999).

Early diversification of microboring organisms, which was initiated during Proterozoic, continued throughout the Phanerozoic. In Middle Cambrian, both body and trace fossils of microbial endoliths were present (Stockfors and Peel, 2005), and entire microboring assemblages appear to

be well-established by the Ordovician times (Figure 14), with about 35% of fossil taxa that survived to the present; this record also includes the first occurrence of borings attributable to euendolithic green alga *Ostreobium* and chytrid fungi (Glaub and Vogel, 2004). In general, microboring traces constitute attractive markers for identification of paleoenvironments (bio- and litho-facies), but are less reliable as stratigraphic markers. Although microborers in their protected niches persisted through periods of major extinction, they seem to show bursts of diversification at the base of the Mesozoic and Cenozoic eras (Glaub and Vogel, 2004).

Evidence of predatory macroboring has been found in late Proterozoic strata associated with the evolution of the earliest exoskeletal organisms (Bengtson and Zhao, 1992). The diversification of macroboring organisms proceeded during the early Paleozoic when representatives of most animal phyla appeared in the fossil record (Palmer and Plewes, 1993; Wilson, 2007). The sheltered habitat that macroendoliths occupy does offer an advantage and protection from predators and may have been a strategy favored by the early selective pressure. An overview of the evolutionary history of macroborers is provided by Taylor and Wilson (2003), and the use of macroborers in stratigraphy is discussed by Bromley (2004). Boring traces of sponges and worms (e.g., *Tripanites*) were observed from the Cambrian on. Macroborers diversified thereafter and became well-established by early Ordovician times (Ekdale and Bromley, 2001) when ctenostome boring bryozoans also occurred for the first time in addition to boring sponges and worms (Mayoral, 1991). Small stellate *Dendrina*-type borings diversified in Devonian (Vogel et al., 1987) and later in the Cretaceous (Hofmann, 1996). These are now attributed to boring foraminifera (Bromley et al., 2007).

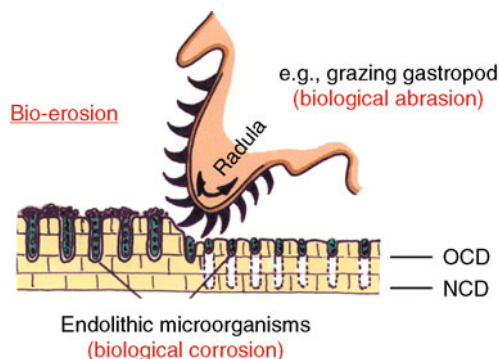


Bioerosion, Figure 14 Resin replicas of borings in late Ordovician brachiopod *Raphinesquiana alternata* from the shallow marine facies of Tanner Formation, Richmond Group, southeastern Indiana, USA. Two out of four boring morphotypes produced by phototrophic euendoliths include: coccoid cyanobacteria (left) and filamentous algae with changing diameter, a possible chlorophytes. Scale bar is 100 μ m long. (From Golubic, S., Knoll, A. H., and Rann, W., 1980. Morphometry of late Ordovician microbial borings, American Association of Petroleum Geologists, Bulletin, 64, 713.)

The first borings attributed to endolithic mussels appeared in the Carboniferous, whereas the first boring cirripedia were documented in Permian strata (Simonsen and Cuffey, 1980). Modern type macroboring communities became established during the Jurassic times (Fürsich et al., 1994).

Synergistic relations among agents of bioerosion

Phototrophic microbial euendoliths are distributed over large areas of coastal limestone, coral rubble, and other carbonate substrates in illuminated marine benthic habitats. Their growth and substrate penetration stabilize at the light level of compensation (where the rates of photosynthetic activity equal respiration). If left alone, the bioerosion by phototrophic euendoliths would remain limited to a few millimeters at the substrate surface. However, as primary producers, euendolithic cyanobacteria and algae attract and support a variety of grazing animals, which periodically remove layers of carbonate substrate together with the resident epiliths and some endoliths. This synergism between euendoliths and their grazers converts an intrinsically self-limited process into a progressive one with significant bioerosive impact (Schneider and Torunski, 1983). For example, the removal of a thin surface layer of the rock by hard, magnetite reinforced radula of a grazing gastropod (Figure 15) causes a displacement of the compensation depth for phototrophs deeper into the rock, leading to a resumption of boring. With continuing grazing, the horizon with euendoliths moves like a front through the rock. The abraded carbonate particles break along cyanobacterial perforations, as they pass through the digestive system and can be recognized in the sediment (Figure 16). The interaction between microborers and their grazers degrade coastal rocks and contribute to

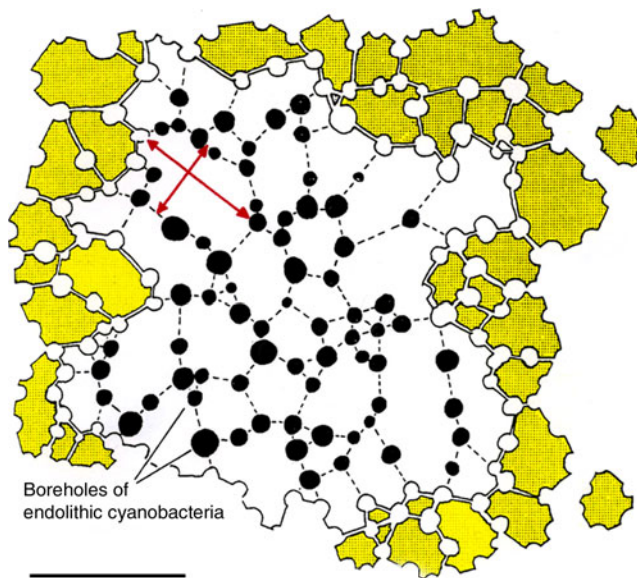


Bioerosion, Figure 15 The synergistic relation between microboring phototrophs and their mollusk grazers. Penetration in the rock is limited by light penetration and stops at the depth of compensation, where respiration equals photosynthesis. As grazers remove a layer of rock the light penetrates deeper, and the boring proceeds from the old depth of compensation (ODC) to a deeper new depth level (NDC), and progresses further with each passage of the grazer. (Modified from Schneider and Torunski, 1983.)

fine-grain sediment accumulation in the environment. A different mechanism with similar results was described for macroboring clionid sponges (Schneider and Torunski, 1983).

Grazing is a normal condition on coral reefs, so that the composition, diversity, and integrity of primary producers, which coevolved with the grazers, also depend on them. The rates of grazing and microbioerosion by euendoliths are positively correlated (Tribollet and Golubic, 2005). Moderate rates of grazing appear to have a regulatory effect by promoting high productivity-to-biomass ratio among the epilithic and endolithic cyanobacteria and algae (Vooren, 1981). In addition, moderate grazing provides new settling grounds for endoliths and coral larvae (Sammarco, 1980). The zone of active microbioerosion at any time contains only the “residual standing crop” of microborers, which does not include the microbial contribution already removed by grazers. These dynamic relations need to be taken into account when rates of bioerosion are calculated and attributed to different components of the system (Chazottes et al., 1995; Tribollet and Golubic, 2005).

Without grazing, the reefs would be overgrown by soft-bodied algae, as it is achieved locally by aggressive territorial damselfish that deter sea urchins and other grazing fish. Damselfishes are known to maintain and weed algal



Bioerosion, Figure 16 Dense distribution of euendolithic cyanobacterial boreholes outlines a pattern, which determines the fragmentation of rock upon being grazed and passed through gastropod's digestive tract. These fragments were recognized in the sediment based on margins scalloped by boreholes, reminiscent of perforations that facilitate separation of postal stamps. Scale bar is 50 μ m long. (Modified from Torunski, H., 1979. Biological erosion and its significance for the morphogenesis of limestone coasts and for nearshore sedimentation (northern Adriatic). (Senckenbergiana maritima, 11: 193–265.)

farms, thus influencing macrobioeroding communities in the process (Sammarco et al., 1987). The results of grazer-exclusion experiments (Wanders, 1977), which showed a successional shift in species composition in favor of macroalgae and in a loss of some normally abundant and structurally important species of coralline algae, confirmed independently the experimentation performed by damselfish.

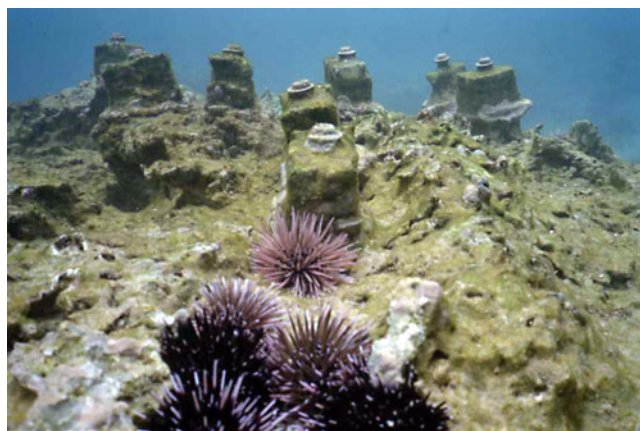
In contrast, excessive grazing is expected to be self-limiting and thus cyclical. When grazing pressure is too intense, as in the case of populations of grazing urchins studied on la Reunion Island, Indian Ocean (Chazottes et al., 2002), or in pollution impacted Faa reef at the airport of Papeete (French Polynesia; Pari et al., 1998), grazing and microbioerosion become negatively correlated (Figure 17). When grazing is too intense, euendoliths may be completely removed, thus reducing grazing while new euendoliths recolonize the barren grounds.

The optimal conditions for the interaction between euendoliths and their grazers are exemplified by the formation of intertidal bioerosional notch (Neumann, 1966) (Figure 18). Tidal ranges on rocky coasts represent an ecotone environment, a transitional zone between ecosystems that are adapted either to marine or to terrestrial environments. This transitional zone may be perceived as extreme for both. It is characterized by a gradient of variable water supply, solar irradiation, and salinity. Organisms along that gradient are arranged in distinct zones (Figure 19). Both, microbial euendoliths and their grazers optimize their activity in dependence on local water supply. Their activity, in turn, improves local water retention. The upper tidal ranges are harsh environments dominated by euendolithic cyanobacteria, often by a single species. Accordingly, the rates of bioerosion are modest. In contrast, the lower ranges with regular water supply by tides are more diversified, inviting competing microboring

and grazing taxa. The coastal profile reflects the resulting bioerosion like a diagram, with the location of the notch corresponding to maximum bioerosion. The pattern of bioerosional notch formation is repeated around the margins of flat-bottom intertidal and supratidal rockpools although the species composition of the euendolith and grazer populations of the pools may be distinct from those of the large-scale coastal notches (Radtke et al., 1996).

In the supratidal ranges, the distribution of wave spray, water retention, and solar irradiation selects and controls the combined boring and grazing activity of darkly pigmented euendolithic cyanobacteria and small grazing gastropods. Consequently, the coastal limestone rocks are biodegraded selectively, a process that enhances the relief of the coastal rock surface, leading to formation of elaborate sharp-edged rock forms called biokarst (Figure 20). The term emphasizes the biological modification of the limestone dissolution forms that are characteristic of world's karstic regions (Schneider and Torunski, 1983).

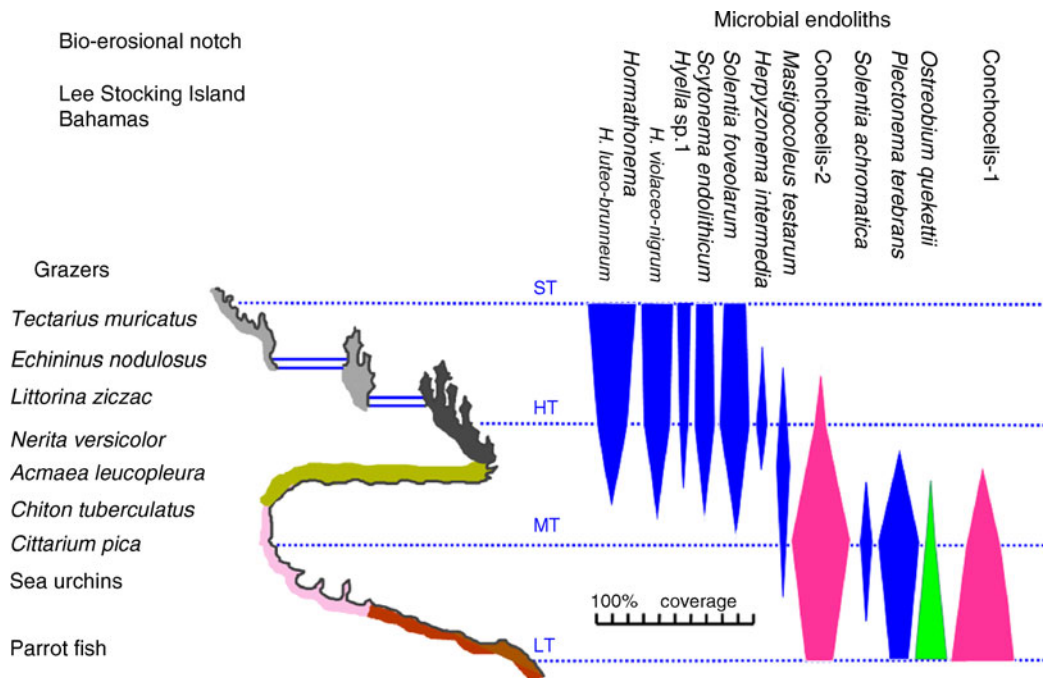
Macroborers are orders of magnitude larger than microborers (compare Figures 6 and 7) and can excavate significantly larger volumes of carbonate than microbial euendoliths but, unlike the microborers, the macroborers are not subjected to grazing pressure. Macroborers self-stabilize once they reach their adult size and thus are not part of the advancing bioerosion. Macroboring activity may lighten the reef structure but does not necessarily imperil the structural integrity of the reef's framework (Scott and Risk, 1988); although, excessive damage to corals by macroborers makes them vulnerable to physical forces, usually associated with the passage of tropical storms (Clark and Morton, 1999). Macroboring increases porosity of rocks and promotes formation of new settling grounds and additional ecological niches, an activity that can be viewed as a positive contribution to the reef ecosystem.



Bioerosion, Figure 17 Heavy grazing by *Echinometra mathaei* has bypassed the experimental exposure blocks and undermined their basis. Coral reef under the influence of pollution at Faa, near Papeete, French Polynesia. The blocks are about 10 cm wide. (Courtesy of M. Peyrot-Clausade.)



Bioerosion, Figure 18 Bioerosional notch on limestone coast in protected shallow lagoonal environment at Lee-Stocking Island, Bahamas.



Bioerosion, Figure 19 Bioerosional notch carved into a coastal profile by microbial euendoliths and their grazers on a small island on the Bahamas carbonate platform, near Lee Stocking Island. Zonal distribution of phototrophic microborers listed above, comprising cyanobacteria (blue), chlorophytes (green), and Conchoecelis stages of rhodophytes (red) is correlated with zonal distribution of their grazers, listed left, and with the tidal levels (ST = supratidal; HT = high tide; MT = mean tide; LT = low tide). (Modified from Radtke et al., 1996.)



Bioerosion, Figure 20 Biokarst shaping of coastal carbonate rocks caused by combined bioerosion of euendolithic cyanobacteria and small gastropods. Scale bar is 10 cm long.

Production and cementation of sediment is another process that re-enforces reef framework, and is in part facilitated by endolithic organisms. There is good evidence that some of the carbonate dissolved by microbial euendoliths is re-precipitated, thus contributing to formation of micritic encrustations in marine (Kobluk and Risk, 1977; MacIntyre et al., 2000) and freshwater

environments (Schneider et al., 1983). *Ostreobium quekettii* dissolves carbonate, which then precipitates around its filaments when the alga exits the substrate and inhabits the coral pore spaces as cryptoendolith (Figure 21). Precipitation of carbonate on filaments of *Ostreobium*, was observed first in corals (Schroeder, 1972) and later in stromatolites and live corals (Feldmann and McKenzie, 1998; Nothdurft et al., 2007).

Rates of bioerosion

Quantification of bioerosion rates includes direct observations of behavioral patterns of particular organisms such as grazers (Hoey and Bellwood, 2008) and their selective impact (Rojtan and Lewis, 2005). A different approach involves measuring grazers' carbonate consumption rates (Mokady et al., 1996). Other approaches involve assessment of long-term substrate removal from experimentally exposed carbonate blocks without (Kiene and Hutchings, 1992), or with identification of participating bioeroders (Chazottes et al., 1995, 2002; Tribollet and Golubic, 2005; Tribollet, 2008a). The latter approaches assess the total bioerosion rates (macrobioerosion + microbioerosion + grazing) by measuring changes in substrate size and density over time (Chazottes et al., 1995; Tribollet and Golubic, 2005). The techniques involve measurements of eroded areas in surface projection and in vertical sections, using light and scanning electron microscopy with



Bioerosion, Figure 21 View of the fractured skeleton of the coral *Montastrea*, invaded by *Ostreobium quekettii*. The euendolithic filaments of this chlorophyte produced the perforation in the skeleton (arrow), whereas the cryptoendolithic ones are covered by precipitate. Scale bar is 10 μm long.

image analysis. Carbonate removal rates are calculated for each dominant species of euendoliths as described by Tribollet (2008a). Variations on analytical methods are described in Chazottes et al. (1995), Kiene et al. (1995), and Vogel et al. (2000). The rates expressed in kg or $\text{mol CaCO}_3 \text{ m}^{-2}$ of total surface area of substrate exposed to bioerosion or m^{-2} of reef planar area per unit of time are recommended, but not always implemented in the literature, which makes comparisons of the results of different studies difficult.

Bioeroding organisms are ubiquitous in carbonate ecosystems and their efficiency of carbonate removal in coral reefs varies between 0.002 and $1.4 \text{ kg CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ (Chazottes et al., 1995; Vogel et al., 2000; Carreiro-Silva et al., 2005; Tribollet and Golubic, 2005). Dissolution of carbonate by microbial euendoliths, measured in a tropical lagoon by exposing sand-sized particles to euendolith settlement and excluding grazers by a $250 \mu\text{m}$ size nylon mesh, was estimated to be $350 \text{ g m}^{-2} \text{ y}^{-1}$ (Tudhope and Risk, 1985). These rates should vary with depth (Vogel et al., 2000), type of substrate (Perry, 1998; Zubia and Peyrot-Clausade, 2001), grazing pressure (Reaka-Kudla et al., 1996; Chazottes et al., 1995, 2002), sedimentation (Tribollet and Golubic, 2005), and eutrophication (Carreiro-Silva et al., 2005).

Tribollet and Golubic (2005) showed that carbonate dissolution by euendoliths in synergy with grazers along an inshore–offshore profile across the Great Barrier Reef was the dominant bioerosion process over time. They also quantified the combined yearly rates of grazing per 1 m^2 of exposed surface area to be between 0.04 and 1.8 kg of CaCO_3 . Macroborers as filter-feeders were important only

on inshore reefs where waters were rich in particulate matter (Risk et al., 1995). Macroborers eroded between 0.03 and $0.38 \text{ kg m}^{-2} \text{ y}^{-1}$. Combined interactive activity of grazing and microbioerosion contributes more to the total bioerosion of the reefs than macrobioerosion (Tribollet and Golubic, 2005).

Grazing rates vary depending on the organism. Rates by sea urchins are often very high. Grazing rates measured on Galapagos Islands reach up to $22 \text{ kg CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ (Reaka-Kudla et al., 1996). Mokady et al. (1996) measured consumption of grazing echinoids in the Red Sea. They observed that a meal of a sea urchin contained on the average three times more carbonate per weight than organic food. According to their calculations, removal of carbonate by a single sea urchin could reach $1 \text{ kg CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$, depending on the size of the animal. The actual removal per area then depends on grazer population densities, which are commonly reciprocal to the average size of the animal. Grazing by extremely dense populations of small gastropods has been studied in the intertidal ranges (Schneider and Torunski, 1983), but has largely been ignored in the coastal subtidal ranges and on the reefs where there are also large numbers of small gastropods.

Summary: bioerosion and the carbonate budget of coral reefs

A carbonate ecosystem with a positive carbonate budget occurs when carbonate production, due to growth of calcifying organisms and precipitation of CaCO_3 , is higher than carbonate dissolution and/or exportation of carbonate sediments to the open ocean (e.g., Le Campion-Alsumard et al., 1993). An unbalanced carbonate budget in favor of destructive forces can be observed in coral reef ecosystems, which are mostly made of carbonates (Peyrot-Clausade et al., 1995; Reaka-Kudla et al., 1996; Pari et al., 1998). Such a disequilibrium between reef constructive forces (production of carbonates) and reef destructive forces (mainly bioerosion) can lead to physical losses of reef framework and consequently to a loss of reef diversity (Hallock, 2005; Wilkinson, 2004).

The balance between constructive and destructive forces is critical for coral reefs, particularly in view of global warming and the expected rise in sea level. It is predicted that the rising atmospheric pCO_2 due to human activities and the consequent ocean acidification (Tribollet et al., 2006), in conjunction with the rising seawater surface temperature, overfishing, and eutrophication (Szmant, 2002), will increase the rate of coral morbidity and mortality (Mumby et al., 2001; Hallock, 2005). This may lead to a shift from coral-dominated to algal-dominated reefs, with a loss of corals as one of the main frame-builders in the reefs (Szmant, 2002; Hallock, 2005). Although the effects of the above combined factors on bioerosion processes are poorly known, the integrity of coral reefs is increasingly in jeopardy (Hoegh-Guldberg, 1999; Wilkinson, 2004; Kleypas et al., 2006).

Coral reefs protect tropical coastlines and oceanic islands (Spencer and Viles, 2002). In the past they were able to grow and keep up with changes in seawater level. In order to better monitor and to preserve coral reefs it is essential to quantify carbonate budgets accurately. Different techniques have been used to achieve this goal, including alkalinity method of seawater passing over the reefs, which measure both constructive and destructive effects simultaneously. The alkalinity method provides an instantaneous measurement of reef net calcification rate ($\text{kg CaCO}_3 \text{ m}^{-2}$ of planar reef area d^{-1}). This provides a fast estimate of whether the reef is accreting or dissolving (Gattuso et al., 1999; Langdon et al., 2000). However, this method does not show which organisms are responsible for carbonate production, and which for bioerosion, nor does it predict the reef's carbonate budget over time. Additional techniques were introduced, which include monitoring of chemical and biological variables such as coral cover, coral recruitment and settlement, coral growth and calcification rates, and rates of macrobioerosion (Edinger et al., 2000). These studies have shown that inshore reefs, disturbed by sedimentation and eutrophication, had a negative carbonate budget, even without considering microbioerosion and grazing rates. However, budget calculations of "pristine coral reefs" according to the same study (Edinger et al., 2000) have to be considered with caution, because grazing and microbioerosion were not quantified, and these processes are often greater in "pristine" oligotrophic reefs than at the "disturbed" inshore reefs (Tribollet and Golubic, 2005). The studies of carbonate budget in reefs stress the importance of simultaneous analyzes of constructive and destructive forces over time and under changing environmental conditions if the state of health of coral reefs, and more generally of carbonate coastal ecosystems, has to be maintained and improved.

Bibliography

- Al-Thukair, A. A., and Golubic, S., 1991. New endolithic cyanobacteria from the Arabian Gulf. I. *Hyella immanis* sp. nov. *Journal of Phycology*, **27**, 766–780.
- Al-Thukair, A. A., Golubic, S., and Rosen, G., 1994. New euendolithic cyanobacteria from the Bahama Bank and the Arabian Gulf: *Hyella racemus* sp. nov. *Journal of Phycology*, **30**, 764–769.
- Anagnostidis, K., and Pantazidou, A., 1988. *Hyella kalligrammos* sp. nov., *Hyella maxima* (Geitl.) comb. nov., and other freshwater morphotypes of the genus *Hyella* Born. et Flah. (Chroococcales, Cyanophyceae). *Algological Studies*, **50**(53), 227–247.
- Bengtson, S., and Zhao, Y., 1992. Predatorial borings in late Precambrian mineralized exoskeletons. *Science*, **257**, 367–369.
- Bentis, C. J., Kaufman, L., and Golubic, S., 2000. Endolithic fungi in reef-building corals (Order: Scleractinia) are common, cosmopolitan, and potentially pathogenic. *Biological Bulletin*, **198**, 254–260.
- Bergman, K. M., Elner, R. W., and Risk, M. J., 1982. The influence of *Polydora websteri* borings on the strength of the shell of the sea scallop, *Placopecten magellanicus*. *Canadian Journal of Zoology*, **60**, 2551–2556.
- Bertling, M., Braddy, S. J., Bromley, R. G., Demathieu, G. R., Genise, J., Mikuláš, R., Nielsen, J., Nielsen, K., Rindsberg, A. K., Schlirf, M., and Uchman, A., 2006. Names for trace fossils: a uniform approach. *Lethaia*, **39**, 265–286.
- Beuck, L., López Correa, M., and Freiwald, A., 2008. Biogeographical distribution of *Hyrrokin* (Rosalinidae, Foraminifera) and its host-specific morphological and textural trace variability. In Wisshak, M., and Tapanila, L. (eds.), *Current Developments in Bioerosion*. Berlin: Springer, pp. 329–360.
- Boucot, A. J., 1990. *Evolutionary Paleobiology of Behavior and Coevolution*. Heidelberg, Berlin: Elsevier.
- Bromley, R. G., 1970. Borings as trace fossils and *Entobia cretacea* Portlock, as an example. In Crimes, T. P., and Harper, J. C. (eds.), *Trace fossils – Geological Journal, Special Issue 3*, pp. 49–90.
- Bromley, R. G., 1990. *Trace Fossils: Biology and Taphonomy*. London: Hyman.
- Bromley, R. G., 2004. A stratigraphy of marine bioerosion. In McIlroy, D. (ed.), *The Application of Ichnology to Palaeoenvironmental and Stratigraphic Analysis*, Geological Society of London, Special Publication, **228**, pp. 455–481.
- Bromley, R. G., and D'Alessandro, A., 1989. Ichnological study of shallow marine endolithic sponges from the Italian coast. *Rivista Italiana di Paleontologia e Stratigrafia*, **95**, 279–314.
- Bromley, R. G., Wisshak, M., Glaub, I., and Boutquelen, A., 2007. Ichnotaxonomic review of dendriniform borings attributed to foraminifera: *Semidendrina* gen. nov., In Miller III, W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier, pp. 508–520.
- Bruggemann, J. H., van Oppen, M. J. H., and Breeman, A. M., 1994. Foraging by the stoplight parrotfish *Sparisoma viride*. I. Food selection in different, socially determined habitats. *Marine Ecology Progress Series*, **106**, 41–55.
- Campbell, S. E., 1980. *Palaeoconchocelis starmachii*, a carbonate boring microfossil from the Upper Silurian of Poland (425 million years old): implications for the evolution of the Bangiaceae (Rhodophyta). *Phycologia*, **19**, 25–36.
- Campbell, S. E., 1982. Precambrian endoliths discovered. *Nature*, **299**, 429–431.
- Cantrell, D. L., and Hagerty, R. M., 1999. Microporosity in Arab Formation carbonates, Saudi Arabia. *GeoArabia*, **4**, 129–154.
- Carreiro-Silva, M., and McClanahan, T. R., 2001. Echinoid bioerosion and herbivory on Kenyan coral reefs: the role of protection from fishing. *Journal of Experimental Marine Biology and Ecology*, **262**, 133–153.
- Carreiro-Silva, M., McClanahan, T. R., and Kiene, W. E., 2005. The role of inorganic nutrients and herbivory in controlling microbioerosion of carbonate substratum. *Coral Reefs*, **24**, 214–221.
- Carriker, M. R., Smith, E. H., and Wilce, R. T., (eds.), 1969. *Penetration of Carbonate Substrates by Lower Plants and Invertebrates*. American Zoologist, **9**, 629–1020.
- Carriker, M. R., 1969. Excavation of boreholes by the gastropod, *Urosalpinx*: an analysis by light and scanning electron microscopy. *American Zoologist*, **9**, 917–933.
- Cedhagen, T., 1994. Taxonomy and biology of *Hirrokkin sarcophaga* gen. et sp. n., a parasitic foraminiferan (Rosalinidae). *Sarsia*, **79**, 65–82.
- Chazottes, V., Le Campion-Alsumard, T., and Peyrot-Clausade, M., 1995. Bioerosion rates on coral reefs: interaction between macroborers, microborers and grazers (Moorea, French Polynesia). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **113**, 189–198.
- Chazottes, V., Le Campion-Alsumard, T., Peyrot-Clausade, M., and Cuet, P., 2002. The effects of eutrophication-related alterations to coral reef communities on agents and rates of bioerosion (Reunion Island, Indian Ocean). *Coral Reefs*, **21**, 375–390.

- Cherchi, A., and Schroeder, R., 1991. Perforations branchues dues à des Foraminifères cryptobiotiques dans les coquilles actuelles et fossiles. *Comptes Rendus de l'Académie des Sciences, Paris*, **312**, 111–115.
- Clark, T., and Morton, B., 1999. Relative roles of bioerosion and typhoon-induced disturbance on the dynamics of a high latitude scleractinian coral community. *Journal of Marine Biological Association of United Kingdom*, **79**, 803–820.
- Clokic, J. J. P., Scoffin, T. P., and Boney, A. D., 1981. Depth maxima of *Conchocelis* and *Phymatolithon rudulosum* on the N.W. Shelf and Rockall Plateau. *Marine Ecology Progress Series*, **4**, 131–133.
- Crimes, T. P., and Harper, J. C., 1970. Trace fossils. *Geological Journal, Special Issue*, **3**, 1–547.
- Domart-Coulon, I. J., Sinclair, C. S., Hill, R. T., Tambutté, S., Puverel, S., and Ostrander, G. K., 2004. A basidiomycete isolated from the skeleton of *Pocillopora damicornis* (Scleractinia) selectively stimulates short-term survival of coral skeletogenic cells. *Marine Biology*, **144**, 583–592.
- Edinger, E. N., Limmon, G. V., Jompa, J., Widjatmoko, W., Heikoop, J. M., and Risk, M. J., 2000. Normal coral growth rates on dying reefs: Are coral growth rates good indicators of reef health? *Marine Pollution Bulletin*, **40**, 404–425.
- Ekdale, A. A., and Bromley, R. G., 2001. Bioerosional innovation in the Early Ordovician of Sweden: a revolutionary adaptation for living in carbonate hardgrounds. *Lethaia*, **34**, 1–12.
- Feldmann, M., and McKenzie, J., 1998. Stromatolite-thrombolite associations in modern environment, Lee Stocking Island, Bahamas. *Palaios*, **13**, 201–212.
- Fine, M., Roff, G., Ainsworth, T. D., and Hoegh-Guldberg, O., 2006. Phototrophic microendoliths bloom during coral “white syndrome”. *Coral Reefs*, **25**, 577–581.
- Fork, D. C., and Larkum, A. W. D., 1989. Light harvesting in the green alga *Ostreobium* sp., a coral symbiont adapted to extreme shade. *Marine Biology*, **103**, 381–385.
- Freiwald, A., Reitner, J., and Kruttschinn, J., 1997. Microbial alteration of the deep-water coral *Lophelia pertusa*: Early postmortem processes. *Facies*, **36**, 223–226.
- Frey, R. W., 1975. *The Study of Trace Fossils, A Synthesis of Principles, Problems, and Procedures in Ichnology*. Heidelberg, Berlin: Springer.
- Fürsich, F. T., Palmer, T. J., and Goodyear, K. L., 1994. Growth and disintegration of bivalve-dominated patch reefs in the Upper Jurassic of southern England. *Palaeontology*, **37**, 131–171.
- Garcia-Pichel, F., 2006. Plausible mechanisms for the boring on carbonates by microbial phototrophs. *Sedimentary Geology*, **185**, 205–213.
- Gattuso, J.-P., Franquignoulle, M., and Smith, S. V., 1999. Measurement of community metabolism and significance in the coral reef CO₂ source-sink debate. *Proceedings of the National Academy of Science, PNAS*, **96**, 13017–13022.
- Gherardi, D. F. M., and Bosence, D. W. J., 2001. Composition and community structure of the coralline algal reefs from Atol das Rocas, Suth Atlantic, Brazil. *Coral Reefs*, **19**, 205–219.
- Ghirardelli, L. A., 2002. Endolithic microorganisms in live and dead thalli of coralline red algae (Corallinales, Rhodophyta) in the northern Adriatic Sea. *Acta Geologica Hispanica*, **37**, 53–60.
- Glaub, I., Golubic, S., Gektidis, M., Radtke, G., and Vogel, K., 2007. Microborings and microbial endoliths: geological implications. Miller III., W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier Scientific Publishing Co., pp. 368–381.
- Glaub, I., and Vogel, K., 2004. The stratigraphic record of microborings. *Fossils and Strata*, **51**, 126–135.
- Glaub, I., Vogel, K., and Gektidis, M., 2001. The role of modern and fossil cyanobacterial borings in bioerosion and bathymetry. *Ichnos*, **8**, 185–195.
- Golubic, S., Brent, G., and Le Campion-Alsumard, T., 1970. Scanning electron microscopy of endolithic algae and fungi using a multipurpose casting-embedding technique. *Lethaia*, **3**, 203–209.
- Golubic, S., Campbell, S. E., Drobne, K., Cameron, B., Balsam, W. L., Cimerman, F., and Dubois, L., 1984. Microbial endoliths: a benthic overprint in the sedimentary record, and a paleobathymetric cross-reference with foraminifera. *Journal of Paleontology*, **58**, 351–361.
- Golubic, S., Friedmann, I., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**, 475–478.
- Golubic, S., Perkins, R. D., and Lukas, K. J., 1975. Boring microorganisms and microborings in carbonate substrates. In Frey, R. W. (ed.), *The Study of Trace Fossils*. Heidelberg-Berlin: Springer, pp. 229–259.
- Golubic, S., and Radtke, G., 2008. The trace *Rhopalia clavigera* isp. nov. reflects the development of its maker *Eugomontia sacculata* Kormann 1960. In Wisshak, M., Tapanila, L. (eds.), *Current Developments in Bioerosion*, Berlin, Heidelberg: Springer, pp. 95–108.
- Golubic, S., Radtke, G., and Le Campion-Alsumard, T., 2005. Endolithic fungi in marine ecosystems. *Trends in Microbiology*, **13**, 229–235, Oxford: Elsevier.
- Golubic, S., and Schneider, J., 1979. Carbonate dissolution. In Trudinger, P. A., and Swaine, D. J. (eds.), *Biogeochemical Cycling of Mineral-forming Elements*. Amsterdam: Elsevier Scientific Publishing Co., pp. 107–129.
- Golubic, S., and Schneider, J., 2003. Microbial endoliths as internal biofilms. In Krumbein, W. E., Dornieden, T., and Volkmann, M. (eds.), *Fossil and Recent Biofilms*. Dordrecht: Kluwer Academic Publishers, pp. 249–263.
- Günther, A., 1990. Distribution and bathymetric zonation of shell-boring endoliths in Recent reef and shelf environments: Cozumel, Yucatan (Mexico). *Facies*, **22**, 233–262.
- Hallock, P., 2005. Global change and modern coral reefs: new opportunities to understand shallow-water carbonate depositional processes. *Sedimentary Geology*, **175**, 19–33.
- Harris, P. M., Halley, R. B., and Lukas, K. J., 1979. Endolith microborings and their preservation in Holocene-Pleistocene (Bahama-Florida) ooids. *Geology*, **7**, 216–220.
- Highsmith, R. C., 1981. Lime-boring algae in hermatypic coral skeleton. *Journal of Experimental Marine Biology and Ecology*, **55**, 267–281.
- Hoegh-Guldberg, O., 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Marine Freshwater Research*, **50**, 839–866.
- Hoey, A. S., and Bellwood, D. R., 2008. Cross-shelf variation in the role of parrotfishes on the Great Barrier Reef. *Coral Reefs*, **27**, 27–47.
- Hofmann, K., 1996. Die mikroendolithischen Spurenfossilien der borealen Oberkreide. *Geologisches Jahrbuch, A*, **136**, 3–153.
- Hutchings, P. A., 1986. Biological destruction of coral reefs. *Coral Reefs*, **4**, 239–252.
- Kaehler, S., and McQuaid, C. D., 1999. Lethal and sublethal effects of phototrophic endoliths attacking the shell of the intertidal mussel *Perna perna*. *Marine Biology*, **13**, 497–503.
- Kazmierczak, J., and Golubic, S., 1976. Oldest organic remains of boring algae from Polish Upper Silurian. *Nature*, **261**, 404–406.
- Kendrick, B. W., Risk, M. J., Michaelides, J., and Bergman, K., 1982. Amphibious microborers: bioeroding fungi isolated from live corals. *Bulletin of Marine Science*, **32**, 862–867.
- Kiene, W. E., and Hutchings, P. A., 1992. Long-term bioerosion of experimental coral substrates from Lizard Island, Great Barrier Reef. *Proceedings of the 7th International Coral Reef Symposium*, **2**, 397–403.

- Kiene, W., Radtke, G., Gektidis, M., Golubic, S., and Vogel, K., 1995. Factors controlling the distribution of microborers in Bahamian reef environments. *Facies*, **32**, 176–188.
- Kleemann, K. H., 1996. Biocorrosion by bivalves. *Marine Ecology*, **17**, 145–158.
- Kleypas, J. A., Feely, R. A., Fabry, V. J., Langdon, C., Sabine, C. L., and Robbins, L. L., 2006. Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research, Report of a workshop held 18–20 April 2005, St Petersburg, FL, sponsored by NSF, NOAA and the US Geological Survey, pp. 1–88.
- Knoll, A. H., Golubic, S., Green, J., and Swett, K., 1986. Organically preserved microbial endoliths from the Late Proterozoic of East Greenland. *Nature*, **321**, 856–857.
- Kobluk, D. R., and Risk, M. J., 1977. Calcification of exposed filaments of endolithic algae, micrite envelope formation and sediment production. *Journal of Sedimentary Petrology*, **47**, 517–528.
- Koehne, B., Elli, G., Jennings, R., Wilhelm, C., and Trissl, H.-W., 1999. Spectroscopic and molecular characterization of long wavelength absorbing antenna of *Ostreobium* sp. *Biochimica et Biophysica Acta*, **1412**, 94–107.
- Kolbasov, G. A., 2000. *Lithoglyptes cornutes*, new species (Cirripedia: Acrothoracica), a boring barnacle from the Seychelles, with some data on its ultrastructure. *Hydrobiologia*, **438**, 185–191.
- Langdon, C., and Atkinson, M. J., 2005. Effect of elevated pCO₂ on photosynthesis and calcification of corals and interaction with seasonal change in temperature/radiance and nutrient enrichment. *Journal of Geophysical Research-Oceans*, **110**, C09S07.
- Langdon, C., Takahashi, T., Marubini, F., Atkinson, M. J., Sweeney, C., Aceves, H., Barnett, H., Chipman, D., and Goddard, J., 2000. Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochemical Cycles*, **14**, 639–654.
- Le Campion-Alsumard, T., 1979. Les cyanophycées endolithes marines. Systématique, ultrastructure, écologie et biodestruction. *Oceanologica Acta*, **2**, 143–156.
- Le Campion-Alsumard, T., and Golubic, S., 1985. *Hyella caespitosa* Bornet et Flahault and *Hyella balani* Lehman (Pleurocapsales, Cyanophyta): a comparative study. *Algological Studies*, **38**, 39: 119–148.
- Le Campion-Alsumard, T., Romano, J.-C., Peyrot-Clausade, M., Le Campion, J., and Paul, R., 1993. Influence of some coral reef communities on the calcium carbonate budget of Tiahura reef (Moorea, French Polynesia). *Marine Biology*, **115**, 685–693.
- Le Campion-Alsumard, T., Golubic, S., and Hutchings, P., 1995a. Microbial endoliths in skeletons of live and dead corals: *Porites lobata* (Moorea, French Polynesia). *Marine Ecology. Progress Series*, **117**, 149–157.
- Le Campion-Alsumard, T., Golubic, S., and Priess, K., 1995b. Fungi in corals: symbiosis or disease? Interaction between polyps and fungi causes pearl-like skeleton biomineralization. *Marine Ecology. Progress Series*, **117**, 137–147.
- Lukas, K. J., 1973. Taxonomy and Ecology of the Endolithic Microflora of Reef Corals, with a Review of the Literature on Endolithic Microphytes. Unpublished PhD thesis, University Rhode Island, 159 pp.
- Lukas, K. J., 1974. Two species of the Chlorophyte genus *Ostreobium* from skeletons of Atlantic and Caribbean reef corals. *Journal of Phycology*, **10**, 331–335.
- Lukas, K. J., 1978. Depth distribution and form among common microboring algae from the Florida continental shelf. *Geological Society of America, Abstracts and Programs*, **10**, 448.
- MacIntyre, I. G., Prufert-Bebout, L., and Reid, R. P., 2000. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology*, **47**, 915–921.
- Mao-Che, L., Le Campion-Alsumard, T., Boury-Esnault, N., Payri, C., Golubic, S., and Bézac, C., 1996. Biodegradation of shells of the black pearl oyster, *Pinctada margaritifera* var. *cumingii*, by microborers and sponges of French Polynesia. *Marine Biology*, **126**, 509–519.
- Mariani, S., Uriz, M.-J., and Turon, X., 2000. Larval bloom of the oviparous sponge *Cliona viridis*: coupling of larval abundance and adult distribution. *Marine Biology*, **137**, 783–790.
- Mayoral, E., 1991. Actividad bioerosiva de briozoos ctenostomados en el Ordovícico Superior de la Zona Cantábrica del Macizo Hespérico (Cabo Vidrias, Oviedo). *Revista Española de Paleontología*, **6**, 27–36.
- Miller III, W., (ed.), 2007. *Trace Fossils, Concepts, Problems, Prospects*. Amsterdam: Elsevier.
- Mokady, O., Lazar, B., and Loya, Y., 1996. Echinoid bioerosion as a major structuring force of Red Sea coral reefs. *Biological Bulletin*, **190**, 367–372.
- Mumby, P. J., Chisholm, J. R. M., Edwards, A. J., Clark, C. D., Roark, E. B., Andrefouet, S., and Jaubert, J., 2001. Unprecedented bleaching-induced mortality in *Porites* spp. At Rangiroa Atoll, French Polynesia. *Marine Biology*, **139**, 183–189.
- Neumann, A. C., 1966. Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge, *Cliona lampa*. *Limnology and Oceanography*, **11**, 92–108.
- Nothdurft, L. D., Webb, G. E., Bostrom, T., Rintoul, L., 2007. Calcite-filled borings in the most recently deposited skeleton in live-collected *Porites* (Scleractinia): Implications for trace element archives. *Geochimica et Cosmochimica Acta*, **71**, 5423–5438.
- Palmer, T., and Plewes, C., 1993. Borings and bioerosion in fossils, *Geology Today*, **9**, 138–142.
- Pantazidou, A., Belegreatis, M. R., Louvrou, I., and Enonomou-Amilli, A., 2000. Euendolithic algae and patterns of diatom colonization on biotic carbonate substrates. In Economou-Amilli, A. (ed.), *Proceedings of 16th International Diatom Symposium*, University of Athens, Greece, pp. 381–392.
- Pari, N., Peyrot-Clausade, M., Le Campion-Alsumard, T., Hutchings, P. A., Chazottes, V., Golubic, S., Le Campion, J., and Fontaine, M. F., 1998. Bioerosion of experimental substrates on high islands and atoll lagoons (French Polynesia) after two years of exposure. *Marine ecology Progress Series*, **166**, 119–130.
- Perry, C. T., 1998. Grain susceptibility to the effects of microboring: implication for the preservation of skeletal carbonates. *Sedimentology*, **45**, 39–51.
- Perry, C. T., and Hepburn, L. J., 2008. Syn-depositional alteration of coral reef framework through bioerosion, encrustation and cementation: taphonomic signatures of reef accretion and reef depositional events. *Earth-Science Reviews*, **86**, 106–144.
- Peyrot-Clausade, M., Le Campion-Alsumard, T., Hutchings, P. A., Le Campion, J., and Fontaine, F., 1995. Initial bioerosion and bioaccretion on experimental substrates in high island and atolls lagoons (French Polynesia). *Oceanologica Acta*, **18**, 531–541.
- Plewes, C. R., 1994. Jurassic boring phoronids – non-boring insights into fossil record of some soft bodied worms. *Palaeontology Newsletter*, **24**, 24.
- Plewes, C. R., Palmer, T. J., and Haynes, J. R., 1993. A boring foraminiferan from the Upper Jurassic of England and northern France. *Journal of Micropalaeontology*, **12**, 83–89.
- Pohowsky, R. A., 1978. The boring ctenostomate Bryozoa: taxonomy and paleobiology based on cavities in calcareous substrata. *Bulletins of American Paleontology*, **73**, 1–192.
- Pomponi, S. A., 1980. Cytological mechanisms of calcium carbonate excavation by boring sponges. *International Review of Cytology*, **65**, 301–319.
- Priess, K., Le Campion-Alsumard, T., Golubic, S., Gadel, F., and Thomassin, B. A., 2000. Fungi in corals: black bands and

- density-banding of *Porites lutea* and *P. lobata* skeleton. *Marine Biology*, **136**, 19–27.
- Radtke, G., 1991. Die mikroendolithischen Spurenfossilien im Alt-Tertiär West-Europas und ihre palökologische Bedeutung. *Courier des Forschungsinstitut, Senckenberg*, **138**, 1–185.
- Radtke, G., and Golubic, S., 2005. Microborings in mollusk shells, Bay of Safaga, Egypt: morphometry and ichnology. *Facies*, **51**, 118–134.
- Radtke, G., Hofmann, K., and Golubic, S., 1997. A bibliographic overview of micro- and macroscopic bioerosion. *Courier Forschungsinstitut Senckenberg*, **201**, 307–340.
- Radtke, G., Le Campion-Alsumard, T., and Golubic, S., 1996. Microbial assemblages of the bioerosional “notch” along tropical limestone coasts. *Algological Studies*, **83**, 469–482.
- Rasmussen, K. A., and Frankenberg, E. W., 1990. Intertidal bioerosion by the chiton *Acanthopleura granulata*; San Salvador, Bahamas. *Bulletin of Marine Science*, **47**, 680–695.
- Reaka-Kudla, M. L., Feingold, J. S., and Glynn, W., 1996. Experimental studies of rapid bioerosion of coral reefs in the Galapagos Islands. *Coral Reefs*, **15**, 101–107.
- Risk, M. J., and Muller, H. R., 1983. Porewater in coral heads: evidence for nutrient regeneration. *Limnology and Oceanography*, **28**, 1004–1008.
- Risk, M. J., Sammarco, P. W., and Edinger, E. N., 1995. Bioerosion in *Acropora* across the continental shelf of the Great Barrier Reef. *Coral Reefs*, **14**, 79–86.
- Rojtan, R. D., and Lewis, S. M., 2005. Selective predation by parrotfishes on the reef coral *Porites astreoides*. *Marine Ecology Progress Series*, **305**, 193–201.
- Rützler, K., 2002. Impact of crustose clionid sponges on Caribbean reef corals. *Acta Geologica Hispanica*, **37**, 61–72.
- Sammarco, P. W., 1980. Geographically specific recruitment and postsettlement mortality as influences on coral communities: the cross-continental shelf transplant experiment. *Limnology and Oceanography*, **36**, 496–514.
- Sammarco, P. W., and Risk, M. J., 1990. Large-scale patterns in internal bioerosion of *Porites*: cross continental shelf trends on the Great Barrier Reef. *Marine Ecology Progress Series*, **59**, 145–156.
- Sammarco, P. W., Risk, M. J., and Rose, C., 1987. Effects of grazing damselfish territoriality on internal bioerosion of dead coral: indirect effects. *Journal of Experimental Marine Biology and Ecology*, **112**, 185–199.
- Schlichter, D., Kampmann, H., and Conradi, S., 1997. Trophic potential and photoecology of endolithic algae living within coral skeletons. *P.S.Z.N.: Marine Ecology*, **18**, 299–317.
- Schneider, J., and Le Campion-Alsumard, T., 1999. Construction and destruction of carbonates by marine and freshwater cyanobacteria. *European Journal of Phycology*, **34**, 417–426.
- Schneider, J., and Torunski, H., 1983. Biokarst on limestone coasts, morphogenesis and sediment production. *Marine Ecology*, **4**, 45–63.
- Schneider, J., Schröder, H.-G., and Le Campion-Alsumard, T., 1983. Algal micro-reefs: coated grains from freshwater environments. In Peryt, T. M. (ed.), *Coated Grains*. Berlin: Springer-Verlag, pp. 284–298.
- Schönberg, C., 2008. A history of sponge erosion: from past myths and hypotheses to recent approaches. In Wisshak, M., and Tapanila, L. (eds.), *Current Developments in Bioerosion*. Berlin: Springer, pp. 165–202.
- Schroeder, J. H., 1972. Calcified algal filaments of an endolithic alga in Recent Bermuda reefs. *Neues Jahrbuch der Geologie und Paläontologie, Monatsheft*, **1**, 16–33.
- Scoffin, T. P., and Bradshaw, C., 2000. The taphonomic significance of endoliths in dead-versus live-coral skeletons. *Palaaios*, **15**, 248–254.
- Scott, P. J. B., and Risk, M. J., 1988. The effect of *Lithophaga* (Bivalvia: Mytilidae) boreholes on the strength of the coral *Porites lobata*. *Coral Reefs*, **7**, 145–151.
- Shashar, N., and Stambler, N., 1992. Endolithic algae within corals – life in an extreme environment. *Journal of Experimental Marine Biology and Ecology*, **163**, 277–286.
- Simonsen, A. H., and Cuffey, R. J., 1980. Fenestrate, pinnate and ctenostome bryozoans and associated barnacle borings in the Wreford Megacyclothem (Lower Permian) of Kansas, Oklahoma and Nebraska. *University of Kansas Paleontological Contribution, Paper*, **101**, 11–38.
- Smyth, M. J., 1988. The foraminifer *Cymbaloporella tabellaeformis* (Brady) bores into gastropod shells. *Journal of Foraminiferal Research*, **18**, 277–285.
- Spencer, T., and Viles, H., 2002. Bioconstruction, bioerosion, and disturbance on tropical coasts: coral reefs and rocky limestone shores. *Geomorphology*, **48**, 23–50.
- Stockfors, M., and Peel, J. S., 2005. Euendolithic cyanobacteria from the Middle Cambrian of North Greenland. *Geologiska Föreningens i Stockholm Förhandlingar, IFF*, **127**, 179–185.
- Szmant, A. M., 2002. Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? *Estuaries*, **25**, 743–766.
- Tapanila, L., 2008. The medium is the message: imaging a complex microboring (*Pyrodendrina cupra* n. igen. and isp.) from the early Paleozoic of Anticosti Island, Canada. In Wisshak, M., and Tapanila, L. (eds.), *Current Developments in Bioerosion*. Berlin, Heidelberg: Springer, pp. 123–146.
- Taylor, P. D., and Wilson, M. A., 2003. Palaeoecology and evolution of marine hard substrate communities. *Earth-Science Reviews*, **62**, 1–103.
- Todd, J. A., 2000. The central role of ctenostomes in bryozoan phylogeny. In Herrera Cubilla, A., and Jackson Jeremy, B. C. (eds.), *Proceedings of the 11th International Bryozoology Association Conference*. Balboa, RP: Smithsonian Tropical Research Institute, pp. 104–135.
- Tribollet, A., 2008a. Dissolution of dead corals by euendolithic microorganisms across the northern Great Barrier Reef (Australia). *Microbial Ecology*, **55**, 569–580.
- Tribollet, A., 2008b. The boring microflora in modern coral reef ecosystems: a review of its roles. In Wisshak, M., and Tapanila, L. (eds.), *Current Developments in Bioerosion*. Berlin-Heidelberg: Springer, pp. 67–94.
- Tribollet, A., and Golubic, S., 2005. Cross-shelf differences in the pattern and pace of bioerosion of experimental carbonate substrates exposed for 3 years on the northern Great Barrier Reef, Australia. *Coral Reefs*, **24**, 422–434.
- Tribollet, A., and Payri, C., 2001. Bioerosion of the crustose coralline alga *Hydrolithon onkodes* by microborers in the coral reefs of Moorea, French Polynesia. *Oceanologica Acta*, **24**, 329–342.
- Tribollet, A., Atkinson, M., and Langdon, C., 2006. Effects of elevated pCO₂ on epilithic and endolithic metabolism of reef carbonates. *Global Change Biology*, **12**, 2200–2208.
- Tudhope, A. W., and Risk, M. J., 1985. Rate of dissolution of carbonate sediments by microboring organisms, Davies Reef, Australia. *Journal of Sedimentary Petrology*, **55**, 440–447.
- Underwood, C. J., Mitchell, S. F., and Veltkamp, C. J., 1999. Microborings in mid-Cretaceous fish teeth. *Proceedings of the Yorkshire Geological Society*, **52**, 269–274.
- Vogel, K., Golubic, S., and Brett, C. E., 1987. Endolith associations and their relation to facies distribution in the Middle Devonian of New York State, U.S.A. *Lethaia*, **20**, 263–290.
- Vogel, K., Gektidis, M., Golubic, S., Kiene, W. E., and Radtke, G., 2000. Experimental studies on microbial bioerosion at Lee Stocking Island, Bahamas and One Tree Island, Great Barrier Reef, Australia: implications for paleoecological reconstructions. *Lethaia*, **33**, 190–204.

- Vooren, C. M., 1981. Photosynthetic rates of benthic algae from the deep coral reef of Curaçao. *Aquatic Botany*, **10**, 143–154.
- Walker, S. E., 2007. Traces of gastropod predation on molluscan prey in tropical reef environments. In Miller III, W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier, pp. 324–344.
- Wanders, J. B. W., 1977. The role of benthic algae in the shallow reef of Curaçao (Netherlands Antilles) III: the significance of grazing. *Aquatic Botany*, **3**, 357–390.
- Wielgus, J., Glassom, D., and Chadwick, N. E., 2006. Patterns of polychaete worm infestation of stony corals in the northern Red Sea and relationships to water chemistry. *Bulletin of Marine Science*, **78**, 377–388.
- Wilhelm, C., and Jakob, T., 2006. Uphill energy transfer from long-wavelength absorbing chlorophylls to PS II in *Ostreobium* sp. is functional in carbon assimilation. *Photosynthesis Research*, **87**, 323–329.
- Wilkinson, C., 2004. *Status of Coral Reefs of the World: 2004*. Townsville, Australia: Australian Institute of Marine Science, 557 pp.
- Wilson, M. A., 2007. Macroborings and the evolution of marine bioerosion. In Miller III, W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier Scientific Publishing Co., pp. 356–367.
- Wisshak, M., 2006. High-latitude bioerosion. In Bhattacharji, S., Neugebauer, H. J., Reitner, J., and Stüwe, K. (eds.), *Lecture Notes in Earth Sciences*, **109**, 1–202. Berlin-Heidelberg: Springer-Verlag.
- Wisshak, M., Gektidis, M., Freiwald, A., and Lundälv, T., 2005. Bioerosion along a bathymetric gradient in cold-temperate setting (Kosterfjord, SW Sweden): an experimental study. *Facies*, **51**, 93–117.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from Early Proterozoic stromatolites, Hebei, China, *Acta Micropaleontologica Sinica*, **4**, 1–12.
- Zubia, M., and Peyrot-Clausade, M., 2001. Internal bioerosion of *Acropora formosa* in Reunion (Indian Ocean): microborer and macroborer activities. *Oceanologica Acta*, **24**, 251–262.
- Zundelovich, A., Lazar, B., and Ilan, M., 2007. Chemical versus mechanical bioerosion of coral reefs by boring sponges – lessons from *Pione cf. vastifica*. *The Journal of Experimental Biology*, **210**, 91–96.

Cross-references

Algae (Eukaryotic)
 Animal Skeletons, Advent
 Basalt (Glass, Endoliths)
 Biodeterioration (of Stone)
 Biogeochemical Cycles
 Biomineral (Mineral Bioleaching, Mineral Biooxidation)
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Carbon Cycle
 Carbonate Environments
 Carbonates
 Cyanobacteria
 Endoliths
 Extracellular Polymeric Substances (EPS)
 Extreme Environments
 Foraminifera
 Fungi and Lichens
 Glass
 Geomycology
 Ichnology
 Karst Ecosystems
 Microbial Biomineralization

Microbiocorrosion

Reefs

Sediment Diagenesis – Biologically Controlled
 Sponges (Porifera) and Sponge Microbes
 Trace Fossils: Neoproterozoic

Additional references

- <http://www.sbg.ac.at/ipk/avstudio/pierofun/transcript/bioeros.pdf> (Bioerosion)
<http://bioweb.usc.edu/documents/bisc469-outlines.pdf>
<http://paleopolis.rediris.es/phoronida/ref/ref90.htm>
<http://www.wooster.edu/geology/Bioerosion/Bioerosion.html>
<http://www.wooster.edu/geology/Bioerosion/BioerosionBiblio.pdf> (Wilson, M.A.)
<http://en.wikipedia.org/wiki/Bioerosion> (Wilson, M.A.)
<http://www.diva-portal.org> (urn_nbn_se_uu_diva-5726-2_full-text.pdf)
<http://www.havforsker.dk/hjemmeside.php?id=4&forskning> (Benthic Foraminifera)

BIOFILMS

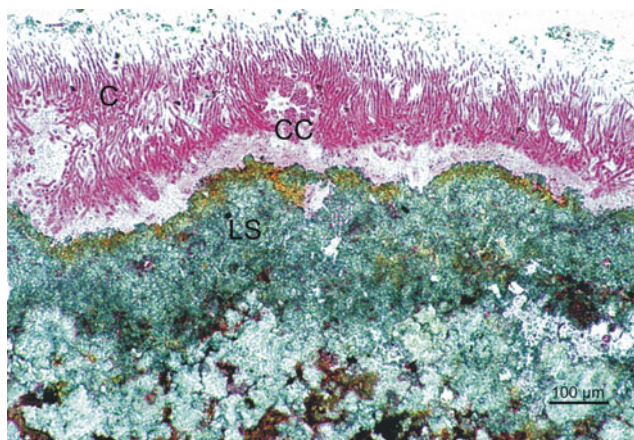
Joachim Reitner

University of Göttingen, Göttingen, Germany

Definition

Nearly all hard surfaces exhibit biofilms, organic coatings of microbial cells which are enclosed in extracellular polymeric substances (EPS). Biofilms interact between hard substrates such as minerals and rocks, water bodies, and the atmosphere. Metabolic exchange processes controlled by biofilms, globally influence bio-geochemical cycles.

Biofilms and microbial mats are benthic systems formed by different microbial communities which secrete hydrated mucus substances (EPS) (e.g., Characklis and Wilderer, 1989; Costerton et al., 1987, 1995) in various amounts. These EPS substances are mainly polysaccharides, glycoproteins, exoenzymes, and nucleic acids (Sutherland, 1977; Decho, 1990; Wingender et al., 1999). Biofilms and microbial mats display a continuous spectrum ranging from thin micrometer-sized coatings of mono-specific and poly-specific microbial populations up to centimeter thick communities of various microbes such as archaea, bacteria, eukaryotic algae, fungi, and protozoa. In many cases, these thick microbial mats exhibit a clearly visible ecological zoning owing to the presence of different microbial communities which are often characterized by colored pigments. The geobiological importance of microbial mats was recognized early by geoscientists (Walcott, 1914; Black, 1933; Pia, 1933) in contrast to the hardly visible biofilms which are globally much more widespread. Engineers and microbiologists, for example, Characklis and Wilderer (1989); Flemming (1991); Muyzer et al. (1993); Costerton et al. (1987), (1995); Neu and Lawrence (1999), have recognized the enormous importance and potential of biofilms in



Biofilms, Figure 1 Cyanobacterial biofilm forming a modern aragonitic stromatolite (Walker Lake, Nevada). Biofilm is dominated by filamentous cyanobacteria – *Calothrix* (C). Few coccoid cyanobacteria are also present (CC). Within the youngest lithified part, remains of cyanobacterial sheets are visible (LS).

many common processes. Geoscientists have increasingly investigated the rock- and mineral-forming potentials of biofilms in the 90s of the last century (e.g., Ferris, 1991; Pedley, 1992; Reitner, 1993). Biofilms were also found in deep subsurface environments and, significantly for the Deep Biosphere, up to the upper known temperature limit of life (110–120°C) (Pedersen, 1993; Fyfe, 1996; Gold, 1999; Stetter, 1994, 1996).

Organo-films are special organic coatings which are formed by various macromolecules and do not contain living cells. Organo-films are taphonomic remains of biofilms or newly formed from biogenic degradation products (geopolymers sensu Killops and Killops, 1997; Gold, 1999). Adsorption on mineral surfaces and self organizing catalytic processes play a central role during formation (Ferris et al., 1996; Reitner, 1993).

Stromatolites, thrombolites, and generally microbialites, are lithified products of bio- and organofilms, as well as thick microbial mats (Figure 1).

Bibliography

- Black, M., 1933. *The Algal Sediments of Andros Island, Bahamas*. London: Philosophical Transactions of the Royal Society of London, Vol. B222, pp. 165–192.
- Characklis, W. G., and Wilderer, P. A., (eds.), 1989. *Structure and Function of Biofilms*. Life sciences research reports, 46: 387 S. Chichester: Wiley.
- Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. J., Nickel, J. C., Dasgupta, M., and Marrie, T., 1987. Bacterial biofilms in nature and disease. *Annual Reviews in Microbiology*, **41**, 435–464.
- Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., and Lappin-Scott, H. M., 1995. Microbial biofilms. *Annual Reviews in Microbiology*, **49**, 711–745.
- Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review*, **28**, 73–153 (Aberdeen).

- Ferris, F. G., 1991. Biogeochemical importance of microbial biofilms. *Geological Association of Canada Program with Abstracts*, **37**, Waterloo.
- Ferris, J. P., Hill, A. R., Liu, R., and Orgel, L. E., 1996. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature*, **381**, 59–61.
- Flemming, H. C., 1991. Biofilms as a particular form of microbial life. In Flemming, H. C., and Geesey, G. G. (eds.), *Biofouling and Biocorrosion in Industrial Water Systems*. Heidelberg: Springer, pp. 3–9.
- Fyfe, W. S., 1996. The biosphere is going deep. *Science*, **273**, 448 (Washington).
- Godderis, Y., and Veizer, J., 2000. Tectonic control of chemical and isotopic composition of ancient oceans; the impact of continental growth. *American Journal of Science*, **300**(5), 434–461.
- Gold, T., 1999. *The Deep Hot Biosphere*. New York: Copernicus, p. 235 S.
- Killops, S. D., and Killops, V. J., 1997. *Einführung in die organische Geochemie*. Stuttgart: Enke.
- Muyzer, G., de Waal, E. C., and Uitterlinden, A. G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, **59**, 695–700.
- Neu, T. R., and Lawrence, J. R., 1999. Lectin-binding analysis in biofilm systems. *Methods in Enzymology*, **310**, 145–152.
- Pedersen, K., 1993. The deep subterranean biosphere. *Earth Science Reviews*, **34**, 243–260 (Amsterdam).
- Pedley, M., 1992. Freshwater (phytoherm) reefs. The role of biofilms and their bearing on marine reef cementation. *Sedimentary Geology*, **79**, 255–274 (Amsterdam).
- Pia, J. v., (1933): *Die rezenten Kalksteine*. - 420 S., Leipzig (Akademische Verlagsgesellschaft).
- Reitner, J., 1993. Modern cryptic microbialite/Metazoan facies from lizard island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40 (Erlangen).
- Stetter, K. O., 1994. The lesson of archaeobacteria. In Bengtson, S. (ed.), *Early Life on Earth*. Nobel Symposium, 84, New York: Columbia University Press, pp. 143–151.
- Stetter, K. O., 1996. Hyperthermophilic procaryotes. *FEMS Microbiology Reviews*, **18**, 149–158 (Amsterdam).
- Sutherland, I. W. (ed.), 1977. *Surface Carbohydrates of the Prokaryotic Cell*. London: Academic Press, p. 472 S.
- Walcott, C. D., 1914. Prekambrian algonkian flora. *Smithsonian Miscellaneous Collections*, **64**, 77–156.
- Wingender, J., Neu, T. R., and Flemming, H. C. (eds.), 1999. *Microbial Extracellular Polymeric Substances. Characterization, Structure and Function*. Berlin: Springer, p. 258 S.

Cross-references

[Biofilms and Fossilization](#)
[Calcified Cyanobacteria](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Desert Varnish](#)
[Detachment](#)
[Ediacaran Biota](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Frutexites](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Moonmilk](#)
[Organomineralization](#)
[Stromatolites](#)
[Thrombolites](#)

BIOFILMS AND FOSSILIZATION

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Carbonaceous bacterial biofilms; Hautschatten

Definition

Microbial biofilms cover normally in aquatic environments dead organisms and control partly the process of decay. The soft tissues of the body outline are preserved as a layered structure which comprises a carbonaceous microbial biofilm, in the literature often called “Hautschatten” (Figure 1). Fossils with soft part preservation are extremely rare, but under certain circumstances, like suboxic or anaerobic conditions, remains of their soft tissues with detailed histological fabrics are found. The evidence of non-biomineralized soft tissue normally degrading completely during decay is linked with special microbial processes. This exceptional preservation requires soft tissues to be replicated by authigenic minerals or pseudomorphed by bacterial biofilms (e.g., Wuttke, 1983; Toporski et al., 2002; Briggs, 2003). The different organs in many cases often exhibit different microbially controlled lithified biofilms with an unusual preservation of involved microbes.

Geobiological implications and examples

Understanding the relationship between decay and mineralization is of fundamental importance to palaeontologists

interested in the record of soft-bodied organisms. Soft-bodied fossils also provide insights into early diagenetic processes in sediments and give basic information on related organic geochemistry. The lack of biomineralized hard parts in many organisms is the normal condition in the fossil record. However, the mode of soft-tissue decay is far from uniform and the sum of various processes results in a preservation in so-called Konservat-Lagerstätten (Seilacher, 1970; Seilacher et al., 1985).

Derek Briggs and his group have made fundamental experiments on how the preservation of soft-tissues works (e.g., Briggs, 1999, 2003; Briggs and Kear, 1993; Briggs and Wilby, 1996; Grimes et al., 2001; Gupta et al., 2006). For example, they could demonstrate that initial pyritization of a plant tissue can be an extremely rapid process (within 80 days) and is driven by anaerobic bacterially mediated decay. They have made comparable experiments mainly with arthropods. Toporski's and coworkers' (Toporski et al., 2002) investigation on the Oligocene Enspel Maar black shale is a milestone in the research on soft-tissue preservation via microbial biofilms. The multitask biogeochemical analyses give the deepest insight into the taphonomy of soft tissue and microbial-related decay processes. The most famous locality with preserved soft tissues is the Eocene Messel locality. From this locality, soft tissues covered with lithified biofilms were first described by Wuttke (1983). Thick microbial biofilms are generally much darker than the surrounding black-shale and form the so-called “Hautschatten” and were for a long time believed to represent fossilized soft tissues. The biofilms trace back the decaying soft tissue in an anaerobic environment and “casting” of the former soft tissues. Often the digestive



Biofilms and Fossilization, Figure 1 A very well-preserved mouse (*Apodemus atavus*) from the late Pliocene fossil lagerstaette Willershausen (Lower Saxony, Germany) with perfectly preserved biofilm-related “Hautschatten” with visible remains of the former fur (Geoscience Museum University of Göttingen).

system of large fossils is excellently preserved (Franzen, 1994). Fundamental studies on biofilm soft-tissue relationship were carried out by Richter (1994) and Liebig (1997). Another intriguing example is the “soft-tissue” preservation in dinosaur bones (Kaye et al., 2008). Kaye and coworkers have found microbially altered soft tissue in a dinosaur bone and they give a critical explanation of this fact. Within their paper they discuss the processes of tissue preservation and also demonstrate the importance of recent microbial biofilm invasion and late diagenetic alteration of primarily formed pyrite framboids. The clou of this investigation is also that there is no real soft-tissue preservation – however, preserved biofilms cast the former tissue and give an idea about the original structures.

All “Hautschatten” soft-tissue preservations are probably preserved lithified biofilms and not real soft tissues.

Conclusion

Biofilms play a significant role in suboxic and anaerobic milieus preserving soft tissue remains. The body outline of dead organisms is exactly traced by the margins of the mineralised biofilms and give a perfect image of the entire fossil. These structures are often called after the German word “Hautschatten”-perservation and is commonly found under black shale condition.

Bibliography

- Briggs, D. E. G., 1999. Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis. *Philosophical Transactions of Royal Society Series B*, **354**, 7–16.
- Briggs, D. E. G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences*, **31**, 275–301.
- Briggs, D. E. G., and Kear, A. J., 1993. Fossilization of soft-tissue in the laboratory. *Science*, **259**, 1439–1442.
- Briggs, D. E. G., and Wilby, P. R., 1996. The role of calcium carbonate – calcium phosphate switch in the mineralization of soft bodied fossils. *Journal of the Geological Society, London*, **153**, 665–668.
- Franzen, J. L., 1994. Bacterial preservation of the digestive system in Messel Equoids. *Kaupia*, **4**, 3–11.
- Grimes, S. T., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Gupta, N. S., Michels, R., Briggs, D. E. G., Evershed, R. P., and Pancost, R. D., 2006. The organic preservation of fossil arthropods: an experimental study. *Proceedings of the Royal Society of London, Series B*, **273**, 2777–2783.
- Kaye, T. G., Gaugler, G., and Sawlowicz, Z., 2008. Dinosaur soft tissues interpreted as bacterial biofilms. *PLOS One*, **3**(7), e2808.
- Liebig, K., 1997. *Fossil Microorganisms from the Eocene Messel Oil Shale of Southern Hessen*. University Dissertation, Heidelberg, Germany.
- Richter, G., 1994. Bacteria and bacteria-like structures from the oil-shale of Messel. *Kaupia*, **4**, 21–28.
- Seilacher, A., 1970. Begriff und Bedeutung der Fossil-Lagerstätten. *Neues Jahrbuch Fur Geologie und Paläontologie, Monatshefte*, **1970**, 34–39.
- Seilacher, A., Reif, W. E., and Westphal, F., 1985. Sedimentological, ecological and temporal patterns of fossil lagerstätten.

Philosophical Transactions of the Royal Society of London, Series B, **311**, 5–23.

Toporski, J. K. W., Steele, A., Westall, F., Avet, R., Martill, D. M., and McKay, D. S., 2002. Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany. *Geochimica et Cosmochimica Acta*, **66**, 1773–1791.

Wuttke, M., 1983. “Weichteilerhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

[Bacteria](#)
[Beggiatoa](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Black Shales](#)
[Detachment](#)
[Ediacaran Biota](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Thiotrophic Bacteria](#)
[Whale and Wood Falls](#)

BIOGEOCHEMICAL CYCLES

A biogeochemical cycle defines the pathways by which chemical elements occurring in organisms are cycled between different living and nonliving compartments on the Earth (e.g., biosphere, atmosphere, hydrosphere, geosphere, etc.). Typical driving forces of a biogeochemical cycle are the metabolisms of living organisms, geological processes, or chemical reactions. For specific reading on major biogeochemical cycles, please refer to “[Carbon Cycle](#),” “[Nitrogen](#),” “[Sulfur Cycle](#),” “[Phosphorus](#),” “[Phosphorites](#).”

BIOLOGICAL CONTROL ON DIAGENESIS: INFLUENCE OF BACTERIA AND RELEVANCE TO OCEAN ACIDIFICATION

Fred T. Mackenzie¹, Andreas J. Andersson²

¹University of Hawaii, Honolulu, HI, USA

²Bermuda Institute of Ocean Sciences, Ferry Reach, St. George's, Bermuda

Introduction

Soon after carbonate mineral particles are deposited, a series of chemical, physical, and biological processes start that can significantly alter their mineralogy, isotopic composition and chemistry, and the fabric of the accumulating sediment. These processes include dissolution and precipitation reactions, abrasion, and transport, and

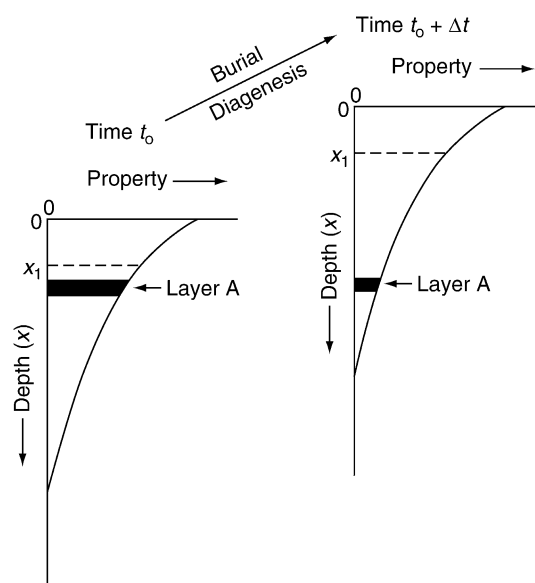
ingestion and boring by organisms. The processes occurring after deposition fall into the category of diagenesis. In particular, carbonate sediments consisting of the minerals low (<4 mol% MgCO_3) and high (>4 mol% MgCO_3) biogenic and inorganic magnesian calcite and aragonite and organic matter deposited in shallow waters are repeatedly deposited, resuspended, and then deposited again during periods of elevated turbulence, which are common especially during storms. Consequently, carbonate minerals may be subjected to several depositional environments before being permanently buried in a sediment. The study of carbonate diagenesis is further complicated by the fact that significant amounts of shallow-water carbonates are commonly associated with reefs, for which traditional models of sediment diagenesis are not always appropriate.

Soon after deposition, chemical and physical changes occur in carbonate sediments as a result of a myriad of diagenetic processes. Among the most important are dissolution-precipitation reactions and major alterations of pore water chemistry by bacterially mediated oxidation of organic matter. These changes generally occur most rapidly in the upper few to tens of centimeters of sediment. It is this topic that is most fully addressed in this article. In addition, transport within the sediment of both solid and dissolved components is also of fundamental importance in diagenesis. It can be caused by physical stirring resulting from the movement of infaunal organisms (e.g., worms, bivalves, shrimp), and is called bioturbation. Closely associated with bioturbation is bioirrigation, which is a result of organisms pumping water through their burrows. Deeper within the sediment and thus later in its history, advection of pore water and molecular diffusion in pore water are generally the dominant transport processes.

The diagenetic equation

Since the early 1960s, considerable effort has gone into modeling the diagenesis of sediments. A central theme of most models is that diagenetic change results from the sum of transport and chemical and biological reactions of various types. Diagenetic models can be divided into those assuming constant conditions (steady-state models) and those taking into account factors that may vary with time (e.g., temperature, salinity, material input rates, water depth). The primary idea in the steady-state models is that a property in the sediment, at a given depth below the sediment-water interface, is time-independent (Figure 1). Thus, if one were to measure the pore water acidity (pH) in a sediment at 20 cm depth and come back 10 years later and again measure the pH at the 20 cm depth, it would be the same. This statement would be true even though the sediment was not physically the same at the 20 cm depth because of deposition during the intervening 10 years.

The general diagenetic equation from which many important models have been derived (e.g., Berner, 1980) is:



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 1 Diagram illustrating under steady-state (constant) conditions how a property such as hydrogen ion concentration varies with depth as the sediment column accumulates. Note that a property at fixed depth x_1 does not change in magnitude as the sediment accumulates.

$$(\partial C_i / \partial t)_x = -\partial F_i / \partial x + \sum R_i \quad (1)$$

where C_i = concentration of solid or liquid component i in mass per unit volume of total sediments (solids plus pore water); F_i = flux of component i in terms of mass per unit area of total sediment per unit time; and R_i = rate of each diagenetic chemical, biochemical, and radiogenic reaction affecting i in terms of mass per unit volume of total sediment per unit time. An example of application of this equation would be the change in hydrogen ion concentration (pH) with time at a given depth in a sediment due to diffusive transport and the dissolution of calcium carbonate in the sediment. For a steady-state condition, Equation 1 is set equal to 0, because at a given depth x , the hydrogen ion concentration does not change with time. Steady-state models are generally more amenable to a mathematical solution than non-steady-state models. However, diagenesis in many shallow-water carbonate sediments is significantly influenced or even dominated by non-steady-state processes.

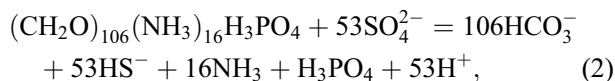
Dissolution of carbonates

Continental shelves and slopes comprise about 20% of the Earth's surface, and contain more than half of the sediments in the ocean. Recent estimates of marine carbonate burial rates indicate that between 35% and 70% of Holocene carbonate deposition has taken place on continental shelves and today's production of shallow-water biogenic carbonate ("calcareous") skeletons and tests represents about 28% of the total carbonate produced in the world's oceans.

A substantial fraction (>50%) of shallow-water produced CaCO_3 is at present time permanently buried in the sediments. In contrast, most of the CaCO_3 produced in the open ocean mainly by planktonic organisms such as coccolithophorids, forams, and pteropods dissolves before it reaches the ocean floor. As much as 50–90% of the material may dissolve in the upper 1,000 m owing to microbial decomposition of organic matter creating microenvironments corrosive to CaCO_3 particles. Typical examples of such environments include inside the guts of zooplankton or within aggregates of CaCO_3 and organic material referred to as “marine snow.” Carbonate material making it past the depth of the saturation horizon, the thermodynamic equilibrium boundary between the forward and backward reactions of precipitation and dissolution, will consequently become subject to dissolution regardless of the activity of microbes and the formation of microenvironments.

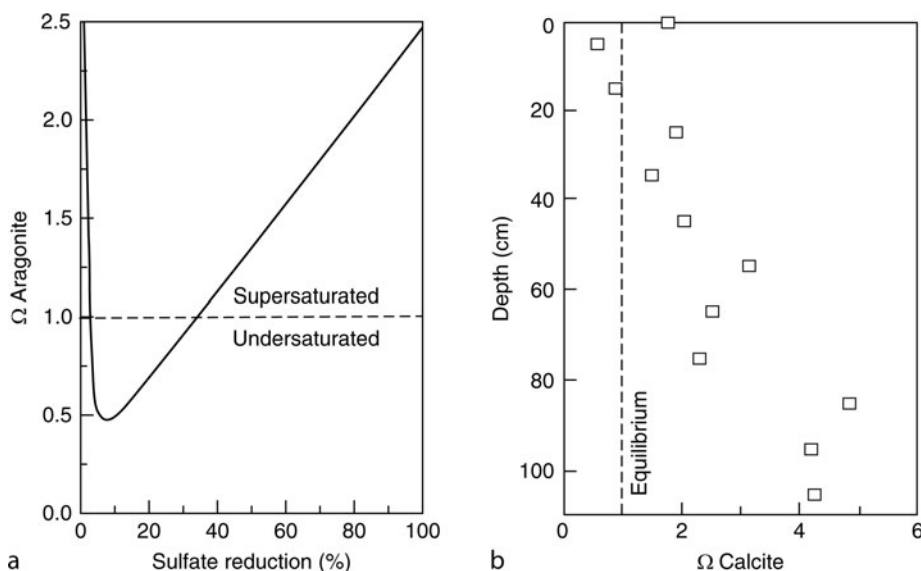
Since most shallow-water environments (coastal zone, continental shelf, banks, reefs, etc.) at present time are immersed in seawater supersaturated with respect to calcite, aragonite, and most Mg-calcite phases, dissolution of sedimentary CaCO_3 minerals in these environments is completely driven by microbially mediated processes associated with oxidation of organic material in the pore water-sediment system or boring autotrophic or heterotrophic microorganisms referred to as endoliths. In the presence of oxygen, the extent of CaCO_3 dissolution is almost completely controlled by the amount and the extent of organic matter decomposed or respired, but under anoxic conditions, oxidation of organic matter accompanied by reduction of sulfate can produce radically different

results. Most studies of the impact of chemical diagenesis on the carbonate chemistry of anoxic sediments have focused primarily on the fact that sulfate (SO_4^{2-}) reduction results in the production of alkalinity (mostly dissolved bicarbonate [HCO_3^-], carbonate [CO_3^{2-}] and carbonic acid [H_2CO_3] and CO_2 chemical species), which can cause precipitation of carbonate minerals (e.g., Berner, 1971). The reduction of the sulfate is promoted by bacteria, particularly *Desulfovibrio desulfuricans*, splitting the sulfur–oxygen bond and leading to the accumulation of dissolved chemical species of reduced sulfur (mainly H_2S and HS^- ; these sulfur species may contribute to the total alkalinity of the pore waters) in the pore waters and in sediments containing iron as ferrous sulfide minerals (mainly pyrite, “fool’s gold,” FeS_2) and free solid sulfur. Although the reaction is complex, this process can be schematically represented, using the average composition of organic matter of phytoplankton as substrate material, as:



which results in the commonly observed decrease in sulfate ion, and pH (measure of hydrogen ion concentration, H^+), and increase in total alkalinity, hydrogen sulfide (H_2S), phosphate (PO_4^{3-}), ammonia (NH_3), and total dissolved inorganic carbon (DIC) in sediment pore waters with increasing depth below the sediment–water interface.

During the early stages of sulfate reduction (~2 to 35%) (Figure 2), this reaction may not cause precipitation,

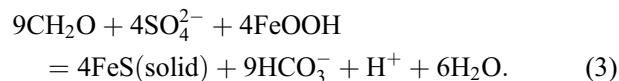


Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 2 The saturation state with respect to aragonite (Ω aragonite) of marine pore waters as a function of the amount of sulfate reduced to sulfide based on the model of Ben Yakoov (a). Saturation state with respect to calcite (Ω calcite) as a function of depth as observed in Mangrove Lake, Bermuda sediment pore waters. (From Morse and Mackenzie, 1990.)

but rather results in undersaturation conditions with respect to carbonate minerals because the impact of lowered pH (increasing H^+) has a larger negative effect on the total alkalinity than the positive effect on this chemical property arising from reduction of sulfate. Carbonate ion activity decreases rapidly as it is "titrated" by CO_2 from organic matter decomposition abetted by bacteria leading to a decrease in pore water saturation state. This process is evident in observational data for Fe-poor shallow water carbonate sediments from the Bahamas and has been confirmed in recent studies of sediments from Florida Bay, in the Checker Reef of Kaneohe Bay, Oahu, Hawaii, and in organic-rich sediments of Mangrove Lake, Bermuda (Morse and Mackenzie, 1990) and elsewhere. In Mangrove Lake, it was shown based on model calculations that the degree of undersaturation of anoxic pore waters with respect to carbonate mineral compositions can depend significantly on the carbon to nitrogen ratio (C/N) of the labile organic matter undergoing bacterial oxidation in the sediment pore water (Figure 3).

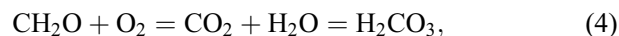
This sulfate reduction reaction in anoxic carbonate sediments has potential importance for carbonate dissolution in shallow-water, marine environments, but its global significance remains a question. An observation of interest is that even complete sulfate reduction can return the saturation state of the pore water only to about half of its original value. Thus, the sulfate reduction reaction by itself may not promote carbonate precipitation as total alkalinity increases and partial sulfate reduction may result in carbonate dissolution. The combination of the sulfate reduction reaction and reactions primarily with iron and

manganese oxide minerals in the sediments can lead to significant calcium carbonate precipitation. This type of net process can be represented schematically as (where CH_2O is a simplified formula for organic matter):

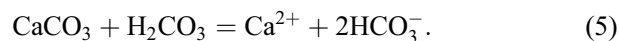


Reaction 3 results in the pH of the waters, in the absence of carbonate precipitation, being buffered at higher pH values. It is, therefore, reasonable to expect that the effectiveness of sulfate reduction in producing carbonate dissolution or precipitation may depend in part on the availability of reactive iron. This conclusion has been demonstrated in a study of the pore water geochemistry of aluminosilicate and carbonate-rich sediments from Kaneohe Bay, Oahu, Hawaii. The aluminosilicate sediments contain abundant pyrite whereas the pyrite content of the carbonate sediments in the bay is low. Pore waters collected from the aluminosilicate sediments have higher pH values than those collected from the carbonate-rich sediments. This observation is a result of the pH values in the pore waters of the aluminosilicate sediments being buffered at higher values than those for carbonate sediments because of Reaction 3 (Mackenzie et al., 1981).

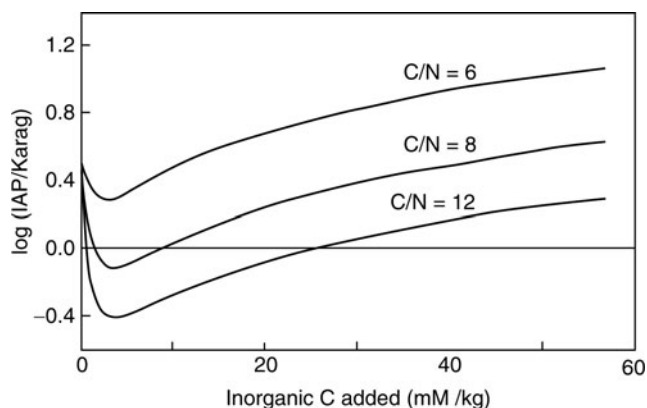
There are several processes that have been identified that can cause undersaturation of pore waters with respect to carbonate minerals, in addition to the previously discussed undersaturation conditions that may result during the early stages of sulfate reduction. These are early post-death microenvironments within organisms, oxidation of organic matter by processes preceding sulfate reduction, and oxidation of sulfides. In addition, both autotrophic and heterotrophic endoliths actively penetrate carbonate substrates by creating corrosive conditions with respect to these mineral phases. These processes commonly will be most important near the sediment–water interface. The most important process occurring prior to the onset of bacterial sulfate reduction is extensive organic matter degradation due to bacterially mediated oxygen reduction, a process important in both shallow-water and deep-sea carbonate sediments. The influence of benthic bacterial activity under aerobic conditions on carbonate mineral dissolution was nicely demonstrated by Moulin et al. (1985) for pore waters from sediments of the Gulf of Calvi, Corsica. Under aerobic conditions, the oxidation of organic matter may be written:



and if carbonate minerals were to dissolve because of the CO_2 produced by organic matter decomposition, then the reaction:

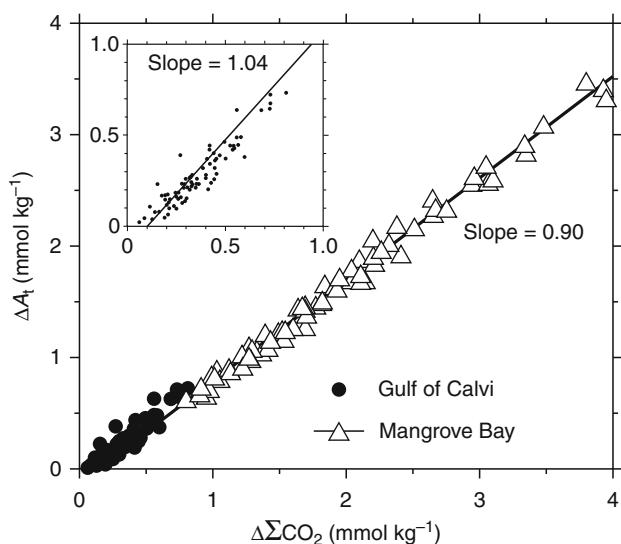


The overall net Reaction of 4 + 5 is:



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 3 Diagram illustrating how the C/N ratio of the organic matter deposited influences the saturation state with respect to aragonite ($\log(IAP/Karag)$; 0.0 = saturation) of the pore waters of Mangrove Lake, Bermuda organic-rich sediments. The saturation state is shown as a function of the amount of dissolved inorganic carbon (DIC) added to the pore waters from the oxidation of organic matter by aerobic oxygen consumption and anaerobic sulfate reduction processes. (From Morse and Mackenzie, 1990.)

Reaction 6 is possible only if the pore waters are undersaturated with a carbonate phase, and there are no other competing reactions, like significant ammonia release from decaying organic matter, to increase pore water saturation state. **Figure 4** shows the increase in total alkalinity versus increase in total CO_2 (DIC) as observed in shallowly buried pore waters in the Gulf of Calvi *Lithothamnion* sp. algal-rich sediments and in low and high magnesian calcite- and aragonite-rich sediments from Mangrove Bay, Bermuda. The slopes of the regression lines for the relationships are close to 1. The slope indicates that **Reaction 6** most likely gives rise to the increased total alkalinity observed in these pore waters; that is, the H_2CO_3 produced by organic matter oxidation is stoichiometrically utilized in dissolution of a carbonate mineral phase in the sediment. Close inspection of the regression line for the Gulf of Calvi sediments in **Figure 4** shows that it does not pass through the origin. This is because about 0.1 mmol kg^{-1} of H_2CO_3 has been added to the pore waters from aerobic bacterial respiration prior to carbonate dissolution. This initial input of H_2CO_3 lowers the saturation state of the shallowly buried pore waters to that required for carbonate mineral dissolution. These pore water observations have been confirmed in experiments using a biological reactor,



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 4 Increase in total alkalinity (ΔA_t , change in sediment pore water total alkalinity from that of overlying water column) and total DIC ($\Delta \Sigma \text{CO}_2$, change in sediment pore water total DIC from that of overlying water column) in pore waters of Gulf of Calvi, Corsica (insert, Moulin et al., 1985), and Mangrove Bay, Bermuda. Notice the slope of the lines of nearly 1 as predicted from **Reaction 6**. At zero total alkalinity the total DIC is not zero due to the addition of dissolved carbon species to the pore water column from the biological, mainly bacterial, remineralization of organic matter at very shallow depths (several centimeters) in the sediments above the depth, at which the waters become undersaturated with some magnesian calcite compositions and carbonate minerals dissolve with increasing depth.

and from solid phase mineralogical studies. High magnesian calcites are dissolved preferentially in these sediments, although the sediment contains a mixture of nearly pure calcite, aragonite, and magnesian calcites with up to 20 mol% MgCO_3 . In the Gulf sediments composed principally of the red alga *Lithothamnion* sp., this early diagenetic reaction has resulted in dissolution of 75% of the carbonate initially deposited. Furthermore, in the case of the study of Mangrove Bay, the pore waters were sampled every few hours during a complete, 24 h, diel cycle. The trend and relationship between changes in total alkalinity and total DIC are essentially the same as observed in the sediments of Gulf of Calvi. In addition, sea grasses, such as *Thalassia*, which covers vast areas of the bottom in Mangrove Bay probably enhances carbonate dissolution in these sediments by releasing oxygen through its roots and rhizomes into the sediments that consequently enhances decomposition of organic matter and production of H_2CO_3 . Evidence from the Bahamas bank has clearly demonstrated enhanced calcium carbonate dissolution owing to this process (Burdige and Zimmerman, 2002).

Other reactions of less importance than those above leading to undersaturated conditions with respect to calcium carbonate near the sediment–water interface include nitrate reduction and fermentation (e.g., Aller, 1980). Such reactions may also be important near the sediment–water interface of continental shelf and slope sediments, where bioturbation and bioirrigation can result in enhanced transport of reactants. Generally, as the water depth increases over continental slope sediments, the depths increase within the sediment at which significant sulfate reduction and reactions other than sulfate reduction influence carbonate chemistry.

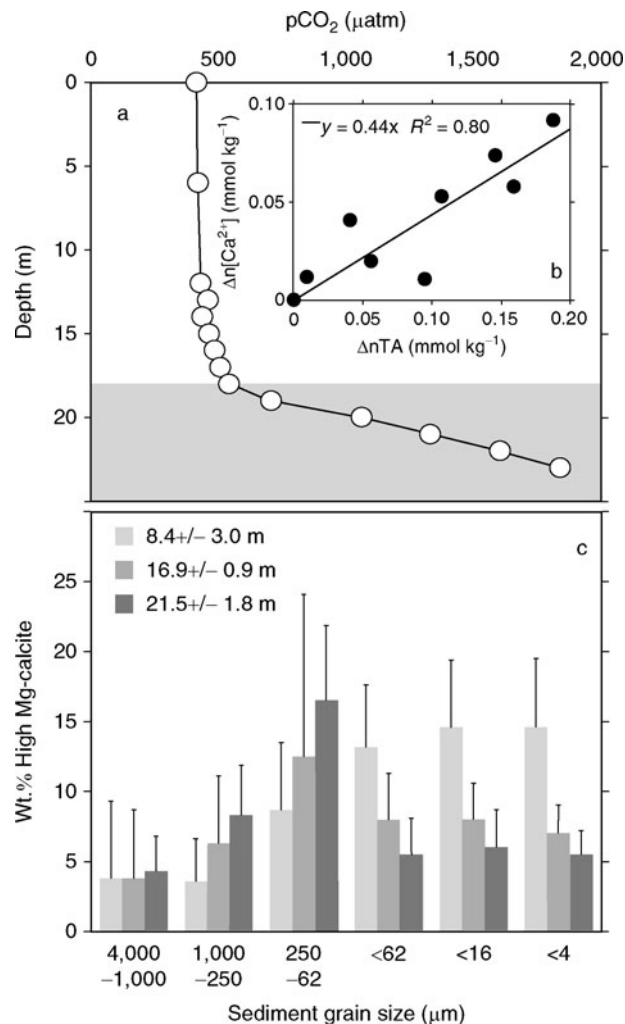
Carbonate dissolution and ocean acidification

Future increases in atmospheric CO_2 owing to human activities, such as burning of fossil fuels and land-use changes, result in increased uptake of part of this CO_2 by ocean surface seawater and formation of carbonic acid. As the term implies, dissociation of carbonic acid produces hydrogen ions, which results in increasing acidity and decreasing pH of seawater and therefore the name ocean acidification. Surface seawater pH has already decreased by approximately 0.1 pH units since the onset of the Industrial Revolution and is anticipated to decrease by another 0.2–0.3 pH units by the end of the twenty-first century (e.g., Caldeira and Wickett, 2003). Oceanic uptake of CO_2 also produces an increase in the seawater concentration of bicarbonate ions (HCO_3^-) and a decrease in the carbonate ion concentration (CO_3^{2-}). As a consequence, the saturation state with respect to calcium carbonate minerals (Ω), which is determined by dividing the in situ ion concentration product of calcium and carbonate ions by an experimentally determined solubility product ($\Omega = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K_{sp}^*$), will decrease. A lower carbonate saturation state of surface seawater implies that the initial composition of marine sediment pore waters will be closer to undersaturation with

respect to carbonate minerals, and thus, microbial decomposition of less organic material is required to produce undersaturated conditions and subsequent dissolution of carbonate mineral phases in the pore water–sediment system. Furthermore, while most shallow-water environments at present time are supersaturated with respect to calcite, aragonite, and most Mg-calcite phases (in general, Mg-calcite containing 8–12 mol% MgCO_3 or more is more soluble than both calcite and aragonite), surface seawater in high and intermediate latitude environments could become undersaturated with respect to most Mg-calcite phases and even aragonite during the present century. Consequently, as a result of human-induced ocean acidification, dissolution of carbonate minerals and sediments in shallow-water ocean environments is most likely to increase.

Increased dissolution of calcium carbonate minerals and sediments owing to human-induced ocean acidification will most likely occur selectively according to mineral stability. This could lead to a change in the average mineral composition of contemporary carbonate sediments in favor of stable mineral phases such as calcite and low Mg-calcite phases over metastable phases such as aragonite and high Mg-calcite. Although such a transition is slow relative to the rate at which seawater chemistry is changing owing to human activities, evidence for increasing carbonate dissolution and changing sediment composition exists based on numerical model simulations, saturation, and dissolution experiments, and mesocosm experiments, as well as from observations from natural environments. One such environment is Devil's Hole, located within Harrington Sound, Bermuda. During summertime, the water column of Devil's Hole becomes significantly stratified owing to the exponential decrease of solar irradiance as a function of depth. Organic matter produced in the surface mixed layer sinks and is decomposed by microbes in the subthermocline layer. As a consequence, the CO_2 , pH and the seawater saturation state with respect to carbonate minerals in this layer reach values typically not observed for most shallow-water environments today, but similar to values anticipated during the next several decades of the twenty-first century as a result of ocean acidification. In spite of significant production of fine grained Mg-calcite minerals throughout Harrington Sound, for example, by coralline algae, forams, and echinoderms, as well as the abundant occurrence of these mineral particles in sediments located at depths above the thermocline, little evidence exists for their presence in the sediments of Devil's Hole. These mineral phases are most likely selectively dissolved, a conclusion that is also supported by observed changes in seawater carbonate chemistry in the subthermocline layer in Devil's Hole (Figure 5; Neumann, 1965; Andersson et al., 2007).

In addition to the chemical changes imposed on surface seawater owing to rising atmospheric CO_2 , future dissolution of shallow-water carbonate minerals could also increase owing to increased deposition and subsequent increased microbial decomposition of organic matter in marine coastal sediments. Human activities on land have



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 5 (a) Partial pressure of CO_2 (pCO_2) in the water column of Devil's Hole, Harrington Sound, Bermuda. The pCO_2 of the subthermocline layer (gray) is significantly higher than anticipated from equilibrium with the atmosphere owing to microbial decomposition of organic matter. As a result, calcium carbonate minerals are selectively dissolved, which is demonstrated in (b) and (c). The former shows changes in salinity normalized ($S = 36$) calcium concentration ($\Delta[\text{Ca}^{2+}]$) as a function of total alkalinity (ΔnTA) in the subthermocline layer. The observed relationship clearly demonstrates that carbonate dissolution is taking place and the slope of a best fit line (0.44) suggests that the average composition of the dissolving phases has an Mg-content of 12 mol% MgCO_3 . (c) Relative weight percent high Mg-calcite as a function of sediment grain size and depth in Devil's Hole and Harrington Sound. For small grain sizes (<62 μm), the high Mg-calcite content decreases as a function of depth. The figure has been compiled based on data from Neumann (1965), Balzer and Wefer (1981), and Andersson et al. (2007).

significantly increased the amount of organic matter transported via rivers and runoff to the global coastal ocean relative to preindustrial conditions, and the total amount is expected to continue to increase with increased

deposition of this material to the sediments. Furthermore, input of nutrients to the global coastal ocean region have also significantly increased, which may stimulate increased production of organic matter and subsequent deposition of this material to the sediments within this region. As long as surface seawaters are supersaturated with respect to most carbonate phases, microbial decomposition of organic material is the main driver of carbonate dissolution. Consequently, even if the organic material deposited to the sediment–water remained constant, but the microbial activity increased, for example, owing to increasing temperature, carbonate dissolution would also increase. For the next several decades, human activities are likely to continue to lead to acidification of surface seawater and continuous increases in the amount of organic material being deposited to shallow-water sediments, and concurrently with increasing atmospheric concentrations of CO₂, surface and sea surface temperatures are likely to increase with potential increases in microbial and endolithic activity. As a result, dissolution of calcium carbonate minerals, particularly in coastal ocean environments, probably will increase due to all of these forcings. It is possible that under a business as usual scenario of fossil fuel CO₂ emissions, the production of carbonates in the global coastal ocean will be exceeded by their dissolution by year 2150 or earlier.

Summary

Diagenesis refers to those physical, chemical and biological processes that alter the original physical and chemical characteristics and properties of minerals and sediments. Calcium carbonate minerals constitute a substantial fraction of shallow-water sediments and may be subject to significant diagenetic alterations after deposition including dissolution and precipitation reactions. These reactions are largely mediated by microbial processes including oxidation of organic matter under both oxic and anoxic conditions as well as by endoliths, which actively penetrate carbonate substrates. The extent of calcium carbonate dissolution is to a large degree controlled by the amount of organic matter decomposed. Carbonate dissolution is likely to increase in the future in response to human-induced ocean acidification arising from the burning of fossil fuels, but also due to increased riverine transport of organic matter and nutrients to the coastal ocean, enhanced production in situ of organic matter in coastal waters, and subsequent deposition and microbial decomposition of the organic matter in marine sediments.

Acknowledgments

This work was supported by the US National Science Foundation grant OCE-0749401.

Bibliography

Aller, R. C., 1980. Diagenetic processes near the sediment–water interface of Long Island Sound. I. Decomposition and nutrient element geochemistry (S, N, P). *Advances in Geophysics*, **22**, 237–350.

- Andersson, A. J., Bates, N. R., and Mackenzie, F. T., 2007. Dissolution of carbonate sediments under rising pCO₂ and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, **13**, 237–264.
- Balzer, W., and Wefer, G., 1981. Dissolution of carbonate minerals in a subtropical shallow marine environment. *Marine Chemistry*, **10**, 545–558.
- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.
- Berner, R. A., 1980. *Early Diagenesis, A Theoretical Approach*. Princeton, NJ: Princeton University Press.
- Burdige, D. J., and Zimmerman, R. C., 2002. Impact of sea grass density on carbonate dissolution in Bahamian sediments. *Limnology and Oceanography*, **47**, 1751–1763.
- Caldeira, K., and Wickett, M. E., 2003. Anthropogenic carbon and ocean pH. *Nature*, **425**, 365.
- Mackenzie, F. T., Ristvet, B. L., Thorstenson, D. C., Lerman, A., and Leeper, R. H., 1981. Reverse weathering and chemical mass balance in a coastal environment. In Martin, J. M., Burton, J. D., and Eisma, D. (eds.), *River Inputs to Ocean Systems*. Switzerland: UNEP-UNESCO, pp. 152–187.
- Morse, J. W., and Mackenzie, F. T., 1990. *Geochemistry of Sedimentary Carbonates*. Amsterdam: Elsevier.
- Moulin, E., Jordens, A., and Wollast, R., 1985. Influence of the aerobic bacterial respiration on the early dissolution of carbonates in coastal sediments. In *Proceedings Progress in Belgium Oceanographic Research*, Brussels, Belgium, pp. 196–208.
- Neumann, A. C., 1965. Processes of recent carbonate sedimentation in Harrington Sound Bermuda. *Bulletin of Marine Science*, **15**, 987–1035.

Cross-references

[Alkalinity](#)
[Bioerosion](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbon \(Organic, Degradation\)](#)
[Carbonate Environments](#)
[Carbonates](#)
[Endoliths](#)
[Iron Sulfide Formation](#)
[Microbial Biomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)

BIOLOGICAL VOLCANIC ROCK WEATHERING

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The biologically induced breakdown of volcanic rocks, including crystalline rocks and glasses.

Volcanic rock weathering and global processes

The weathering of volcanic minerals is known to make a significant contribution to the global silicate weathering budget (Dessert et al., 2003), thus influencing carbon dioxide drawdown and climate control, since carbon

dioxide is consumed in mineral weathering reactions. For example, on land, the Deccan Traps in India, with an estimated area of 10^6 km^2 , is thought to account for about 5% of the global silicate weathering flux (Dessert et al., 2003). In total, basalt rocks may account for over 30% of the global carbon dioxide drawdown in silicate weathering (Gaillardet et al., 1999). The weathering of volcanic rocks also contributes a significant flux of nutrients into the biosphere, and through the weathering of rocks and the formation of clays and secondary minerals, microorganisms accelerate the rate of production of volcanic soils (Andisols) from their parent minerals. Thus, understanding how volcanic rocks weather and what role a biota might play in these processes has importance for understanding climate control, soil formation, and the mechanisms governing the release and distribution of nutrients in the biosphere.

The biological role

The role of plants in accelerating the weathering of volcanic rocks has been established, primarily from studies in Hawaii and Iceland. In Hawaii, plants have been estimated to increase weathering rates by over an order of magnitude compared to unvegetated areas (Cochran and Berner, 1996). In geological terms, the acceleration of weathering caused by plants following their early evolution has been proposed as one mechanism for carbon dioxide drawdown in the late Silurian, resulting in a general cooling trend (Berner, 1992). Plants increase weathering rates by, *inter alia*, physically disaggregating the rock with their roots, the production of rock weathering organic acids, the removal of ions from the subsurface (which changes chemical equilibrium in favor of rock dissolution), and by indirectly producing nutrients and favorable microenvironments for prokaryotes and fungi within the rhizosphere, which themselves weather rocks (see below).

A microbiological involvement in volcanic rock weathering has been established. The primary effect of microorganisms is to accelerate the breakdown of the rock both physically and chemically, often with the formation of secondary minerals such as poorly ordered aluminosilicates and clays (Figure 1). Early studies on the biological weathering of volcanic rocks focused on the role of lichens (Jackson and Keller, 1970; Adamo and Violante, 1991, 2000; Banfield et al., 1999). The earliest study of biological volcanic rock weathering was the seminal study by Fry (1927) who showed that lichens can cause etching, fragmentation, and weakening of obsidian, a silica-rich volcanic glass. Lichens accelerate the physical disaggregation of volcanic rocks as the lichen thallus penetrates the rock substrate. The production of organic acids, organic complexing agents, and the excretion of oxalic acids are just some of the mechanisms by which lichens contribute to the chemical dissolution of the rock substrate (Adamo and Violante, 2000). Oxalic acids are produced by the fungal component in the lichen symbiosis. Calcium oxalate at the lichen–rock interface, although not



Biological Volcanic Rock Weathering, Figure 1 A 1913 basalt flow in Iceland covered in lichens, which play an important role in rock weathering. Image is 20 cm across.

ubiquitous, is one widely reported compound formed on lichen-weathered rocks, which suggests the role of oxalic acids reacting with mineral-derived calcium, although other oxalates, such as copper- and manganese-rich compounds, have been reported.

Lichens have been shown to induce the formation of considerably thicker weathering rinds on crystalline basalts in Hawaii compared to control rocks, which in addition to directly contributing to weathering, may secondarily accelerate the rate of abiotic chemical weathering (Jackson and Keller, 1970). Lichens may also accelerate the dissolution of the silicate from the rock matrix, causing it to be more susceptible to being washed away, promoting the preferential formation of oxides and other secondary minerals from iron. Iron oxide formation is reported at the interface between lichens and basaltic rocks in Hawaii and near Vesuvius, Italy.

Early studies on lichen weathering of volcanic rocks were the first to elucidate two important factors in microbial rock weathering in general: (1) the role of changes in the microenvironment induced by microorganisms at the rock surface, which include changes in pH, water retention, and redox state that alter the equilibrium chemistry of rock dissolution or secondary mineral formation, accelerating rock weathering; and (2) the differential susceptibility of minerals to microbial rock weathering. Calcium-containing plagioclase tends to weather faster than minerals, such as K-feldspars, and these differential effects are determined by the susceptibility of minerals to weathering agents produced by microorganisms or the changes in chemical equilibria.

Since early studies on lichens, many other microorganisms have been examined and found to be capable of accelerating rock weathering. Mechanisms of microbial weathering include: the production of organic acids; the secretion of protons, which changes the local pH at the

rock surface and accelerates weathering; and the production of complexing ligands, such as siderophores, which chelate oxidized iron. It is also becoming clear that these are not merely serendipitous processes resulting from microbial metabolic activity, but that microorganisms actively excrete substances to gain access to nutrients within rocks that might otherwise be limiting, such as major nutrients, including Fe, P, Na, and Ca and trace elements such as K, Ni, or Mn. In addition to the secretion of weathering-promoting substances, by oxidizing or reducing metals during energy acquisition microorganisms can accelerate chemical dissolution or alteration of the rock surface, particularly important examples being iron-oxidizing or reducing bacteria, which use iron as an electron donor or acceptor respectively, and may therefore change the oxidation state of the iron in iron-containing minerals, including olivines and pyroxenes, which form an important component of basaltic mineralogies.

The considerations above apply to both fungi and prokaryotes. In the case of fungi, the penetration of the rock by the hyphae is one primary means by which fungi can invade the interior of a rock and accelerate weathering. Fungi, in particular, are well-known to produce organic acids by which they contribute to rock weathering (Mehta et al., 1978; Gadd, 2006).

Microorganisms do not merely accelerate the weathering of all elements from volcanic rocks. A number of studies have shown that biotic weathering can cause preferential leaching or enrichment of elements at the weathering front or within the sediments produced by volcanic rock weathering. Staudigel et al. (1998), in a study of basaltic glass weathering using a microbial enrichment culture from Loihi seamount, Hawaii, showed that the population of microorganisms, which included heterotrophic bacteria, cyanobacteria, and diatoms, induced an enrichment of calcium in the sediments produced by weathering, but a loss of magnesium, in contrast to the controls which showed the opposite trend. These results, and many others, show that microorganisms can cause a differential rate of leaching of different elements from rocks. Bacteria on their own have been shown to induce the release of iron and manganese. Investigations using a single bacterial species (*Burkholderia fungorum*) (Wu et al., 2007) showed that iron release is induced by changes in the pH at the rock surface. Phosphorus released from the apatite within the basalt rock apparently also helped sustain bacterial growth. All of these experiments show that microorganisms potentially have an important role in modulating the release of different elements from the crust. The oxidation of iron by bacteria in the deep-sea probably gives them an important role in iron cycling and thus weathering of the basalt crust (Edwards et al., 2003).

These data also highlight the general point that although the role of microorganisms in enhancing mineral dissolution has been a major focus of research, it is also important to understand that the weathering of elements from rocks might be influenced by the retardation of elemental release caused by microbial action. For example,

the production of oxidized iron from reduced iron in basaltic minerals such as olivine has been suggested to retard leaching of elements from the rocks (Santelli et al., 2001). Thus, through changes in oxidation states of elements and the production of secondary minerals at rock surfaces, the microbial involvement in volcanic rock weathering may be much more complex than merely enhancing weathering rates, instead changing the leaching rates of specific elements in ways that are either retarded or accelerated compared to abiotic conditions.

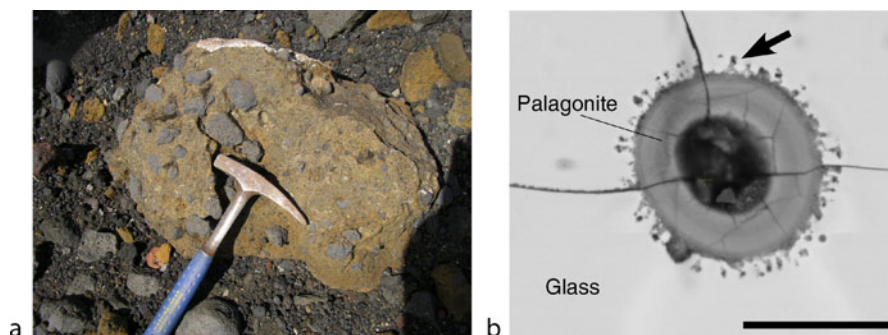
The formation of clays and poorly ordered aluminosilicates, such as smectites and allophane-like materials, has been reported around bacterial aggregates in volcanic ash near Sakurajima volcano, Japan (Kawano and Tomita, 2001). The authors, by carrying out batch culture experiments in the laboratory using bacteria isolated from the ash, suggest that the natural populations are involved in biomineralization and therefore play an important part in bacterial alteration of the weathered volcanic ash. In this case mineral formation is likely to have been facilitated by the bacterial cell surface. Although this work did not show active weathering of the primary rock minerals, it illustrates that the formation of clays and other minerals from weathered volcanic ash by microorganisms can play an important role in defining the chemical characteristics of the weathering environment, which itself will alter the environment for other members of the microbial community which may be directly involved in weathering. The work underlines the important link between rock weathering (dissolution and alteration) and mineral formation (biomineralization) during the transition of primary volcanic minerals to soils such that both processes must be considered to truly understand biological volcanic rock weathering and soil neogenesis.

As a logical development of the work of Fry, Herrera et al. (2008) examined bacterial communities within obsidian in Iceland and showed a diverse population of bacteria, which by the use of hybridization probes were shown to be associated with weathering alteration fronts in the rock. Basaltic rocks in terrestrial environments, as with the oceanic environments described above, have been shown to harbor a high prokaryotic diversity (Gomez-Alvarez et al., 2007).

The mechanisms by which microorganisms weather rocks are still based primarily on chemical measurements such as organic acid production or changes in solution pH. New molecular methods are likely to elucidate pathways by which microorganisms can weather rocks and strengthen our understanding of the link between the organisms and the weathering process.

Microbial borings in volcanic glass

Other evidence for a direct role of microorganisms in volcanic rock weathering has been presented in the form of channels or pitted and corroded granular textures, in reactive volcanic glasses. The most commonly studied of these are endolithic borings into volcanic glasses, including



Biological Volcanic Rock Weathering, Figure 2 (a) An outcrop of subglacial basalt glass at Valafell, Iceland. The glass turns conspicuous brown as it transforms into palagonite, a weathering product, (b) “elongate” (tunnellike) weathering features in a vesicle within the outcrop, features (arrow) are at the interface between the palagonite weathering rind (center) and the glass (scale bar 40 μm). The biological origin of such features in different environments is an ongoing controversy.

deep ocean pillow lavas (Fisk et al., 1998; Torsvik et al., 1998; Thorseth et al., 2003; Storrie-Lombardi and Fisk, 2004). Furnes and Staudigel (1999) suggest a dominant role for bioalteration of volcanic glasses in the upper 300 m of oceanic crust. Glass recovered by drilling from more than 250 m below the sea floor shows channels into altered zones that are 1–10 μm wide and penetrate to a depth of 150 μm . In other settings, larger tubes up to 20 μm in diameter and with a depth of 0.5 mm have been observed. The provenance of some of these borings has been controversial since the organisms responsible for the borings have not been cultured. Abiotic ambient inclusion trails, generated by the pressure forcing of mineral grains, such as sulfides, through glass have been suggested as a mechanism for the production of channel features (Figure 2). To address this possibility a number of approaches have been used to show an involvement of biological activity. These include isotopic and elemental analysis. Binding of the nucleic acid stain DAPI (4',6-diamidino-2-phenylindole) to DNA and fluorescence signals obtained using fluorescent in situ hybridization (FISH) probes against both archaea and bacteria in the channels suggest that organisms were responsible for the weathering. Microprobe analysis shows the presence of carbon not associated with carbonates. Low carbon isotope values were also suggested as evidence supporting a biological role in glass alteration.

Early Earth studies and astrobiology

Volcanic terrains would have dominated the landscape of the Archean Earth when life emerged about 3.5 billion years ago. One reason for the keen interest in weathering textures in volcanic glass is the discovery of similar features in ancient Archean volcanic rocks (Furnes et al., 2004). Insofar as channels are morphologically more distinctive than, for example, irregular pitted features, then they might offer evidence for life in early Earth environments. The age dating of materials with biological isotopic signatures has been used to argue that fossils of putative

ancient microbial volcanic glass borers are the same as the age of the rocks (Banerjee et al., 2006). The study of microbial volcanic rock weathering and the textures and minerals left by such interactions may also offer a means to assay ancient Martian terrains for evidence of life (McLoughlin et al., 2007), particularly since Mars, in the absence of plate tectonics, is a planet dominated by volcanic terrains. On a more general level, understanding the interaction of microorganisms with volcanic rocks may provide a means to understand whether the formation of clays and other secondary minerals from basalts on Mars is a purely abiotic process or whether it could ever have been biologically mediated.

Conclusion

Both field and laboratory observations show that plants and microorganisms play an important role in volcanic weathering. The mechanisms by which they achieve this are diverse, but reduce to some common principles which include physical disruption, production of compounds that attack minerals, alteration of chemical equilibria in favor of dissolution, and changes in redox states of metals which favors dissolution. Through their actions, the biota generally accelerates rock weathering, contributing to the release of rock nutrients into the environment and silicate weathering reactions which themselves contribute to long-term carbon dioxide drawdown.

Bibliography

- Adamo, P., and Violante, P., 1991. Weathering of volcanic rocks from Mt. Vesuvius associated with the lichen *Stereocaulon vesuvianum*. *Pedobiologia*, **35**, 209–217.
- Adamo, P., and Violante, P., 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science*, **16**, 229–256.
- Banerjee, N. R., Furnes, H., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2006. Preservation of ~3.4–3.5 Ga microbial biomarkers in pillow lavas and hyaloclastites from the Barberton Greenstone Belt, South Africa. *Earth and Planetary Science Letters*, **241**, 707–722.

- Banfield, J. F., Barker, W. W., Welch, S. A., and Taunton, A., 1999. Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. *Proceedings of the National Academy of Sciences*, **96**, 3404–3411.
- Berner, R. A., 1992. Weathering, plants and the long-term carbon cycle. *Geochimica et Cosmochimica Acta*, **56**, 3225–3231.
- Cochran, M. F., and Berner, R. A., 1996. Promotion of chemical weathering by higher plants: field observations on Hawaiian basalts. *Chemical Geology*, **132**, 71–77.
- Dessert, C., Dupré, B., Gaillardet, J., François, L. M., and Allègre, C. J., 2003. Basalt weathering laws and the impact of basalt weathering on the global carbon cycle. *Chemical Geology*, **202**, 257–273.
- Edwards, K. J., Bach, W., and Rogers, D. R., 2003. Geomicrobiology of the ocean crust: a role for chemoautotrophic Fe-bacteria. *Biological Bulletin*, **204**, 180–185.
- Fisk, M. R., Giovannoni, S. J., and Thorseth, I. H., 1998. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science*, **281**, 978–980.
- Fry, E. J., 1927. The mechanical action of crustaceous lichens on substrata of shale, schist, gneiss, limestone, and obsidian. *Annals of Botany*, **151**, 437–460.
- Furnes, H., and Staudigel, H., 1999. Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth and Planetary Science Letters*, **166**, 97–103.
- Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- Gadd, G. M., 2006. *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press.
- Gaillardet, J., Dupre, P., Louvat, C. J., and Allègre, C. J., 1999. Global silicate weathering and CO₂ consumption rates deduced from the chemistry of the large rivers. *Chemical Geology*, **159**, 3–30.
- Gomez-Alvarez, V., King, G. M., and Nüsslein, K., 2007. Comparative bacterial diversity in recent Hawaiian volcanic deposits of different ages. *FEMS Microbiology Ecology*, **60**, 60–73.
- Herrera, A., Cockell, C. S., Self, S., Blaxter, M., Reitner, J., Arp, G., Dröse, W., Thorsteinsson, T., and Tindle, A. G., 2008. Bacterial colonization and weathering of terrestrial obsidian in Iceland. *Geomicrobiology Journal*, **25**, 25–37.
- Jackson, T. A., and Keller, W. D., 1970. A comparative study of the role of lichens and “inorganic” processes in the chemical weathering of recent Hawaiian lava flow. *American Journal of Science*, **269**, 446–466.
- Kawano, M., and Tomita, K., 2001. Microbial biomineralization in weathered volcanic ash deposit and formation of biogenic minerals by experimental incubation. *American Mineralogist*, **86**, 400–410.
- McLoughlin, N., Brasier, M. D., Wacey, D., Green, O. R., and Perry, R. S., 2007. Formulating biogenicity criteria for endolithic microborings on early Earth and beyond. *Astrobiology*, **7**, 10–26.
- Mehta, A., Torma, A. E., Murr, L. E., and Berry, V. K., 1978. An SEM characterization of biodegradation of an aluminium-bearing (basalt) rock by fungi. *Scanning Electron Microscopy*, **1**, 171–176.
- Santelli, C. M., Welch, S. A., Westrich, H. R., and Banfield, J. F., 2001. The effect of Fe-oxidizing bacteria on Fe-silicate mineral dissolution. *Chemical Geology*, **180**, 99–115.
- Staudigel, H., Yayanos, A., Chastain, R., Davies, G., Verdurmen, E. A. T., Schiffman, P., Boucier, R., and de Baar, H., 1998. Biologically mediated dissolution of volcanic glass in seawater. *Earth and Planetary Science Letters*, **164**, 233–244.
- Storrie-Lombardi, M. C., and Fisk, M. R., 2004. Elemental abundance distributions in suboceanic basalt glass: evidence of biogenic alteration. *Geochemistry, Geophysics and Geosystems*, **5**, 1–15.
- Thorseth, I. H., Pedersen, R. B., and Christie, D. M., 2003. Microbial alteration of 0–30-Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic discordance. *Earth and Planetary Science Letters*, **215**, 237–247.
- Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I. H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters*, **162**, 165–176.
- Wu, L., Jacobson, A. D., Chen, H., and Hausner, M., 2007. Characterisation of elemental release during microbe-basalt interactions at $T = 28^{\circ}\text{C}$. *Geochimica et Cosmochimica Acta*, **71**, 2224–2239.

Cross-references

[Astrobiology](#)
[Basalt \(Glass, Endoliths\)](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biosignatures in Rocks](#)
[Fluorescence In Situ Hybridisation \(FISH\)](#)
[Glass](#)

BIOMARKERS (MOLECULAR FOSSILS)

Jochen J. Brocks¹, Kliti Grice²

¹The Australian National University, Canberra, ACT, Australia

²Curtin University of Technology, Perth, WA, Australia

Synonyms

Biological marker molecules; Geochemical fossils; Molecular fossils

Definition

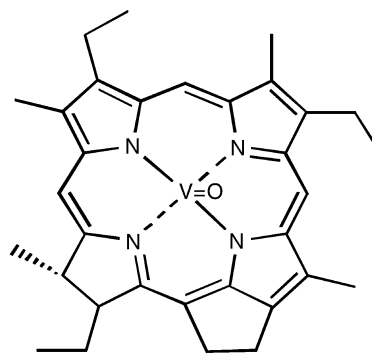
Biomarkers are the molecular fossils of lipids and other natural products. In sedimentary environments, lipids that escape the remineralization process are commonly chemically reduced to hydrocarbon skeletons. Encased in sedimentary rocks, these skeletons can remain intact over hundreds of millions of years. The structure of biomarkers is often directly related to their precursor lipids and may be diagnostic for a specific group or groups of organisms. Diagnostic biomarkers are used to obtain information about the composition of past (microbial) ecosystems or to determine the first occurrence of organisms in the geological record. As many organisms prefer specific habitats, and as lipid compositions of individual organisms are frequently adjusted to changing physical and chemical conditions, biomarkers may also serve as paleoenvironmental proxies, for example for salinity, temperature, and oxygen availability. In the petroleum industry, biomarkers are utilized to elucidate the extent of biodegradation of crude oil and to obtain information about the thermal history, lithology, depositional environment, and geological age of hydrocarbon source rocks.

Other meanings of "biomarker"

Environmental studies: intact and predominantly functionalized biomolecules in recent sediments, including ancient DNA. *Medicine/pharmacology:* a natural or introduced molecule or substance in an organism that indicates the presence or state of a specific disease. *Toxicology:* a molecule or substance that indicates exposure of an organism to a toxin. *Cell biology:* a molecule that facilitates the detection and isolation of specific cell types. *Genetics:* a DNA fragment that causes disease or contains information about susceptibility to a disease. *Paleobiology:* mineralogical, elemental, isotopic, and morphological indicators for the presence and activity of life in the geological record. *Astrobiology:* anything that could yield experimental evidence for the existence, past or present, of life in our solar system and beyond.

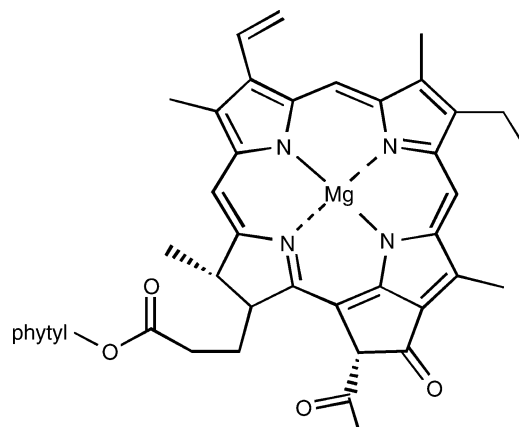
History

Today, crude oil is indisputably recognized as a concentrate of biomarkers and other molecular alteration products of biological organic matter. The hypothesis about a biological origin of petroleum was probably first formulated by the Russian scholar Mikhail Lomonosov in 1757. However, in the nineteenth century, partly based on observations that organic molecules can be generated in the laboratory from inorganic ingredients, most scientists supported an inorganic theory of petroleum formation. The prominent French chemist Marcellin Berthelot suggested in 1866 that petroleum is the product of the reaction of water with inorganic carbides (Berthelot, 1866 and references therein), and Dmitri Mendeleev (1878) insisted that petroleum is abiogenic and forms deep in the Earth's crust. The first studies that yielded systematic evidence for an organic origin of crude oil were carried out by petroleum geologists in the late nineteenth to early twentieth century (see Durand, 2003). Geologists found correlations between the location of petroleum reservoirs and the thermal maturity of associated sedimentary organic matter, and chemists were able to generate petroleum-like oils by heating animal products and black shales under the exclusion of oxygen (pyrolysis). As a consequence of these studies, it was well accepted among North American geologists in the 1930s that petroleum is generated by thermal degradation of organic matter in sedimentary rocks. In Russia in the 1920s, the organic theory of petroleum formation was defended by Vladimir Vernadsky. Vernadsky, clearly ahead of his time and now regarded as one of the founders of organic geochemistry, had already integrated his concepts of petroleum formation into a general framework of biogeochemical cycles (e.g., Vernadsky, 1934). However, the first undeniable proof that bitumen in sedimentary rocks contains molecules of biological origin was presented by Alfred Treibs. In 1934, Treibs isolated a vanadylporphyrin complex (vanadyl-deoxyphylloerythroetioporphyrin **I**)



I Vanadyl-deoxyphylloerythroetioporphyrin

from a bituminous black shale. He found that the nitrogen-carbon skeleton of the red-colored sedimentary porphyrin was virtually identical to the green plant pigment chlorophyll *a* **II**. He showed that the fossil pigment had been



II Chlorophyll *a*

generated from a biogenic precursor through replacement of the central magnesium ion with vanadyl oxide and loss of several functional groups. Alfred Treibs (1936) subsequently solved the degradation pathway of chlorophyll *a* in sedimentary environments, and is now celebrated as the father of biomarker geochemistry.

Crude oil is a complex mixture of thousands of different hydrocarbons and heteroatomic components containing C, S, N, and O. In the 1960s and 1970s, the analysis of such complex systems was facilitated by the rapid development of chemical separation and detection techniques such as gas chromatography (GC), gas chromatography-mass spectroscopy (GC-MS), and elemental analysis. The earliest biomarker studies were strongly inspired by the search for clues to the evolution of early life on the Earth (Barghoorn, 1957; Eglinton et al., 1964; Meinschein et al., 1964; Barghoorn et al., 1965; Burlingame et al.,

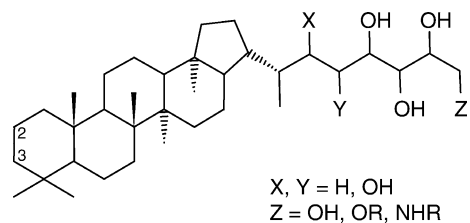
1965; Hoering, 1965; Oró and Nooner, 1967; Kvenvolden and Hodgson, 1969; for a review see Brocks et al., 2003b). However, the main driving force of biomarker research later in the twentieth century was the petroleum industry. Organic geochemists employed biomarkers to understand the transformation of organic matter into petroleum, to estimate the thermal maturity and geological age of reservoir oil, and to correlate crude oil with potential source rocks (e.g., Philippi, 1965; Albrecht and Ourisson, 1969; Hunt, 1979; Tissot and Welte, 1984; Peters and Moldowan, 1993). Flowing from applications in the petroleum industry, and in the spirit of Vernadsky, biomarkers also became of increasing interest in the understanding of remineralization and burial of organic matter as crucial aspects of the global carbon cycle (e.g., Hedges et al., 1997; Eglinton and Repeta, 2004), the study of organisms and metabolisms that otherwise rarely leave a fossil record (e.g., Moldowan and Talyzina, 1998; Hinrichs et al., 1999; Summons et al., 1999; Kuypers et al., 2001), and as paleoenvironmental proxies (e.g., Brassell et al., 1986; Summons and Powell, 1986; Schouten et al., 2002).

Declining oil prices in 1998 led to the closure of most biomarker laboratories in the petrochemical industry, and biomarker research strongly shifted to universities with a focus on paleoenvironmental and geomicrobiological applications. In these applications, an integration with modern microbiological techniques and genomics will be a crucial aspect of biomarker geochemistry in the future (e.g., Pearson et al., 2003; Sinninghe Damsté et al., 2004b; Rashby et al., 2007).

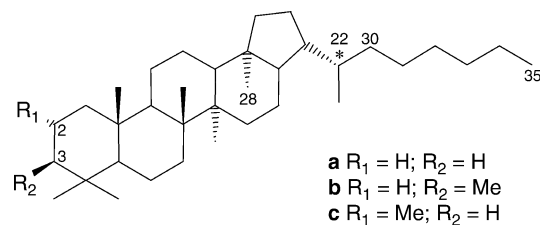
Formation and alteration of sedimentary organic matter (diagenesis)

In most planktonic environments, organic carbon from primary producers is nearly quantitatively remineralized back to carbon dioxide and water. On an average less than 0.1% permanently accumulate in bottom sediments (Holser et al., 1988). Most biogenic compounds such as carbohydrates, proteins, nucleic acids, and small metabolites are prone to biological degradation under most conditions and are recycled rapidly back to carbon dioxide and water. Molecules that escape the mineralization process are commonly altered by diagenetic processes in the water column and subsequently, in the sediment. Diagenesis includes a multitude of chemical and biological reactions that lead to the formation of macromolecular aggregates from smaller molecules through condensation and cross-linking reactions, but also to fragmentation, oxidation, reduction, isomerization, and epimerization products (Tissot and Welte, 1984). The relative concentration of different alteration products depends on prevailing physical and chemical conditions in the sediment during and after burial. Analysis of the diagenetic products may, thus, yield information for example, about redox conditions and the presence of catalytic substances such as active mineral surfaces. Under prevailing reducing conditions and over

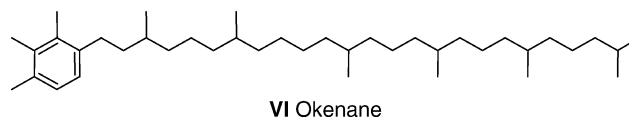
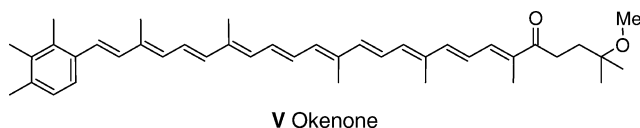
geological periods of time, biolipids eventually lose their functional groups and are transformed to hydrocarbon



III Bacteriohopanepolyols (BHP)



IV Pentakishomohopane



skeletons (e.g., hopanepolyols **III** to hopanes **IV**, or okenone **V** to okenane **VI**). The hydrocarbon products may remain stable over hundreds of millions of years encased in sedimentary rock.

The exact chemical nature of organic matter in sediments primarily depends on the composition of the source organisms and the mechanisms of preservation. In the so-called selective preservation pathway, molecules that are highly resistant to chemical and biological degradation, such as aliphatic algal biopolymers or cuticular waxes of plant leaves, have a high potential to escape remineralization and may form a major fraction of sedimentary organic matter (Tegelaar et al., 1989; De Leeuw et al., 2006). Smaller molecular building blocks, including monomers generated by hydrolysis of biopolymers, may undergo random condensation to a highly cross-linked macromolecular product that is resistant to further degradation (degradation/recondensation pathway) (e.g., Larter and Douglas, 1980; Tissot and Welte, 1984). Under transitory oxidizing conditions, fatty acids and other lipids may also become cross-linked via ether and carbon-carbon bonds to form resistant aliphatic geomacromolecules ("oxidative polymerization pathway") (e.g., Kuypers et al., 2002; Versteegh et al., 2004). One of the most

significant pathways for the preservation of biomarkers in sediments is natural sulfurization (or natural vulcanization) (Sinninghe Damsté and De Leeuw, 1990). In anoxic environments, bacterial sulfate reduction may lead to the accumulation of hydrogen sulfide and polysulfides. These reduced sulfur species can react with functional groups of organic molecules during early stages of diagenesis, either already in an anoxic water column or in the sediment. Abiotic addition of hydrogen sulfide to double bonds followed by reductive desulfurization is a crucial mechanism in the transformation of labile, oxygen-sensitive lipids into their more stable reduced counterparts (Hebting et al., 2006). Intramolecular incorporation of inorganic sulfur may also lead to the generation of small sulfur-containing molecules that are more resistant to biodegradation, and intermolecular vulcanization may protect smaller molecules by incorporation into a macromolecular network via sulfide and polysulfide bridges (e.g., Adam et al., 1993; Kohnen et al., 1993; Schaeffer et al., 1995; Wakeham et al., 1995). Finally, lithology also has an important influence on the presence and preservation of biomarkers. High concentrations of well-preserved organic matter are most commonly found in fine grained sedimentary rocks such as shales and marls. This has several reasons. Firstly, very fine suspended mineral particles and particulate organic matter have similar hydrodynamic properties and are likely to be deposited at the same location. Secondly, organic matter has a high affinity to clay minerals, and adsorption to mineral surfaces protects organic matter against attack by heterotrophic microorganisms and digestive enzymes, a phenomenon described as the “sorpitive protection pathway.” Thirdly, the fineness of clay particles prevents the circulation of oxygenated water in the sediment, promoting development of anoxic and sulfidic conditions that exclude aerobic heterotrophic organisms and support natural sulfurization.

Thermal alteration of biomarkers and sedimentary organic matter (catagenesis)

The aggregates that form through degradation/recondensation, oxidative polymerization, and natural sulfurization, coalesce with degradation-resistant biopolymers to form kerogen. Kerogen is a highly complex, amorphous macromolecular network of organic matter in sedimentary rocks. Viewed in thin section, it commonly has a yellow, brown, or black color, depending on thermal maturity (De Leeuw and Largeau, 1993). With accumulation of sediment over the top and increasing depth of burial, geothermal heat will induce catagenesis, the thermal alteration and degradation of sedimentary organic matter. The cracking of chemical bonds in kerogen releases smaller molecular fragments that may form a liquid bitumen phase. Formally, bitumen is defined as the fraction of organic matter in sediments and sedimentary rocks that can be extracted using organic solvents. Bitumen released by the thermal decomposition of kerogen typically consists of saturated and aromatic hydrocarbons, including biomarkers, and

lower concentrations of polar (NSO) compounds. Carbon–sulfur and sulfur–sulfur bonds in kerogen are cleaved at relatively low temperatures ($\sim 60^{\circ}\text{C}$), while biomarkers covalently attached to kerogen via carbon–oxygen and carbon–carbon bonds are released at progressively higher temperatures (e.g., Koopmans et al., 1997). Increasing pressure, partially caused by the release of carbon dioxide and methane from kerogen, may eventually lead to the expulsion of liquid hydrocarbons from the source rock and their possible release at hydrocarbon seeps or accumulation in oil reservoirs.

At catagenic temperatures, hydrocarbon biomarkers also undergo thermal isomerization, epimerization, and cracking reactions, and the relative concentration of the thermal alteration products can be used to elucidate the thermal history of bitumen and crude oil. Thus, maturity parameters based on biomarkers and other compounds are used in the petroleum industry to estimate the oil- and gas-generation potential of sedimentary basins. A large variety of different maturity parameters for different temperature stages is available and is summarized in Peters et al. (2004). For instance, the ratio of $22S/(22S + 22R)$ isomers of $17\alpha(\text{H})$ -homohopanes measures the gradual transformation of the biological $22R$ configuration to the more stable $22S$ epimer (see carbon atom marked “*” in **IV**). The $22S/(22S + 22R)$ ratio reaches a thermodynamic equilibrium at a value of ~ 0.6 , indicating a thermal maturity roughly corresponding to the early phase of oil generation (e.g., Zumberge, 1987). However, it is not possible to translate biomarker maturity parameters into absolute temperatures, as the rates of molecular isomerization and degradation also strongly depend on the availability of catalytically active mineral surfaces and reactive species such as free radicals. Therefore, biomarker maturity parameters may be widely different in rocks of different lithology and need to be recalibrated for different basins and formations.

Destruction of biomarkers at high temperatures (metagenesis)

One of the most intriguing topics in biogeology is the examination of early microbial ecosystems using molecular fossils extracted from Precambrian rocks (>542 Ma [million years]). The oldest clearly indigenous bitumens come from the ~ 2.5 Ga (billion years) old Mt McRae Shale of the Hamersley Group in northwestern Australia (Brocks et al., 2003a). However, these bitumens were severely altered by pyrolytic temperatures and predominantly contain polycyclic aromatic hydrocarbons. The oldest molecules of proven provenance that contain biological information come from the 1.64 Ga old Barney Creek formation of the McArthur Basin in northern Australia (Summons et al., 1988). The distribution of biomarkers in the Barney Creek formation is distinct from younger bitumens and strongly dominated by bacterial lipids and pigments (Brocks et al., 2005). While the biomarkers of the Barney Creek formation are clearly

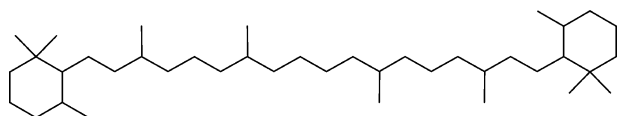
indigenous (Brocks et al., 2008), reports of biomarkers from older successions have to be regarded with caution as the concentration of extractable hydrocarbons are commonly minute and molecular maturity parameters are inconsistent with the thermal history of the rock. Moreover, the extracted biomarkers are often found concentrated on rock surfaces (Brocks et al., 2008) and their composition is always conspicuously similar to Phanerozoic petroleum products (e.g., Brocks et al., 2003b).

All known sedimentary successions older than ~1.8 Ga have suffered burial metamorphism to zeolite facies or higher, probably at minimum temperatures of 175–280°C. At these temperatures, metagenetic processes transform kerogen to a black, hydrogen-poor, and highly aromatic carbon phase, and residual bitumen is cracked to gas and expelled from the rock. However, the existence of residual biomarkers in rocks heated to about 220°C is theoretically not impossible. Modern deep-subsurface petroleum reservoirs produce commercial quantities of oil at present-day temperatures of up to 200°C, and these oils apparently still contain intact biomarkers. Biomarkers were also observed in bitumens extracted from sedimentary rocks that currently exist at temperatures of 200–223°C (for a review see Brocks and Summons, 2004). The persistence of aliphatic hydrocarbons over geological periods of time at up to 250°C is also supported by kinetic models of petroleum degradation (Pepper and Dodd, 1995; Domíné et al., 2002). Therefore, if pockets of organic-rich sedimentary rocks of Paleoproterozoic to Archean age have escaped temperatures above ~220°C, it should still be possible to discover intact molecular fossils to reconstruct the Earth's oldest microbial ecosystems.

Biological interpretation of biomarkers

Most fossil hydrocarbons have multiple biological precursors that are distinguished by different functional groups and stereochemistries. Moreover, most of these biolipids may occur in a wide range of organisms that are not necessarily closely related. Therefore, for a meaningful interpretation of molecular fossils, it is crucial to identify all extant biological sources.

Some biomarkers contain little biological information because they are abundant across all domains of life. For example, the fossil hydrocarbon β -carotane **VII** contains

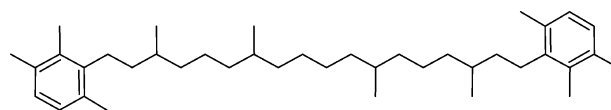


VII β -Carotane

virtually no taxonomic information. There are several hundred different biological carotenoids with a β -carotane skeleton that are distinguished by different combinations of functional groups such as double and triple bonds, oxo-, hydroxy-, epoxy-, and carboxy-groups and functional ornamentations such as carbohydrate side chains (e.g., Liaaen-Jensen, 1979). These carotenoids

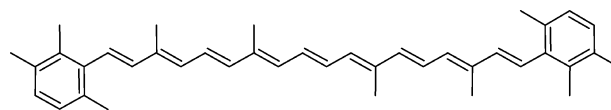
occur across a vast array of lineages in all three domains of life, and most are likely to yield β -carotane **VII** during diagenesis. Therefore, without additional information, β -carotane can not be assigned to any particular biological source.

In contrast to the ubiquitous occurrence of carotenoids with the β -carotane skeleton, the only known potential precursor of okenane **VI** is the purple-red colored carotenoid okenone **V**. As okenone is exclusively known from planktonic species of Chromatiaceae, a family of purple sulfur bacteria, it is interpreted as a diagnostic marker for this group of organisms (Brocks and Schaeffer, 2008). Isorenieratene **VIII**, an aromatic carotenoid like okenane



VIII Isorenieratene

VI, is an intermediate case. The known biological precursor isorenieratene **IX** is the major accessory pigment in



IX Isorenieratene

brown strains of green sulfur bacteria (Chlorobiaceae). Chlorobiaceae are anoxygenic phototrophs and strictly require anaerobic and sulfidic conditions in the presence of light. In planktonic environments, Chlorobiaceae inhabit a zone in the chemocline of stratified waters wherever sulfidic conditions arise into the light penetrated zone (photic zone euxinia). Therefore, isorenieratene is commonly interpreted as a biomarker for Chlorobiaceae and photic zone euxinia (see below). However, a second potential microbial source of aromatic carotenoids with the isorenieratene skeleton are genera of the actinobacterial order Actinomycetales, such as *Mycobacterium*, *Streptomyces*, and *Brevibacterium*. For instance, the orange-colored growths on the surface of cheese is often due to isorenieratene, 3-hydroxyisorenieratene, and 3,3'-dihydroxyisorenieratene from *Brevibacterium linens*, an actinomycete that belongs to the cheese ripening flora (Guyomarc'h et al., 2000). Generally, Actinomycetales are regarded as a microbial group that is restricted to terrestrial environments, particularly soils. However, the group is also known to be ubiquitous, and occasionally abundant, in marine environments (Ward and Bora, 2006). Actinomycetales were detected in the marine water column, in marine snow, in sea sediments, including deep sea sediments, and as symbionts in vertebrates and invertebrates, particularly sponges. It is not yet known just how widespread aromatic carotenoids in marine actinomycetes are. However, at least *Streptomyces griseus*, an actinomycete known from terrestrial as well as marine environments, has the capacity to biosynthesize

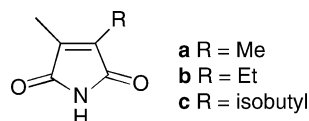
isorenieratene (Krügel et al., 1999). Therefore, the fraction of isorenieratene in marine sediments that is derived from terrestrial and marine actinomycetes may be significant, and an actinomycetal origin of isorenieratene in sedimentary rocks from marine depositional environments can not be ruled out.

The occurrence of isorenieratene in distantly related groups of bacteria, Actinomycetales and Chlorobiaceae, highlights our lack of understanding of the distribution of genes involved in carotenoid biosynthesis in extant organisms. For a full interpretation of isorenieratene, we have to learn whether isorenieratene biosynthesis was present in the common ancestor of Chlorobiaceae and Actinomycetales, whether the biosynthetic capacity was transferred horizontally between the two groups, or whether the pathways developed independently. While these questions may best be answered in the future using genomic information, isorenieratene derived from Chlorobiaceae can principally be distinguished from other sources by a diagnostic isotopic enrichment in ^{13}C (Summons and Powell, 1987; Grice et al., 1996a) (also see *Biomarkers (Organic, Compound-Specific Isotopes)*).

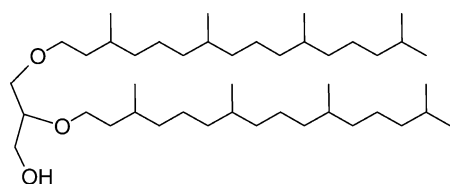
In summary, strictly, biomarkers have to be interpreted as indicators for biosynthetic pathways, not as markers for taxonomic groups. In the above example, isorenieratene is a biomarker for β -carotene desaturase, the enzyme responsible for the convergence of β -carotene to isorenieratene, and for *crtU*, the gene that codes for this enzyme. In some cases, it is possible to discriminate between different biological sources of a biomarker using carbon isotopic compositions (see also *Biomarkers (Organic, Compound-Specific Isotopes)*).

Biomarkers for the domains bacteria, archaea, and eukarya

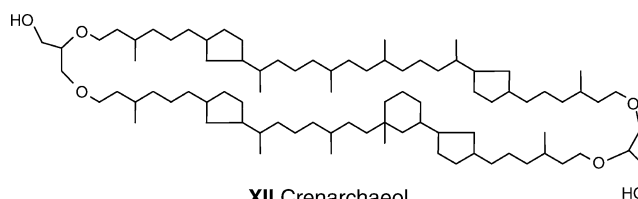
A selection of biologically informative molecular fossils, their potential biological origins and environmental interpretations are summarized in Table 1 (more comprehensive accumulations can be found in Peters and Moldowan [1993], Brocks and Summons [2004], and Peters et al. [2004]). Table 1 also includes biomarkers that are not hydrocarbons but are stable over geological periods of time such as maleimides **X** and archaeal



X Maleimides



XI Archaeal

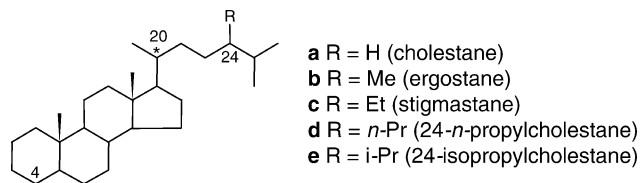


XII Crenarchaeol

glycerol ethers (**XI** and **XII**). Molecules with limited or no biological information were not included.

Biomarkers of eukaryotes

Next to bacterial hopanoids, eukaryotic steroids are probably the most common polycyclic biomarkers in the geological record. Sterols, such as cholesterol, ergosterol, and sitosterol, are essential membrane components of all eukaryotic organisms. Eukaryotes produce a large variety of different sterols that are distinguished by the number and position of double bonds, hydroxy-, oxo- and alkyl-groups and other, often complex, substituents. Functionalized sterols detected in recent sediments can be diagnostic for a wide range of taxonomic groups, particularly algae (e.g., Volkman, 2003). However, the number of different diagenetic hydrocarbon products is comparatively low (Table 1). The most abundant steranes in bitumens and oils from the late Neoproterozoic to the Cenozoic are cholestane **XIIIa** (C_{27}), ergostane **XIIIb**



XIII Steranes

(24-methylcholestane, C_{28}) and stigmastane **XIIIc** (24-ethylcholestane, C_{29}). Biological precursors of cholestane are abundant in animals and red algae (Rhodophyceae), and precursors of ergostane occur in fungi and several groups of algae, including coccolithophorids and diatoms, while sterols with the stigmastane skeleton are very widespread in higher plants, eustigmatophytes, chrysophytes, and green algae (Chlorophyceae) (Volkman, 1986; Volkman, 2003). However, C_{27} to C_{29} sterols are not restricted to these organisms and occur across all major eukaryotic clades. Even individual organisms may contain sterols with all three carbon skeletons. Thus, cholestanes, ergostanes, and stigmastanes in sedimentary rocks are not specific for any particular group of eukarya. However, the relative abundance of these three sterols shows systematic variations through the Phanerozoic and can be used to obtain an estimate of the age of crude oil (Grantham and Wakefield, 1988).

The biosynthetic capacity to produce steroids also occurs in at least three independent groups of bacteria, the Myxococcales, Methylococcales, and Planctomycetales.

Biomarkers (Molecular Fossils), Table 1 Diagnostic biomarkers and their biological and environmental interpretation

Group of organisms	Biomarker or biomarker pattern	Environmental interpretation and age constraints	Other known potential sources	Further information ^a
Eukarya				
Eukaryotes in general	Ergostane XIIIb , stigmastane XIIIc and their aromatic analogs Cholestane XIIIa and aromatic analogs	Most environments, Neoproterozoic to present	Possible minor contribution from myxococcales (myxobacteria)	(Volkman et al., 1998; Volkman, 2003; Summons et al., 2006) (Kohl et al., 1983; Bode et al., 2003)
Pelagophyte algae ("brown tides" and Sarcinochrysidales)	24- <i>n</i> -Propylcholestane XIIIId	Commonly found only in marine environments		(Moldowan et al., 1990)
Dinoflagellates	4-Methylergostane, 4-methylstigmastane Dinosterane XIV	Mostly Mesozoic and Cenozoic (minor concentrations in Paleozoic, possibly of "protodinoflagellate" origin)	Minor component in other eukaryotes Rare in diatoms	(Volkman, 2003) (Robinson et al., 1984; Volkman et al., 1993; Moldowan and Talyzina, 1998)
Diatoms	24-Norcholestane (C ₂₆)	Cretaceous to Cenozoic		(Holba et al., 1998a, b)
Centric diatoms (genus <i>Rhizosolenia</i>)	C ₂₅ XV and C ₃₀ highly branched isoprenoid (HBI) alkanes	Marine environments, Upper Turonian to present	C ₂₅ HBI in pennate diatoms	(Nichols et al., 1988; Volkman et al., 1994; Sinninghe Damsté et al., 2004b)
Pennate diatoms (phylogenetic cluster including <i>Haslea</i> , <i>Pleurosigma</i> , <i>Navicula</i>)	C ₂₅ HBI alkanes XV	Marine environments	Centric diatoms (genus <i>Rhizosolenia</i>)	(Nichols et al., 1988; Sinninghe Damsté et al., 2004b)
Prymnesiophyte algae	C ₃₇ -C ₃₉ di- to tetra-unsaturated alkenones	The degree of unsaturation changes with water temperature, and this is used in the photic zone temperature proxy U _k ³⁷		(Brassell et al., 1986)
<i>Botryococcus braunii</i> (Chlorophyte alga)	Botryococcanes XVI , cyclobotryococcanes, and polymethylsqualanes Macrocyclic C ₁₅ -C ₃₄ alkanes without carbon number preference	Fresh to brackish water, Tertiary Fresh to brackish water, Tertiary		(Metzger et al., 1985; Huang et al., 1988; Metzger and Largeau, 1999; Summons et al., 2002) (Audino et al., 2002)
<i>Gloeocapsomorpha prisca</i> (uncertain affinity, possibly an alga)	Outstanding concentrations of n-C ₁₅ , n-C ₁₇ , and n-C ₁₉	Cambrian to Devonian		(Fowler, 1992; Blokker et al., 2001)
Ciliates	Gammacerane XVII	The main source is possibly bacterivorous ciliates inhabiting the chemocline of stratified waters	Gammacerane precursors were also observed in a fungus, a fern, and the ubiquitous α -proteobacterium <i>Rhodopseudomonas</i>	(Ten Haven et al., 1989; Sinninghe Damsté et al., 1995)
Terrestrial plants in general	<i>n</i> -Alkanes > C ₂₅ with odd-over-even carbon number preference, derived from higher plant waxes	Proximity to terrestrial organic matter sources; post-Silurian age	Non-marine algae	(Hedberg, 1968; Tissot and Welte, 1984)

Biomarkers (Molecular Fossils), Table 1 (Continued)

Group of organisms	Biomarker or biomarker pattern	Environmental interpretation and age constraints	Other known potential sources	Further information ^a
	Lupane, various plant sources	Common in coal and lignites, Cretaceous to Cenozoic		(Wang and Simoneit, 1990)
	Isopimarane, retene, simonellite, eudesmane, and fichtelite		Possible occurrence in algae and microorganisms	(Peters et al., 2004)
Angiosperms	Oleanane XVIII , precursors are betulins, taraxerenes, and others triterpenoids	Predominantly Upper Cretaceous to Cenozoic	Precursors also occur in lichens and ferns in minor concentrations	(Moldowan et al., 1994)
Angiosperms (e.g., dipterocarpaceae)	Cadinanes, bicadinanes XIX (from degradation of polycadinene resins)	Carboniferous to Cenozoic		(Cox et al., 1986; Van Aarssen et al., 1992)
Conifers	Phyllocladanes, beyeran, kaurane, and atisane	Devonian to Cenozoic, often abundant in coal	Lower concentrations in other land plants and possibly algae	(Noble et al., 1985)
Sponges (demospongiae)	24-Isopropylcholestane XIIIe	Neoproterozoic to present, with higher relative abundance in the late Neoproterozoic to early Cambrian		(McCaffrey et al., 1994; Love et al., 2009)
Archaea				
Archaea in general	C ₃₁ -C ₄₀ head-to-head linked acyclic isoprenoids (e.g., XXI), archaeol XI , and other glycerol ethers Abundant squalane XX , abundant regular acyclic isoprenoids		Bacteria and eukaryotes, but commonly in lower concentrations	
Marine pelagic Crenarchaeota	2,6,15,19-Tetramethylicosane (TMI) Crenarchaeol XII	Only reported from a Mid-Cretaceous oceanic anoxic event First appearance in the Cretaceous		(Vink et al., 1998; Kuypers et al., 2001) (Kuypers et al., 2001)
Haloarchaea	Regular acyclic isoprenoids with 21–30 carbon atoms	Evaporitic environments, salt lakes	Other archaea	(Grice et al., 1998b)
Anaerobic methane oxidizers (ANME)	Crocetane XXII	Typical habitats are subsea gas sources, gas hydrates, and mud volcanoes	Degradation product of diaromatic carotenoids	(Thiel et al., 1999; Bian et al., 2001)
Methanogenic and methanotrophic archaea	2,6,10,15,19-Pentamethylicosane (PMI)			(Schouten et al., 1997; Elvert et al., 1999; Thiel et al., 1999)
Bacteria				
Bacteria in general	C ₃₀ -hopanes		Some cryptogams, ferns, mosses, lichens, filamentous fungi, and protists	(Rohmer et al., 1984)
	Hopanes with extended side chain (homohopanes (C ₃₁) – pentakishomohopane IVa (C ₃₅))			(Rohmer et al., 1984; Ourisson and Albrecht, 1992)

Biomarkers (Molecular Fossils), Table 1 (Continued)

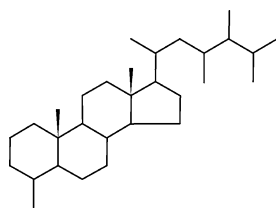
Group of organisms	Biomarker or biomarker pattern	Environmental interpretation and age constraints	Other known potential sources	Further information ^a
Cyanobacteria	Abundant monomethylalkanes and dimethylalkanes		Other bacteria, sponges	(Shiea et al., 1990; Kenig et al., 1995; Köster et al., 1999; Dembitsky et al., 2001)
	2-Methylhopanes IVc		Pseudomonadales, rhizobiales	(Summons et al., 1999; Rashby et al., 2007)
Type-I methanotrophic bacteria (methylococcaceae)	3 β -Methylhopanes IVb		Methylotrophs, acetic acid bacteria	(Zundel and Rohmer, 1985a, b, c; Summons and Jahnke, 1992; Farrimond et al., 2000)
	4-Methylcholestanes, 4,4-dimethylcholestanes		Dinoflagellates, minor component in all eukaryotes	(Bird et al., 1971; Brocks et al., 2005)
Green sulfur bacteria (Chlorobiaceae)	Chlorobactane XXIII (green pigmented species)	Anoxic and sulfidic conditions in the presence of light in microbial mats or planktonic environments (photic zone euxinia)		(Schaeffer et al., 1997; Grice et al., 1998a, 2005; Brocks et al., 2005)
	Isorenieratane VIII (brown pigmented species)	Photic zone euxinia	Some sponges, Actinomycetales	(Hartgers et al., 1993; Grice et al., 1996a; Koopmans et al., 1996b; Bosch et al., 1998; Pancost et al., 1998; Putschew et al., 1998; Simons and Kenig, 2001; Sinninghe Damsté et al., 2001)
	β -Isorenieratane (brown pigmented species)	Photic zone euxinia		(Grice et al., 1998a; Brocks and Schaeffer, 2008)
	2,3,6-Trimethyl aryl isoprenoids – (cleavage products of VIII and XXIII)	Photic zone euxinia	Aromatization and degradation of β -carotene	(Summons and Powell, 1986; Requejo et al., 1992; Summons and Powell, 1992; Hartgers et al., 1993; Koopmans et al., 1996a)
	Palaerenieratane XXIV and 3,4,5- trimethyl aryl isoprenoids	Photic zone euxinia; restricted to the Paleozoic?		(Requejo et al., 1992; Hartgers et al., 1993)
	3-Isobutyl-4-methylmaleimide Xc		Chloroflexaceae	(Grice et al., 1996b, 1997; Pancost et al., 2002)
Purple sulfur bacteria (Chromatiaceae)	Okenane VI	Photic zone euxinia, planktonic conditions		(Brocks et al., 2005; Brocks and Schaeffer, 2008)
	2,3,4-Trimethyl aryl isoprenoids	Photic zone euxinia	Cyanobacterial synechocanthin?	(Brocks et al., 2005; Graham and Bryant, 2008)
	Renieratane	Photic zone euxinia	Sponges or sponge symbionts	(Brocks and Schaeffer, 2008)
	Renierapurpurane	Photic zone euxinia	Sponges or sponge symbionts, cyanobacterial synechocanthin?	(Graham and Bryant, 2008; Brocks and Schaeffer, 2008)
Gemmata (Planctomycetales)	Lanostane		Minor in all eukaryotes and steroid producing bacteria (Pearson et al., 2003)	(Pearson et al., 2003)
Phototrophs in general	Pristane, phytane	From chlorophylls of cyanobacteria and phototrophic eukaryotes, BChl <i>a</i> and <i>b</i> of phototrophic bacteria	Tocopherols and other acyclic isoprenoids, archaeal membrane lipids	(Peters et al., 2004)

Biomarkers (Molecular Fossils), Table 1 (Continued)

Group of organisms	Biomarker or biomarker pattern	Environmental interpretation and age constraints	Other known potential sources	Further information ^a
	3,4-Dimethyl Xa and 3-ethyl-4-methylmaleimides Xb	Chlorophylls and bacteriochlorophylls		(Grice et al., 1996b)
<i>Anthropogenic contaminants</i>				
Polyethylene plastic bags	5,5-Diethylalkanes with odd-over-even carbon number preference, and other branched alkanes with quaternary carbon (BAQCs) Alkylcyclopentanes with even-over-odd carbon number preference			(Grosjean and Logan, 2007; Brocks et al., 2008) (Grosjean and Logan, 2007; Brocks et al., 2008)
Detergents	Phenylalkanes			(Harvey et al., 1985)
Plasticizers	Phthalates			(Mayer et al., 1972)

^aadditional information taken from Peters et al. (2004) and Brocks and Summons (2004)

Phylogenetic trees of oxidosqualene cyclase (OSC), the first enzyme in the sterol biosynthetic pathway, show that sterol biosynthesis was laterally transferred between eukaryotes and the three bacterial groups (Pearson et al., 2003). However, bacteria do not possess the enzymes required to alkylate the steroid side chain, and fossils steroids alkylated at C-23 or C-24 (e.g., ergostane **XIIIb**, stigmastane **XIIIc** and dinosterane **XIV**) must still be regarded as diagnostic biomarkers for eukaryotes.

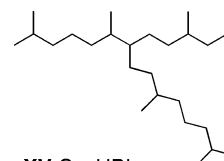


XIV Dinosterane

Pelagophytes and dinoflagellates. In contrast to C₂₇ to C₂₉ steranes, steroids with 30 carbon atoms can be highly specific. For example, 24-*n*-propylcholestane **XIIIId** is a diagnostic marker for algae of the order Sarcinochrysidales and the so-called brown tide algae (Moldowan et al., 1990). These organisms are strictly marine, and 24-*n*-propylcholestane is, thus, regarded as a reliable indicator for marine depositional conditions. Another steroid with a unique carbon skeleton is dinosterane **XIV** (4,23,24-trimethylcholestane). The biological precursors of dinosterane **XIV**, dinosterol, and related compounds (Robinson et al., 1984), are the dominant sterols in many dinoflagellate species (see references in Volkman, 2003). Only one other organism, a diatom species, is known to contain sterols with the same carbon skeleton. Therefore, dinosterane is regarded as

a relatively reliable biomarker for dinoflagellates (Summons et al., 1987). In the microfossil record, the first modern dinoflagellates appeared in the Mesozoic, and this is also reflected by a significant increase in dinosterane concentrations in contemporaneous rocks (Moldowan et al., 1996; Moldowan and Talyzina, 1998; Summons et al., 1987, 1992). Traces of dinosteranes in Paleozoic sedimentary rocks were identified as the likely molecular remains of ancestral dinoflagellates (Moldowan et al., 1996; Talyzina et al., 2000). Dinoflagellates are probably also the dominant source of other steranes and aromatic steroid that bear a methyl group at C-4 (4-methylcholestane, 4-methylergostane, and 4-methylstigmastane; compare structure **XIII**). However, these biomarkers are less specific, and multiple other biological sources are known (Volkman, 2003).

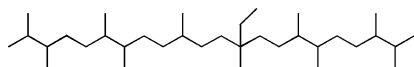
Diatoms. An unusual sterane common in post-Jurassic marine sediments is 24-norcholestane. Although the biological precursor lipids remain unknown, indirect evidence points to diatoms as the major source (Holba et al., 1998a). Consistent with this assignment, the relative concentration of 24-norcholestanes in crude oils rises in concert with the radiation of diatoms from the Jurassic to the Tertiary (Holba et al., 1998b). Other specific biomarkers for diatoms are “highly branched isoprenoid” (HBI) alkanes **XV** with 25 and 30 carbon atoms



XV C₂₅ HBI

(e.g., Volkman et al., 1994). The unsaturated precursors of HBI alkanes are found in two discrete phylogenetic clusters of diatoms. C_{25} and C_{30} HBIs occur in centric diatoms of the genus *Rhizosolenia*, and a second cluster, comprising three genera of pennate diatoms, produces C_{25} HBI alkenes (Sinninghe Damsté et al., 2004b). Remarkably, the C_5 building blocks of HBI alkenes in the centric diatoms are biosynthesized using the mevalonate (MVA) pathway, while the isoprene building blocks of HBI alkenes in pennate diatoms are constructed following the methylerythritol (MEP) route (Massé et al., 2004). The distribution of C_{25} HBIs **XV** in marine sediments and petroleum constrains the radiation of rhizosolenid diatoms quite accurately to the Upper Turonian, 91.5 ± 1.5 Ma ago (Sinninghe Damsté et al., 2004b). The biological origin of HBIs in marine sediments with 20, 21, 22, and 26 carbon atoms is still unknown, but diatoms are also a likely source (Sinninghe Damsté et al., 2005). Although C_{20} HBI has been identified in field samples of the green macro algae *Chara* and *Enteromorpha* (Rowland et al., 1985; Jaffé et al., 2001), it is likely derived from epiphytic micro algae colonizing the macrophytes (Volkman et al., 1998; Atahan et al., 2007) (see also *Biomarkers (Organic, Compound-Specific Isotopes)*).

Green algae. The green unicellular microalga *Botryococcus braunii* is found in freshwater, brackish lakes, and reservoirs at varying latitudes (Tyson, 1995 and references therein). Three distinct races (A, B, and L) of this alga are recognized and are classified according to their hydrocarbon composition. The A race biosynthesizes n -alkadienes and trienes with an odd carbon number between C_{25} and C_{31} (Metzger et al., 1986, 1991). The B race produces C_{30} to C_{37} branched isoprenoidal hydrocarbons called botryococcenes giving rise to botryococcanes (e.g., **XVI**) (Maxwell et al., 1968;



XVI Botryococene

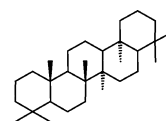
Metzger and Largeau, 1999) and a wide variety of cyclobotryococcenes (Metzger et al., 1985; David et al., 1988), and polymethylsqualenes (Summons et al., 2002). The L race makes a C_{40} isoprenoid hydrocarbon, lycop-14(*E*),18(*E*)-diene (Metzger and Casadevall, 1987; Metzger et al., 1991). Macrocyclic alkane biomarkers with 15-34 carbon atoms are derived from *Botryococcus* cell wall material (Audino et al., 2002). Although botryococcanes are quite rare in petroleum, they are among the most specific biomarkers and indicate a lacustrine origin and Tertiary age.

Straight chain alkanes (n -alkanes) are the most abundant class of saturated hydrocarbons in all bitumens and oils that have not suffered biodegradation. n -Alkanes have a vast variety of biological precursors such as saturated and unsaturated fatty acids and their esters, alcohols, alkenes, plant waxes, and polymethylenic biopolymers.

As biochemicals with n -alkyl skeletons occur in all branches across the three domains of life, the structure of single n -alkanes has no taxonomic value (although their carbon and hydrogen isotopic composition may yield valuable environmental and biological clues - see *Biomarkers (Organic, Compound-Specific Isotopes)*). However, some n -alkane profiles, i.e., the relative concentration of n -alkanes with different carbon numbers, can be taxonomically informative. For instance, bitumens and oils from the early Paleozoic often show elevated abundances of n -alkanes with odd carbon numbers in the range n - C_{15} and n - C_{19} . This age diagnostic n -alkane profile is attributed to *Gloeocapsomorpha prisca*, an extinct marine alga (or other organism) of unknown affinity (Hoffmann et al., 1987; Fowler, 1992).

The cell wall of many marine and lacustrine chlorophytes and marine eustigmatophytes is composed of algaenan, an insoluble, polymethylenic macromolecule (Derenne et al., 1992; e.g., Gelin et al., 1997; Blokker et al., 2000). Cracking of algaenan during burial and catagenesis releases large volumes of n -alkanes that contribute significantly to the formation of liquid petroleum (Tegelaar et al., 1989). There is evidence that a major radiation of chlorophytes in the late Neoproterozoic also contributed to the formation of the oldest commercial reservoirs of petroleum (Grantham et al., 1988). These oils are characterized by high concentrations of long-chain n -alkanes derived from the degradation of algaenan or related macromolecules (Klomp, 1986; Fowler and Douglas, 1987; Höld et al., 1999). The same oils contain unprecedented high relative concentrations of stigmastanes **XIIIc**, the major steroid derived from green algae (Volkman, 2003).

Ciliates. Some bacterivorous ciliates produce the pentacyclic triterpenoid tetrahymanol (Harvey and McManus, 1991). The fossil product of tetrahymanol, gammacerane **XVII**, is often abundant in sedimentary rocks

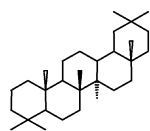


XVII Gammacerane

that were deposited under stratified and anoxic waters (Sinninghe Damsté et al., 1995). In these environments, predatory ciliates may inhabit the chemocline feeding on microaerophilic and anaerobic microorganisms. Gammacerane can reflect the often unusual carbon isotopic composition of the microbial food source (see *Biomarkers (Organic, Compound-Specific Isotopes)*) (Sinninghe Damsté et al., 1995; Grice et al., 1998a).

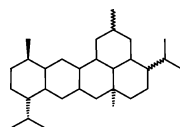
Terrestrial plants. In the geological record, plant-derived biomarkers start to appear in bitumens and oils from the Devonian, and can be exceedingly abundant in sediments from the late Mesozoic to Cenozoic. The most common plant signature is a suite of n -alkanes derived from plant waxes with more than 27 carbon atoms and a

predominance of odd-over-even carbon numbers (i.e., C_{27} , C_{29} , C_{31} etc.) (Hedberg, 1968). In coals and other sediments with significant input of terrestrial organic matter, high-molecular-weight alkanes with more than 40 and up to 110 carbon atoms are the degradation products of cuticular waxes and plant-derived biopolymers such as cutan and suberan (e.g., Nip et al., 1986; Tegelaar et al., 1995; Del Rio and Philp, 1999). One of the most common polycyclic terpenoids derived from plants is oleanane **XVIII**. Oleanane is a biomarker for flowering plants



XVIII Oleanane

(angiosperms), and the relative concentration of oleanane in bitumens from the early Cretaceous to Tertiary parallels the radiation of angiosperm families, as documented in the pollen record (Moldowan et al., 1994). The thermal degradation of polycadinene resins of tropical angiosperms generates terpenoids of the cadinane family. Cadinane, bicadinanes **XIX**, and tricadinanes are found



XIX Bicadinane

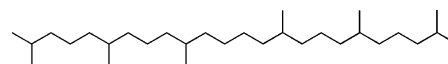
in sedimentary rocks from the Carboniferous to Cenozoic and are potentially diagnostic for Dipterocarpaceae, a group that today comprises mainly tropical lowland rainforest trees (Cox et al., 1986; Van Aarssen et al., 1990). The degradation of conifer resins releases a large variety of bicyclic and tricyclic terpenoids such as phyllocladane, beyerane, kaurane, atisane, retene, and simonellite that are abundant in coal (Table 1). However, many of these biomarkers are also found in lower concentrations in marine sedimentary rocks or rock of pre-Devonian age, and it appears likely that they also have algal or prokaryotic sources. For instance, the triaromatic hydrocarbon retene can be abundant in Tertiary shales and coals, and used to be interpreted as the degradation product of conifer resins (Villar et al., 1988). However, retene was also detected in marine rocks of late Neoproterozoic to early Paleozoic age (Jiang et al., 1995), and in pyrolysis products of algal and cyanobacterial tissue (Wen et al., 2000).

Sponges. Surprisingly, 24-isopropylcholestane **XIIIe**, indicative of the class Demospongiae, is the only known hydrocarbon biomarker that appears to be specific for any group of animals. In the geological record, the concentration of 24-isopropylcholestane **XIIIe** (relative to algal 24-*n*-propylcholestane **XIIIId**) is particularly high

from the latest Neoproterozoic to early Phanerozoic age, a distribution that may reflect the radiation and abundance of early sponges during this time (McCaffrey et al., 1994; Love et al., 2009).

Biomarkers of archaea

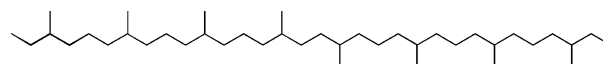
Archaea play a key role in the geochemical cycles of hydrogen, nitrogen, methane, and sulfur in a wide variety of marine, lacustrine and terrestrial environments. Although many archaea inhabit "extreme" environments such as hot springs and crystallizer ponds, they are also cosmopolitan inhabitants of the open ocean (e.g., Delong, 1992; Karner et al., 2001). Therefore, it is not unexpected that archaeal biomarkers can be found in a large number of oils and bitumens of all ages. Membrane lipids of archaea are very distinct from eukaryotes and bacteria. They are predominantly based on regular or irregular, acyclic or cyclic isoprenoids commonly bound to a glycerol backbone via ether links. The most common hydrocarbons derived from archaea in mature bitumens and oils are C_{21+} regular acyclic isoprenoids, and squalane **XX**, an



XX Squalane

irregular isoprenoid that comprises two tail-tail linked C_{15} isoprenoid units. Although precursors of these isoprenoids also occur in other organisms, archaea are presumed to be the major source. The abundance of archaeal biomarkers in bitumens is usually low in comparison to eukaryotic and bacterial lipids. However, under specific environmental conditions, archaeal organic matter may constitute a significant fraction of total sedimentary organic carbon, including marine (Kuypers et al., 2001) and hypersaline (Grice et al., 1998b) depositional environments.

Marine Crenarchaeota. According to cell counts based on whole-cell rRNA-targeted fluorescent in situ hybridization, pelagic crenarchaeota constitute ~20% of picoplankton cells in the world ocean, and may even outnumber the bacteria in the mesopelagic and bathypelagic zones (Karner et al., 2001). Cell numbers computed from the concentration of archaeal membrane lipids at different water depth in the Arabian Sea confirm this estimate (Sinninghe Damsté et al., 2002). The membrane lipids of crenarchaeota are typically glycerol dialkyl glycerol tetraethers (GDGTs) (De Rosa and Gambacorta, 1988). GDGTs consist of two glycerol units linked by two tetraetherpenoid (C_{40}) chains that may be acyclic (biphytane **XXI**) or contain one to three cyclopentane moieties.



XXI Biphytane

GDGTs are also biosynthesized by methanogenic and methanotrophic euryarchaeotes, and occur in a

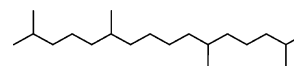
wide variety of environments, including hot springs and cold lakes. However, crenarchaeol **XII**, which is distinguished by the presence of an additional cyclohexane moiety, appears to be a diagnostic biomarker for marine pelagic crenarchaeotes (Sinninghe Damsté et al., 2002). GDGTs are exceptionally stable and have been detected in 140 Ma old rocks from the Upper Jurassic age (Carrillo-Hernandez et al., 2003). During the mid-Cretaceous Oceanic Anoxic Event OAE1b, the ocean may have been dominated by pelagic crenarchaeota (Kuypers et al., 2001). The sediments of OAE1b contain acyclic and cyclic biphytanes as well as intact GDGTs, including crenarchaeol (see also *Biomarkers (Organic, Compound-Specific Isotopes)*).

The rings incorporated into the biphytanyl chains of GDGTs are considered to be a mechanism for temperature adaptation in archaea. Schouten et al. (2002) found that the number of pentacyclic rings in GDGTs (expressed as the biomarker ratio TEX₈₆) is a linear function of annual mean sea surface temperatures. The correlation $T = 10.8 + 56.2 * \text{TEX}_{86}$ is calibrated for temperatures from 5 to 35°C (Kim et al., 2008) and possibly 40°C (Schouten et al., 2007). As an example, TEX₈₆ measurements revealed that average sea surface temperatures in the late Cretaceous were very high, ~15°C in the Arctic ocean (Jenkyns et al., 2004), and >32–36°C at low latitudes in the North Atlantic (Schouten et al., 2003). Temperature measurements on GDGTs also contributed significantly to the understanding of the Paleocene/Eocene thermal maximum (PETM) 55 Ma ago. The PETM was one of the most rapid global heating events in Earth's history. It was accompanied by environmental changes and a sharp global negative carbon isotopic excursion, although the precise order of events remained uncertain for a long time. Sluijs et al. (2007) obtained a high-resolution record of sea surface temperatures across the PETM based on TEX₈₆ from sediments in New Jersey. The record indicated that sea surface temperatures and ecological changes preceded the carbon isotopic excursion by ~3,000 years. This discovery is consistent with the hypothesis that increasing temperatures of marine bottom waters caused dissociation of submarine gas hydrates and injection of isotopically light methane carbon into the atmosphere/ocean system. TEX₈₆ measurements also indicated that sea surface temperatures near the North Pole increased during the PETM from 18 to 23°C, temperatures that are more than 10°C higher than predicted by previous models (Sluijs et al., 2006).

Halophilic archaea. Archaea of the order Halobacteriales are frequently the dominant organisms in hypersaline environments such as salt lakes and pools of evaporating sea water (Oren, 2002). The membrane lipids of extremely halophilic archaea that may thrive at halite concentrations as high as saturation levels, contain glycerol ethers with C₂₀ and C₂₅ regularly branched acyclic isoprenoid side chains (e.g., **XI**). The degradation products of these glycerol ethers are often preserved in sedimentary rocks

associated with halite deposits. For example, the dominant hydrocarbons in Miocene/Pliocene sediments from the Dead Sea Basin, Israel, are regular isoprenoids with 19–25 carbon atoms (Grice et al., 1998b). Halophilic archaea also biosynthesize carotenoids with highly characteristic skeletons with 50 carbon atoms such as bacterioruberin. However, the fossil equivalents of these carotenoids have not yet been detected in the geological record.

Methanogenic and methanotrophic archaea. Methane metabolizing archaea biosynthesize generic glycerol diethers and tetraethers, and diagnostic hydroxyarchaeols, macrocyclic archaeols, and irregularly branched acyclic isoprenoid hydrocarbons such as 2,6,10,15,19-pentamethylcosane (PMI). Methanotrophic archaea oxidize methane under anaerobic conditions in consortium with sulfate reducing bacteria. The sulfate reducers make the process thermodynamically viable by removing the hydrogen that is generated as a by-product of the methanotrophic process. Crocetane **XXII**, an irregular



XXII Crocetane

tail-to-tail linked C₂₀ isoprenoid, is commonly found at methane seeps, and is diagnostic for methanotrophic archaea if it has a light carbon isotopic composition (Greenwood and Summons, 2003). For more details about biomarkers at methane venting sites and of methane metabolizing Archaea, see *Biomarkers (Organic, Compound-Specific Isotopes)*.

Biomarkers of bacteria

By mass, bacterial hopanoids stored in sedimentary organic matter and oil deposits were described as the most abundant natural products on the Earth (Ourisson and Albrecht, 1992). Fossil hopanes **IV** and aromatic hopanoids can be found in virtually all thermally well-preserved bitumens and oils from the Paleoproterozoic to the present. The biological precursors in bacteria are bacteriohopanepolyols **III** with a hydrophilic side chain that is biosynthetically derived from a pentose sugar. The polar side chain commonly carries three to five hydroxy groups and may additionally be ornamented with a terminal amino group, peptidic derivatives, carbohydrates, glucosamines, nitrogenous bases, and many more (Ourisson et al., 1987). Bacteriohopanepolyols are thought to play a role as membrane modifiers analogous to sterols in eukaryotes, but the exact function of the large structural variety of hopanoids remains a major area for future research. Although terpenoids with a C₃₀ hopane skeleton have been observed in some cryptogams, ferns, mosses, lichens, filamentous fungi, and protist, C₃₅ bacteriohopanoids **III** with a pentose-derived side chain appear to be diagnostic for bacteria. Although most hopanoid producers are aerobic, there is now a growing number of reports of anaerobic bacteria that contain

hopanols as well (Sinninghe Damsté et al., 2004a; Fischer et al., 2005). Generally, bacterial lineages that produce hopanoids are widely distributed across phylogenetic trees without apparent systematic patterns, and less than 10% of all species contain the genes required for hopanoid biosynthesis (Pearson et al., 2007). Thus, hopanes in sediments cannot be attributed to any specific bacterial source without additional information (Rohmer et al., 1984; Farrimond et al., 1998). However, hopanoids with alkyl substituents at ring A appear to be limited to a restricted number of groups. For instance, 3-methylhopanoids appear to be exclusive to microaerophilic proteobacteria (acetic acid bacteria, methanotrophs, methylotrophs) (Summons and Jahnke, 1992), and 2-methylhopanoids are abundant in, although not exclusive to, cyanobacteria (Summons et al., 1999).

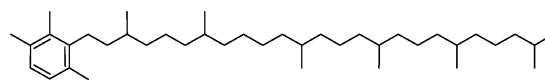
Cyanobacteria. Many cyanobacteria produce relatively high concentrations of hopanepolyols that are methylated at C-2. The hydrocarbon derivatives of these lipids, 2 α -methylhopanes **IVc**, are found in most oils and bitumens of all ages. To gauge the contribution of cyanobacterial debris to the sedimentary carbon pool, Summons et al. (1999) introduced the 2 α -methylhopane index (2-MHI) that expresses the concentration of C₃₁ 2 α -methylhopane relative to general bacterial C₃₀ hopane. On an average, the 2-MHI is higher in the Proterozoic than the Phanerozoic, consistent with the hypothesis that cyanobacteria played a more significant role as primary producers in the Precambrian (Summons et al., 1999). However, not all cyanobacteria produce hopanoids (Rohmer et al., 1984), and hopanoid biosynthesis in marine cyanobacteria appears to be rare (Pearson et al., 2007). Thus, absence or low concentrations of 2-methylhopanes should not be used as evidence for low activity of these organisms. Moreover, 2-methylbacteriohopanepolyols also occur in methylotrophic and nitrogen fixing proteobacteria. 2-Methylhopanoid biosynthesis in the γ -proteobacterium *Rhodospseudomonas palustris* occurs under anaerobic conditions, and this further complicates the interpretation of 2-MHI as an indicator for oxygenic photosynthesis (Rashby et al., 2007).

Low-molecular-weight (C₁₄–C₁₉) monomethyl alkanes and other acyclic alkanes with one or more alkyl groups occur in all oils and bitumens, but are particularly abundant in the Precambrian and in sediments with remnant microbial mat assemblages (Summons and Walter, 1990). Although methylalkyl compounds occur in many microorganisms, the predominant sources are probably the mono- to tri-methyl branched alkanes that are abundant in extant cyanobacteria (Köster et al., 1999).

Methanotrophs, methylotrophs, and acetic acid bacteria. Several groups of microaerophilic proteobacteria - type-I methanotrophs, methylotrophs, and acetic acid bacteria - biosynthesize hopanoids with an additional methyl group in 3 β -position (Zundel and Rohmer, 1985a; Summons and Jahnke, 1992). Although 3 β -methylhopanes **IVb** in sedimentary rocks and oils may be derived from any of

the above groups, hopanoids of methanotrophs are probably the major source and can be distinguished by a pronounced depletion in ¹³C compared to co-occurring compounds (see *Biomarkers (Organic, Compound-Specific Isotopes)*) (Collister et al., 1992; Jahnke et al., 1999). A second unusual group of lipids produced by type-I methanotrophs are sterols with a cholestane **XIIIa** skeleton (C₂₇) that carry one or two additional methyl groups at C-4 (Bird et al., 1971; Summons and Capon, 1988). 4-methyl and 4,4-dimethyl cholesteroids are also widespread in eukaryotes because they are the early intermediates in the biosynthesis of higher sterols. However, as intermediates, concentrations in eukaryotes are commonly very low, and very high relative concentrations of 4-methyl- and 4,4-dimethylcholestanes and their aromatic analogs in bitumens and oils likely indicate activity of methanotrophic bacteria. The oldest biomarkers of type-I methanotrophic bacteria come from the 1.6 Ga Barney Creek Formation in the McArthur Basin, northern Australia (Brocks et al., 2005). The well-preserved, organic-rich dolostones of the Barney Creek Formation were deposited in a deep rift basin that was, at least intermittently, stratified and sulfidic. In these ancient bitumens, 4-methylated triaromatic cholesteroids constituted >90% of the entire steroid population and, together with high relative concentrations of 3 β -methylhopanes, suggest that aerobic methanotrophic bacteria were abundant members of the microbial community. As aerobic methanotrophs are usually only abundant in sulfate-starved environments (<0.5 mM) (Hoehler et al., 1998), the biomarkers suggest that sulfate concentrations in this Paleoproterozoic basin were well below the present marine level of about 28 mM.

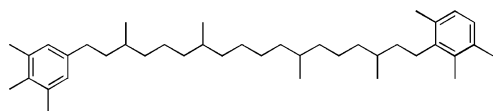
Green sulfur bacteria (Chlorobiaceae). Green sulfur bacteria (Chlorobiaceae) are strictly anaerobic, obligately phototrophic organisms that exclusively utilize photosystem I (PS I). They require, with few exceptions, reduced sulfur species such as sulfide and sulfur as an electron donor. Thus, biomarkers of Chlorobiaceae, including specific aromatic carotenoids and bacteriochlorophyll breakdown products, are proxies for anoxic and sulfidic conditions in the presence of light, either in microbial mats or marine and lacustrine stratified waters (photic zone euxinia). Green pigmented Chlorobiaceae contain the carotenoids chlorobactene and hydroxychlorobactene, and the diagenetic product of these pigments, chlorobactane **XXIII**, is regarded as a highly diagnostic



XXIII Chlorobactane

biomarker. The major carotenoids of brown pigmented strains of Chlorobiaceae are isorenieratene **IX** and β -isorenieratene that form the geologically stable fossil analogs isorenieratane **VIII** and β -isorenieratene. Isorenieratene **IX** also occurs in Actinomycetes, bacteria

that predominantly inhabit soil but have also been observed in marine sediments. However, Chlorobiaceae and Actinomycetes follow different carbon fixation pathways, and the carbon isotopic compositions of isorenieratane **VIII** can be used to determine the exact biological source (see *Biomarkers (Organic, Compound-Specific Isotopes)*). An unusual isomer of isorenieratane **VIII**, palaerenieratane **XXIV** with a 3,4,5-trimethyl aryl



XXIV Palaerenieratane

substitution pattern, is exclusively known from sedimentary rocks of Paleozoic age. A biological precursor of palaerenieratane **XXIV** has not yet been discovered in extant organisms, but a strong carbon isotopic enrichment indicative of carbon assimilation via the reversed tricarboxylic acid (TCA) suggests that it is derived from unknown Chlorobiaceae (Hartgers et al., 1993).

Other biomarkers that are diagnostic for Chlorobiaceae are degradation products of specific bacteriochlorophylls (BChl). BChl *c* and *d* exclusively occur in green filamentous bacteria (Chloroflexaceae) and Chlorobiaceae. BChl *e* (as a major pigment) is restricted to brown strains of Chlorobiaceae. During diagenesis, oxidative degradation of the tetrapyrrole skeleton of chlorophylls can lead to the formation of maleimides (1H-pyrrole-2,5-diones) that are highly stable under geological conditions (Grice et al., 1996b). While Chl *a* found in oxygenic phototrophs yields 3,4-dimethyl **Xa** and 3-ethyl-4-methylmaleimides **Xb** upon oxidation, BChls *c*, *d* and *e* yield the diagnostic biomarker 3-isobutyl-4-methylmaleimide **Xc**. Whether **Xc** in a particular sample is derived from Chlorobiaceae or Chloroflexaceae can be distinguished by compound-specific carbon isotopic measurements (Grice et al., 1996b) (see also *Biomarkers (Organic, Compound-Specific Isotopes)*).

Purple sulfur bacteria (Chromatiales). Purple sulfur bacteria (families Chromatiaceae and Ectothiorhodospiraceae) are anoxygenic phototrophs that form a well-separated group in the γ -subgroup of Proteobacteria. Although purple sulfur bacteria are generally metabolically more versatile than green sulfur bacteria, they also predominantly utilize sulfide and sulfur as their electron source. In microbial mats and stratified water bodies, they commonly inhabit a layer above green sulfur bacteria within the light penetrated zone directly beneath the oxic–anoxic interface. Twelve known genera of Chromatiaceae contain the purple coloured carotenoid okenone **V** that possesses a unique carbon skeleton with a 2,3,4-trimethyl aryl substitution pattern (Brocks and Schaeffer, 2008). The molecular fossil, okenane **VI**, is believed to be a diagnostic biomarker for Chromatiaceae (Brocks et al., 2005). Okenone has not been detected in microbial mat environments, and it may function as

a specific accessory pigment for planktonic Chromatiaceae, adjusting the light harvesting systems to lighting conditions at relatively shallow water depth of less than ~25 m. Thus, okenane can be regarded as a biomarker for euxinic water systems with particularly shallow stratification.

The oldest biomarkers of green and purple sulfur bacteria were discovered in the Paleoproterozoic Barney Creek Formation in the McArthur Basin in northern Australia (Brocks et al., 2005). The 1.64 Ga old dolostones were deposited in a deep intracratonic rift basin that probably only had restricted connection to the ocean. Extracts of sedimentary rocks yielded high relative concentrations of okenane **VI** and chlorobactane **XXIII**, but only minor amounts of isorenieratane **VIII**. In comparison with modern ecosystems with similar pigment distributions, the biomarkers probably indicate a basin with a very shallow thermocline (~12 m). Chromatiaceae probably formed major blooms in a layer directly beneath the anoxic–oxic boundary, underlain by a layer of green pigmented Chromatiaceae and, yet deeper, minor communities of brown pigmented strains. In modern ecosystems, a strong activity of Chromatiaceae and green pigmented Chlorobiaceae is often associated with a low abundance of oxygenic phototrophs in the overlying water column. In the Barney Creek Formation, this is confirmed by a paucity of biomarkers diagnostic for eukaryotic algae (Brocks and Schaeffer, 2008).

Conclusions

The development of new metabolic pathways that permitted microorganisms to utilize previously untapped energy sources, such as iron reduction, oxygenic photosynthesis, and methanotrophy probably had profound effects on the chemical evolution of Earth's atmosphere, crust, and oceans. It is a major aim of geobiology to correlate these chemical and isotopic changes in the geological record with the appearance of new microbial physiologies. One of the most informative methodologies to recognize the radiation of a new microbial group in the geological record is molecular fossils. To assign molecular fossils to specific organisms, or groups of organisms, we rely on information about the occurrence of precursor lipids in extant organisms, and almost all this information is derived from organisms that were grown in culture. However, according to some estimates, more than 99% of microorganisms currently defy isolation, and their physiologies, ecological roles, and lipid biosynthetic capacities remain largely unknown. This gap in knowledge is the main limitation for the application and interpretation of biomarkers. Promising ways to fill this gap are offered by cultivation-independent genomic and microbiological techniques such as 16S rRNA clone libraries, group-specific fluorescence in situ hybridization (FISH), and simultaneous community genomic (Tyson et al., 2004) and proteomic (Ram et al., 2005) studies. The technologies will help to describe which organisms are present in natural environments,

which groups are quantitatively important, and what are their major lipid products under specific conditions (Brocks and Banfield, 2009). Ideally, it should become possible to collate quantitative data about the rate of horizontal gene transfer and convergent evolution in lipid biosynthetic pathways, and this should make it possible to estimate the probability with which a biomarker from an ancient rock can be assigned to a specific biological source.

Bibliography

- Adam, P., Schmid, J. C., Mycke, B., Strazielle, C., Connan, J., Huc, A., Riva, A., and Albrecht P., 1993. Structural investigation of non-polar sulfur cross-linked macromolecules in petroleum. *Geochimica et Cosmochimica Acta*, **57**, 3395–3419.
- Albrecht, P., and Ourisson, G., 1969. Diagenèse des hydrocarbures saturés dans une série sédimentaire épaisse (Douala, Cameroun). *Geochimica et Cosmochimica Acta*, **33**, 138–142.
- Atahan, P., Grice, K., and Dodson, J., 2007. Human influence on holocene environmental change in the Yangtze river delta: a combined biomarker, $\delta^{13}\text{C}$, ^{14}C , pollen and charcoal approach. *The Holocene*, **17**, 507–515.
- Audino, M., Grice, K., Alexander, R., and Kagi, R. I., 2002. Macrocyclic alkanes in crude oils from the algaenan of *Botryococcus braunii*. *Organic Geochemistry*, **33**, 979–984.
- Barghoorn, E. S., 1957. Origin of life. *Geological Society of America* (Memoir), **67**, 75–85.
- Barghoorn, E. S., Meinschein, W. G., and Schopf, J. W., 1965. Paleobiology of a Precambrian shale. *Science*, **148**, 461–472.
- Berthelot, M., 1866. Sur l'origine des carbures et des combustibles minéraux. *Annales de Chimie Physique*, **9**, 481–483.
- Bian, L., Hinrichs, K. U., Xie, T., Brassell, S. C., Iversen, N., Fossing, H., Jørgensen, B. B., Sylva, S. P., and Hayes, J. M., 2001. Algal and archaeal polyisoprenoids in a recent marine sediment: molecular isotopic evidence for anaerobic oxidation of methane. *Geochemistry, Geophysics, Geosystems*, **2**, 2000GC000112.
- Bird, C. W., Lynch, J. M., Pirt, F. J., Reid, W. W., Brooks, C. J. W., and Middleditch, B. S., 1971. Steroids and squalene in *Methylococcus capsulatus* grown on methane. *Nature*, **230**, 473–474.
- Blokker, P., Schouten, S., De Leeuw, J. W., Sinninghe Damsté, J. S., and Van Den Ende, H., 2000. A comparative study of fossil and extant algaenans using ruthenium tetroxide degradation. *Geochimica et Cosmochimica Acta*, **64**, 2055–2065.
- Blokker, P., Van Bergen, P. F., Pancost, R. D., Collinson, M. E., Sinninghe Damsté, J. S., and De Leeuw, J. W., 2001. The chemical structure of *Gloeocapsamorpha prisca* microfossils: implication for their origin. *Geochimica et Cosmochimica Acta*, **65**, 885–900.
- Bode, H. B., Zeggel, B., Silakowski, B., Wenzel, S. C., Hans, R., and Müller, R., 2003. Steroid biosynthesis in prokaryotes: identification of myxobacterial steroids and cloning of the first bacterial 2,3(S)-oxidosqualene cyclase from the myxobacterium *Stigmatella aurantiaca*. *Molecular Microbiology*, **47**, 471–481.
- Bosch, H. J., Sinninghe Damsté, J. S., and De Leeuw, J. W., 1998. Molecular palaeontology of eastern Mediterranean sapropels: evidence for photic zone euxinia. *Proceedings of the Ocean Drilling Program, Scientific Results*, **160**, 285–295.
- Brassell, S. C., Eglinton, G., Marlowe, I. T., Pflaummann, U., and Sarnthein, M., 1986. Molecular stratigraphy: a new tool for climatic assessment. *Nature*, **320**, 129–133.
- Brocks, J. J., and Banfield, J., 2009. Unravelling ancient microbial history with community proteogenomics and lipid geochemistry. *Nature Reviews Microbiology*, **7**, 601.
- Brocks, J. J., and Schaeffer, P., 2008. Okenane, a biomarker for purple sulfur bacteria (Chromatiaceae), and other new carotenoid derivatives from the 1,640 Ma Barney Creek formation. *Geochimica et Cosmochimica Acta*, **72**, 1396–1414.
- Brocks, J. J., and Summons, R. E., 2004. Sedimentary hydrocarbons, biomarkers for early life. In Schlesinger, W. H. (ed.), *Treatise on Geochemistry, Vol 8, Biogeochemistry*. Oxford: Elsevier-Pergamon, pp. 63–115.
- Brocks, J. J., Summons, R. E., Buick, R., and Logan, G. A., 2003a. Origin and significance of aromatic hydrocarbons in giant iron ore deposits of the late Archean Hamersley Basin in Western Australia. *Organic Geochemistry*, **34**, 1161–1175.
- Brocks, J. J., Buick, R., Logan, G. A., and Summons, R. E., 2003b. Composition and syngeneity of molecular fossils from the 2.78–2.45 billion year old Mount Bruce Supergroup, Pilbara Craton, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4289–4319.
- Brocks, J. J., Love, G. D., Summons, R. E., Knoll, A. H., Logan, G. A., and Bowden, S. A., 2005. Biomarker evidence for green and purple sulphur bacteria in a stratified Paleoproterozoic sea. *Nature*, **437**, 866–870.
- Brocks, J. J., Grosjean, E., and Logan, G. A., 2008. Assessing biomarker syngeneity using branched alkanes with quaternary carbon (BAQCs) and other plastic contaminants. *Geochimica et Cosmochimica Acta*, **72**, 871–888.
- Burlingame, A. L., Haug, P., Belsky, T., and Calvin, M., 1965. occurrence of biogenic steranes and pentacyclic triterpanes in an Eocene shale and in an early Precambrian shale (2.7×10^9 years). *Proceedings of the National Academy of Sciences*, **54**, 406.
- Carrillo-Hernandez, T., Schaeffer, P., Adam, P., Albrecht, P., Derenne, S., and Largeau, C., 2003. Remarkably well preserved archaeal and bacterial membrane lipids in 140 million years old sediments from the Russian platform (Kashpir Oil Shales, Upper Jurassic). *21st International Meeting on Organic Geochemistry*. Poland: Kraków, pp. 77–78.
- Collister, J. W., Summons, R. E., Lichtfouse, E., and Hayes, J. M., 1992. An isotopic biogeochemical study of the Green River oil shale. *Organic Geochemistry*, **19**, 265–276.
- Cox, H. C., De Leeuw, J. W., Schenk, P. A., Van Koningsveld, H., Jansen, J. C., Van De Graaf, B., Van Geerstein, V. J., Kanters, J. A., Kruk, C., and Jans, A. W. H., 1986. Biscadinane, a C_{30} pentacyclic isoprenoid hydrocarbon found in crude oil. *Nature*, **319**, 316–318.
- David, M., Metzger, P., and Casadevall, E., 1988. Two cyclobotryococenes from the B race of the green alga *Botryococcus braunii*. *Phytochemistry*, **27**, 2863.
- De Leeuw, J. W., and Largeau, C., 1993. A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation. In Engel, M. H., and Macko, S. A. (ed.), *Organic Geochemistry, Principles and Applications*. New York: Plenum Press, pp. 23–72.
- De Leeuw, J., Versteegh, G., and Van Bergen, P., 2006. Biomacromolecules of algae and plants and their fossil analogues. *Plant Ecology*, **182**, 209.
- De Rosa, M., and Gambacorta, A., 1988. The lipids of archaeobacteria. *Progress in Lipid Research*, **27**, 153–175.
- Del Rio, J. C., and Philp, R. P., 1999. Field ionization mass spectrometric study of high molecular weight hydrocarbons in a crude oil and a solid bitumen. *Organic Geochemistry*, **30**, 279–286.
- Delong, E. F., 1992. Archaea in coastal marine environments. *Proceedings of the National Academy of Sciences*, **89**, 5685–5689.
- Dembitsky, V. M., Dor, I., Shkrob, I., and Aki, M., 2001. Branched alkanes and other apolar compounds produced by the

- cyanobacterium *Microcoleus vaginatus* from the Negev Desert. *Russian Journal of Bioorganic Chemistry*, **27**, 110–119.
- Derenne, S., Le Berre, F., Largeau, C., Hatcher, P. G., Connan, J., and Raynaud, J. F., 1992. Formation of ultralaminae in marine kerogens via selective preservation of thin resistant outer walls of microalgae. *Organic Geochemistry*, **19**, 345–350.
- Dominé, F., Bounaceur, R., Scacchi, G., Marquaire, P. M., Dessort, D., Pradier, B., and Brevart, O., 2002. Up to what temperature is petroleum stable? new insights from a 5200 free radical reaction model. *Organic Geochemistry*, **33**, 1487–1499.
- Durand, B., 2003. A history of organic geochemistry. *Oil & Gas Science and Technology*, **58**, 203–231.
- Eglinton, G., Scott, P. M., Belsky, T., Burlingame, A. L., and Calvin, M., 1964. Hydrocarbons of a biological origin from a one-billion-year-old sediment. *Science*, **145**, 263–264.
- Eglinton, T. I., and Repeta, D. J., 2004. Organic matter in the contemporary ocean. In Schlesinger, W. H. (ed.), *Treatise on Geochemistry* (Vol 6). Oxford: Elsevier-Pergamon, pp. 145–180.
- Elvert, M., Suess, E., and Whiticar, M. J., 1999. Anaerobic methane oxidation associated with marine gas hydrates: superlight C-isotopes from saturated and unsaturated C₂₀ and C₂₅ irregular isoprenoids. *Naturwissenschaften*, **31**, 1175–1187.
- Farimond, P., Fox, P. A., Innes, H. E., Miskin, I. P., and Head, I. M., 1998. Bacterial sources of hopanoids in recent sediments: improving our understanding of ancient hopane biomarkers. *Ancient Biomolecules*, **2**, 147–166.
- Farimond, P., Head, I. M., and Innes, H. E., 2000. Environmental influence on the biohopanoid composition of recent sediments. *Geochimica et Cosmochimica Acta*, **64**, 2985–2992.
- Fischer, W. W., Summons, R. E., and Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. *Geobiology*, **3**, 33–40.
- Fowler, M. G., 1992. The influence of *Gloeocapsomorpha prisca* on the organic geochemistry of oils and organic-rich rocks of late Ordovician age from Canada. In Schidlowski, M., Golubic, S., Kimberley, M. M., McKirdy, D. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Mineral and Energy Resources*. Berlin: Springer, pp. 336–356.
- Fowler, M. G., and Douglas, A. G., 1987. Saturated hydrocarbon biomarkers in oils of late Precambrian age from Eastern Siberia. *Organic Geochemistry*, **11**, 201–213.
- Gelin, F., Boogers, I., Noordeloos, A. A. M., Sinninghe Damsté, J. S., Riegman, R., and De Leeuw, J. W., 1997. Resistant biomacromolecules in marine microalgae of the classes eustigmatophyceae and chlorophyceae: geochemical implications. *Organic Geochemistry*, **26**, 659–675.
- Graham, J. E., and Bryant, D. A., 2008. The biosynthetic pathway for synechoxanthin, an aromatic carotenoid synthesized by the euryhaline, unicellular cyanobacterium *Synechococcus* sp. Strain PCC 7002. *The Journal of Bacteriology*, **190**, 7966–7974.
- Grantham, P. J., and Wakefield, L. L., 1988. Variations in the sterane carbon number distribution of marine source rock derived oils through geological time. *Organic Geochemistry*, **12**, 61–73.
- Grantham, P. J., Lijmbach, G. W. M., Postuma, J., Hughes-Clark, M. W., and Willink, R. J., 1988. Origin of crude oils in Oman. *Journal of Petroleum Geology*, **11**, 61–80.
- Greenwood, P. F., and Summons, R. E., 2003. GC-MS detection and significance of crocetane and pentamethylcosane in sediments and crude oils. *Organic Geochemistry*, **34**, 1211–1222.
- Grice, K., Schaeffer, P., Schwark, L., and Maxwell, J. R., 1996a. Molecular indicators of palaeoenvironmental conditions in an immature Permian shale (Kupferschiefer, Lower Rhine Basin, north-west Germany) from free and S-bound lipids. *Organic Geochemistry*, **25**, 131–147.
- Grice, K., Gibbison, R., Atkinson, J. E., Schwark, L., Eckardt, C. B., and Maxwell, J. R., 1996b. Maleimides (1*H*-pyrrole-2,5-diones) as molecular indicators of anoxygenic photosynthesis in ancient water columns. *Geochimica et Cosmochimica Acta*, **60**, 3913–3924.
- Grice, K., Schaeffer, P., Schwark, L., and Maxwell, J. R., 1997. Changes in palaeoenvironmental conditions during deposition of the Permian Kupferschiefer (Lower Rhine Basin, northwest Germany) inferred from molecular and isotopic compositions of biomarker components. *Organic Geochemistry*, **26**, 677–690.
- Grice, K., Schouten, S., Peters, K. E., and Sinninghe Damsté, J. S., 1998a. molecular isotopic characterisation of hydrocarbon biomarkers in palaeocene-eocene evaporitic, lacustrine source rocks from the Jiangnan Basin, China. *Organic Geochemistry*, **29**, 1745–1764.
- Grice, K., Schouten, S., Nissenbaum, A., Charrach, J., and Sinninghe Damsté, J. S., 1998b. Isotopically heavy carbon in the C₂₁ to C₂₅ regular isoprenoids in halite-rich deposits from the Sdom Formation, Dead Sea Basin, Israel. *Organic Geochemistry*, **28**, 349–359.
- Grice, K., Cao, C., Love, G. D., Böttcher, M. E., Twitchett, R. J., Grosjean, E., Summons, R. E., Turgeon, S. C., Dunning, W., and Jin, Y., 2005. Photoc zone euxinia during the Permian-Triassic superanoxic event. *Science*, **307**, 706–709.
- Grosjean, E., and Logan, G. A., 2007. Incorporation of organic contaminants into geochemical samples and an assessment of potential sources: examples from Geoscience Australia marine survey S282. *Organic Geochemistry*, **38**, 853.
- Guyomarc'h, F., Binet, A., and Dufossé, L., 2000. Production of carotenoids by *Brevibacterium linens*: variation among strains, kinetic aspects and HPLC profiles. *Journal of Industrial Microbiology and Biotechnology*, **74**, 64.
- Hartgers, W. A., Sinninghe Damsté, J. S., Requejo, A. G., Allan, J., Hayes, J. M., Ling, Y., Tiang-Min, X., Primack, J., and De Leeuw, J. W., 1993. A molecular and carbon isotopic study towards the origin and diagenetic fate of diaromatic carotenoids. *Organic Geochemistry*, **22**, 703–725.
- Harvey, G. R., Sinninghe Damsté, J. S., and De Leeuw, J. W., 1985. On the origin of alkylbenzenes in geochemical samples. *Marine Chemistry*, **16**, 187–188.
- Harvey, H. R., and McManus, G. B., 1991. Marine ciliates as a widespread source of tetrahymanol and hopan-3 β -ol in sediments. *Geochimica et Cosmochimica Acta*, **55**, 3387–3390.
- Hebting, Y., Schaeffer, P., Behrens, A., Adam, P., Schmitt, G., Schneckenburger, P., Bernasconi, S. M., and Albrecht, P., 2006. Biomarker evidence for a major preservation pathway of sedimentary organic carbon. *Science*, **312**, 1627–1631.
- Hedberg, H. D., 1968. Significance of high-wax oils with respect to genesis of petroleum. *American Association of Petroleum Geologists Bulletin*, **52**, 736–750.
- Hedges, J. I., Keil, R. G., and Benner, R., 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry*, **27**, 195–212.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and Delong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoehler, T. M., Alperin, M. J., Albert, D. B., and Martens, C. S., 1998. Thermodynamic control on hydrogen concentrations in anoxic sediments. *Geochimica et Cosmochimica Acta*, **62**, 1745–1756.
- Hoering, T. C., 1965. The extractable organic matter in Precambrian rocks and the problem of contamination. *Carnegie Institution of Washington Yearbook*, **64**, 215–218.
- Hoffmann, C. F., Foster, C. B., Powell, T. G., and Summons, R. E., 1987. Hydrocarbon biomarkers from Ordovician sediments and

- the fossil alga *Gloeocapsomorpha prisca* Zalesky 1917. *Geochimica et Cosmochimica Acta*, **51**, 2681–2697.
- Holba, A. G., Tegelaar, E. W., Huizinga, B. J., Moldowan, J. M., Singletary, M. S., McCaffrey, M. A., and Dzou, L. I. P., 1998a. 24-norcholestanes as age-sensitive molecular fossils. *Geology*, **26**, 783–786.
- Holba, A. G., Dzou, L. I. P., Masterson, W. D., Hughes, W. B., Huizinga, B. J., Singletary, M. S., Moldowan, J. M., Mello, M. R., and Tegelaar, E., 1998b. Application of 24-norcholestanes for constraining source age of petroleum. *Organic Geochemistry*, **29**, 1269–1283.
- Höld, I. M., Schouten, S., Jellema, J., and Sinninghe Damsté, J. S., 1999. Origin of free and bound mid-chain methyl alkanes in oil, bitumens and kerogens of the marine, Infracambrian Huqf Formation (Oman). *Organic Geochemistry*, **30**, 1411–1428.
- Holser, W. T., Schidlowski, M., Mackenzie, F. T., and Maynard, J. B., 1988. Biogeochemical cycles of carbon and sulfur. In Gregor, C. B., Garreis, R. M., Mackenzie, F. T., and Maynard, J. B. (eds.), *Chemical Cycles in the Evolution of the Earth*. New York: Wiley, pp. 105–173.
- Huang, Z., Poulter, C. D., Wolf, F. R., Somers, T. C., and White, J. D., 1988. Braunicene, a novel cyclic C₃₂ isoprenoid from *Botryococcus braunii*. *Journal of American Chemical Society*, **110**, 3959–3964.
- Hunt, J. M., 1979. *Petroleum Geochemistry and Geology*. San Francisco: Freeman.
- Jaffé, R., Mead, R., Hernandez, M. E., Peralba, M. C., and Diguida, O. A., 2001. Origin and transport of sedimentary organic matter in two subtropical estuaries: a comparative, biomarker-based study. *Organic Geochemistry*, **32**, 507.
- Jahnke, L. L., Summons, R. E., Hope, J. M., and Des Marais, D. J., 1999. Carbon isotopic fractionation in lipids from methanotrophic bacteria II: the effects of physiology and environmental parameters on the biosynthesis and isotopic signatures of biomarkers. *Geochimica et Cosmochimica Acta*, **63**, 79–93.
- Jenkyns, H. C., Forster, A., Schouten, S., and Sinninghe Damsté, J. S., 2004. High temperatures in the Late Cretaceous Arctic Ocean. *Nature*, **432**, 888–892.
- Jiang, N., Tong, Z., Ren, D., Song, F., Yang, D., Zhu, C., and Yijun, G., 1995. The discovery of retene in Precambrian and lower Paleozoic marine formations. *Chinese Journal of Geochemistry*, **14**, 41–51.
- Karner, M. B., Delong, E. F., and Karl, D. M., 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature*, **409**, 507–510.
- Kenig, F., Sinninghe Damsté, J. S., Kock-Van Dalen, A. C., Rijpstra, W. I. C., Huc, A. Y., and De Leeuw, J. W., 1995. Occurrence and origin of mono-, di-, and trimethylalkanes in modern and holocene cyanobacterial mats from Abu Dhabi, United Arab Emirates. *Geochimica et Cosmochimica Acta*, **59**, 2999–3015.
- Kim, J. H., Schouten, S., Hopmans, E. C., Donner, B., and Sinninghe Damsté, J. S., 2008. Global sediment core-top calibration of the TEX₈₆ paleothermometer in the ocean. *Geochimica et Cosmochimica Acta*, **72**, 1154.
- Klomp, U. C., 1986. The chemical structure of a pronounced series of iso-alkanes in South Oman Crudes. *Organic Geochemistry*, **10**, 807–814.
- Kohl, W., Gloe, A., and Reichenbach, H., 1983. Steroids from the myxobacterium *Nannocystis exedens*. *Journal of General Microbiology*, **129**, 1629–1635.
- Kohnen, M. E. L., Sinninghe Damsté, J. S., Baas, M., Kock-Van Dalen, A. C., and De Leeuw, J. W., 1993. Sulphur-bound steroid and phytane carbon skeletons in geomacromolecules: implications for the mechanism of incorporation of sulphur into organic matter. *Geochimica et Cosmochimica Acta*, **57**, 2515–2528.
- Koopmans, M. P., Schouten, S., Kohnen, M. E. L., and Sinninghe Damsté, J. S., 1996a. Restricted utility of aryl isoprenoids for photic zone anoxia. *Geochimica et Cosmochimica Acta*, **60**, 4873–4876.
- Koopmans, M. P., Köster, J., Van Kaam-Peters, H. M. E., Kenig, F., Schouten, S., Hartgers, W. A., De Leeuw, J. W., and Sinninghe Damsté, J. S., 1996b. Diagenetic and catagenetic products of isorenieratene: molecular indicators for photic zone anoxia. *Geochimica et Cosmochimica Acta*, **60**, 4467–4496.
- Koopmans, M. P., De Leeuw, J. W., Lewan, M. D., and Sinninghe Damsté, J. S., 1997. Impact of dia- and catagenesis on sulphur and oxygen sequestration of biomarkers as revealed by artificial maturation of an immature sedimentary rock. *Organic Geochemistry*, **25**, 391–426.
- Köster, J., Volkman, J. K., Rullkötter, J., Scholz-Böttcher, B. M., Rethmeier, J., and Fischer, U., 1999. Mono-, di- and trimethyl-branched alkanes in cultures of the filamentous cyanobacterium *Calothrix scopulorum*. *Organic Geochemistry*, **30**, 1367–1379.
- Krügel, H., Krubasik, P., Weber, K., Saluz, H. P., and Sandmann, G., 1999. Functional analysis of genes from *Streptomyces griseus* involved in the synthesis of isorenieratene, a carotenoid with aromatic end groups, revealed a novel type of carotenoid desaturase. *Biochimica et Biophysica Acta*, **1439**, 57–64.
- Kuypers, M. M. M., Blokker, P., Erbacher, J., Kinkel, H., Pancost, R. D., Schouten, S., and Sinninghe Damsté, J. S., 2001. Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. *Science*, **293**, 92–94.
- Kuypers, M. M. M., Blokker, P., Hopmans, E. C., Kinkel, H., Pancost, R. D., Schouten, S., and Sinninghe Damsté, J. S., 2002. Archaeal remains dominate marine organic matter from the early Albian oceanic anoxic event 1b. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **185**, 211.
- Kvenvolden, K. A., and Hodgson, G. W., 1969. Evidence for porphyrins in early Precambrian Swaziland system sediments. *Geochimica et Cosmochimica Acta*, **33**, 1195–1202.
- Larter, S. R., and Douglas, A. G., 1980. Melanoidins-kerogen precursors geochemical lipid sinks: a study using pyrolysis gas chromatography (PGC). *Geochimica et Cosmochimica Acta*, **44**, 2087–2095.
- Liaen-Jensen, S., 1979. Carotenoids - a chemosystematic approach. *Pure and Applied Chemistry*, **51**, 661–675.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–721.
- Massé, G., Belt, S. T., Rowland, S. J., and Rohmer, M., 2004. Isoprenoid biosynthesis in the diatoms *Rhizosolenia setigera* (Brightwell) and *Haslea ostrearia* (Simonsen). *Proceedings of the National Academy of Sciences*, **101**, 4413–4418.
- Maxwell, J. R., Douglas, A. G., Eglinton, G., and McCormick, A., 1968. The botryococcenes - hydrocarbons of novel structure from the Alga *Botryococcus braunii*, Kützinger. *Phytochemistry*, **7**, 2157.
- Mayer, F. L., Stalling, D. L., and Johnson, J. L., 1972. Phthalate esters as environmental contaminants. *Nature*, **238**, 411.
- McCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Paleoenvironmental implications of novel C₃₀ steranes in Precambrian to Cenozoic Age petroleum and bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Meinschein, W. G., Barghoorn, E. S., and Schopf, J. W., 1964. Biological remnants in a Precambrian sediment. *Science*, **145**, 262–263.
- Mendeleev, D. I., 1878. *Revue Scientifique*, **13**, 409.

- Metzger, P., and Casadevall, E., 1987. Lycopadiene, a tetraterpenoid hydrocarbon from new strains of the green alga *Botryococcus braunii*. *Tetrahedron Letters*, **28**, 3931.
- Metzger, P., and Largeau, C., 1999. Chemicals of *Botryococcus braunii*. In Cohen, Z. (ed.), *Chemicals from Microalgae*. London: Taylor & Francis, pp. 205–260.
- Metzger, P., Casadevall, E., Pouet, M. J., and Pouet, Y., 1985. Structures of some *Botryococcus* branched hydrocarbons from the B-race of the green alga *Botryococcus braunii*. *Phytochemistry*, **24**, 2995–3002.
- Metzger, P., Templier, J., Largeau, C., and Casadevall, E., 1986. An *n*-alkatriene and some *n*-alkadienes from the A race of the green alga *Botryococcus braunii*. *Phytochemistry*, **25**, 1869.
- Metzger, P., Largeau, C., and Casadevall, E., 1991. Lipids and macromolecular lipids of the hydrocarbon-rich microalga *Botryococcus braunii*. chemical structure and biosynthesis. geochemical and biotechnological importance. In Herz, W., Kirby, G. W., Steglich, W., Tamm, C. (eds.), *Progress in the Chemistry of Organic Natural Products* Vol. 57. Berlin: Springer, pp. 1–70.
- Moldowan, J. M., and Talyzina, N. M., 1998. Biogeochemical evidence for dinoflagellate ancestors in the early Cambrian. *Science*, **281**, 1168–1170.
- Moldowan, J. M., Fago, F. J., Lee, C. Y., Jacobson, S. R., Watt, D. S., Slougui, N. E., Jeganathan, A., and Young, D. C., 1990. Sedimentary 24-*n*-propylcholestanes, molecular fossils diagnostic of marine algae. *Science*, **247**, 309–312.
- Moldowan, J. M., Dahl, J. E. P., Huizinga, B. J., Fago, F. J., Hickey, L. J., Peakman, T. M., and Taylor, D. W., 1994. The molecular fossil record of oleanane and its relation to angiosperms. *Science*, **265**, 768–771.
- Moldowan, J. M., Dahl, J. E. P., Jacobson, S. R., Huizinga, B. J., Fago, F. J., Shetty, R., Watt, D. S., and Peters, K. E., 1996. Chemostratigraphic reconstruction of biofacies: molecular evidence linking cyst-forming dinoflagellates with pre-Triassic ancestors. *Geology*, **24**, 159–162.
- Nichols, P. D., Volkman, J. K., Palmisano, A. C., Smith, G. A., and White, D. C., 1988. Occurrence of an isoprenoid C₂₅ diunsaturated alkene and high neutral lipid content in Antarctic sea-ice diatom communities. *Journal of Phycology*, **24**, 90–96.
- Nip, M., Tegelaar, E. W., Brinkhuis, H., De Leeuw, J. W., Schenck, P. A., and Holloway, P. J., 1986. Analysis of modern and fossil plant cuticles by curie-point Py-GC and curiepoint Py-GC-MS: recognition of a new highly aliphatic and resistant biopolymer. *Organic Geochemistry*, **10**, 769–778.
- Noble, R. A., Alexander, R., Kagi, R. I., and Knox, J., 1985. Tetracyclic diterpenoid hydrocarbons in some Australian coals, sediments and crude oils. *Geochimica et Cosmochimica Acta*, **49**, 2141–2147.
- Oren, A., 2002. Diversity of halophilic microorganisms: environments, phylogeny, physiology, and applications. *Journal of Industrial Microbiology and Biotechnology*, **28**, 56.
- Oró, J., and Nooner, D. W., 1967. Aliphatic hydrocarbons in Precambrian rocks. *Nature*, **213**, 1082–1085.
- Ourisson, G., and Albrecht, P., 1992. Hopanoids 1. Geohopanoids: the most abundant natural products on Earth? *Accounts of Chemical Research*, **25**, 398–402.
- Ourisson, G., Rohmer, M., and Poralla, K., 1987. Prokaryotic hopanoids and other polyterpenoid sterol surrogates. *Annual Review of Microbiology*, **41**, 301–333.
- Pancost, R. D., Freeman, K. H., Patzkowsky, E., Wavrek, D. A., and Collister, J. W., 1998. Molecular indicators of redox and marine photoautotroph composition in the late Middle Ordovician of Iowa, U.S.A. *Organic Geochemistry*, **29**, 1649–1662.
- Pancost, R. D., Crawford, N., and Maxwell, J. R., 2002. Molecular evidence for basin-scale photic zone euxinia in the Permian Zechstein Sea. *Chemical Geology*, **188**, 217–227.
- Pearson, A., Budin, M., and Brocks, J. J., 2003. Phylogenetic and biochemical evidence for sterol synthesis in the bacterium *Gemmata obscuriglobus*. *Proceedings of the National Academy of Sciences*, **100**, 15352–15357.
- Pearson, A., Flood Page, S. R., Jorgenson, T. L., Fischer, W. W., and Higgins, M. B., 2007. Novel hopanoid cyclases from the environment. *Environmental Microbiology*, **9**, 2175–2188.
- Pepper, A. S., and Dodd, T. A., 1995. Simple kinetic models of petroleum formation. Part II: oil-gas cracking. *Marine and Petroleum Geology*, **12**, 321–340.
- Peters, K. E., and Moldowan, J. M., 1993. *The Biomarker Guide*. Englewood Cliffs: Prentice Hall.
- Peters, K. E., Walters, C. C., and Moldowan, J. M., 2004. *The Biomarker Guide*. Englewood Cliffs: New Jersey.
- Philippi, G. T., 1965. On the depth, time and mechanism of petroleum generation. *Geochimica et Cosmochimica Acta*, **29**, 1021–1049.
- Putschew, A., Schaeffer, P., Schaeffer-Reiss, C., and Maxwell, J. R., 1998. Carbon isotope characteristic of the diaromatic carotenoid, isorenieratene (intact and sulfide bound) and a novel isomer in sediments. *Organic Geochemistry*, **28**, 1849–1856.
- Ram, R. J., Verberkmoes, N. C., Thelen, M. P., Tyson, G. W., Baker, B. J., Blake, R. C. II, Shah, M., Hettich, R. L., and Banfield, J. F., 2005. Community proteomics of a natural microbial biofilm. *Science*, **308**, 1915–1920.
- Rashby, S. E., Sessions, A. L., Summons, R. E., and Newman, D. K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of the National Academy of Sciences*, **104**, 15099–15104.
- Requejo, A. G., Creaney, S., Allan, J., Gray, N. R., and Cole, K. S., 1992. Aryl isoprenoids and diaromatic carotenoids in paleozoic source rocks and oils from the Western Canada and Williston Basins. *Organic Geochemistry*, **19**, 245–264.
- Robinson, N., Eglinton, G., and Brassell, S. C., 1984. Dinoflagellate origin for sedimentary 4 α -methylsteroids and 5 α (H)-stanols. *Nature*, **308**, 439–442.
- Rohmer, M., Bouvier-Navé, P., and Ourisson, G., 1984. Distribution of hopanoid triterpenes in prokaryotes. *Journal of General Microbiology*, **130**, 1137–1150.
- Rowland, S. J., Yon, D. A., Lewis, C. A., and Maxwell, J. R., 1985. Occurrence of 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane and related hydrocarbons in the green alga *Enteromorpha prolifera* and sediments. *Organic Geochemistry*, **8**, 207.
- Schaeffer, P., Reiss, C., and Albrecht, P., 1995. Geochemical study of macromolecular organic matter from sulfur-rich sediments of evaporitic origin (Messinian of Sicily) by chemical degradations. *Organic Geochemistry*, **23**, 567–581.
- Schaeffer, P., Adam, P., Wehrung, P., and Albrecht, P., 1997. Novel aromatic carotenoid derivatives from sulfur photosynthetic bacteria in sediments. *Tetrahedron Letters*, **38**, 8413–8416.
- Schouten, S., Van Der Maarel, M. J., Huber, R., and Sinninghe Damsté, J. S., 1997. 2,6,10,15,19-pentamethylcosenes in *Methanobrevibacter smithii*, a marine methanogenic archaeon, and in *Methanosarcina mazei*. *Organic Geochemistry*, **26**, 409–414.
- Schouten, S., Hopmans, E. C., Schefuss, E., and Sinninghe Damsté, J. S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea water temperatures? *Earth and Planetary Science Letters*, **204**, 265–274.
- Schouten, S., Hopmans, E. C., Forster, A., Van Breugel, Y., Kuenen, J. G., and Sinninghe Damsté, J. S., 2003. Extremely high sea-surface temperatures at low latitudes during the Middle Cretaceous as revealed by archaeal membrane lipids. *Geology*, **31**, 1069–1072.
- Schouten, S., Forster, A., Panoto, F. E., and Sinninghe Damsté, J. S., 2007. Towards calibration of the TEX₈₆ palaeothermometer for

- tropical sea surface temperatures in ancient greenhouse worlds. *Organic Geochemistry*, **38**, 1537.
- Shiea, J., Brassell, S. C., and Ward, D. M., 1990. Mid-chain branched mono- and dimethyl alkanes in hot spring cyanobacterial mats: a direct biogenic source for branched alkanes in ancient sediments? *Organic Geochemistry*, **15**, 223–231.
- Simons, D. J. H., and Kenig, F., 2001. Molecular fossil constraints on the water column structure of the Cenomanian-Turonian Western Interior Seaway, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **169**, 129–152.
- Sinninghe Damsté, J. S., and De Leeuw, J. W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. *Organic Geochemistry*, **16**, 1077–1101.
- Sinninghe Damsté, J. S., Kenig, F., Koopmans, M. P., Köster, J., Schouten, S., Hayes, J. M., and De Leeuw, J. W., 1995. Evidence for gammacerane as an indicator of water column stratification. *Geochimica et Cosmochimica Acta*, **59**, 1895–1900.
- Sinninghe Damsté, J. S., Schouten, S., and Van Duin, A. C. T., 2001. Isorenieratene derivatives in sediments: possible controls on their distribution. *Geochimica et Cosmochimica Acta*, **65**, 1557–1571.
- Sinninghe Damsté, J. S., Rijpstra, W. I. C., Hopmans, E. C., Prahl, F. G., Wakeham, S. G., and Schouten, S., 2002. Distribution of membrane lipids of planktonic crenarchaeota in the Arabian Sea. *Applied and Environmental Microbiology*, **68**, 2997–3002.
- Sinninghe Damsté, J. S., Rijpstra, W. I. C., Schouten, S., Fuerst, J. A., Jetten, M. S. M., and Strous, M., 2004a. The occurrence of hopanoids in planctomycetes: implications for the sedimentary biomarker record. *Organic Geochemistry*, **35**, 561–566.
- Sinninghe Damsté, J. S., Muyzer, G., Abbas, B., Rampen, S. W., Massé, G., Allard, W. G., Belt, S. T., Robert, J. M., Rowland, S. J., Moldowan, J. M., Barbanti, S. M., Fago, F. J., Denisevich, P., Dahl, J., Trindade, L. A. F., and Schouten, S., 2004b. The rise of the rhizosolenid diatoms. *Science*, **304**, 584–587.
- Sinninghe Damsté, J. S., Baas, M., Geenevasen, J. A. J., and Kenig, F., 2005. Structural identification of sedimentary C₂₁ and C₂₂ highly branched isoprenoid alkanes. *Organic Geochemistry*, **36**, 511–517.
- Sluijs, A., Schouten, S., Pagani, M., Woltering, M., Brinkhuis, H., Sinninghe Damsté, J. S., Dickens, G. R., Huber, M., Reichert, G. J., Stein, R., Matthiessen, J., Lourens, L. J., Pedentchouk, N., Backman, J., Moran, K., and The Expedition Scientists, 2006. Subtropical Arctic Ocean temperatures during the Palaeocene/Eocene thermal maximum. *Nature*, **441**, 610.
- Sluijs, A., Brinkhuis, H., Schouten, S., Bohaty, S. M., John, C. M., Zachos, J. C., Reichert, G. J., Sinninghe Damsté, J. S., Crouch, E. M., and Dickens, G. R., 2007. Environmental precursors to rapid light carbon injection at the Palaeocene/Eocene boundary. *Nature*, **450**, 1218.
- Summons, R. E., and Capon, R. J., 1988. Fossil steranes with unprecedented methylation in ring-A. *Geochimica et Cosmochimica Acta*, **52**, 2733–2736.
- Summons, R. E., and Jahnke, L. L., 1992. Hopanes and hopanes methylated in ring-A: correlation of the hopanoids from extant methylotrophic bacteria with their fossil analogues. In Moldowan, J. M., Albrecht, P., and Philip, R. P. (eds.), *Biological Markers in Sediments and Petroleum*. Englewood Cliffs, NJ: Prentice Hall, pp. 182–200.
- Summons, R. E., and Powell, T. G., 1986. Chlorobiaceae in paleozoic seas revealed by biological markers, isotopes and geology. *Nature*, **319**, 763–765.
- Summons, R. E., and Powell, T. G., 1987. Identification of aryl isoprenoids in source rocks and crude oils: biological markers for the green sulphur bacteria. *Geochimica et Cosmochimica Acta*, **51**, 557–566.
- Summons, R. E., and Powell, T. G., 1992. hydrocarbon composition of the late Proterozoic oils of the Siberian Platform: implications for the depositional environment of source rocks. In Schidlowski, M., Golubic, S., Kimberley, M. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Mineral and Energy Resources*. Berlin: Springer, pp. 296–307.
- Summons, R. E., and Walter, M. R., 1990. Molecular fossils and microfossils of prokaryotes and protists from proterozoic sediments. *American Journal of Science*, **290-A**, 212–244.
- Summons, R. E., Powell, T. G., and Boreham, C. J., 1988. Petroleum geology and geochemistry of the middle Proterozoic McArthur Basin, northern Australia: III. Composition of extractable hydrocarbons. *Geochimica et Cosmochimica Acta*, **52**, 1747–1763.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Summons, R. E., Metzger, P., Largeau, C., Murray, A. P., and Hope, J. M., 2002. Polymethylsqualanes from *Botryococcus braunii* in lacustrine sediments and oils. *Organic Geochemistry*, **33**, 99–109.
- Summons, R. E., Bradley, A. S., Jahnke, L. L., and Waldbauer, J. R., 2006. Steroids, triterpenoids and molecular oxygen. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **361**, 951.
- Summons, R. E., Thomas, J., Maxwell, J. R., and Boreham, C. J., 1992. Secular and environmental constraints on the occurrence of dinosterane in sediments. *Geochim. Cosmochim. Acta*, **56**, 2437–2444.
- Summons, R. E., Volkman, J. K., and Boreham, C. J., 1987. Dinosterane and other steroidal hydrocarbons of dinoflagellate origin in sediments and petroleum. *Geochim. Cosmochim. Acta*, **51**, 3075–3082.
- Talyzina, N. M., Moldowan, J. M., Johannisson, A., and Fago, F. J., 2000. Affinities of Early Cambrian acritarchs studied by using microscopy, fluorescence flow cytometry and biomarkers. *Review of Palaeobotany and Palynology*, **108**, 37–53.
- Tegelaar, E. W., De Leeuw, J. W., Derenne, S., and Largeau, C., 1989. A reappraisal of kerogen formation. *Geochimica et Cosmochimica Acta*, **53**, 3103–3106.
- Tegelaar, E. W., Hollman, G., Van Der Vegt, P., De Leeuw, J. W., and Holloway, P. J., 1995. Chemical characterization of the periderm tissue of some angiosperm species: recognition of an insoluble, non-hydrolyzable, aliphatic biomacromolecule (Suberan). *Organic Geochemistry*, **23**, 239–251.
- Ten Haven, H. L., Rohmer, M., Rullkötter, J., and Bissleret, P., 1989. Tetrahymanol, the most likely precursor of gammacerane, occurs ubiquitously in marine sediments. *Geochimica et Cosmochimica Acta*, **53**, 3073–3079.
- Thiel, V., Peckmann, J., Seifert, R., Wehrung, P., Reitner, J., and Michaelis, W., 1999. Highly isotopically depleted isoprenoids: molecular markers for ancient methane venting. *Geochimica et Cosmochimica Acta*, **63**, 3959–3966.
- Tissot, B. P., and Welte, D. H., 1984. *Petroleum Formation and Occurrence*. Berlin: Springer.
- Treibs, A., 1936. Chlorophyll and hemin derivatives in organic mineral substances. *Angewandte Chemie*, **49**, 682–686.
- Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., and Banfield, J. F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, **428**, 37–43.
- Tyson, R. V., 1995. *Sedimentary Organic Matter. Organic Facies and Palynofacies*, New York: Chapman and Hall, pp. 309–315.
- Van Aarssen, B. G. K., Cox, H. C., Hoogendoorn, P., and De Leeuw, J. W., 1990. A cadinene biopolymer in fossil and extant dammar

- resins as a source for cadinanes and bicadinanes in crude oils from South East Asia. *Geochimica et Cosmochimica Acta*, **54**, 3021–3031.
- Van Aarssen, B. G. K., Hessels, J. K. C., Abbink, O. A., and De Leeuw, J. W., 1992. The occurrence of polycyclic sesqui-, tri-, and oligoterpenoids derived from a resinous polymeric cadinene in crude oils from Southeast Asia. *Geochimica et Cosmochimica Acta*, **56**, 1231–1246.
- Vernadsky, V. I., 1934. Outlines of geochemistry ONTI. *Gornogeolog. Neft. Izd.*
- Versteegh, G. J. M., Blokker, P., Wood, G. D., Collinson, M. E., Sinninghe Damsté, J. S., and De Leeuw, J. W., 2004. An example of oxidative polymerization of unsaturated fatty acids as a preservation pathway for dinoflagellate organic matter. *Organic Geochemistry*, **35**, 1129–1139.
- Villar, H. J., Püttmann, W., and Wolf, M., 1988. Organic geochemistry and petrography of tertiary coals and carbonaceous shales from Argentina. *Organic Geochemistry*, **13**, 1011–1021.
- Vink, A., Schouten, S., Sephton, S., and Sinninghe Damsté, J. S., 1998. A newly discovered norisoprenoid, 2,6,15,19-tetramethylcosane, in Cretaceous black shales. *Geochimica et Cosmochimica Acta*, **62**, 965–970.
- Volkman, J. K., 1986. A review of sterol markers for marine and terrigenous organic matter. *Organic Geochemistry*, **9**, 83–99.
- Volkman, J. K., 2003. Sterols in microorganisms. *Applied Microbiology and Biotechnology*, **60**, 496–506.
- Volkman, J. K., Barrett, S. M., Dunstan, G. A., and Jeffrey, S. W., 1993. Geochemical significance of the occurrence of dinosterol and other 4-methyl sterols in a marine diatom. *Organic Geochemistry*, **20**, 7–15.
- Volkman, J. K., Barrett, S. M., and Dunstan, G. A., 1994. C₂₅ and C₃₀ highly branched isoprenoid alkanes in laboratory cultures of two marine diatoms. *Organic Geochemistry*, **21**, 407–413.
- Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., and Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. *Organic Geochemistry*, **29**, 1163–1179.
- Wakeham, S. G., Sinninghe Damsté, J. S., Kohnen, M. E. L., and De Leeuw, J. W., 1995. Organic sulfur compounds formed during early diagenesis in Black Sea sediments. *Geochimica et Cosmochimica Acta*, **59**, 521–533.
- Wang, T. G., and Simoneit, B. R. T., 1990. Organic geochemistry and coal petrology of tertiary brown coal in the Zhoujing Mine, Baise Basin, South China. 2. biomarker assemblage and significance. *Fuel*, **69**, 12–20.
- Ward, A. C., and Bora, N., 2006. Diversity and biogeography of marine actinobacteria. *Current Opinion in Microbiology*, **9**, 279–286.
- Wen, Z., Ruiyong, W., Radke, M., Qingyu, W., Guoying, S., and Zhili, L., 2000. Retene in pyrolysates of algal and bacterial organic matter. *Organic Geochemistry*, **31**, 757–762.
- Zumberge, J. E., 1987. Terpenoid biomarker distributions in low maturity crude oils. *Organic Geochemistry*, **11**, 479.
- Zundel, M., and Rohmer, M., 1985a. Prokaryotic triterpenoids 1. 3-methylhopanoids from *Acetobacter* sp. and *Methylococcus capsulatus*. *European Journal of Biochemistry*, **150**, 23–27.
- Zundel, M., and Rohmer, M., 1985b. Prokaryotic triterpenoids 3. the biosynthesis of 2 β -methylhopanoids and 3 β -methylhopanoids of *Methylobacterium organophilum* and *Acetobacter pasteurianus* spp. *pasteurianus*. *European Journal of Biochemistry*, **150**, 35–39.
- Zundel, M., and Rohmer, M., 1985c. Hopanoids of the methylotrophic bacteria *Methylococcus capsulatus* and *Methylomonas* sp. as possible precursors for the C₂₉ and C₃₀ hopanoid chemical fossils. *FEMS Microbiology Letters*, **28**, 61–64.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Astrobiology
 Bacteria
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Cyanobacteria
 Diatoms
 Isotopes and Geobiology
 Methane, Origin
 Nitrogen
 Origins of the Metazoa
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOMARKERS (ORGANIC, COMPOUND-SPECIFIC ISOTOPES)

Kliti Grice¹, Jochen J. Brocks²

¹Curtin University of Technology, Perth, WA, Australia

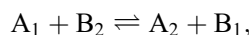
²The Australia National University, Canberra, ACT, Australia

Synonyms

Stable isotopes of individual biomarkers

Definition

Stable isotopes as opposed to radiogenic (unstable) isotopes do not decay; thus, their natural abundances stay relatively constant, even over geological time. However, variations in stable isotopic composition, or isotopic fractionation, occur in nature as a consequence of chemical and physical processes, due to different isotopes of an element having different physical and chemical properties. Equilibrium processes and kinetic processes can lead to isotopic fractionation. Equilibrium isotope effects occur as a result of temperature-dependent equilibrium isotope-exchange reactions resulting in a change of the isotope distribution between different chemical materials, between varying phases, or between individual molecules (Hoefs, 1987). For example, the equilibrium reaction:

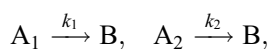


Subscripts indicate that A and B contain either the light (1) or heavy (2) isotope.

In these cases, there is no net variation in the chemical process, i.e., the products are chemically similar to the reactants, with the exception that the stable isotopes are distributed differently between them. An example is the evaporation/condensation cycle of water as shown in the following equation (D stands for “deuterium,” the heavy hydrogen isotope, ²H)

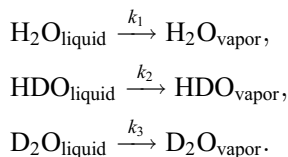


This physical process results in a change in the distribution of hydrogen and oxygen isotopes in both liquid and vapor phases. Kinetic fractionation occurs when water vapor is removed before condensation occurs. Kinetic isotope effects occur when an irreversible chemical reaction takes place resulting in a change in the chemical process. As a simple example, a unidirectional chemical reaction always shows a depletion of the heavy isotope in the reaction products relative to the reactants. This occurs due to the slower reaction rate of the heavier isotopic species. For example, in a reaction of reactant A to product B, there may be two (or more) competing unidirectional isotopic reactions:



where k is the rate constant.

In this theoretical example, the rate-determining step involves the cleavage of a bond. It is generally easier to cleave the bonds of molecules that contain the lighter isotopes because the vibrational frequency of such bonds tends to be elevated. Thus, in this case, $k_1 > k_2$, and therefore more of the lighter isotope will be incorporated into the product. A similar, although physical, process is the evaporation of water where the vapor is removed immediately. This process involves the following three competing unidirectional isotopic reactions:



In this case, $k_1 > k_2 > k_3$ because the lighter isotopic species of water evaporate preferentially. Thus, the net process results in water vapor that is enriched in protium (^1H).

Standards and notation

Stable isotopic compositions are expressed as a delta (δ) value (e.g., $\delta^{13}\text{C}$ for carbon, δD for hydrogen) in units of per mil (‰) or parts per thousand (Equation 1). Stable carbon and hydrogen isotopic compositions are determined not as absolute isotopic abundances, but as ratios of a heavy isotope (e.g., ^{13}C , D) to a light isotope (e.g., ^{12}C , H) relative to an international standard.

$$\delta^{13}\text{C} = [(R_{\text{sample}})/(R_{\text{standard}}) - 1] \times 1000\text{‰}, \quad (1) \\ R = ^{13}\text{C}/^{12}\text{C} \text{ or } \text{D}/\text{H}.$$

For example, stable carbon isotopic compositions are determined as a ratio of ^{13}C to a ^{12}C relative to the international Vienna Pee Dee Belemnite (VPDB) calibrated to the

original marine limestone from the Pee Dee formation in South Carolina (USA). This original PDB standard was exhausted and has been replaced by calibrating another carbonate standard (NBS-19) relative to PDB (Urey et al., 1951; Craig, 1957). The new calibration scale is called “VPDB” (Vienna PDB). For stable hydrogen isotopic composition, the ratio is deuterium ^2H , or D, to protium relative to Standard Mean Ocean Water (SMOW). SMOW is thought to represent an average isotopic composition of the world’s oceans based on data collected over a decade (Werner and Brand, 2001). However, the standard is no longer available as a reference material, and therefore was defined relative to an additional water standard (NBS-1, also exhausted) which differs by 47.6‰ from the present scale (Werner and Brand, 2001). Thus a new reference material was developed with an isotopic composition equivalent to that of SMOW, which led to the current “VSMOW” (Vienna SMOW) scale. An alternative primary international standard for δD determinations is Standard Light Antarctic Precipitation (SLAP, δD of -428‰).

Compound-specific isotopes

The stable isotopic composition of organic matter is determined by its genetic origin. The elements hydrogen and carbon are the main constituents of organic matter and play key roles in many biochemical, ecological, environmental, hydrologic, and atmospheric processes. Therefore, stable isotopic compositions preserved in organic matter can provide powerful insights into these processes. Early isotopic investigations were chiefly based on bulk carbon isotope analysis. Continuous flow isotope ratio monitoring gas-chromatography mass spectrometry (irm-GCMS) also commonly referred to as compound-specific isotope analysis (CSIA) is important for determining the stable isotopic compositions of individual organic components in complex mixtures (e.g., petroleum, natural gases, sediments, soils, groundwater, potable waters, and extracts from plants and other media). As with gas-chromatography-mass spectrometry (GC-MS), compounds are separated according to mass differences. In an isotope ratio mass spectrometer, different isotopes of the same compound are separated based on relative mass differences. A gas analyte stream is prepared and it is then ionized by an ion source. The electron multiplier detects the ions of different isotope masses over a range of mass/charge ratios. This technique has been applied for several decades to the analysis of biomarkers of lipids (also see Chapter *Biomarkers (Molecular Fossils)*) and other natural products such as chlorophylls/bacteriochlorophylls found in intricate mixtures of petroleum, and in bitumens and kerogens extracted from sediments and sedimentary rocks. John Hayes pioneered the development of CSIA in the field of organic geochemistry based on his practice and principles of isotopic measurement and calibration, inter- and intramolecular isotopic distributions and

a fundamental understanding of carbon and hydrogen isotopic fractionation within biosynthetic pathways (Matthews and Hayes, 1978; Hayes, 1983, 1993, 2001; Summons et al., 2008). The first carbon isotopic measurements of individual fossil hydrocarbons was performed by Summons and Powell in 1986, and within the fields of organic geochemistry and biogeochemistry such measurements have become important for palaeoclimate reconstruction and are also used to elucidate aspects of biogeochemical cycling within a broad range of microbially mediated processes (Freeman et al., 1990; Summons et al., 1994; Summons et al., 1996).

Instrumentation

The irm-GCMS, whereby the gas chromatograph (GC) is linked to an isotope ratio mass spectrometer via a combustion interface for determining $^{13}\text{C}/^{12}\text{C}$ of individual organic components, in complex mixtures of petroleum and organic extracts from sedimentary material (Matthews and Hayes, 1978). The combustion interface either consists of a quartz tube containing CuO pellets (850°C) or a ceramic tube containing twisted CuO/Pt wires (850°C) (Matthews and Hayes, 1978; Hayes, 1983) yielding a gas analyte of CO_2 and by-product H_2O for each GC-separated component. H_2O is removed either with a Nafion trap or a liquid N_2 trap at -100°C . The isotope ratio mass spectrometer measures the abundances of the ions m/z 44 ($^{12}\text{CO}_2$), 45 ($^{13}\text{CO}_2$) and 46 ($^{12}\text{C}^{18}\text{O}^{16}\text{O}_2$). D/H CSIA requires that a GC is linked to an isotope ratio mass spectrometer via a pyrolysis furnace – either ceramic with a glassy carbon coating ($1,400^{\circ}\text{C}$) or quartz packed with sieved chromium pellets ($1,050^{\circ}\text{C}$) yielding a gas analyte of H_2 for each GC-separated component (e.g., Prosser and Scrimgeour, 1995; Burgøyne and Hayes, 1998). For this application the mass spectrometer measures the abundances of the ions m/z 3 (^2DH) and 2 ($^1\text{H}_2$). Contributions from H_3^+ produced in the ion source are corrected following m/z 3 analyses at two different pressures of the H_2 reference gas to determine the H_3^+ correction factor. Developments also include irm-GCMS instruments capable of measuring $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ (Merrit, 1993; Brand et al., 1994; Kashiyaama et al., 2007).

In brief, biomarkers are the molecular fossils of biolipids and other natural products and are diagnostic for certain organisms such as algae, higher plants (e.g., Brassell et al., 1986), methanotrophic bacteria (Summons et al., 1994), and a wide range of other heterotrophic organisms (Grice et al., 1998a and references therein). Many biomarkers can be detected by GC-MS, and stable isotopic values are now routinely measured with the advent of CSIA. The identity, isomeric arrangement and stable isotopic composition of biomarkers have been used in studies of petroleum and sedimentary organic matter to assess biological sources, and paleoenvironmental and depositional information.

In the petroleum industry, CSIA has been used for source rock–oil, oil–gas, and gas–gas correlation studies (e.g., Fusetti et al., 2010a, b).

Separation techniques

To obtain accurate and precise isotope ratios, components must be amenable to analysis using GC. However, biomarkers may be chemically bound to kerogen, or they may be highly functionalized, making them difficult to resolve by GC, thus presenting significant analytical challenges for CSIA. With the advent of hydropyrolysis (HyPy) (e.g., Love et al., 2005 and references therein) and micro-scale-sealed-vessel pyrolysis (MSSV) (Horsfield and Dueppenbecker, 1991; Beramendi-Orosco et al., 2006; Greenwood et al., 2006; Meredith et al., 2006) we can now release biomarkers from kerogen under mild conditions and convert highly functionalized lipids into hydrocarbons that are more amenable to GC-MS and CSIA.

Sample preferences for CSIA include: (1) low-molecular weight compounds, (2) at least 60 ng component concentration, and (3) complete chromatographic separation (i.e., to baseline) of component peaks of interest. CSIA has much more critical requirements for the GC resolution of products than standard molecular characterization by GC-MS. Compounds containing a higher propensity of ^{13}C atoms elute slightly earlier than their isotopically lighter counterparts. Thus, incomplete chromatographic resolution results in an overlap between $^{12}\text{CO}_2$ and $^{13}\text{CO}_2$ analyte signals from the co-eluting compounds, making it impossible to accurately determine their individual isotopic values. To avoid co-elution, separation techniques such as molecular sieving isolate fractions based on molecular size (e.g., 5A, ZSM-5, 13X, Armanios et al., 1993; Ellis et al., 1994; West et al., 1990; Grice et al., 2001; Audino et al., 2004; Tolosa and Ogrinc, 2007; Grice et al., 2008a). Other separation techniques include preparative thin layer chromatography (TLC), liquid chromatography (LC), gel permeation chromatography (Inaba and Suzuki, 2003), and High Performance LC-MS (HPLC-MS) (e.g., Smittenberg and Sachs, 2007). However, in petroleum, there are many minor components that are not routinely analyzed by CSIA due to lack of techniques to isolate suitable fractions. Even after fractionation of petroleum using state-of-the-art separation techniques, the resulting mixtures can still be extraordinarily complex and may not be amenable to CSIA. It is also important to establish that separation procedures do not cause isotopic fractionation.

For polar components, it is usually necessary to derivatize and improve chromatographic separation using derivatization agents such as BSTFA (N-O-*bis*(trimethylsilyl) trifluoroacetamide) (Jones et al., 1991) or MTBSTFA (N-(*tert*-butyldimethylsilyl)-N-methyltrifluoroacetamide) (e.g., Grice et al., 1996a). For CSIA, choice of the derivatization reagent is crucial to ensure accurate $\delta^{13}\text{C}$ analysis

(see Rieley, 1994 for a review). Imprecision is introduced into the δ value of the compound with derivatization groups of unknown $^{13}\text{C}/^{12}\text{C}$ contribution. A derivatization reagent which only introduces a small number of carbon atoms is desirable for minimizing the imprecision (Rieley, 1994). The $\delta^{13}\text{C}$ value for the derivatization reagent can be determined by $\delta^{13}\text{C}$ analyses of standards before and after derivatization and applying the mass balance equation as described by Jones et al. (1991). Monosaccharides can be derivatized using methylboronic acid and BSTFA (van Dongen et al., 2001). This method has proven to be useful for $\delta^{13}\text{C}$ analyses of arabinose, xylose, fucose, fructose, and glucose.

Isotopic interpretation of biomarkers

The $\delta^{13}\text{C}$ and δD of individual biomolecules in sediments and organisms depends on the complex isotopic fractionation associated with the biosynthetic network that is responsible for their formation (e.g., Hayes, 1993; Collister et al., 1994; Hayes, 2001; Monson and Hayes, 1982; Melzer and Schmidt, 1987; Schwender et al., 1996; Sessions et al., 1999; Chikaraishi et al., 2004a, b; Liu and Huang, 2005; Chikaraishi and Naraoka, 2006; Rommerskirchen et al., 2006). Lipids produced by different biosynthetic pathways can differ in $\delta^{13}\text{C}$ by up to 20‰ within an individual organism (e.g., Summons et al., 1994; Schouten et al., 1998; and van der Meer et al., 1998). However, the exact mechanisms leading to these isotopic differences are not well-understood (Hayes, 2001). Other factors that impose carbon isotopic variation in living organisms include the partial pressure of CO_2 ($p\text{CO}_2$), cell size and geometry (Goericke et al., 1994; Popp et al., 1998), the growth rate of phytoplanktonic cells (Laws et al., 1995; Bidigare et al., 1997), and in land plants, water-use efficiency as, e.g., observed in C_3 , C_4 , and CAM plants (Ehleringer et al., 1993).

When reconstructing environments of deposition based on CSIA $^{13}\text{C}/^{12}\text{C}$ of biomarkers it is important to determine an isotopic reference point against which values for various components can be compared. C_{27} steranes and the C_{20} regular isoprenoid phytane are especially useful components since they are commonly reported in lacustrine and marine settings and mostly deriving from C_{27} sterols and chlorophyll *a*, respectively. Hence, $\delta^{13}\text{C}$ of sedimentary steranes and phytane provide a reference point for lipid components predominantly biosynthesized by the algae in the upper water column. In sediment samples from the Permian Kupferschiefer, the $\delta^{13}\text{C}$ of phytane was found to be similar to co-occurring algal-derived cholestane, consistent with a common algal origin for both biomarkers. However, other sources of phytane cannot be excluded, such as chlorophyll *a* of cyanobacteria (Grice et al., 1996b), and C_{27} sterols are also a significant component in the cell membranes of most animals. The Permian Kupferschiefer also contains the biomarker methyl ethyl maleimide, an oxidative degradation product predominantly derived from the macrocycle of chlorophyll *a* (Grice et al., 1996b, 1997). The maleimide was

3.5–4.5‰ more enriched in ^{13}C than phytane from the phytol side chain, a magnitude of difference to be expected from a common chlorophyll origin (Grice et al., 1996b, 1997). When using CSIA $^{13}\text{C}/^{12}\text{C}$ of biomarkers in sedimentary organic matter to reconstruct paleoenvironments, it is understood that stable carbon isotopic fractionation effects associated with the biosynthesis of sterols and isoprenoids as well as cell sizes and geometry of the phytoplankton community, may be averaged out for sediment samples covering hundreds to millions of years. CSIA $^{13}\text{C}/^{12}\text{C}$ has also been applied to feeding studies. Zooplankton feeding on algae does not alter the stable carbon isotopic compositions of the algal lipids, which these organisms consume, retain, metabolize to cholesterol, and/or excrete (e.g., Grice et al., 1998a). Zooplanktons are selective feeders and the preferred algae may have distinct $\delta^{13}\text{C}$. $\delta^{13}\text{C}$ signals of sterols (sterols and steranes) in sediments are probably representative of a phytoplankton mixture, although individual communities of the mixture have different $\delta^{13}\text{C}$ compositions. This limitation should be borne in mind when using $\delta^{13}\text{C}$ of sterols and steranes as isotopic reference points. Furthermore, a co-occurrence of algal lipids can complicate the interpretation of $\delta^{13}\text{C}$ of phytoplankton biomarkers, since individual algal species are known to biosynthesize lipids with quite different carbon isotopic compositions (see Schouten et al., 1998). Thus, differences between phytane and cholestane in organic matter may not be due to different source organisms, since there may be distinct effects associated with the biosynthesis of each by a single organism. Schouten et al. (1998), however, still suggest cholestane is one of the most suitable isotopic reference points of the primary producers living in the euphotic zone of ancient water columns. Until recently, phytol and its diagenetic alteration products were thought to be useful indicators of phytoplanktonic isotopic reference points (e.g., Freeman et al., 1990; Hayes et al., 1990; Grice et al., 1998a). However, recent phytol isotopic data reported from a range of algae, land plants, and bacteria suggest that the disparities in carbon isotopic value of phytane and of a given degradation (e.g., the C_{19} isoprenoid pristane) may be due to isotopic heterogeneity within molecules (Schouten et al., 2008). However, the stable carbon isotopic composition will depend on whether pristane and phytane are exclusively derived from phytol or if there are additional sources (e.g., archaeal diether lipids, tocopherols, bacteriochlorophylls *a/b*) with potentially different stable carbon isotopic compositions (see below).

It is well-established that diagenetic and catagenetic processes over geological time (millions of years) promote significant hydrogen exchange between organic biomolecules in the surrounding environment with formation water (Rigby et al., 1981; Alexander et al., 1984; Schimmelmann et al., 1999; Leif and Simoneit, 2000; Schimmelmann et al., 2001; Sessions et al., 2004; Dawson et al., 2005). D/H studies of extant organisms have shown that biosynthetically produced

lipids are depleted in D relative to the total biomass (e.g., Estep and Hoering, 1980). Furthermore, polyisoprenoid lipids are depleted in D relative to *n*-alkyl lipids (Estep and Hoering, 1980; Sessions et al., 1999). The magnitude of the fractionation between growth water and *n*-alkyl lipids is ca. -150‰ , while the fractionation between water and isoprenoid lipids is ca. -235‰ (e.g., Smith and Epstein, 1970; e.g., Estep and Hoering, 1980; Sessions et al., 1999).

D/H of biomarkers can be strongly influenced by thermal maturation reflecting a strong relationship with the alteration of indigenous D/H signatures. Dawson et al. (2005) demonstrated the use of D/H ratios of sedimentary hydrocarbons (*n*-alkanes, pristane, and phytane) to evaluate the maturity of source-rocks and crude oils from the Perth Basin (WA). Pristane and phytane were significantly depleted in D compared to *n*-alkanes, although this difference reduces with increasing maturity due to thermally promoted isotopic exchange. The *n*-alkane–isoprenoid D/H signature of crude oils of different source facies was consistent with values associated with mature–late mature sediments, i.e., representative of the peak oil-generative window. The average D/H ratios for Pr and Ph correlate well with % equivalent vitrinite reflectance, which further supports a relationship between maturation and isotopic enrichment in isoprenoids. The work by Dawson et al. (2005, 2007) and Pedentchouk et al. (2006) suggests that the D/H measurement of sedimentary hydrocarbons may represent a useful maturity parameter which also accounts for different source-effects.

Green algae

Biomarkers derived from *Botryococcus braunii* (also see Chapter *Biomarkers (Molecular Fossils)*) have been shown to be significantly enriched in ^{13}C compared with other phytoplanktonic biomarkers in crude oils (Dowling et al., 1995) and sediments (Boreham et al., 1994; Huang et al., 1995; Grice et al., 1998b; Huang et al., 1999; Audino et al., 2001a; Summons et al., 2002). High $\delta^{13}\text{C}$ have also been demonstrated in *Botryococcus braunii* cultures (Summons et al., 1996), and although the reasons for this anomaly remain ambiguous, there are several potential explanations proposed, including (1) isotopic fractionation accompanying photosynthesis in *Botryococcus braunii* may not be fully expressed due to limiting internal $p\text{CO}_2$, (2) the thick outer walls of these microalgae may limit the diffusion rates for CO_2 , thereby enriching biomass in ^{13}C (Boreham et al., 1994), and (3) *Botryococcus braunii* utilize a ^{13}C -rich bicarbonate source (Huang et al., 1999 and references therein). A biomarker of *Botryococcus braunii* in a sediment dated to the last glacial maximum (Huang et al., 1999) was found to be significantly enriched in ^{13}C ($\delta^{13}\text{C} = -5\text{‰}$) which was attributed to low atmospheric $p\text{CO}_2$ leading to depletion of dissolved CO_2 and therefore causing *Botryococcus braunii* to assimilate isotopically heavier bicarbonate in its lake environment. In

some hypersaline sediments of Miocene/Pliocene age from the Mount Sdom Formation, Dead Sea, Israel (Grice et al., 1998b) a novel series of organosulfur compounds (OSC) derived from the functionalized lipids of *Botryococcus braunii* races B and L were recognized. The B and L race derived components were 13–20‰ and 5–7‰, respectively more enriched in ^{13}C than marine phytoplanktonic biomarkers pointing to differing seasonal blooms for each race. Other unique biomarkers from *Botryococcus braunii* include at least four homologous series of monomethylalkanes (MMAs) ranging from C_{23} to C_{31+} in the bitumens and kerogens of a number of torbanites (Audino et al., 2001a). Stable carbon isotopic data support a source derived directly from botryals of *Botryococcus braunii* race A. These compounds are also symptomatic of extreme heterotrophic reworking of ^{13}C -rich *Botryococcus braunii* algal biomass. Macrocyclic alkanes (Audino et al., 2001a, 2002a, b; Grice et al., 2001) have been previously identified in torbanites and Indonesian crude oils containing remains of the freshwater alga. Based on $\delta^{13}\text{C}$ data it has been suggested that these compounds are derived from the algaenan of *Botryococcus braunii* formed by an olefin metathesis reaction. The hydrogen isotopic ratios of lipids from *Botryococcus braunii*, including alkadienes, botryococenes, heptadecenes, fatty acids, and phytadiene, closely follow the δD of the assimilated water (Zhang et al., 2007). A δD saw-toothed profile for odd and even *n*-alkanes in the torbanites is attributed to a dual-source system, a predominant *B. braunii* input with a second minor contribution from land plants (Dawson et al., 2004). δD of *n*-alkanes also reflected the depositional palaeolatitude/palaeoclimate of the sediments, attributed to the δD composition of meteoric waters in the environment where the sediments were deposited. For example, Torbanites deposited in a mid-latitude region under cool-temperate conditions contain *n*-alkanes with δD values falling in between those of *n*-alkanes from tropical (isotopically heavier) and glacial (isotopically lighter) environments (Dawson et al., 2004).

Photosynthetic sulfur bacteria

Green sulfur bacteria (Chlorobiaceae) fix CO_2 via the reversed tricarboxylic acid cycle (Evans et al., 1966) leading to an enrichment of ^{13}C in biomass (Quandt et al., 1977; Sirevag et al., 1977) by around 15‰ relative to oxygenic phototrophic organisms utilizing the Calvin–Benson cycle for carbon fixation (e.g., Summons and Powell, 1986, 1987; Sinninghe Damsté et al., 1993; Hartgers et al., 1994a, b; Grice et al., 1996a, 1997). Chlorobiaceae also produce a range of relatively specific biomarkers such as the carotenoid derivatives isorenieratane and chlorobactane, certain aryl isoprenoids, methyl isobutyl maleimide, and farnesane (see Chapter *Biomarkers (Molecular Fossils)*). Detection of carbon isotopically strongly enriched lipids of this kind

in the rock record is evidence for the activity of green sulfur bacteria. As Chlorobiaceae are strictly anaerobic phototrophs and utilize reduced sulfur species as electron donor, isotopically heavy isorenieratane and related aromatic compounds in geological samples have been widely used as molecular indicators for the presence of H₂S in the zone of light penetration ("PZE" photic zone euxinia). In addition, more recently the biomarker crocetane in thermally mature samples has been attributed to carotenoids of Chlorobiaceae (Maslen et al., 2009).

The characteristic isotopic enrichment of Chlorobiaceae has also been observed in chlorobactane derived from the accessory pigment chlorobactene (Grice et al., 1998d) and in certain bacteriochlorophylls (Grice et al., 1996a, b). The structurally specific bacteriochlorophylls (BChl) of Chlorobiaceae (BChl *c*, *d* (*e*)) give rise to biomarkers such as 3-methyl-4-isobutylmaleimide and farnesane derived from the bacteriochlorophyll macrocycle and lipid farnesyl side chain, respectively. Farnesane has been reported to be depleted in ¹³C by about 3‰ compared to methyl isobutyl maleimide consistent with an origin of bacteriochlorophylls of Chlorobiaceae (Grice et al., 1996a, b). These data are supported by a recent study of the isotopic composition of lipids at different depths of saline meromictic Lake Kaiike, Japan (Ohkouchi et al., 2007). The carbon isotopic compositions of tetrapyrroles (chlorophyllides) were considerably enriched in ¹³C relative to the side chains (phytol or farnesol) of chlorophyll *a*, bacteriochlorophyll *a*, and bacteriochlorophylls *e*.

Along with S isotopic evidence from sedimentary pyrite minerals, Chlorobiaceae-derived biomarkers have provided evidence for the presence of PZE conditions during the largest extinction event of the Phanerozoic, at the end-Permian mass extinction (Grice et al., 2005a; Nabbefeld et al., 2010a, b). It has been proposed that the release of toxic H₂S to the ocean surface and atmosphere played a critical role in the extinction and protracted recovery (Knoll et al., 1996). Similar conditions during the end-Permian have been reported in the contemporaneous seas off southern China, W. Canada, E. Greenland, and Kashmir-Tibet (Summons et al., 2006). It has been proposed by Kump et al. (2005) that a chemocline upward excursion probably provided a trigger for the extinction, releasing toxic H₂S to the ocean surface and atmosphere.

Lipids derived from purple sulfur bacteria of the family Chromatiaceae, such as the carotenoid pigment okenone (Schaeffer et al., 1997; Brocks and Schaeffer, 2008), may be significantly depleted in ¹³C compared to phytoplankton markers. Carbon fixation in both Chromatiaceae and phytoplankton follows the Calvin–Benson Cycle and should, in principle, lead to similar carbon isotopic compositions of organic matter. Chromatiaceae inhabit deeper layers just beneath the thermocline of stratified euxinic waters where they may utilize CO₂ derived from remineralized organic matter, and a CO₂ source that is typically strongly depleted in ¹³C.

Dinoflagellates

CSIA ¹³C/¹²C has been used to distinguish dinoflagellates and methanotrophic bacterial sources of sedimentary 4-methyl and 4, 4-dimethylcholesteroids (Bird et al., 1971). The δ¹³C values of the 4-methylsteroids in Chinese hypersaline lacustrine sediments (Grice et al., 1998d) are significantly enriched in ¹³C compared to co-occurring phytoplankton markers. These data are inconsistent with an origin from methanotrophic bacteria (see below).

In a study by Ramos et al. (2003) CSIA of individual fatty acids was performed to establish the transfer of primary photosynthate from the euphotic zone to benthic/hyperbenthic environments in Conception Bay (Newfoundland, Canada). δ¹³C of fatty acids were highest at the height of the phytoplanktonic bloom, and became ¹³C-depleted at the end of the bloom cycle.

Diatoms

Highly branched C₂₅ and C₃₀ isoprenoid (HBI) alkane biomarkers of sedimentary organic matter (Robson and Rowland, 1986; Sinninghe Damsté et al., 1989) are sourced from diatoms (Nichols et al., 1988; Volkman et al., 1992, 1994). The δ¹³C of these components are heavier than other phytoplankton biomarkers (e.g., Freeman et al., 1994; Summons et al., 1993), which have been attributed to a seasonal blooming of certain diatoms. Non-blooming diatoms are also reported to use a bicarbonate pumping mechanism leading to their biomass being ¹³C-rich (Summons et al., 1993). In samples of particulate organic matter from the water column and sediments from Ellis Fjord (eastern Antarctica) the C_{25:2} HBI alkene-assumed precursor of C₂₅ HBI was found to have an enriched stable carbon isotopic composition consistent with a diatom source, probably belonging to the *Navicula* genus in the sea ice (Sinninghe Damsté et al., 2007).

Epiphytic algae

C₂₀ HBI and its monoene have been reported in both marine and freshwater sediments (Rowland et al., 1985). The structural similarity of the C₂₀ HBI to C₂₅ and C₃₀ HBIs, led Sinninghe Damsté et al. (2005) to propose a common diatom source for each of these. The C₂₀ HBI has not been identified in cultured diatoms (Volkman et al., 1998; Sinninghe Damsté et al., 2005), but has been identified in field samples of macrophytes *Chara* sp. and *Enteromorpha prolifera* (Rowland et al., 1985; Jaffe et al., 2001) pointing toward an epiphytic algal source (Volkman et al., 1998). Similar δ¹³C and δD of the C₂₀ HBI to the C₃₁ *n*-alkane attributed to land plants waxes in a series of Holocene sediments from China (Atahan et al., 2007; Grice et al., 2008b, 2009) implies a common source organism, or organisms utilizing similar carbon sources. Therefore, an epiphytic algal source is suggested for C₂₀ HBI.

Haptophytes

Long-chain C_{37} and C_{38} alkenones (de Leeuw et al., 1980; Volkman et al., 1980; Rechka and Maxwell, 1988a, b; Thiel et al., 1997) are biosynthesized by certain haptophytes (e.g., Marlowe et al., 1990). The individual stable carbon isotopic compositions of alkenones, in combination with other data, have been used to assess ancient pCO_2 levels (Jasper and Hayes, 1990; Jasper et al., 1994). CSIA $^{13}C/^{12}C$ data for haptophytes are consistent with active uptake of bicarbonate, followed by intracellular carbonic anhydrase-catalyzed conversion of bicarbonate to CO_2 for cellular utilization by these organisms (Werne and Hollander, 2004). The ratio of long-chain alkenones and their stable carbon isotopic compositions are unaffected by zooplankton herbivory (Grice et al., 1998a). $\delta^{13}C$ (ca. -36‰) of abundant C_{37} and C_{38} n -alkanes in a Chinese hypersaline lacustrine (Eocene-Paleocene) sediment have been found to be significantly depleted in ^{13}C compared to phytoplankton markers pointing to a specific origin from alkenones in haptophytes (Grice et al., 1998a), ascribed either to different algal bloom periods of the haptophytes to other algae. D/H of long-chain alkenones have also been used to record freshwater flooding of the eastern Mediterranean during sapropel deposition over the last 10 Myr, with their δD reflecting that of source water and sea surface salinity.

Ciliates

Unusual $\delta^{13}C$ of the biomarker gammacerane can provide information on microbial food sources has been reported. Thus, gammacerane of heterotrophic ciliates feeding on methanotrophic bacteria or archaea can be strongly depleted in ^{13}C (Werne et al., 2002), while gammacerane from ciliates that consume green sulfur bacteria may be enriched in ^{13}C relative to biomass of oxygenic phototrophic organisms (Sinninghe Damsté et al., 1995; Grice et al., 1998d; Santos Neto et al., 1998). Thus, while tetrahymanol occurs in a variety of different organisms, gammacerane derived from bacterivorous ciliates may be recognized by an unusual carbon isotopic composition (Sinninghe Damsté et al., 1995; Werne et al., 2002). Also the carbon isotopic composition of C_{30} 17α , 21β (H) hopane has been found to be similar to gammacerane in certain samples (Grice et al., 1998d) consistent with the notion that C_{30} hopane- 3β -ol occurs together with tetrahymanol in certain bacterivorous marine sauticociliates (Harvey and McManus, 1991).

Terrestrial plants

Long-chain n -alkyl compounds (n -alkanoic acid, n -alkane, and n -alkanol) are key components of epicuticular waxes extracted from the leaves of higher plants (e.g., Eglinton and Hamilton, 1967). Such compounds are ubiquitous found in soils, sediments, atmospheric dust, petroleum, and coal samples. CSIA $^{13}C/^{12}C$ of n -alkyl compounds have been determined in many geochemical studies (Freeman et al., 1990; Hayes et al., 1990), to

establish vegetation inputs. For example, vegetation source assessments (C3 and C4) based on $^{13}C/^{12}C$ of n -alkyl compounds has been widely applied to soils (e.g., Lichtfouse, 1995; Lichtfouse, 1997; Lichtfouse, 1998; Conte et al., 2003; Wiesenberg et al., 2004; Quénéa et al., 2006), marine and terrestrial sediments (Ficken et al., 1998; Huang et al., 2000; Zhao et al., 2000; Schefuß et al., 2003; Zhao et al., 2003; Feakins et al., 2005; and Huang et al., 2006), and atmospheric dust particles (Simoneit, 1997; Huang et al., 2000; and Conte et al., 2003). More recently, CSIA D/H of n -alkyl compounds in geological samples has become an important proxy for paleohydrological cycles (Xie et al., 2000; Andersen et al., 2001; Sauer et al., 2001; Huang et al., 2002; Grice et al., 2003; Yang and Huang, 2003; Dawson et al., 2004; Sachse et al., 2004; Xie et al., 2004; Liu and Huang, 2005; Hou et al., 2006; Smith and Freeman, 2006; Sachse et al., 2006; Sessions, 2006; Pagani et al., 2006; Grice et al., 2008c; Zhou et al., 2010).

It is generally accepted that n -alkyl compounds show $\delta^{13}C$ in the $-35 \pm 5\text{‰}$ range for C3 plants and $-20 \pm 5\text{‰}$ range for C4 plants. A significant difference in $\delta^{13}C$ also exists between C3 angiosperm and gymnosperm, where angiosperm biomass is ^{13}C depleted compared to gymnosperm biomass by ca. 5‰ (Chikaraishi et al., 2004b). δD of n -alkyl compounds are quite similar for all types of higher plants, spanning a range of -250 to -100‰ . Terrestrial plants are more exposed to the external environment, and use water produced by the complex hydrological cycle. An additional source of isotopic fractionation associated with terrestrial plants is evapotranspiration, which leads to the evaporative loss of the light isotopic species of water on leaf surfaces, resulting in an enrichment of D in the remaining water (Dongmann et al., 1974; Liu and Huang, 2005; Smith and Freeman, 2006; Sachse et al., 2006). Several studies on biosynthetic isotopic fractionation during photosynthesis and production of n -alkyl compounds have been carried out (Sessions et al., 1999; Hayes, 2001; Grice et al., 2003; Chikaraishi et al., 2004a, b; Sessions, 2006; Chikaraishi and Naraoka, 2006). The small differences in δD for various lipids reported between different plant types are mainly attributed to variations in physiology (e.g., Smith and Freeman, 2006). In general, terrestrially derived organic matter has been shown to display a wide variation in δD (e.g., Schimmelmann et al., 2004; Xiong et al., 2005). While temperature, humidity, and latitude (Xie et al., 2000; Xie et al., 2004; Pagani et al., 2006) are the most important determining factors, the D/H of meteoric waters in the terrestrial hydrological cycle are also affected by altitude and continentality (e.g., Dawson et al., 2004). These effects are highly changeable, leading to the large isotopic variations observed in terrigenous organic matter. Schimmelmann et al. (2004) measured the δD of the individual fractions of 75 terrestrially derived crude oils and found that the δ values of saturated fractions covered a range from -245 to -62‰ . Xiong et al. (2005) determined the δD of individual n -alkanes in terrestrial Chinese

source rocks from the Turpan and Liaohe Basins, which covered a range from -250 to -140 ‰. Furthermore, the bulk δD of saturated hydrocarbon fractions of marine-evaporitic, lacustrine, and mixed oils reported by Santos Neto and Hayes (1999) showed that the lacustrine oils were enriched in D (by 10–26‰) relative to other oils in the basin.

$\delta^{13}C$ and δD of leaf waxes depends on understanding the relative contribution and isotopic signatures of angiosperm and conifer plants in the rock record, due to the variable isotopic fractionation of CO_2 and water associated with differences in their stomatal structure (Smith et al., 2007). In a study by Pedentchouk et al. (2008) $\delta^{13}C$ and δD compositions of n - C_{27} alkane data were consistent with a lower stomatal conductance for CO_2 and water vapor in conifers relative to angiosperms.

Few studies have focused on $\delta^{13}C$ of aromatic components derived from land plants. In torbanite deposits, alkyphenanthrenes were found to have similar $\delta^{13}C$ to the higher-plant biomarker retene in the same samples (Grice et al., 2001) consistent with a land plant origin for these (e.g., Simoneit, 1997; Alexander et al., 1987, 1988). The proposed mechanism of formation involves rearrangement of phyllocladane precursors yielding 1-methylphenanthrene and retene (Simoneit, 1997; Alexander et al., 1987). The reported isotopic data for retene and alkyphenanthrenes are consistent with a 6 β (H)-phyllocladane (ca. -23 ‰) source also reported in Australian Gippsland Basin oils and close to the average for class I conifer resinites reported by Murray et al. (1998). 1,7-dimethylphenanthrene in the torbanites is thought to be derived from a different precursor, being slightly more depleted in ^{13}C compared with retene possibly deriving from a pimarane type precursor. A multidisciplinary study using molecular and organic isotope geochemistry, petrology, and palynology have been used to establish palaeoenvironmental conditions of fluvio-deltaic deposits from the Delambre-1 well, Western Australia (Triassic–Jurassic) (Grice et al., 2005b). The $\delta^{13}C$ of retene ranging from -26.9 to -24.9 ‰ was also consistent with a source resinite from class I conifers (Murray et al., 1998) and was related to samples rich in Araucariacian conifers.

δD analyses have also been extended to diterpenoid hydrocarbons from land plants in coal and carbonaceous mudstone samples from the Liaohe Basin, China (Tuo et al., 2006). δD for a majority of the n -alkanes range from -150 to -220 ‰. Pristane is reported to be 34–69‰ depleted in D relative to phytane. Diterpenoid hydrocarbons were found to be also depleted in D relative to the n -alkanes indicating a different source for these compounds, although hydrogen exchange could not be fully excluded as a result of thermal maturity (see above). $\delta^{13}C$ of terpenoid hydrocarbons (-24.5 ‰) in samples from the Middle Miocene second Lusatian lignite seam (Lubstów open cast mine, Poland) are attributed fossil wood fragments of gymnosperms (most probably

a species of the coniferales families Taxodiaceae/Cupressaceae) (Bechtel et al., 2007).

Archaea

Marine crenarchaeota

Based on the carbon isotopic composition of kerogen and archaeal lipids, Kuypers et al. (2001) estimated that the dead biomass of marine Crenarchaeota and possibly other Archaea constitutes up to 80% of sedimentary organic matter deposited during the Cretaceous Oceanic Anoxic Event (OAE1b event). The unusually heavy carbon isotopic composition (10‰ vs. PDB) of archaeal lipids suggests that the marine Crenarchaeota had a chemoautotrophic metabolism.

Halophilic archaea

The C_{21} to C_{25} regular isoprenoids extracted from Dead Sea halite deposits (Miocene/Pliocene) were reported to be 7‰ enriched in ^{13}C relative to lipids from phototrophic organisms (Grice et al., 1998d), consistent with the heterotrophic metabolism of Halobacteriales. In the Dead Sea today, *Dunaliella* sp. is the main primary producer and Halobacteriales feed off their biosynthetic products, such as glycerol (Oren, 1993). It is suggested that *Dunaliella*, capable of growing rapidly under hypersaline conditions, may be responsible for producing isotopically heavy products. The consumption of this isotopically heavy glycerol by Halobacteriales would in turn lead to a ^{13}C enrichment of their biomass (Grice et al., 1998d and references therein).

Andersen et al. (2001) reported δD of n -alkanes and isoprenoids attributed to rapid climatic changes during the Messinian salinity crisis, their isotopic data showing that enrichment in D followed climatically driven hydrologic changes in response to intense evaporation.

Sources of methane

Methane can be biogenically produced from CO_2 and H_2 , acetate, or methylated compounds such as trimethylamine and dimethylsulfide (Galimov, 2006 for a review). In marine sediments methane is produced almost entirely via CO_2 reduction via microbes. In terrestrial settings (e.g., freshwater, marsh) acetate fermentation prevails. It has been well-established that methane produced in anaerobic settings is extremely depleted in the ^{13}C . $\delta^{13}C$ of -60 ‰ upward are characteristic of microbial-produced methane (Games and Hayes, 1978). Cross plots of $\delta^{13}C$ and δD measurements (Schoell, 1980; Galimov and Kvenvolden, 1983; Woltemate et al., 1984; Whiticar et al., 1986) are used to establish (a) CO_2 reduction (b) Acetate fermentation pathway. Isotopic shifts have been reported in methane evolved in both freshwater ($\delta^{13}C$ ca. -55 ‰ to -90 ‰) and marine ($\delta^{13}C$ ca. -40 ‰ to -55 ‰) environments (Whiticar et al., 1986). As a result of such fractionations, organisms utilizing isotopically light methane are typically highly depleted in ^{13}C (Summons et al., 1994).

Methanogenic archaea and methane oxidizing archaea

Due to their isotopically light carbon source, lipids of methanotrophic and methane-oxidizing archaea and euryarchaea are commonly characterized by very negative carbon isotopic values. Biomarkers of these organisms include, phytane, 2,6,10,15,19-pentamethylcosane (PMI), crocetane, archaeol, biphytane, hydroxyarchaeol, acyclic biphytanic diacids, and various macrocyclic diphytanyl glycerol diethers (Birgel et al., 2006; Birgel and Peckmann, 2008). Biphytane has also been reported to be enriched in ^{13}C compared to phytoplankton biomarkers by about 4–5‰ in pelagic sediments and in the water column (Hoefs et al., 1997). This difference was ascribed to either (1) the use of dissolved inorganic carbon as carbon source, but with biosynthetic pathways within methanogens discriminating less against ^{13}C than phytoplankton, or (2) archaea obtaining their carbon source from low-molecular weight organic substrates (acetate/methylated amines) generated during decomposition of algal-derived particulate or dissolved organic matter. The methane produced by methanogens is isotopically light compared to their bulk biomass. $\delta^{13}\text{C}$ of PMI in some black shales (Cretaceous) has been found to share a similar $\delta^{13}\text{C}$ to phytane (Vinke et al., 1998). However, other presumed algal biomarkers were in fact lighter by about 10‰, indicating that phytane is probably related to the same source as PMI (an archaeal origin).

The anaerobic oxidation of methane (AOM) with sulfate is the most significant sink for methane in marine settings (Niemann and Elvert, 2008). This process is performed by a consortium of methanotrophic archaea and sulfate reducing bacteria (e.g., Hinrichs et al., 1999; Boetius et al., 2000; Orphan et al., 2001; Reitner et al., 2005; Birgel and Peckmann, 2008).

AOM accounts for an increase in alkaline conditions, causing subsequent precipitation of authigenic carbonates (Ritger et al., 1987; Aloisi et al., 2002). In modern seep environments, biomarkers and their $\delta^{13}\text{C}$ have provided detailed information on microbial activity related to seeps. ^{13}C -depleted archaeal biomarkers reported in limestones associated with ancient methane seeps have linked AOM back Jurassic times (Peckmann et al., 1999; Peckmann and Thiel, 2004; Birgel et al., 2006). Markers of sulfate-reducing bacteria (*iso*- C_{15} -fatty acids and *anteiso*- C_{15} -fatty acids) are characterized by very negative carbon isotopic values (Birgel and Peckmann, 2008).

Coolen et al. (2004) reported lipids of methanogenic archaea and ^{13}C depleted $\Delta^{8(14)}$ sterols supporting an active methane cycle in Ace Lake during the last 3,000 years; extant methanotrophs existed when it became a marine inlet.

Methane oxidizing bacteria

The isotopically light methane produced by methanogenic archaea (above) can be further fractionated by

methanotrophic bacteria leading to biomass becoming depleted in ^{13}C (ca. –90‰) and their sedimentary hopanoid analogues are similarly highly depleted in ^{13}C (Freeman et al., 1990). The presence of ^{13}C -depleted hopanoids in non-marine sediments has been related to bacterial methane cycling in the Messel Shale of Eocene age (ca. –65‰; Freeman et al., 1990), the Green River Formation (ca. –80‰; Collister et al., 1992) and in a recent lake sediment (ca. –50‰; Spooner et al., 1994). Methanotrophic bacteria are probably the major source of 3 β -methylhopanes in sedimentary rocks and oils, as these biomarkers may show a pronounced depletion in ^{13}C compared to co-occurring compounds (see Chapter *Biomarkers (Organic, Compound-Specific Isotopes)*) (Collister et al., 1992; Jahnke et al., 1999).

Apart from ^{13}C depleted isoprenoids derived from methanotrophic archaea, ^{13}C depleted triterpenoids (3 β -methylated hopanoids, $\delta^{13}\text{C}$ –100‰; lanostanes, $\delta^{13}\text{C}$ –80 to –70‰) have also been reported from a number of ancient seep deposits (Birgel and Peckmann, 2008). These data are consistent with contributions of ^{13}C depleted lipids from aerobic methanotrophic bacteria.

Cyanobacteria

Monomethyl and dimethyl branched alkanes, in particular 7-methylheptadecane, 8-methylheptadecane, and 7,11-dimethylheptadecane are known to be biosynthesized by cyanobacteria (e.g., Summons et al., 1998). For example, it has been reported by Summons et al. (1998) that cyanobacteria are able to synthesize normal or branched alkanes of different $\delta^{13}\text{C}$ compositions depending upon the environmental conditions. Cyanobacteria also biosynthesize extended bacteriohopanetetrol derivatives. $\delta^{13}\text{C}$ of extended hopanoids (> C_{30}) determined in sediments (Grice et al., 1996b, 1997; Schouten et al., 1997) generally have similar $\delta^{13}\text{C}$ to algal-derived cholestane and/or phytane, indicating a possible origin from cyanobacteria inhabiting upper regions of a water column. However, Schoell et al. (1994) found $\delta^{13}\text{C}$ differences between the cyanobacterial hopanoids (–29.5 to –31.5‰) and algal-derived steranes (–25‰) in the Middle Miocene which were interpreted as marking the interglacial–glacial transition from a well-mixed to a highly thermally stratified ocean. Schoell et al. (1994) attributed these isotopic differences to cyanobacteria living in deeper parts of the water column of a stratified oceanic environment utilizing a more depleted inorganic carbon source than the algae inhabiting the upper water column. Sinninghe Damsté, et al. (2001) identified two novel glycolipids, docosanyl 3-*O*-methyl- α -rhamnopyranoside and docosanyl 3-*O*-methylxylopyranoside in Ace Lake from Antarctica. $\delta^{13}\text{C}$ measurements showed that the sugar moieties of these glycolipids were ca. 8–9‰ enriched relative to the alkyl chains. Since structurally similar glycolipids have been reported to occur in nitrogen-fixing cyanobacteria, a cyanobacterial origin is proposed for these glycolipids.

Based on empirical isotopic relationships between the tetrapyrrole nuclei of chlorophylls and photoautotrophic cells, it has been concluded that diazotrophic cyanobacteria were the dominant components of phytoplankton communities during the mid-Cretaceous oceanic anoxic events (OAEs) in the western Tethys Sea (Kashiyama et al., 2007). N_2 -fixation was an important and major process in phytoplankton production during these OAEs.

Conclusion

Molecular carbon and hydrogen isotopic compositions of organic matter determined using irm-GCMS, provide significantly more useful information on palaeoenvironments than one gains from $\delta^{13}C$ bulk data. The groundbreaking research into CSIA of biomarkers in geochemistry research has further led to applications in a multiple of microbiological, environmental, medical, geochemical, ecological or biochemical, and forensic research areas. Future areas of interest lie in radiocarbon measurements at the molecular level and the complexity of the N_2 fixation processes in our oceans.

Bibliography

- Alexander, R., Kagi, R. I., and Larcher, A., 1984. Clay catalysis of alkylhydrogen exchange reactions - reaction mechanisms. *Organic Geochemistry*, **6**, 755–760.
- Alexander, R., Larcher, A. V., Kagi, R. I., and Price, P. L., 1988. The use of plant-derived biomarkers for correlation of oils with source rocks in the Cooper/Eromanga Basin system, Australia. *APEA Journal*, **28**, 310–323.
- Alexander, R., Noble, R. A., and Kagi, R. I., 1987. Fossil resin biomarkers and their application to oil-source rock correlation, Gippsland Basin, Australia. *APEA Journal*, **27**, 63–72.
- Aloisi, G., Bouloubassi, I., Heijis, S. K., Pancost, R. D., Pierre, C., Damste, J. S. S., Gottschal, J. C., Forney, L. J., and Rouchy, J. M., 2002. CH_4 -consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters*, **203**, 195–203.
- Andersen, N., Paul, H. A., Bernasconi, P., and Albrecht, P., 2001. Large and rapid climate variability during the Messinian salinity crisis: evidence from deuterium concentrations of individual biomarkers. *Geology*, **29**, 799–802.
- Armanios, C., Alexander, R., Kagi, R. I., and Sosrowidjojo, I., 1993. Molecular sieving of higher plant derived pentacyclics from crude oil. In: Oygard, K. (ed.), *Organic Geochemistry*. Hurlitrykk, Norway: Falch, pp. 745–746.
- Asif, M., Grice, K., and Fazeelat, T., 2009. Assessment of petroleum biodegradation using stable hydrogen isotopes and polycyclic aromatic hydrocarbons. *Organic Geochemistry*, **40**, 301–311.
- Atahan, P., Grice, K., and Dodson, J., 2007. Human influence on Holocene environmental change in the Yangtze River delta: a combined biomarker, $\delta^{13}C$, ^{14}C , pollen and charcoal approach. *The Holocene*, **17**, 507–515.
- Audino, M., Grice, K., Alexander, R., Boreham, C. J., and Kagi, R. I., 2001a. An unusual distribution of monomethylalkanes in *Botryococcus Braunii*-rich sediments. *Geochimica et Cosmochimica Acta*, **65**, 1995–2006.
- Audino, M., Grice, K., Alexander, R., and Kagi, R. I., 2001b. Macrocylic alkanes: a new class of biomarker. *Organic Geochemistry*, **32**, 759–763.
- Audino, M., Grice, K., Alexander, R., and Kagi, R. I., 2002a. Macrocylic alkanes: markers for the freshwater alga *Botryococcus braunii* in the Gippsland Basin. *APPEA*, **1**, 67–71.
- Audino, M., Grice, K., Alexander, R., and Kagi, R. I., 2002b. Macrocylic alkanes in crude oils from the algaenan of *Botryococcus braunii*. *Organic Geochemistry*, **33**, 978–984.
- Audino, M., Grice, K., Alexander, R., and Kagi, R. I., 2004. Macrocylic alkanes in crude oils and sediment extracts: enrichment using molecular sieves. *Organic Geochemistry*, **35**, 661–663.
- Bechtel, A., Widera, M., Sachsenhofer, R. F., Gratzner, R., Lucke, A., and Woszczyk, M., 2007. Biomarker and stable carbon isotope systematics of fossil wood from the second Lusatian lignite seam of the Lubstow deposit (Poland). *Organic Geochemistry*, **38**, 1850–1864.
- Beramendi-Orosco, L., Snape, C. E., and Large, D. J., 2006. Stable carbon isotope analysis of wood hydrolysis residues: a potential indicator for the extent of cross-linking between lignin and polysaccharides. *Organic Geochemistry*, **37**, 64–71.
- Bidigare, R. R., Fluegge, A., Freeman, K. H., Hanson, K. L., Hayes, J. M., Hollander, D., Jasper, J. P., King, L. L., Laws, E. A., Milder, J., Millero, F. J., Pancost, R., Popp, B. N., Steinberg, P. A., and Wakeham, S. G., 1997. Consistent fractionation of ^{13}C in nature and in the laboratory: Growth-rate effects in some haptophyte algae. *Global Biogeochemical Cycles*, **11**, 279–292.
- Bird, C. W., Lynch, J. M., Pirt, F. J., Reid, W. W., Brooks, C. J. W., and Middleditch, B. S., 1971. Steroids and squalene in *Methylococcus capsulatus* grown on methane. *Nature*, **230**, 473–474.
- Birgel, D., and Peckmann, J., 2008. Aerobic methanotrophy at ancient marine methane seeps: a synthesis. *Organic Geochemistry*, in press.
- Birgel, D., Thiel, V., Hinrichs, K.-U., Elvert, M., Campbell, K. A., Reitner, J., Farmer, J. D., and Peckmann, J., 2006. Lipid biomarker patterns of methane-seep microbialites from the Mesozoic convergent margin of California. *Organic Geochemistry*, **37**, 1289–1302.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Boreham, C. J., Summons, R. E., Roksandic, Z., Dowling, L. M., and Hutton, A. C., 1994. Chemical, molecular and isotopic differentiation of organic facies in the Tertiary lacustrine Duaringa oil shale deposit. *Organic Geochemistry*, **21**, 685–712.
- Brand, W. A., Tegtmeier, A. R., and Hilkert, A., 1994. Compound specific isotope analysis: extending toward $^{15}N/^{14}N$ and $^{18}O/^{16}O$. *Organic Geochemistry*, **21**, 585–594.
- Brassell, S. C., Eglinton, G., Marlowe, I. T., Pflaumann, U., and Sarntheim, M., 1986. Molecular stratigraphy: a new tool for climatic assessment. *Nature*, **320**, 129–133.
- Brocks, J. J., and Schaeffer, P., 2008. Okenane, a biomarker for purple sulfur bacteria (Chromatiaceae), and other new carotenoid derivatives from the 1,640 Ma Barney Creek Formation. *Geochimica et Cosmochimica Acta*, **72**, 1396–1414.
- Burgoyne, T. W., and Hayes, J. M., 1998. Quantitative production of H_2 by pyrolysis of gas chromatographic effluents. *Analytical Chemistry*, **70**, 5136–5141.
- Chikaraishi, Y., and Naraoka, H., 2006. Carbon and hydrogen isotope variation of plant biomarkers in a plant–soil system. *Chemical Geology*, **231**, 190–202.
- Chikaraishi, Y., Naraoka, H., and Poulson, S. R., 2004a. Carbon and hydrogen isotopic fractionation during lipid biosynthesis in a higher plant (*Cryptomeria japonica*). *Phytochemistry*, **65**, 323–330.
- Chikaraishi, Y., Naraoka, H., and Poulson, S. R., 2004b. Hydrogen and carbon isotopic fractionations of lipid biosynthesis among

- terrestrial (C_3 , C_4 and CAM) and aquatic plants. *Phytochemistry*, **65**, 1369–1381.
- Collister, J. W., Rieley, G., Stern, B., Eglinton, G., and Fry, B., 1994. Compound-specific $\delta^{13}C$ analyses of leaf lipids from plants with different carbon dioxide metabolisms. *Organic Geochemistry*, **21**, 619–627.
- Collister, J. W., Summons, R. E., Lichtfouse, E., and Hayes, J. M., 1992. An isotopic biogeochemical study of the Green River oil shale. *Organic Geochemistry*, **19**, 265–276.
- Conte, M. H., Weber, J. C., Carlson, P. J., and Flanagan, L. B., 2003. Molecular and carbon isotopic composition of leaf wax in vegetation and aerosols in a northern prairie ecosystem. *Oecologia*, **135**, 67–77.
- Coolen, M. J. L., Hopmans, E. C., Rijpstra, W. I. C., Muyzer, G., Schouten, S., Volkman, J. K., and Damste, J. S. S., 2004. Evolution of the methane cycle in Ace Lake (Antarctica) during the Holocene: response of methanogens and methanotrophs to environmental change. *Organic Geochemistry*, **35**, 1151–1167.
- Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochimica et Cosmochimica Acta*, **12**, 133–149.
- Dawson, D., Grice, K., Wang, S. X., Alexander, R., and Radke, J., 2004. Stable hydrogen isotopic composition of hydrocarbons in torbanites (Late Carboniferous to Late Permian) deposited under various climatic conditions. *Organic Geochemistry*, **35**, 2189–2197.
- Dawson, D., Grice, K., and Alexander, R., 2005. Effect of maturation on the indigenous δD signatures of individual hydrocarbons in sediments and crude oils from the Perth Basin (Western Australia). *Organic Geochemistry*, **36**, 95–104.
- Dawson, D., Grice, K., Edwards, D. S., and Alexander, R., 2007. The effect of source and maturity on the stable isotopic compositions of individual hydrocarbons in sediments and crude oils from the Vulcan Sub-basin, Timor Sea, Northern Australia. *Organic Geochemistry*, **38**, 1015–1038.
- de Leeuw, J. W., van der Meer, F. W., Rijpstra, W. I. C., and Schenck, P. A., 1980. On the occurrence and structural identification of long chain unsaturated ketones and hydrocarbons in sediments. In Douglas, A. G., and Maxwell, J. R. (eds.), *Advances in Organic Geochemistry 1979*. Oxford: Pergamon Press, pp. 211–217.
- Dongmann, G., Number, H. W., Forstel, H., and Wagner, K., 1974. The contribution of land photosynthesis to the stationary enrichment of ^{18}O in the atmosphere. *Radiation and Environmental Biophysics*, **11**, 41–52.
- Dowling, L. M., Boreham, C. J., Hope, J. M., Murray, A. P., and Summons, R. E., 1995. Carbon isotopic composition of hydrocarbons in ocean-transported bitumens from the coastline of Australia. *Organic Geochemistry*, **23**, 729–737.
- Eglinton, G., and Hamilton, R. J., 1967. Leaf epicuticular waxes. *Science*, **156**, 1322–1335.
- Ehleringer, J. R., Hall, A. E., and Farquhar, G. D., 1993. *Stable Isotopes and Plant-Carbon Water Relations*. San Diego: Academic Press.
- Ellis, L., Alexander, R., and Kagi, R. I., 1994. Separation of petroleum hydrocarbons using dealuminated mordenite molecular sieve-II. Alkyl naphthalenes and alkylphenanthrenes. *Organic Geochemistry*, **21**, 849–855.
- Estep, M. F., and Hoering, T. C., 1980. Biogeochemistry of the stable hydrogen isotopes. *Geochimica et Cosmochimica Acta*, **44**, 1197–1206.
- Evans, M. C. W., Buchanan, B. B., and Arnon, D. I., 1966. A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium. *Biochemistry*, **55**, 928–934.
- Feakins, S. J., deMenocal, P. B., and Eglinton, T. I., 2005. Biomarker records of late Neogene change in northeast Africa vegetation. *Geology*, **33**, 977–980.
- Ficken, K. J., Barber, K. E., and Eglinton, G., 1998. Lipid biomarker, $\delta^{13}C$ and plant macrofossil stratigraphy of a Scottish montane peat bog over the last two millennia. *Organic Geochemistry*, **28**, 217–237.
- Freeman, K. H., Hayes, J. M., Trendel, J. M., and Albrecht, P., 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature*, **343**, 254–256.
- Freeman, K. H., Wakeham, S. G., and Hayes, J. M., 1994. Predictive isotope biogeochemistry: hydrocarbons from anoxic marine basins. *Organic Geochemistry*, **21**, 629–644.
- Fusetti, L., Behar, F., Grice, R. K., and Derenne, S., 2010a. New insights into secondary gas generation from oil thermal cracking. A kinetic approach using 1,2,4-trimethylbenzene. Part II: A lumped kinetic scheme. *Organic Geochemistry*, **41**, 168–176.
- Fusetti, L., Behar, F., Grice, K., and Derenne, S., 2010b. New insights into secondary gas generation from oil thermal cracking. A kinetic approach using 1,2,4-trimethylbenzene. Part III. *Organic Geochemistry*, **41**, 431–436.
- Galimov, E. M., 2006. Isotope organic geochemistry. *Organic Geochemistry*, **37**, 1200–1262.
- Galimov, E. M., and Kvenvolden, K. A., 1983. Concentrations and carbon isotopic composition of CH_4 and CO_2 in gas from sediments of the Blake Outer Ridge. In Sheridan, R. E., and Gradstein, F. M. et al. (eds.), *Initial Reports of the Deep Sea Drilling Project*, 76. Washington: US Government Printing Office, pp. 403–417.
- Games, L. M., and Hayes, J. M., 1978. Methane-producing bacteria: natural fractionations of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **42**, 1295–1297.
- Goericke, R., Montoya, J. P., and Fry, B., 1994. Physiology of isotopic fractionation in algae and cyanobacteria. In Lajtha, K., and Micener, R. H. (eds.), *Stable Isotopes in Ecology and Environmental Science*. Oxford: Blackwell Scientific Publications, pp. 187–221.
- Greenwood, P. F., Leenheer, J. A., McIntyre, C., Berwick, L., and Franzmann, P. D., 2006. Bacterial biomarkers thermally released from dissolved organic matter. *Organic Geochemistry*, **37**, 597–609.
- Grice, K., Audino, M., Alexander, R., Boreham, C. J., and Kagi, R. I., 2001. Distributions and stable carbon isotopic compositions of biomarkers in torbanites from different palaeogeographical locations. *Organic Geochemistry*, **32**, 195–2110.
- Grice, K., Backhouse, J., Alexander, R., Marshall, N., and Logan, G. A., 2005b. Correlating terrestrial signatures from biomarker distributions, $\delta^{13}C$, and palynology in fluvio-deltaic deposits from NW Australia (Triassic-Jurassic). *Organic Geochemistry*, **36**, 1347–1358.
- Grice, K., Cao, C., Love, G. D., Bottcher, M. E., Twitchett, R., Grosjean, E., Summons, R., Turgeon, S., Dunning, W. J., and Jin, Y., 2005a. Photic zone euxinia during the Permian-triassic superanoxic event. *Science*, **307**, 706–709.
- Grice, K., De Mesmay, R., Wang, S., and de Glucina, A., 2008a. New 5A molecular sieving method for CSIA. *Organic Geochemistry*, **39**, 284–288.
- Grice, K., Gibbison, R., Atkinson, J. E., Eckardt, C. B., Schwark, L., Schwark, L., and Maxwell, J. R., 1996a. 1H-Pyrrole-2,5-diones (maleimides) as indicators of anoxygenic photosynthesis in palaeowater columns. *Geochimica et Cosmochimica Acta*, **60**, 3913–3924.
- Grice, K., Klein Breteler, W. C., Schouten, S., Grossi, V., De Leeuw, J. W., and Sinninghe-Damsté, J. S., 1998a. The effects of zooplankton herbivory on biomarker proxy records. *Paleoceanography*, **13**, 686–693.
- Grice, K., Lu, H., Atahan, P., James, E., Hallmann, C., Greenwood, P. F., Maslen, E., and Dodson, J., 2008b. A molecular marker for lignin degradation and the evolution of vascular plants. *Earth and Planetary Science Letters*, submitted.

- Grice, K., Schaeffer, P., Schwark, L., and Maxwell, J. R., 1996b. Molecular indicators of palaeoenvironmental conditions in an immature Permian shale (Kupferschiefer, Lower Rhine Basin, N.W. Germany) from free and sulfide-bound lipids. *Organic Geochemistry*, **25**, 131–147.
- Grice, K., Schaeffer, P., Schwark, L., and Maxwell, J. R., 1997. Changes in palaeoenvironmental conditions during deposition of the Permian Kupferschiefer (Lower Rhine Basin, N.W. Germany) inferred from molecular and isotopic compositions of biomarker components. *Organic Geochemistry*, **26**, 677–690.
- Grice, K., Schouten, S., Blokker, P., Derenne, S., Largeau, C., Nissenbaum, A., Sinninghe-Damsté, J. S., 2003. Structural and isotopic analysis of kerogens in sediments rich in free sulfurised *Botryococcus braunii* biomarkers. *Organic Geochemistry*, **34**, 471–482.
- Grice, K., Schouten, S., Nissenbaum, A., Charach, J., and Sinninghe-Damsté, J. S., 1998b. A remarkable paradox: freshwater algal (*Botryococcus braunii*) lipids in an ancient hypersaline euxinic ecosystem. *Organic Geochemistry*, **28**, 195–216.
- Grice, K., Schouten, S., Nissenbaum, A., Charach, J., and Sinninghe-Damsté, J. S., 1998c. Isotopically heavy carbon in the C₂₁ to C₂₅ regular isoprenoids in halite-rich deposits from the Sdom Formation, Dead Sea Basin, Israel. *Organic Geochemistry*, **28**, 349–359.
- Grice, K., Schouten, S., Peters, K. E., and Sinninghe-Damsté, J. S., 1998d. Molecular isotopic characterisation of hydrocarbon biomarkers in Palaeocene-Eocene evaporitic lacustrine source rocks from the Jiangnan Basin, China. *Organic Geochemistry*, **29**, 1397–1406.
- Grice, K., Schouten, S., Wong, C., Stuart-Williams, H., and Farquhar, G. D., 2003. Compound specific D/H isotopes of lipids and carbohydrates from extant plants grown under controlled conditions, 21st International Meeting on Organic Geochemistry. Krakow: Society of Research on Environmental Changes "Geosphere", pp. 47–48.
- Grice, K., Lu, H., Zhou, Y., Stuart Williams, H., and Farquhar, G. D., 2008c. Biosynthetic and environmental effects on the stable carbon isotopic compositions of the anteiso- (3-methyl) and iso- (2-methyl) alkanes in tobacco leaves. *Phytochemistry*, **69**, 2807–2814.
- Grice, K., Lu, H., Atahan, P., Hallmann, C., Asif, M., Greenwood, P. F., Tulipani, S., Maslen, E., Williford, K. H., and Dodson, J., 2009. New insights into the origin of perlyene in geological samples. *Geochimica et Cosmochimica Acta*, **73**, 6531–6543.
- Hartgers, W. A., Sinninghe Damsté, J. S., and de Leeuw, J. W., 1994a. A molecular and carbon isotopic study towards the origin and diagenetic fate of diaromatic carotenoids. *Organic Geochemistry*, **22**, 703–725.
- Hartgers, W. A., Sinninghe Damsté, J. S., Requejo, A. G., Allan, J., Hayes, J. M., and de Leeuw, J. W., 1994b. Evidence for only minor contributions from bacteria to sedimentary organic carbon. *Nature*, **369**, 224–227.
- Harvey, H. R., and McManus, G. B., 1991. Marine ciliates as a widespread source of tetrahymanol and hopan-3- β -ol in sediments. *Geochimica et Cosmochimica Acta*, **55**, 3387–3390.
- Hayes, J. M., 1983. In Meinschein, W. G. (ed.), *Organic Geochemistry of Contemporaneous and Ancient Sediments*, Great Lakes Section. Bloomington, Indiana: Society of Economic Palaeontologists and Mineralogists, pp. 5–31.
- Hayes, J. M., 1993. Factors controlling ¹³C contents of sedimentary organic compounds: principles and evidence. *Marine Geology*, **113**, 111–125.
- Hayes, J. M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In Valley, J. W., and Cole, D. R. (eds.), *Reviews in Mineralogy and Geochemistry* 43, *Stable Isotope Geochemistry*, Washington: The Mineralogical Society of America, pp. 225–277.
- Hayes, J. M., Freeman, K. H., Popp, B. N., and Hoham, C. H., 1990. Compound-specific isotopic analyses: a novel tool reconstruction of ancient biogeochemical processes. *Organic Geochemistry*, **16**, 115–1128.
- Hinrichs, K.-U., Hayes, J. M., Sylva, S. P., Brewer, P. G., and DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature*, **398**, 802–805.
- Hoefs, J., 1987. *Stable Isotope Geochemistry*. Berlin: Springer-Verlag.
- Hoefs, M. E. L., Schouten, S., King, L. L., Wakeham, S. G., de Leeuw, J. W., and Sinninghe Damsté J. S., 1997. Ether lipids of pelagic archaea in marine water columns. *Applied Environmental Microbiology*, **63**, 3090–3095.
- Horsfield, B., and Dueppenbecker, S. J., 1991. The decomposition of posidonia shale and green river shale kerogens using micro-scale sealed vessel (MSSV) pyrolysis. *Journal of Analytical Application of Pyrolysis*, **20**, 107–123.
- Hou, J., Huang, Y., and Wang, Y., 2006. Postglacial climate reconstruction based on compound-specific D/H ratios of fatty acids from Blood Pond, New England. *Geochemistry Geophysics Geosystems*, **7**, Q03008.
- Huang, Y., DuPont, L., Sarnthein, M., Hayes, J. M., and Eglinton, G., 2000. Mapping of C4 plant input from North West Africa into North East Atlantic sediments. *Geochimica et Cosmochimica Acta*, **64**, 3505–3513.
- Huang, Y., Murray, M., and Eglinton, G., 1995. Sacredicene, a novel monocyclic C₃₃ hydrocarbon from sediment of Sacred Lake, a tropical freshwater lake, Mount Kenya. *Tetrahedron Letters*, **36**, 5973–5976.
- Huang, Y., Shuman, B., Wang, Y., and Webb III, T., 2002. Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late Quaternary climate variations. *Geology*, **30**, 1103–1106.
- Huang, Y., Shuman, B., Wang, Y., Webb III, T., Grimm, E. C., and Jacobson, G. L., 2006. Climatic and environmental controls on the variation of C3 and C4 plant abundances in central Florida for the past 62,000 years. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 428–435.
- Huang, Y., Street-Perrott, F. A., Perrott, R. A., Metzger, P., and Eglinton, G., 1999. Glacial-interglacial environmental changes inferred from molecular and compound-specific $\delta^{13}\text{C}$ analyses of sediments from Sacred Lake, Mt. Kenya. *Geochimica et Cosmochimica Acta*, **63**, 1383–1404.
- Inaba, T., and Suzuki, N., 2003. Gel permeation chromatography for fractionation and isotope ratio analysis of steranes and triterpanes in oils. *Organic Geochemistry*, **34**, 635–641.
- Jaffé, R., Mead, R., Hernandez, M. E., Peralba, M. C., DiGuida, O. A., Carter, J. F., and Eglinton, G., 2001. Origin and transport of sedimentary organic matter in two subtropical estuaries: a comparative, biomarker-based study. *Organic Geochemistry*, **32**, 507–526.
- Jahnke, L. L., Summons, R. E., Hope, J. M., and Des Marais, D. J., 1999. Carbon isotopic fractionation in lipids from methanotrophic bacteria II: the effects of physiology and environmental parameters on the biosynthesis and isotopic signatures of biomarkers. *Geochimica et Cosmochimica Acta*, **63**, 79–93.
- Jasper, J. P., and Hayes, J. M., 1990. A carbon isotope record of CO₂ levels during the late Quaternary. *Nature*, **347**, 462–464.
- Jasper, J. P., Hayes, J. M., Mix, A. C., and Prahl, F. G., 1994. Photosynthetic fractionation of ¹³C and concentrations of CO₂ in central equatorial Pacific during the last 225,000 years. *Paleocean*, **9**, 781–898.
- Jones, D. M., Carter, J. F., Eglinton, G., Jumeau, E. J., and Fenwick, C. S., 1991. Determination of $\delta^{13}\text{C}$ values of sedimentary

- straight chain and cyclic alcohols by gas chromatography/isotope ratio mass spectrometry. *Biological Mass Spectrometry*, **20**, 641–646.
- Kashiyama, Y., Ogawa, N. O., Kuroda, J., Shiro, M., Nomoto, S., Tada, R., Kitazato, H., and Ohkouchi, N., 2007. Diazotrophic cyanobacteria as the major photoautotrophs during mid-Cretaceous oceanic anoxic events: nitrogen and carbon isotopic evidence from sedimentary porphyrin. *Organic Geochemistry*, *in press*.
- Knoll, A. H., Bambach, R. K., Canfield, D. E., and Grotzinger, J. P., 1996. Comparative earth history and Late Permian mass extinction. *Science*, **273**, 452–457.
- Kump, L. R., Pavlov, A., and Arthur, M. A., 2005. Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology*, **33**, 397–400.
- Kuypers, M. M. M., Blokker, P., Erbacher, J., Kinkel, H., Pancost, R. D., Schouten, S., and Sinninghe Damsté, J. S., 2001. Massive expansion of marine archaea during a mid-Cretaceous oceanic anoxic event. *Science*, **293**, 92–94.
- Laws, E. A., Popp, B. N., Bigidare, R. R., Kennicutt, M. C., and Macko, S. A., 1995. Dependence of phytoplankton isotopic composition on growth rate and $[\text{CO}_2]_{\text{aq}}$: theoretical considerations and experimental results. *Geochimica et Cosmochimica Acta*, **59**, 1131–1138.
- Leif, R. N., and Simoneit, R. T., 2000. The role of alkenes produced during hydrous pyrolysis of a shale. *Organic Geochemistry*, **31**, 1189–1208.
- Lichtfouse, É., 1995. ^{13}C labeling of soil *n*-hentriacontane (C_{31}) by maize cultivation. *Tetrahedron Letters*, **36**, 529–530.
- Lichtfouse, É., 1997. Heterogeneous turnover of molecular organic substances from crop soils as revealed by ^{13}C labeling at natural abundance with *Zea mays*. *Naturwissenschaften*, **84**, 23–35.
- Lichtfouse, É., 1998. Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soils. *Naturwissenschaften*, **85**, 76–77.
- Love, G. D., Bowden, S. A., Jahnke, L. L., Snape, C. E., Campbell, C. N., Day, J. G., and Summons, R. E., 2005. A catalytic hydrolysis method for the rapid screening of microbial cultures for lipid biomarkers. *Organic Geochemistry*, **36**, 63–82.
- Liu, W., and Huang, Y., 2005. Compound specific D/H ratios and molecular distributions of higher plant leaf waxes as novel paleoenvironmental indicators in the Chinese Loess Plateau. *Organic Geochemistry*, **36**, 851–860.
- Marlowe, I. T., Brassell, S. C., Eglinton, G., and Green, J. C., 1990. Long-chain alkenones and alkyl alkenoates and the fossil coccolith record of marine sediments. *Chemical Geology*, **88**, 349–375.
- Maslen, E., Grice, K., Gale, J., Hallmann, C., and Horsfield, B., 2009. Crocetane: A potential marker of photic zone euxinia in thermally mature sediments and crude oils of Devonian age. *Organic Geochemistry*, **49**, 1–17.
- Matthews, D. E., and Hayes, J. M., 1978. Isotope-ratio monitoring gas chromatography-mass spectrometry. *Analytical Chemistry*, **50**, 1465–1473.
- Melzer, E., and Schmidt, H. L., 1987. Carbon isotope effects on the pyruvate dehydrogenase reaction and their importance relative to the ^{13}C depletion in lipids. *The Journal of Biological Chemistry*, **262**, 8159–8164.
- Meredith, W., Sun, C. G., Snape, C. E., Sephton, M. A., and Love, G. D., 2006. The use of model compounds to investigate the release of covalently bound biomarkers via hydrolysis. *Organic Geochemistry*, **37**, 1705–1714.
- Merrit, D. A., 1993. *Continuous and Highly Precise Analysis of Stable Isotopes of Carbon and Nitrogen in Gas Chromatographic Effluents*. PhD Thesis. Indiana University, Bloomington.
- Monson, K. D., and Hayes, J. M., 1982. Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. *Geochimica et Cosmochimica Acta*, **46**, 139–149.
- Murray, A. P., Edwards, D., Hope, J. M., Boreham, C. J., Boothe, W. E., Alexander, R. A., and Summons, R. E., 1998. Carbon isotope biogeochemistry of plant resins and derived hydrocarbons. *Organic Geochemistry*, **29**, 1199–1214.
- Nabbefeld, B., Grice, K., Schimmelmann, A., Sauer, P. E., Böttcher, M. E., and Twitchett, R. J., 2010a. Significance of $\delta\text{D}_{\text{kerogen}}$, $\delta^{13}\text{C}_{\text{kerogen}}$ and $\delta^{34}\text{S}_{\text{pyrite}}$ from several Permian/Triassic (P/Tr) sections. *Earth and Planetary Science Letters*, **295**, 21–29.
- Nabbefeld, B., Grice, K., Twitchett, R. J., Summons, R. E., Hays, L., Böttcher, M. E., and Muhammad, A., 2010b. An integrated biomarker, isotopic and palaeoenvironmental study through the Late Permian event at Lusitaniadalen, Spitsbergen. *Earth and Planetary Science Letters*, **291**, 84–96.
- Nichols, P. D., Volkman, J. K., Palmisano, A. C., Smith, G. A., and White, D. C., 1988. Occurrence of an isoprenoid C_{25} di-unsaturated alkene and high neutral lipid content in Antarctic sea-ice diatom communities. *Journal of Phycology*, **24**, 90–96.
- Niemann, H., and Elvert, M., 2008. Diagnostic lipid biomarker and stable carbon isotope signatures of microbial communities mediating the anaerobic oxidation of methane with sulphate. *Organic Geochemistry*, *in press*.
- Noble, R. A., Wu, C. H., and Atkinson, C. D., 1991. Petroleum generation and migration from Talang Akar coals and shales offshore N.W. Java, Indonesia. *Organic Geochemistry*, **17**, 363–374.
- Ohkouchi, N., Kashiya, Y., Chikaraishi, Y., Ogawa, N. O., Tada, R., and Kitazato, H., 2006. Nitrogen isotopic composition of chlorophylls and porphyrins in geological samples as tools for reconstructing paleoenvironment. *Geochimica et Cosmochimica Acta*, **70**, A452.
- Ohkouchi, N., Nakajima, Y., Ogawa, N. O., Chikaraishi, Y., Suga, H., Sakai, S., and Kitazato, H., 2007. Carbon isotopic composition of the tetrapyrrole nucleus in chlorophylls from a saline meromictic lake: a mechanistic view for interpreting the isotopic signature of alkyl porphyrins in geological samples. *Organic Geochemistry*, *in press*.
- Oren, A., 1993. Availability, uptake and turnover of glycerol in hypersaline environment. *FEMS Microbiology Ecology*, **12**, 15–23.
- Orphan, V. J., Hinrichs, K.-U., Ussler III, W., Paull, C. K., Taylor, L. T., Sylva, S. P., Hayes, J. M., and DeLong, E. F., 2001. Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic sediments. *Applied Environmental Microbiology*, **67**, 1922–1934.
- Pagani, M., Pedentchouk, N., Huber, M., Sluijs, A., Schouten, S., Brinkhuis, H., Sinninghe Damsté, J. S., and Dickens, G. R., 2006. The Expedition 302 Scientists. Arctic hydrology during global warming at the Palaeocene/Eocene thermal maximum. *Nature*, **442**, 671–675.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Peckmann, D., Thiel, V. W., Clari, P., Gaillard, C., Martire, L., and Reitner, J., 1999. Cold seep deposits of Beauvoisin (Oxfordian; southeastern France) and Marmorito (Miocene; northern Italy): microbially induced authigenic carbonates. *International Journal of Earth Sciences*, **88**, 60–75.
- Pedentchouk, N., Freeman, K. H., and Harris, N. B., 2006. Different response of δD values of *n*-alkanes, isoprenoids, and kerogen during thermal maturation. *Geochimica et Cosmochimica Acta*, **70**, 2063–2072.
- Pedentchouk, N., Sumner, W., Tipple, B., and Pagani, M., 2008. $\delta^{13}\text{C}$ and δD compositions of *n*-alkanes from modern angiosperms and conifers: an experimental set up in central Washington State, USA. *Organic Geochemistry*, *in press*.

- Popp, B. N., Laws, E. A., Bidigare, R. R., Dore, J. E., Hanson, K. L., and Wakeham, S. G., 1998. Effect of phytoplankton cell geometry on carbon isotopic fractionation. *Geochimica et Cosmochimica Acta*, **62**, 69–79.
- Prosser, S. J., and Scrimgeour, C. M., 1995. High-precision determination of $^2\text{H}/^1\text{H}$ in H_2 and H_2O by continuous-flow isotope ratio mass spectrometry. *Analytical Chemistry*, **67**, 992–1997.
- Quandt, L., Gottschalk, G., Ziegler, H., and Stichler, W., 1977. Isotope discrimination by photosynthetic bacteria. *FEMS Microbiology Letters*, **1**, 125–128.
- Quénec, K., Largeau, C., Derenne, S., Spaccini, R., Bradoux, G., and Mariotti, A., 2006. Molecular and isotopic study of lipids in particle size fractions of a sandy cultivated soil (Cestas cultivation sequence, southwest France): Sources, degradation, and comparison with Cestas forest soil. *Organic Geochemistry*, **37**, 20–44.
- Ramos, C. S., Parrish, C. C., Quibuyen, T. A. O., and Abrajano, T. A., 2003. Molecular and carbon isotopic variations in lipids in rapidly settling particles during a spring phytoplankton bloom. *Organic Geochemistry*, **34**, 195–207.
- Rechka, J. A., and Maxwell, J. R., 1988a. Characterisation of alkenone temperature indicators in sediments and organisms. In Mattavelli, L., and Novelli, L (eds.), *Advances in Organic Geochemistry 1987. Organic Geochemistry*, **13**, 727–734.
- Rechka, J. A., and Maxwell, J. R., 1988b. Unusual long chain ketones of algal origin. *Tetrahedron Letters*, **29**, 2599–2600.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Rieley, G., 1994. Derivatisation of organic compounds prior to gas-chromatographic combustion-isotope ratio mass spectrometric analysis - identification of isotope fractionation processes. *Analyt.*, **119**, 915–919.
- Rigby, D., Batts, B. D., and Smith, J. W., 1981. The effect of maturation on the isotopic composition of fossil fuels. *Organic Geochemistry*, **3**, 29–36.
- Ritger, S., Carson, B., and Suess, E., 1987. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon/Washington margin. *Geological Society of America Bulletin*, **98**, 147–156.
- Robson, J. N., and Rowland, S. J., 1986. Identification of novel widely distributed sedimentary acyclic sesterpenoids. *Nature*, **324**, 561–563.
- Rommerskirchen, F., Eglinton, G., and Dupont, L., 2006. Glacial/interglacial changes in southern Africa: Compound-specific delta C-13 land plant biomarker and pollen records from south-east Atlantic continental margin sediments. *Geochemistry, Geophysics, Geosystems*, **7**, Q08010.
- Rowland, S. J., Yon, D. A., Lewis, C. A., and Maxwell, J. R., 1985. Occurrence of 2,6,10-trimethyl-7-(3-methylbutyl)-dodecane and related hydrocarbons in the green alga *Enteromorpha prolifera* and sediments. *Organic Geochemistry*, **8**, 207–213.
- Sachse, D., Radke, J., and Gleixner, G., 2006. δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient – implications for the sedimentary biomarker record. *Organic Geochemistry*, **37**, 469–483.
- Sachse, D., Radke, J., Gaupp, R., Schwark, L., Lüniger, G., and Gleixner, G., 2004. Reconstruction of palaeohydrological conditions in a lagoon during the 2nd Zechstein cycle through simultaneous use of δD values of individual *n*-alkanes and $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of carbonates. *International Journal of Earth Sciences*, **93**, 554–564.
- Santos Neto, E. V., and Hayes, J. M., 1999. Use of hydrogen and carbon stable isotopes characterizing oils from the Potiguar Basin (onshore), Northeastern Brazil. *AAPGB*, **83**, 496–518.
- Santos Neto, E. V., Hayes, J. M., and Takaki, T., 1998. Isotopic biogeochemistry of the Neocomian lacustrine and Upper Aptian marine-evaporitic sediments of the Potiguar Basin, Northeastern Brazil. *Organic Geochemistry*, **28**, 361–381.
- Sauer, P. E., Eglinton, T. I., Hayes, J. M., Schimmelmann, A., and Sessions, A. L., 2001. Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions. *Geochimica et Cosmochimica Acta*, **65**, 213–222.
- Schaeffer, P., Adam, P., Werung, P., Bernasconi, S., and Albrecht, P., 1997. Molecular and isotopic investigation of free and S-bound lipids from an actual meromictic lake (Lake Cadagno, Switzerland). In *Advances of Organic Geochemistry. 18th International Meeting on Organic Geochemistry*. pp. 57–58.
- Schefeuf, E., Schouten, S., Jansen, J. H. F., and Sinninghe Damsté, J. S., 2003. African vegetation controlled by tropical sea surface temperatures in the mid-Pleistocene period. *Nature*, **422**, 418–421.
- Schimmelmann, A., Boudou, J.-P., Lewan, M. D., and Wintsch, R. P., 2001. Experimental controls on D/H and $^{13}\text{C}/^{12}\text{C}$ ratios of kerogen, bitumen and oil during hydrous pyrolysis. *Organic Geochemistry*, **32**, 1009–1018.
- Schimmelmann, A., Lewan, M. D., and Wintsch, R. P., 1999. D/H isotope ratios of kerogen, bitumen, oil, and water in hydrous pyrolysis of source rocks containing kerogen types I, II, IIS, and III. *Geochimica et Cosmochimica Acta*, **63**, 3751–3766.
- Schimmelmann, A., Sessions, A. L., Boreham, C. J., Edwards, D. S., Logan, G. A., and Summons, R. E., 2004. D/H ratios in terrestrially sourced petroleum systems. *Organic Geochemistry*, **35**, 1169–1195.
- Schoell, M., 1980. The hydrogen and carbon isotopic composition of methane from natural gases of various origins. *Geochimica et Cosmochimica Acta*, **44**, 649–662.
- Schoell, M., Schouten, S., Sinninghe-Damsté, J. S., de Leeuw, J. W., and Summons, R. E., 1994. A molecular organic carbon isotope record of Miocene climate changes. *Science*, **263**, 1122–1125.
- Schouten, S., Klein Breteler, W. C. M., Blokker, P., Schogt, N., Rijpstra, W. I. C., Grice, K., Baas, M., and Sinninghe Damsté, J. S., 1998. Biosynthetic effects on the stable carbon isotopic compositions of algal lipids: implications for deciphering the carbon isotopic biomarker record. *Geochimica et Cosmochimica Acta*, **62**, 1397–1406.
- Schouten, S., Schoell, M., Rijpstra, W. I. C., Sinninghe Damsté, J. S., and de Leeuw, J. W., 1997. A molecular stable carbon isotope study of organic matter from the Miocene Monterey Formation (Pismo Basin). *Geochimica et Cosmochimica Acta*, **61**, 2065–2082.
- Schouten, S., Özdirekcan, S., van der Meer, M. T. J., Blokker, P., Baas, M., Hayes, J. M., and Sinninghe Damsté J. S., 2008. Evidence for substantial intramolecular heterogeneity in the stable carbon isotopic composition of phytol in photoautotrophic organisms. *Organic Geochemistry*, **39**, 135–146.
- Schwender, J., Seemann, M., Lichtenthaler, H. K., and Rohmer, M., 1996. Biosynthesis of isoprenoids (carotenoids, sterols, prenol side-chains of chlorophylls and plastoquinone) via a novel pyruvate/glyceraldehyde 3-phosphate non-mevalonate pathway in the green algae *Scenedesmus obliquus*. *Biochemical Journal*, **316**, 73–80.
- Sessions, A. L., 2006. Seasonal changes in D/H fractionation accompanying lipid biosynthesis in *Spartina alterniflora*. *Geochimica et Cosmochimica Acta*, **70**, 2153–2162.
- Sessions, A. L., Burgoyne, T. W., Schimmelmann, A., and Hayes, J. M., 1999. Fractionation of hydrogen isotopes in lipid biosynthesis. *Organic Geochemistry*, **30**, 1193–1200.
- Sessions, A. L., Sylva, S. P., Summons, R. E., and Hayes, J. M., 2004. Isotopic exchange of carbon-bound hydrogen over

- geologic timescales. *Geochimica et Cosmochimica Acta*, **68**, 1545–1559.
- Simoneit, B. R. T., 1997. Compound-specific carbon isotope analysis of individual long-chain alkanes and alkanolic acids in Harmatten aerosols. *Atmospheric Environment*, **31**, 2225–2233.
- Sinninghe Damsté, J. S., Baas, M., Geenevasen, J. A. J., and Kenig, F., 2005. Structural identification of sedimentary C₂₁ and C₂₂ highly branched isoprenoid alkanes. *Organic Geochemistry*, **36**, 511–517.
- Sinninghe Damsté, J. S., Kenig, F., Koopmans, M., Köster, J., Schouten, S., Hayes, J. M., and de Leeuw, J. W., 1995. Evidence for gammacerane as an indicator of water column stratification. *Geochimica et Cosmochimica Acta*, **59**, 1895–1900.
- Sinninghe Damsté, J. S., Rijpstra, W. I. C., Coolen, M. J. L., Schouten, S., and Volkman, J. K., 2007. Rapid sulfurisation of highly branched isoprenoid (HBI) alkenes in sulfidic Holocene sediments from Ellis Fjord, Antarctica. *Organic Geochemistry*, **38**, 128–139.
- Sinninghe Damsté, J. S., Rijpstra, W. I. C., Kock-Van Dalen, A. C., de Leeuw, J. W., and Schenk, P., 1989. Quenching of labile functionalised lipids by inorganic sulfur species. Evidence for the formation of sedimentary organic sulfur compounds at the early stage of diagenesis. *Geochimica et Cosmochimica Acta*, **58**, 1343–1355.
- Sinninghe Damsté, J. S., van Dongen, B. E., Rijpstra, W. I. C., Schouten, S., Volkman, J. K., and Geenevasen, J. A. J., 2001. Novel intact glycolipids in sediments from an Antarctic lake (Ace Lake). *Organic Geochemistry*, **32**, 321–332.
- Sinninghe Damsté, J. S., Wakeham, S. G., Kohnen, M. E. L., Hayes, J. M., and de Leeuw, J. W., 1993. A 6,000-year sedimentary molecular record of chemocline excursions in the Black Sea. *Nature*, **362**, 827–829.
- Sirevag, R., Buchanan, B. B., Berry, J. A., and Troughton, J. H., 1977. Mechanisms of CO₂ fixation in bacterial photosynthesis studied by the carbon isotope fractionation technique. *Archives of Microbiology*, **112**, 35–38.
- Smith, B. N., and Epstein, S., 1970. Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. *Plant Physiology*, **46**, 738–742.
- Smith, F., and Freeman, K. H., 2006. Influence of physiology and climate on δD of leaf wax *n*-alkanes from C3 and C4 grasses. *Geochimica et Cosmochimica Acta*, **70**, 1172–1187.
- Smittenberg, R., and Sachs, J., 2007. Purification of dinosterol for hydrogen isotopic analysis using high-performance liquid chromatography–mass spectrometry. *Journal of Chromatography A*, **1169**, 70–76.
- Smith, F. A., Wing, S. L., and Freeman, K. H., 2007. Magnitude of the carbon isotope excursion at the Paleocene–Eocene thermal maximum: the role of plant community change. *Earth and Planetary Science Letters*, **262**, 50–65.
- Spooner, N., Rieley, G., Collister, J. W., Lander, M., Cranwell, P. A., and Maxwell, J. R., 1994. Stable carbon isotopic correlation of individual biolipids in aquatic organisms and a lake bottom sediment. *Organic Geochemistry*, **21**, 823–827.
- Summons, R. E., and Powell, T. G., 1986. *Chlorobiaceae* in Palaeozoic seas revealed by biological markers, isotopes and geology. *Nature*, **319**, 763–765.
- Summons, R. E., and Powell, T. G., 1987. Identification of aryl-isoprenoids in source rocks and crude oils: biological markers for the green sulfur bacteria. *Geochimica et Cosmochimica Acta*, **51**, 557–566.
- Summons, R. E., Barrow, R. A., Capon, R. J., Hope, J. M., and Stranger, C., 1993. The structure of a new C₂₅ isoprenoid alkane biomarker from diatomaceous microbial communities. *Australian Journal of Chemistry*, **46**, 907–915.
- Summons, R. E., Freeman, K. H., Grice, K., and Pancost, R., 2008. Where would we be without the isotopes? *Organic Geochemistry*, in press.
- Summons, R. E., Hope, J. M., Dowling, L. M., Jahnke, L. L., Largeau, C., and Metzger, P., 1996. Carbon isotope fractionation in lipid biosynthesis by algae and cyanobacteria. In Grimalt, J. O., and Dorronsoro, C. (eds.), *Organic Geochemistry: Developments and Applications to Energy, Climate, Environment and Human History*. San Sebastian, Spain: A.I.G.O.A., pp. 6–7.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1998. Geolipids from cyanobacteria: New studies of old rocks and hot spring microbial mats from Yellowstone National Park. Australian Organic Geochemistry Conference, Canberra: September 28–30, pp. 10–11.
- Summons, R. E., Jahnke, L. L., and Roksandic, Z., 1994. Carbon isotopic fractionation in lipids from methanotrophic bacteria: relevance for interpretation of the geochemical record of biomarkers. *Geochimica et Cosmochimica Acta*, **58**, 2853–2863.
- Summons, R., Love, G. D., Hays, L., Cao, C., Jin, Y., Shen, S. Z., Grice, K., and Foster, C. B., 2006. Molecular evidence for prolonged photic zone euxinia at the Meishan and East Greenland sections of the Permian Triassic Boundary. 16th Annual V.M. Goldschmidt Conference of 2006, Melbourne.
- Summons, R. E., Metzger, P., Largeau, C., Murray, A. P., and Hope, J. M., 2002. Polymethylsqualanes from *Botryococcus braunii* in lacustrine sediments and oils. *Organic Geochemistry*, **33**, 99–109.
- Thiel, V., Jenisch, A., Landmann, G., Reimer, A., and Michaelis, W., 1997. Unusual distributions of long-chain alkenones and tetrahymanol from the highly alkaline Lake Van, Turkey. *Geochimica et Cosmochimica Acta*, **61**, 2053–2064.
- Tolosa, I., and Ogrinc, N., 2007. Utility of 5 Å molecular sieves to measure carbon isotope ratios in lipid biomarkers. *Journal of Chromatography A*, **1165**, 172–181.
- Tuo, J., Zhang, M., Wang, X., and Zhang, C., 2006. Hydrogen isotope ratios of aliphatic and diterpenoid hydrocarbons in coals and carbonaceous mudstones from the Liaohe Basin, China. *Organic Geochemistry*, **37**, 165–176.
- Urey, H. C., Lowenstam, H. A., Epstein, S., and McKinney, C. R., 1951. Measurement of palaeotemperatures and temperatures of the upper Cretaceous of England, Denmark, and Southeastern United States. *AAPGB*, **62**, 399–416.
- van der Meer, M. T. J., Schouten, S., and Sinninghe Damsté, J. S., 1998. The effect of the reversed tricarboxylic acid cycle on the C-13 contents of bacterial lipids. *Organic Geochemistry*, **28**, 527–533.
- van Dongen, B. E., Schouten, S., and Sinninghe Damsté, J. S., 2001. Gas chromatography/combustion/isotope-ratio-monitoring mass spectrometric analysis of methylboronic derivatives of monosaccharides: a new method for determining natural ¹³C abundances of carbohydrates. *Rapid Communications in Mass Spectrometry*, **15**, 496–500.
- Vinke, A., Schouten, S., Sephton, S., and Sinninghe Damsté, J. S., 1998. A newly discovered norisoprenoid, 2,6,15,19-tetramethylcosane, in Cretaceous black shales. *Geochimica et Cosmochimica Acta*, **6**, 965–970.
- Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., and Gelin, F., 1998. Microalgal biomarkers: a review of recent research developments. *Organic Geochemistry*, **29**, 1163–1179.
- Volkman, J. K., Eglinton, G., Corner, E. D. S., and Forsberg, T. E. V., 1980. Long chain alkenes and alkenones in the marine coccolithophorid *Emiliania huxleyi*. *Phytochemistry*, **19**, 2619–2622.
- Volkman, J. K., Barrett, S. M., and Dunstan, G. A., 1994. C₂₅ and C₃₀ highly branched isoprenoid alkanes in laboratory cultures of two marine diatoms. *Organic Geochemistry*, **21**, 407–413.

- Volkman, J. K., Barrett, S. M., Dunstan, G. A., and Jeffrey, S. W., 1992. Advances in the biomarkers chemistry of marine algae. In *Third Conference on Petroleum Geochemistry and Exploration in the Afro-Asian Region*, Abstracts, February 1992, Melbourne.
- Werne, J. P., Baas, M., and Sinninghe Damsté, J. S., 2002. Molecular isotopic tracing of carbon flow and trophic relationships in a methane-supported benthic microbial community. *Limnology and Oceanography*, **47**, 1694–1701.
- Werne, J. P., and Hollander, D. J., 2004. Balancing supply and demand: Controls on carbon isotope fractionation in the Cariaco Basin (Venezuela) Younger Dryas to Present. *Marine Chemistry*, **92**, 1–4, 275–293.
- Werner, R. A., and Brand, W. A., 2001. Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, **15**, 501–519.
- West, N., Alexander, R., and Kagi, R. I., 1990. The use of silicalite for the rapid isolation of branched and cyclic alkane fractions of petroleum. *Organic Geochemistry*, **15**, 499–501.
- Whiticar, M. J., Faber, E., and Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation-Isotope evidence. *Geochimica et Cosmochimica Acta*, **50**, 693–709.
- Wiesenberg, G. L. B., Schwarzbauer, J., Schmidt, M. W. I., and Schwark, L., 2004. Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry*, **35**, 1371–1393.
- Woltemate, I., Whiticar, M. J., and Schoell, M., 1984. Carbon and hydrogen isotopic composition of bacterial methane in a shallow freshwater lake. *Limnology and Oceanography*, **29**, 985–992.
- Xie, S., Nott, C. J., Avsejs, L. A., Maddy, D., Chambers, F. M., and Evershed, R. P., 2004. Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. *Geochimica et Cosmochimica Acta*, **68**, 2849–2862.
- Xie, S., Nott, C. J., Avsejs, L. A., Volders, F., Maddy, D., Chambers, F. M., Gledhill, A., Carter, J. F., and Evershed, R. P., 2000. Palaeoclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. *Organic Geochemistry*, **31**, 1053–1057.
- Xiong, Y., Geng, A., Pan, C., Liu, D., and Peng, P., 2005. Characterization of the hydrogen isotopic composition of individual *n*-alkanes in terrestrial source rocks. *Applied Geochemistry*, **20**, 455–464.
- Yang, H., and Huang, Y., 2003. Preservation of lipid hydrogen isotope ratios in Miocene lacustrine sediments and plant fossils at Clarkia northern Idaho, USA. *Organic Geochemistry*, **34**, 413–423.
- Zhang, Z., Metzger, P., and Sachs, J. P., 2007. Biomarker evidence for the co-occurrence of three races (A, B and L) of *Botryococcus braunii* in El Junco Lake, Galápagos. *Organic Geochemistry*, **38**, 1459–1478.
- Zhao, M., Dupont, L., Eglinton, G., and Teece, M., 2003. *n*-Alkane and pollen reconstruction of terrestrial climate and vegetation for N.W. Africa over the last 160 kyr. *Organic Geochemistry*, **34**, 131–143.
- Zhao, M., Eglinton, G., Haslett, S. K., Jordan, R. W., Sarnthein, M., and Zhang, Z., 2000. Marine and terrestrial biomarker records for the last 35,000 years at ODP site 658C off NW Africa. *Organic Geochemistry*, **31**, 919–930.
- Zhou, Y., Grice, K., Stuart-Williams, H., Farquhar, G. D., Hocart, C. H., Lu, H., and Liu, W., 2010. Biosynthetic origin of the saw-toothed profile in ¹³C and of *n*-alkanes and systematic isotopic differences between-, iso- & anteiso-alkanes in leaf waxes of land plants. *Phytochemistry*, **71**, 388–403.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Astrobiology
 Bacteria
 Biomarkers (Molecular Fossils)
 Cold Seeps
 Cyanobacteria
 Diatoms
 Isotopes and Geobiology
 Methane, Origin
 Methane Oxidation (Aerobic)
 Nitrogen
 Origins of the Metazoa
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOMINING (MINERAL BIOLEACHING, MINERAL BIOOXIDATION)

Douglas Eric Rawlings
 University of Stellenbosch, Matieland, South Africa

Summary

Biomining is the use of autotrophic, acidophilic, iron-, and sulfur-oxidizing microorganisms to produce ferric iron and sulfuric acid. These chemicals are capable of oxidizing a variety of minerals containing insoluble metal sulfides such as Fe₂S, CuS, NiS, and ZnS into their soluble sulfate forms e.g., Fe₂SO₄, CuSO₄, NiSO₄, and ZnSO₄, respectively, resulting in their extraction into water from which they may be more easily recovered. Metal leaching is carried out from mineral waste dumps, or more typically and especially from constructed heap or stirred tank reactors. The metals recovered commercially in the greatest quantities are copper and gold.

Description of the terms bioleaching, biooxidation, and biomining

The term *bioleaching* refers to the conversion of an insoluble metal (usually a metal sulfide, e.g., CuS, NiS, ZnS) into a soluble form (usually the metal sulfate, e.g., CuSO₄, NiSO₄, ZnSO₄). As the metal is extracted into water, this process is called bioleaching. Because this process involves oxidation, it may also be termed *biooxidation*. However, the term biooxidation usually refers to processes in which the recovery of a metal is enhanced by microbial decomposition of the mineral, but the metal being recovered is not solubilized. An example is the recovery of gold from arsenopyrite ores where the gold remains in the mineral after biooxidation and is extracted by cyanide in a subsequent step. The term bioleaching is clearly inappropriate when referring to gold recovery (although arsenic, iron, and sulfur are bioleached from

the mineral). *Biomining* is a general term that may be used to refer to both processes (Rawlings, 2002).

Definition

Biomining is a generic term that refers to a collection of biotechnological processes in which microorganisms are used in the recovery of metals from mineral ores and concentrates. The microbial processes involved are the same as those responsible for acid mine drainage, and where metals are recovered during the treatment of acid mine drainage such a process may be termed biomining. Although the bioadsorption of metals onto biological materials including microorganisms may be used in metal recovery, the term biomining has not been applied to bioadsorption processes.

Metals that are recovered in the greatest quantities in commercial processes are copper, gold, and cobalt. Illustrations of the number and scale of operations that employ microorganisms for metal recovery are to be found in Olsen et al. (2003) and Rawlings et al. (2003). Processes also exist for the treatment of nickel- and zinc-containing ores although recovery of these metals using "biomining" has not been commercialized. For several years uranium was recovered using related processes (McCready, 1988) and the technology has the potential for the recovery of several other types of metals. In general, to be amenable to biologically assisted leaching, the mineral should contain iron or a reduced form of sulfur.

Biomining processes use the action of microbes for one of two purposes (Rawlings, 2005). Either to convert insoluble metal sulfides (or oxides) to water soluble metal sulfates or as a pretreatment process to open up the structure of the mineral thereby permitting other chemicals to better penetrate the mineral and solubilize the desired metal. An example of the first type of process is the conversion of insoluble copper present in minerals such as covellite (CuS) or chalcocite (Cu_2S) to soluble copper sulfate. An example of the second is the removal of iron, arsenic, and sulfur from gold-bearing arsenopyrite so that the gold that remains in the mineral is more easily extracted by subsequent treatment with cyanide. Both are oxidation processes, but when the metal to be recovered is extracted into solution, the process is known as bioleaching, whereas when the metal remains in the mineral, bioleaching is an inappropriate term and the process should strictly be referred to as biooxidation. The term bioleaching is frequently used for both.

Mechanisms of biologically assisted leaching

Current understanding is that biooxidation is primarily a chemical process. Sand and coworkers have proposed that minerals can be divided into two broad categories, acid-insoluble minerals that are solubilized through oxidation by ferric iron (e.g., FeS_2 , MoS_2 , and WS_2) and acid-soluble minerals that are oxidized by the combined action of ferric iron and protons (e.g., ZnS , PbS , FeAsS , CuFeS_2 ,

and MnS_2). The role of the microorganisms is to provide the ferric iron and sulfuric acid leaching agents. Leaching reactions have been presented in Schippers and Sand (1999) and Rohwerder et al. (2003) and are reviewed in Rawlings (2005).

Because bioleaching processes are chemistry-driven, if a mineral (such as chalcopyrite) is recalcitrant to biooxidation at 40°C , the solution to the problem lies primarily in the realm of chemistry rather than biology. For example, an increase in the temperature of the biooxidation process to 80°C will allow the chemical reactions to take place at a much faster rate. However, the biology needs to follow the chemistry, and microorganisms that are capable of iron and sulfur oxidation at 80°C are required to generate or regenerate the lixiviants (leaching chemicals).

Although it is possible to separate the chemistry of metal solubilization from the biology into two different processes, separation is unlikely to be as efficient as when both processes are carried out together. The reason for this is that the role of the microorganisms is considered to be more than the generation of the ferric iron and acid required for the chemistry of mineral biooxidation. Contact between the microbes and the mineral is important (though not essential) because the microorganisms produce an exopolysaccharide layer when attached to a mineral (Sand et al., 1995). This layer serves as a reaction space where mineral dissolution reactions take place more efficiently than in the bulk solution. Increased efficiency is partly because conditions within the exopolysaccharide layer (e.g., concentration of ferric iron, redox potential, and pH) are substantially different and more favorable for leaching compared with conditions in the bulk solution (Rawlings, 2005).

Biomining processes

Heap reactors

Commercial mineral bioleaching can take place using a low technology process such the irrigation of waste ore dumps (Brierley, 1982). The efficiency of metal recovery may be improved by the construction and irrigation of specially designed heaps rather than by the irrigation of an existing, nonengineered dump (Brierley, 1982; Rawlings et al., 2003). During heap construction, agglomerated ore is piled onto an impermeable base and supplied with an efficient leach liquor distribution and collection system. Acidic leaching solution is percolated through the crushed ore, and microbes growing on the surface of the mineral in the heap produce the ferric iron and acid that carry out mineral dissolution and metal solubilization. Good aeration enhances the metal leaching reaction. Aeration can be passive, with air being drawn into the reactor as a result of the flow of liquid or may be active with air blown into the heap through piping installed near the bottom. Metal-containing leach solutions that drain from the heap are collected and sent for metal recovery. Heap reactors can be of enormous size (Rawlings et al., 2003), are

relatively cheap to construct and operate, and are therefore suited to the treatment of lower grade ores. Compared with tank reactors, the metal recovery efficiency of heap reactors tends to be low. Heap reactors are more difficult to aerate efficiently and undesirable gradients of pH, nutrients, and liquor channeling are difficult to manage. In addition, although initial rates of bioleaching can be improved by effective heap inoculation, it is difficult to achieve in practice and nondeliberate inoculation that occurs during irrigation may be the main method by which microorganisms are spread through a heap.

Tank reactors

Continuous-flow stirred tank reactors placed in series are used to treat high-value finely milled mineral concentrate or ore. Such tanks can be of considerable size, for example, the 1,300 m³ tanks used to process cobalt-containing pyrite at Kasere, Uganda. Mineral concentrate is added to the first tank, together with inexpensive inorganic nutrients in the form of ammonia- and phosphate-containing fertilizers. The mineral suspension flows through the series of highly aerated tanks that are pH- (typically pH 1.5–1.6) and temperature-controlled. Stirred tank reactors that operate at 40°C and 50°C have proven to be highly robust, and very little process adaptation is required for the treatment of different mineral types. Mineral solubilization takes place in days rather than the weeks or months required in heap reactors. A constraint on the operation of stirred tank reactors is the quantity of solids (pulp density) that can be maintained in suspension, which is limited to about 20%. At pulp densities >20%, physical mixing and microbial problems occur. The liquid becomes too thick for efficient gas transfer and the shear force induced by the impellers cause physical damage to the microbial cells. The limitation in solids concentration together with higher capital and running costs has restricted the use of stirred reactors to high value minerals or mineral concentrates. Stirred tanks are used as a pretreatment process for gold-containing arsenopyrite concentrates, with the first of these having been built at the Fairview mine, Barberton, South Africa in 1986. The largest stirred tank process is at Sansu in the Ashanti goldfields of Ghana, West Africa, where 960 tonnes of gold concentrate are processed per day.

Types of microorganisms

Both heap and tank biooxidation processes contain a variety of microorganisms, with two different organisms usually dominating: an iron-oxidizer and a sulfur-oxidizer. A wide variety of these microorganisms may be present (Rawlings and Johnson, 2007). Irrespective of the type of process or temperature, these microbes have several features in common that make them suitable for their role in mineral dissolution. They grow autotrophically by fixing CO₂ from the atmosphere which makes it unnecessary to provide them with a fixed carbon source. Their energy is obtained by using either ferrous iron or reduced

inorganic sulfur compounds (some use both) as electron donors. Oxygen is generally used as the electron acceptor. All are acidophiles and grow in low-pH environments (pH 1.4–1.6 is typical) and they tend to be tolerant of a wide range of metal ions (Dopson et al., 2003). These very modest nutritional requirements can generally be met by the aeration of the iron- and/or sulfur-containing mineral suspension in water or the irrigation of a heap. Small quantities of inorganic fertilizer may be added to ensure that nitrogen, phosphate, potassium, and trace element limitation does not occur.

The types of organisms found in heap and tank reactors that operate at different temperatures have been reviewed (Rawlings and Johnson, 2007). In general, bacteria belonging to the genera *Acidithiobacillus* and *Leptospirillum* tend to dominate the processes up to 40°C, with those belonging to the genera *Acidithiobacillus*, *Leptospirillum* and *Sulfobacillus* dominating the processes between 45 and 55°C. In high-temperature processes (60–80°C), archaea such as *Acidianus*, *Metallosphaera*, and *Sulfolobus* are responsible for iron- and sulfur-oxidation rather than bacteria.

Bibliography

- Brierley, C. L., 1982. Microbiological mining. *Scientific American*, **247**(2), 42–51.
- Dopson, M., Baker-Austin, C., Ram Kopponnedi, P., and Bond, P., 2003. Growth in sulfidic mineral environments: metal resistance mechanisms in acidophilic micro-organisms. *Microbiology*, **149**, 1959–1970.
- McCready, R. G. L., 1988. Progress in the bacterial leaching of metals in Canada. In Norris, P. R., and Kelly, D. P. (eds.), *Biohydrometallurgy-87*, Science and Technology Letters. UK: Kew, Surrey, pp. 177–195.
- Olsen, G. J., Brierley, J. A., and Brierley, C. L., 2003. Bioleaching review part B: progress in bioleaching: applications of the microbial processes by the mineral industries. *Applied Microbiology and Biotechnology*, **63**, 249–257.
- Rawlings, D. E., 2002. Heavy metal mining using microbes. *Annual Review of Microbiology*, **56**, 65–91.
- Rawlings, D. E., 2005. Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microbial Cell Factories*, **4**, 13.
- Rawlings, D. E., and Johnson, D. B., 2007. The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. *Microbiology*, **153**, 315–324.
- Rawlings, D. E., Dew, D., and du Plessis, C., 2003. Biomineralization of metal-containing ores and concentrates. *Trends in Biotechnology*, **21**, 38–44.
- Rohwerder, T., Gehrke, T., Kinzler, K., and Sand, W., 2003. Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. *Applied Microbiology and Biotechnology*, **63**, 239–248.
- Sand, W., Gehrke, T., Hallmann, R., and Schippers, A., 1995. Sulfur chemistry, biofilm, and the (in)direct attack mechanism – critical evaluation of bacterial leaching. *Applied Microbiology and Biotechnology*, **43**, 961–966.
- Schippers, A., and Sand, W., 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Applied and Environmental Microbiology*, **65**, 319–321.

Cross-references

Acid Rock Drainage
Archaea
Bacteria
Copper
Extracellular Polymeric Substances (EPS)
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Gold
Microbial Biomineralization
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation
Sulfur Cycle

BIOPROTECTION

Carlos Rodriguez-Navarro, Maria T. González-Muñoz, Concepción Jimenez-Lopez, Manuel Rodriguez-Gallego
Universidad de Granada, Granada, Spain

Synonyms

Bioconservation; Bioconsolidation; Biopreservation; Biorestitution

Definition

Active or passive microbially induced or mediated consolidation, cleaning, and/or protection of (stone) artworks affected by chemical, physical, and/or biological weathering phenomena. Bioprotection includes the microbially mediated removal (cleaning) of deleterious compounds (sulfates, nitrates, and/or organics), and the in situ cementation (consolidation) of ornamental materials via microbially induced biomineralization. Bioprotection also occurs naturally on rock surfaces where microorganisms aid in the formation of cementing phases such as oxalates and carbonates, and biofilms.

Introduction

The term bioprotection has been applied to a range of processes and applications involving microorganisms. They include microbe-induced defense response in plant–soil interactions, plant/crop treatment, food processing, and toxic metal decontamination (Glazer and Nikaido, 2007), prevention of wood decay (Dawson-Andoh and Morrell, 1992), inhibition of steel corrosion (Volkland et al., 2000), natural protection of rock outcrops (Carter and Viles, 2005) and building stones (Di Bonaventura et al., 1999), as well as cultural heritage conservation (Ciferri et al., 2000; Rodriguez-Navarro et al., 2003; Ramirez et al., 2005; Webster and May, 2006). Here, the term bioprotection refers both to the use of microorganisms (mainly bacteria) for the conservation of stone artworks, and to the microbially mediated formation of natural protection layers on rock outcrops and stone surfaces. Such meanings are the most commonly used and accepted in

geobiology, as they involve the interaction between the biosphere and the geosphere.

Although “carved in stone” is meant to be durable or eternal, ornamental stones are subjected to a range of physical, chemical, and biological weathering phenomena that lead to their eventual crumbling and disintegration. Such weathering processes have been exacerbated by anthropogenic emission of pollutants since the Industrial Revolution (Winkler, 1994). As a consequence, stone monuments and statuary are at risk worldwide.

Granites, sandstones, and carbonate rocks – limestone, dolostone, and marble – are among the most common stones used in monuments and statuary. Carbonate stones, made up of calcite (CaCO_3) and/or dolomite ($\text{CaMg}(\text{CO}_3)_2$), are particularly prone to chemical weathering in polluted (urban) environments, as exemplified by the decay of the Parthenon or the Taj Mahal. Wet (acid rain) and dry deposition of atmospheric pollutants contribute to the leaching of these sparingly soluble carbonates and the formation of sulfate – gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) – crusts, also named “black crusts” (Figure 1). Carbonate stones are also prone to physical–mechanical weathering associated to salt crystallization, insolation weathering, frost damage, and hydric expansion/contraction (Winkler, 1994; Gauri and Bandyopadhyay, 1999; Rodriguez-Navarro et al., 2003). Biodeterioration is also a major



Bioprotection, Figure 1 Black crusts developed on a limestone gargoyle at the Royal Chapel, Granada (Spain).

contributor to the decay of stones in general, and carbonate stones in particular (McNamara and Mitchell, 2005).

Conservation of decayed stoneworks typically involves cleaning (by mechanical or chemical means) and/or protection/consolidation. Organic (e.g., acrylic or epoxy resins), organo-inorganic (e.g., ethyl silicates), and inorganic (e.g., $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$ solutions) protection/consolidation treatments have been applied in the past to alter or mitigate stone decay (Lazzarini and Tabasso, 1986; Price, 1996). However, none of these treatments have been proven to be satisfactory (see Rodriguez-Navarro et al., 2003 and references therein). The limitations and drawbacks of these “classic” conservation treatments have prompted the search for new methods to clean, protect and consolidate weathered ornamental stones. One promising line of research has focused on microbial bioprotection as a new means for the restoration and consolidation of ornamental stone.

Microbial-stone interactions: biodeterioration versus bioprotection

It has been known for decades that microbes actively contribute to the degradation of ornamental stone (McNamara and Mitchell, 2005). For instance, the metabolic activity of nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) results in the production of nitric acid, which in turn causes dissolution of stone and formation of deleterious nitrate salts (Fernandez, 2006). Sulfur-oxidizing bacteria such as *Thiobacillus* sp. produce sulfuric acid and contribute to the development of sulfate crusts (Warscheid and Braams, 2000). Biofilms can exacerbate microbial biodeterioration as they provide a shelter for the aforementioned bacteria, and act as a reservoir for nutrient storage (McNamara and Mitchell, 2005). Wetting and drying of the biofilm matrix may lead to swelling/shrinking of the extracellular polymeric substances (EPS), causing mechanical damage to the host stone (Warscheid and Braams, 2000).

Although microbes are often the problem, they can also be the solution (Rinaldi, 2006). For instance, the anaerobic sulfate-reducing bacteria *Desulfovibrio desulfuricans* and *D. vulgaris* have been shown to be effective for the removal of gypsum crusts (Webster and May, 2006; Cappitelli et al., 2007). Similarly, nitrate salts can be eliminated through the application of nitrate-reducing bacterial cultures (Fernandez, 2006), while *Pseudomonas stutzeri* has been successfully applied to eliminate organic residues from frescoes (Ranalli et al., 2005).

There is growing evidence that several microorganisms contribute to the natural bioprotection of rock outcrops (Carter and Viles, 2005) as well as ornamental stone (Di Bonaventura et al., 1999) via the formation of biofilms and cemented surface layers. Some bacterial communities can promote the production of protective calcium oxalates on building stone (Di Bonaventura et al., 1999), or inhibit calcite dissolution (Rinaldi, 2006). Numerous strains of

bacteria isolated on different building stones are able to induce the precipitation of calcium carbonate (Urzi et al., 1999), an effect that may have aided in the natural protection of weathered ornamental stones. This latter ability has opened new ways for the effective protection and consolidation of decayed ornamental stones based on the in situ mineralization of bacterial carbonate cement (Rodriguez-Navarro et al., 2003; Webster and May, 2006; Jimenez-Lopez et al., 2007).

Bacterially mediated biomineralization of carbonates

The precipitation of carbonate minerals mediated or induced by both autotrophic and heterotrophic bacteria have attracted a substantial amount of research (see recent review by Wright and Oren, 2005). Bacterial precipitation of calcium carbonate has important implications in the past and present formation of carbonate rocks and sediments (Rodriguez-Navarro et al., 2003; Wright and Oren, 2005). It is known that cyanobacteria have aided in the formation of mineralized stromatolites since the Precambrian age (Konhauser, 2007), their photosynthetic activity thus contributing to atmosphere oxygen increase and CO_2 budgeting by carbonate precipitation. While early work (until the 1980s) examined the ability of photosynthetic bacteria to precipitate calcium carbonates in marine environments, precipitation of carbonates induced by heterotrophic bacteria in other environments such as caves, soils, and monuments has become the subject of much research in recent decades (Boquet et al., 1973; Urzi et al., 1999).

Bacteria play both passive and active roles in carbonate nucleation (Mitchell and Ferris, 2006). In the first case, the metabolic activity of heterotrophic bacteria (e.g., ammonification of amino acids, urea degradation, and dissimilatory reduction of nitrates and sulfates), or photosynthetic HCO_3^- consumption and OH^- production by cyanobacteria, increases the alkalinity (high pH, and elevated CO_3^{2-}) in the microenvironment surrounding the bacterial cells. This in turn increases supersaturation with respect to calcium carbonate if free Ca^{2+} is present, thus favoring calcification. Bacterial cell walls and/or sheets, as well as EPS, can actively aid in carbonate crystallization by providing heterogeneous nucleation sites (Ercole et al., 2007; Rodriguez-Navarro et al., 2007).

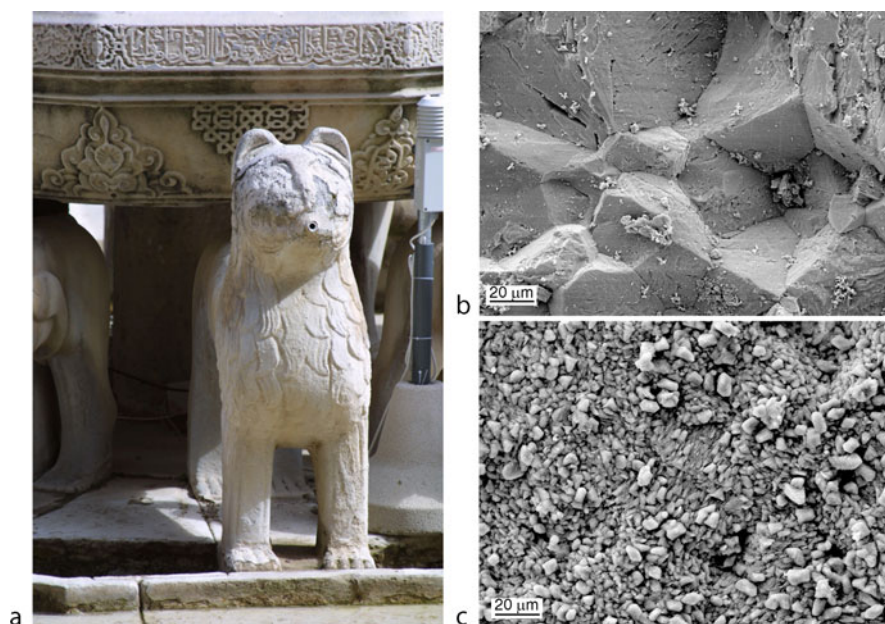
Bioprotection of stone by bacterial biomineralization of carbonates

Based on previous work on the natural ability of many heterotrophic bacteria to induce calcium carbonate precipitation, Adolphe et al. (1990) patented a method, CALCITE, for bacterial bioprotection of ornamental stones. The authors showed that calcinogenic bacteria, such as those typically found in soils (Boquet et al., 1973), were able to precipitate calcite on the surface of weathered limestone, thus contributing to their protection and consolidation. Le Métayer-Levrel et al. (1999) reported the

“successful” application of such a method on a sixteenth century limestone castle. Nonetheless, the authors used *Bacillus cereus* cultures, which may pose a health risk. In addition, potentially harmful biofilm formation and only a few micrometers thick consolidated layer were observed. Tiano et al. (1999) evaluated the effectiveness of such a treatment for bioprotection of a highly porous limestone (Pietra de Lecce) using *Micrococcus* sp. and *B. subtilis*. They observed detrimental obstruction of pores (presumably by biofilm formation), and called attention to other potential drawbacks e.g., reaction of the stone and by-products of bacterial activity, and the formation of stain patches. Rodriguez-Navarro et al. (2003) showed that such limitations could be overcome by using different culture media and a non-pathogenic carbonate-producing heterotrophic soil bacteria, *Myxococcus xanthus*. The treatment resulted in deep consolidation of a porous limestone (calcarenite) with new bacterial calcium carbonate cement, without detrimental pore plugging or extensive biofilm formation. *M. xanthus* metabolic activity results in the release of NH_3 , which leads to a pH rise in the medium surrounding the bacterial cells. This in turn favors carbonate supersaturation and results in CaCO_3 precipitation on different calcareous stones such as limestone and marble (Figure 2). Carbonates typically form on the bacterial cell walls, where heterogeneous nucleation is facilitated, and grow as a coherent cement (often epitaxially) on the stone support. Rodriguez-Navarro et al. (2003) also showed that the new bacterial calcite cement was tougher and more resistant to dissolution than the original calcite

in the stone. They interpreted these results considering that organic by-products of bacterial activity were incorporated into the bacterial calcite, which was in fact a (bio) composite material. Interestingly, Ben Chekroun et al. (2004) and Rodriguez-Navarro et al. (2007) showed that *M. xanthus* induced the precipitation of either calcite or vaterite just by modifying the culture medium. These results demonstrate that polymorph selection in carbonate precipitation is not strain specific. On the other hand, recent research using *B. subtilis* (Perito and Mastromei, 2003), *B. sphaericus* and *B. lentus* (Dick et al., 2006) shows that the range of microorganisms that can contribute to the bioprotection of ornamental stone via the precipitation of carbonates is very wide. In addition, it has been shown that bacteria such as *M. xanthus* can precipitate a range of minerals, other than carbonates, just by changing the composition of the culture medium (Ben Chekroun et al., 2004). These results imply that an accurate selection of growth media and bacterial strains could yield improved protection and consolidation of ornamental stones with disparate composition and textures.

Bioprotection treatments based on bacterial biomineralization are not restricted to ornamental stone. Castanier et al. (1996) used *Pseudomonas* sp. for calcite precipitation as a cementing phase in a biological mortar. Bang et al. (2001) have reported that polyurethane-immobilized *B. pasteurii* (a carbonatogenic ureolytic bacteria) can fill the cracks in concrete. Whiffin et al. (2007) have shown that carbonate precipitation induced by *Sporosarcina pasteurii* contributes to soil strengthening.



Bioprotection, Figure 2 Bacterially mediated calcite biomineralization on Macael marble: (a) Severe damage on a Macael marble lion sculpture at the Lions Court, The Alhambra, Granada, Spain; (b) scanning electron microscopy photomicrographs of a Macael marble sample before and (c) after bacterial conservation treatment resulting in *M. xanthus* mediated calcite cement formation.

Summary

Weathered ornamental stones typically contain a variety of endolithic and epilithic microbial communities (Urzi et al., 1999; McNamara and Mitchell, 2005). Application of bacterial treatments that include a nutritive medium may thus activate, among the microbial community contained in the stones, those bacteria that are able to induce the precipitation of calcium carbonate. In this respect, Jimenez-Lopez et al. (2007) have shown that the application of sterile nutritive media to porous limestone is able to activate carbonatogenic bacteria. As a consequence of the growth of these bacteria, an alkalization occurs that results in calcium carbonate precipitation. The new precipitate is compatible with the substrate and consolidates the stone without pore plugging. Such effects are reinforced by the presence of *M. xanthus*. Therefore, a good candidate to in situ consolidate decayed porous limestone is the application of a sterile culture medium with specific characteristics that favor the development of calcinogenic bacteria, while limiting the proliferation of detrimental microbiota (Jimenez-Lopez et al., 2007). Such an alternative to the direct application of life cells is highly promising. Finally, bacterial biomineralization of silicates as a means to consolidate non-carbonate stones such as granite and sandstone is another topic that should be further studied.

Bibliography

- Adolphe, J. M., Loubière, J. F., Paradas, J., and Soleilhavoup, F., 1990. Procédé de traitement biologique d'une surface artificielle. European Patent No. 90400G97.0 (after French Patent No. 8903517, 1989).
- Bang, S. S., Galinat, J. K., and Ramakrishnan, V., 2001. Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*. *Enzyme and Microbial Technology*, **28**, 404–409.
- Ben Chekroun, K., Gonzalez-Muñoz, M. T., Rodriguez-Navarro, C., Arias, J. M., and Rodriguez-Gallego, M., 2004. Precipitation and growth morphology of calcium carbonate induced by *Myxococcus xanthus*: implications for recognition of bacterial carbonates. *Journal of Sedimentary Research*, **74**, 868–876.
- Boquet, E., Boronat, A., and Ramos-Cormenzana, A., 1973. Production of calcite (calcium carbonate) crystals by soil bacteria is a common phenomenon. *Nature*, **246**, 527–529.
- Cappitelli, F., Toniolo, L., Sansonetti, A., Gulotta, D., Ranalli, G., Zanardini, E., and Sorlini, C., 2007. Advantages of using microbial technology over traditional chemical technology in removal of black crusts from stone surfaces of historical monuments. *Applied and Environmental Microbiology*, **73**, 5671–5675.
- Carter, N. E. A., and Viles, H. A., 2005. Bioprotection explored: the story of a little known earth surface process. *Geomorphology*, **67**, 273–281.
- Castanier, S., Le Métayer-Levrel, G., and Loubière, J. F., 1996. Biological mortar composition. French Patent FR2734261.
- Ciferri, O., Tiano, P., and Mastromei, G., 2000. *Of Microbes and Art: The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage*. New York: Plenum.
- Dawson-Andoh, B. E., and Morrell, J. J., 1992. Enhancing the performance of bioprotection agents by pretreatment with waterborne salts. *Wood and Fiber Science*, **24**, 347–352.
- Di Bonaventura, M. P., Del Gallo, M., Cacchio, P., Ercole, C., and Lepidi, A., 1999. Microbial formation of oxalate films on monument surfaces: bioprotection or biodeterioration? *Geomicrobiology Journal*, **16**, 55–64.
- Dick, J., De Windt, W., De Graef, B., Saveyn, H., van der Meeren, P., De Belie, N., and Verstraete, W., 2006. Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species. *Biodegradation*, **17**, 357–367.
- Ercole, C., Cacchio, P., Botta, A. L., Centi, V., and Lepidi, A., 2007. Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides. *Microscopy and Microanalysis*, **13**, 42–50.
- Fernandez, P., 2006. Applied microbiology and biotechnology in the conservation of stone cultural heritage materials. *Applied Microbiology Biotechnology*, **73**, 291–296.
- Gauri, K. L., and Bandyopadhyay, J. K., 1999. *Carbonate Stone: Chemical Behavior, Durability, and Conservation*. New York: Wiley.
- Glazer, A. N., and Nikaido, H., 2007. *Microbial Biotechnology: Fundamentals of Applied Microbiology*, 2nd edn. Cambridge, MA: Cambridge University Press.
- Jimenez-Lopez, C., Rodriguez-Navarro, C., Piñar, G., Carrillo-Rosúa, F. J., Rodriguez-Gallego, M., and Gonzalez-Muñoz, M. T., 2007. Consolidation of degraded ornamental porous limestone stone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. *Chemosphere*, **68**, 1929–1936.
- Konhauser, K., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Lazzarini, L., and Tabasso, M., 1986. *Il Restauro della Pietra*. Padua: Cedam.
- Le Métayer-Levrel, G., Castanier, S., Orial, G., Loubière, J. F., and Perthuisot, J. P., 1999. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. *Sedimentary Geology*, **126**, 25–34.
- McNamara, C. J., and Mitchell, R., 2005. Microbial deterioration of historic stone. *Frontiers in Ecology and the Environment*, **3**, 445–451.
- Mitchell, A. C., and Ferris, F. G., 2006. The influence of *Bacillus pasteurii* on the nucleation and growth of calcium carbonate. *Geomicrobiology Journal*, **23**, 213–226.
- Perito, B., and Mastromei, G., 2003. Conservation of monumental stones by bacterial biomineralization. *Microbiology Today*, **30**, 113–114.
- Price, C. A., 1996. *Stone Conservation. An Overview of Current Research*. Los Angeles, CA: Getty Conservation Institute.
- Ramirez, J. L., Santana, M. A., Galindo-Castro, I., and Gonzalez, A., 2005. The role of biotechnology in art preservation. *TRENDS in Biotechnology*, **23**, 584–588.
- Ranalli, G., Alfano, G., Belli, C., Lustrato, G., Colombini, M. P., Bonaduce, I., Zanardini, E., Abbruscato, P., Cappitelli, F., and Sorlini, C., 2005. Biotechnology applied to cultural heritage: bioremediation of Frescoes using viable bacterial cells and enzymes. *Journal of Applied Microbiology*, **98**, 73–83.
- Rinaldi, A., 2006. Saving a Fragile Legacy. *EMBO Reports*, **7**, 1075–1079.
- Rodriguez-Navarro, C., Rodriguez-Gallego, M., Chekroun, K. B., and Gonzalez-Muñoz, M. T., 2003. Conservation of ornamental stone by *Myxococcus xanthus*-induced carbonate biomineralization. *Applied and Environmental Microbiology*, **69**, 2182–2193.
- Rodriguez-Navarro, C., Jimenez-Lopez, C., Rodriguez-Navarro, A., Gonzalez-Muñoz, M. T., and Rodriguez-Gallego, M., 2007. Bacterially mediated mineralization of vaterite. *Geochimica et Cosmochimica Acta*, **71**, 1197–1213.
- Tiano, P., Biagiotti, L., and Mastromei, G., 1999. Bacterial bi-mediated calcite precipitation for monumental stones conservation: methods of evaluation. *Journal of Microbiological Methods*, **36**, 139–145.

- Urzi, C., Garcia-Vallez, M., Vendrell, M., and Pernice, A., 1999. Biomineralization processes on rock and monument surfaces observed in field and in laboratory conditions. *Geomicrobiology Journal*, **16**, 39–54.
- Volkland, H. P., Harms, H., Knopf, K., Wanner, O., and Zehnder, A. J. B., 2000. Corrosion of mild steel by bacteria. *Biofouling*, **15**, 287–297.
- Warscheid, T., and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, **46**, 343–368.
- Webster, A., and May, E., 2006. Bioremediation of weathered-building stone surfaces. *Trends in Biotechnology*, **24**, 255–260.
- Whiffin, V. S., van Passen, L. A., and Harkes, M. P., 2007. Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal*, **24**, 417–423.
- Winkler, E. M., 1994. *Stone in Architecture*. Berlin: Springer.
- Wright, D. T., and Oren, A., 2005. Nonphotosynthetic bacteria and the formation of carbonates and evaporites through time. *Geomicrobiology Journal*, **22**, 27–53.

Cross-references

Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Calcite Precipitation, Microbially Induced
 Chemolithotrophy
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Sulfate-Reducing Bacteria

BIOSIGNATURES IN ROCKS

Frances Westall, Barbara Cavalazzi
 Université de Orléans, Orléans, France

Definition

Biosignatures are morphological, chemical (organic, elemental and/or mineral), and isotopic traces of organisms preserved in minerals, sediments, and rocks. They represent the physical presence of the organisms as well as evidence of their metabolic activities and metabolites.

The nature of biosignatures

In this entry, we review (1) the nature of biosignatures in rocks, (2) how they are produced, and (3) the relevance of biosignatures and their astrobiological implications. Examples of biosignatures from throughout the rock record are given. We will limit our discussion to microbial biosignatures.

Microorganisms are characterized by (1) structural components, such as cells, colonies, biofilms/mats, extracellular polymeric substances (EPS); (2) the chemical composition of the different structural elements, including organic molecules and other essential elements; and (3) their living processes, including metabolic activity leading to the fractionation of elements such as C, S, O, N, P, and Fe, as well as cell division. Indirectly, microorganisms are also characterized by (4) the effects of their

physical presence and metabolic products on their immediate environment. These effects may be preserved on a microscopic to macroscopic scale. They include the precipitation of minerals; element leaching from, and dissolution of, minerals and rocks (biocorrosion); influence on mineral grain size and habit; the formation of microbially induced sedimentary structures (MISS; cf. Noffke et al., 2003) and biostabilization of sedimentary surfaces; and the formation of microbialites (e.g., microbial mounds, stromatolites, thrombolites) and microbial fabrics (e.g., clots, peloids, fenestrae). All of these phenomena can leave morphological, chemical, or isotopic traces in rocks (Table 1), but do not always do so. The ultimate fate of biosignatures is dependent upon their immediate preservation and the history of the sediments and rocks during subsequent diagenesis, metamorphism, and weathering.

The various types of biosignatures are discussed in detail elsewhere in this volume. Our aim here is to provide a brief overview of the broad range of biosignatures in rocks and to discuss their relevance and astrobiological implications.

Biosignatures of cell components and metabolic activity

Fossil carbon molecules

All terrestrial life consists of assemblages of macromolecules based on carbon (with water as the universal solvent, Brack, 2002) and the different components of the cell, i.e., cytoplasm, chromosomes, chloroplast, and cell walls and envelopes, etc., are characterized by molecules of different composition and structure. Molecular degradation starts with the death of the organism and may also be aided by the activity of other microorganisms, e.g., the heterotrophs, who obtain their energy and carbon from the breakdown of organic substances. The chemical changes that take place during degradation (diagenesis) are dependent on the redox state and water chemistry of the environment. Degradation of macromolecules to form “fossil” molecules results in the gradual loss of the functional groups. Specific characteristics of fossil molecules that suggest a biological origin include (1) enantiomeric excess (chirality), (2) diastereoisomeric preference, (3) structural isomer preference, (4) repeating constitutional subunits or atomic ratios, (5) systematic isotopic ordering at molecular and intramolecular levels, and (6) uneven distribution patterns or clusters, such as C-number, concentration, or $\delta^{13}\text{C}$, of structurally related compounds (Summons et al., 2008).

Preservation of fossil molecules

Fossil organic molecules or biomarkers are best preserved in anaerobic environments where they are protected from abiotic oxidation or aerobic microbial degradation. Subsequent burial, metamorphism, and heat events (e.g., hydrothermal/volcanic activity) affect the “maturity” or degradation state of the organic matter. This results in the formation of different types of kerogen characterized

Biosignatures in Rocks, Table 1 Morphological, chemical, and isotopic biosignatures in rocks

Major microbial components	Biosignature	Specific component or structure	
Cell components	Organic molecules (biomarkers, kerogen)	Composition chemical structure	Odd/even C number μ -structure
Cell metabolic activity	Biominerals	Direct (e.g., magnetite) indirect (e.g., microcrystalline calcite, aragonite, dolomite)	
	Elements	Concentration Isotopic ratios	e.g., Ni, Cu, Mn, Co, Mo, Se, V, Fe e.g., C, O, S, N, Fe
	Microbial influence on:	Mineral composition, mineral habit, mineral dissolution, mineral size	
Cell colonies, biofilms, mats, EPS	Fossil cells, colonies, mats	Clotted fabrics, microbial mounds, biolaminites	Stromatolites MISS ^a

Source: From Noffke et al. (2003)

^aMicrobially Induced Sedimentary Structures

by H/C ratios ranging from >1.25 for Type I kerogen to <0.5 for type IV kerogen. The small molecular size of some of the degraded molecules means that they are readily mobile and can be transported into other formations with which they are not syngenetic, thus causing contamination. The oldest known biomarkers occur in carbonaceous oil shales from the 2.7 Ga-old Fortescue Group in Australia where molecules such as hopanes and steranes, the degradation products of prokaryotes (specifically aerobic photosynthesizers, cyanobacteria) and eukaryotes, respectively, were interpreted as evidence for oxygenic photosynthetic activity by 2.7 Ga (Brocks et al., 1999) and, more importantly, the early rise of eukaryotes (Summons et al., 1999). These biomarkers have, however, recently given rise to controversy regarding their syngeneticity (Eigenbrode and Freeman, 2006; Rasmussen et al., 2008).

Syngenetic organic molecules occur in even older sediments but the kerogens in the volcanic sediments in the 3.5–3.3 Ga-old Barberton (South Africa) and Pilbara (Australia) greenstone belts are so degraded by age and low-grade metamorphism (prehnite–pumpellyite to lowest greenschist facies) that they no longer contain recognizable compositional biomarkers. The kerogens do, however, retain structural characteristics that are indicative of a biological origin (Westall et al., 2006a; Derenne et al., 2008). Evidence of syngeneticity of the kerogens analyzed is provided by in situ Raman spectra showing D and G peaks indicative of a maturity consistent with the metamorphic grade of the rocks (Westall et al., 2006a), as well as covalent linking of the molecular structures in the kerogen matrix (Derenne et al., 2008).

Traces of cell metabolism and metabolites

The metabolic activity of microorganisms can result in elemental, isotopic, and mineral signatures that can be preserved in rocks.

Bioelements: Unusual concentrations of metals may be associated with microorganisms and their metabolic products. Adsorption of these elements may be either an active or a passive process. Active adsorption includes the chelation of metals to anionic functional groups in the cell walls to neutralize their negative charges, thus contributing to the stability of the cell wall. Microorganisms also require metals, such as Fe, Co, Ni, Cu, Mn, Mo, Se, and V, for certain enzymatic activities (Banfield et al., 2001). They are actively adsorbed onto the cell walls by ligands that are specific for a particular metal (Konhauser, 2007). Microorganisms can also concentrate toxic elements, such as U, Cu, Pb, Al, As, and Cd, as well as precious metals (U, Cu, Pd, Pt, and Au) that are bound to cells and S-layers (Pollmann et al., 2006).

Passive adsorption of metal ions to cell surfaces is an additional kind of metal sequestration (Ledin, 1999). The availability of functional molecules, such as carboxyl or phosphate groups, determines the amount of metals that can be sorbed to the cell surface. With their large quantities of peptidoglycan, Gram positive bacteria can readily scavenge metal ions. Gram negative bacteria, on the other hand, are characterized by only a thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer. The amount of metal that they can bind is an order of magnitude less than that of the Gram positive bacteria (e.g., Beveridge et al., 1982). Microbial EPS is also an important factor in the binding of metals owing to their highly hydrated character that allows the metal ions to freely migrate through the structure (Geesey and Jang, 1989). This characteristic means that microorganisms, such as encapsulated bacteria, are more likely to chelate metals than non-encapsulated varieties. Metal-replaced microorganisms are common and are among the oldest traces of life. Fossil microorganisms preserved as pyritized filaments occur in a 3.2 Ga-old volcanogenic

massive sulfide deposit (the equivalent of a fossilized black smoker) from the Pilbara in Australia (Rasmussen, 2000). A younger example consists of ZnS-replaced filaments in Triassic calcareous rocks in the southern Alps (Kucha et al., 2005).

Concentrations of other biologically important elements, such as P and S, are considered to be significant biosignatures. For example, the combination of graphite associated with apatite inclusions (apatite can be a biomineral, cf. Table 2) and a $\delta^{13}\text{C}$ of -26‰ in a highly metamorphosed rock from the 3.85 Ga-old Akilia province in W. Greenland was interpreted as a biosignature by Mojzsis et al. (1996). However, van Zuilen et al. (2002) demonstrated that abiological processes linked to alteration of the rocks by hydrothermal circulation could have produced carbon via the Fischer–Tropsch process with a similar isotopic signature. On the other hand, phosphatized microorganisms are well-known from the rock record. The spectacular preservation of soft body parts of animals, birds, amphibians, and fish from the Eocene Messel oil shales in Germany is due to phosphatized microbial biofilms that coated the dead organisms (Liebig et al., 1996).

High concentrations of sulfur can be found in kerogen owing to the process of sulfurization during which sulfur, liberated from degrading organic matter by the activity of sulfur-reducing bacteria, is bound to the organic macromolecules. Thus, the identification of the S-molecule thiophene in the organic cell walls of microfossils from the

800 My-old Draken Formation of Svalbard (Lemelle et al., 2008) and even in 2.7 Ga-old kerogen associated with stromatolites from the Tumbiana Formation in the Pilbara (Lepot et al., 2008) has been linked to the degradation of organic matter by sulfur-reducing bacteria.

Biominerals: Minerals of different phases can be formed both actively and passively by microorganisms in biologically controlled and biologically induced processes, respectively. Microbially induced mineralization occurs either as a result of changes in the environment of a microorganism caused by the excretion of metabolic waste products, enzymatic mediated changes in the redox state, or simply the presence of a charged cell surface that attracts mineral nucleation (Banfield et al., 2001; Konhauser, 2007). We have seen above that reactive functional group sites on microbial surfaces readily chelate metal ions. In the same way mineral nuclei in saturated solutions bind to the surfaces of cells and their extrapolymeric substances (EPS), often completely entombing the microorganisms. A non-exhaustive list of microbially induced mineral precipitates is given in Table 2.

Iron hydroxides (ferrihydrite) are among the most common microbially induced deposits and may be formed passively, or by the activities of chemoheterotrophic, photoautotrophic, or chemolithoautotrophic (e.g., *Gallionella*) microorganisms. The ferric hydroxides may act as precursors for Fe oxides, such as hematite. Manganese nodules are an example of microbially induced

Biosignatures in Rocks, Table 2 Non-exhaustive list of microbially induced mineral precipitates

Metal oxides	Fe/Mn hydroxides and oxyde-hydroxides (or oxyhydroxides), e.g., ferrihydrite (ferric hydroxide), $\text{Fe}^{3+}_2\text{O}_3 \cdot 0.5(\text{H}_2\text{O})$, goethite, $\text{Fe}^{3+}\text{O}(\text{OH})$, schwertmannite, $\text{Fe}^{3+}_{16}\text{O}_{16}(\text{OH})_{12}(\text{SO}_4)_2$
Amorphous silica	Fe/Mn/Ti oxides, e.g., magnetite, $\text{Fe}^{3+}_2\text{Fe}^{2+}\text{O}_4$, hematite, $\text{Fe}^{3+}_2\text{O}_3$, anatase, TiO_2
Carbonates	Opal, $\text{SiO}_2 \cdot n(\text{H}_2\text{O})$, e.g., opal-A and opal-A/CT
Carbonates	Ca-carbonates, e.g., calcite, $\text{Ca}(\text{CO}_3)$, magnesian calcite, High-/Low-Mg $\text{Ca}(\text{CO}_3)$, aragonite, CaCO_3 , dolomite, $\text{CaMg}(\text{CO}_3)_2$
Carbonates	Magnesite, $\text{Mg}(\text{CO}_3)$
Carbonates	Strontianite, $\text{Sr}(\text{CO}_3)$
Phosphates	Ca-phosphates, e.g., apatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{F}, \text{Cl})$, carbonate-hydroxylapatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, francolite, or carbonate fluoroapatite, $\text{Ca}_5(\text{PO}_4)_3\text{F}$
Phosphates	Fe-phosphates, e.g., vivianite, $\text{Fe}^{3+}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
Sulfates	Barite, BaSO_4
Sulfates	Celestine, SrSO_4
Sulfates	Gypsum, $\text{Ca}(\text{SO}_4) \cdot 2(\text{H}_2\text{O})$
Sulfates	Fe-sulfates, e.g., jarosite, $\text{KFe}^{3+}_3(\text{SO}_4)_2(\text{OH})_6$
Sulfides	Chalcopyrite, $\text{CuFe}^{2+}\text{S}_2$
Sulfides	Galena, PbS
Sulfides	Greigite, Fe_3S_4
Sulfides	Mackinawite, FeS
Sulfides	Millerite, NiS
Sulfides	Pearceite, $\text{Ag}_{16}\text{As}_2\text{S}_{11}$
Sulfides	Proustite, Ag_3AsS_3
Sulfides	Pyrite, Fe^{2+}S_2
Sulfides	Sphalerite, $(\text{Zn}, \text{Fe})\text{S}$
Silicates	Clay minerals, e.g., phillipsite, $(\text{Ca}, \text{Na}, \text{K})1-2(\text{Si}, \text{Al})_8\text{O}_{16} \cdot 6(\text{H}_2\text{O})$, chamosite, $(\text{Fe}^{2+}, \text{Mg}, \text{Fe}^{3+})_5\text{Al}(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH}, \text{O})_8$, illite, $(\text{K}, \text{H}_3\text{O})(\text{Al}, \text{Mg}, \text{Fe})_2(\text{Si}, \text{Al})_4\text{O}_{10}[(\text{OH})_2, (\text{H}_2\text{O})]$, smectite $(\text{Na}, \text{Ca})\text{Al}_4(\text{Si}, \text{Al})_8\text{O}_{20}(\text{OH})_4 \cdot 2(\text{H}_2\text{O})$

iron-manganese oxides that can form volumetrically important deposits in the deep-sea and lacustrine environments (Mero, 1962).

Amorphous silica commonly nucleates passively onto the surfaces of microorganisms in hot spring environments (Figure 1a) (e.g., Cady and Farmer, 1996) and precipitates from saturated solutions in other environments as well. For example, Monty et al. (1991) and Westall (1994) documented silicified microorganisms in the pore space of 15–10 My-old deep-sea sediments. At the other end of the geological record, silica-saturated seawaters on the early Earth led to the silicification of early Archaean microbes and the preservation of the oldest known traces of life (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b; Westall and Southam, 2006).

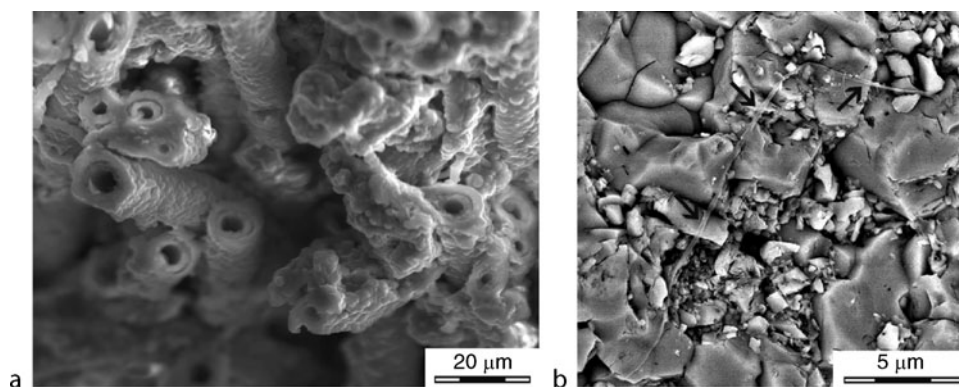
Perhaps the most geologically significant microbially induced mineral deposits are those related to carbonates. The carbonate minerals are produced by processes including mineralization of microbial surfaces, chemical precipitation from supersaturated solutions, and physical erosion of preexisting carbonates (Riding, 2000). Microbial carbonates occur in a wide variety of environments ranging from deep-water cold seeps (Figure 2) (e.g., Cavalazzi et al., 2007), shallow-water platforms (Figure 3a) (e.g., Monty, 1995), lacustrine environments (Figure 3b and c) (e.g., Laval et al., 2000) to carbonate (travertine) springs (e.g., Chafetz and Folk, 1984).

Biologically controlled mineralization occurs when microorganisms exert direct control on the nucleation and growth of the mineral (Mann, 1988). The resulting minerals are characterized by their highly ordered crystal-line structure, size, and morphology. Chains of single-domain magnetite crystals are formed in this way by magnetotactic bacteria (e.g., Bazylinski et al., 1995) and Chang and Kirschvink (1989) record chains of magnetite crystals in the 1.9 Ga-old Gunflint Formation (Canada) that are similar in size and shape to those produced by

recent bacteria. Indeed, the presence of chemically pure magnetite crystals with a specific size/shape range in an ancient Martian meteorite, ALH84001, is one of the key lines of evidence for life on Mars by 3.9 Ga (McKay et al., 1996; Thomas-Keprta et al., 2001, 2002; Friedmann et al., 2001). However, the supposedly biogenic magnetites form only a fraction (<30%) of a larger population that includes magnetites of clearly abiological origin and it is questioned whether this assemblage is purely fortuitous. Moreover, similar magnetites have been produced in the laboratory by abiogenic processes (Golden et al., 2004).

The presence of microbial metabolites plus the physical presence of the organic components can have an enormous effect on the elemental composition and dissolution of minerals, as well as the shape and size of the crystals. Microbial dissolution of nonorganic substrates is a common process, especially in oligotrophic environments where access to essential elements is necessary for survival. Microbial dissolution of silicate minerals, such as feldspars and mafic minerals, contributes strongly to crustal weathering. Microbial colonies and biofilms attach to a mineral surface and obtain their nutrients by physical disaggregation of the minerals, as well as by dissolution through the production of organic acids (Konhauser et al., 1994; Konhauser, 2007). Etching patterns produced in the presence of actively metabolizing cells show clear differences to those produced by inorganic acids (Figure 4) (Welch et al., 2002). Microbial etching of volcanic glass (e.g., Thorseth et al., 1995) has recently received much attention because certain tunnel-like structures in ~3.5 Ga-old pillow lava rims from the Barberton and Pilbara greenstone belts have been interpreted as microbial corrosion biosignatures (Furnes et al., 2004; Banerjee et al., 2006).

Compositional variability can occur in minerals and rocks as a result of microbial metabolic activity. The distribution of elements (major, minor, and trace) in the



Biosignatures in Rocks, Figure 1 Micrometer-scale biosignatures of in rocks. Micrographs of mineralized filamentous structures. (a) SEM image of (sub-)recent silicified microbial filaments from the El Tatio geysers area, northern Chile. (b) Carbonaceous filament (arrows) from the 3.44 Ga-old "Kitty's Gap Chert," Pilbara, Australia. SEM micrograph in backscatter mode.

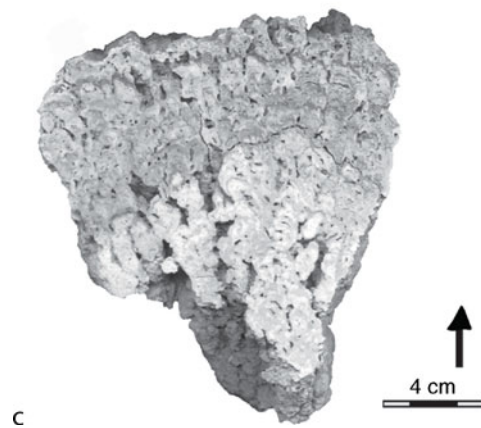
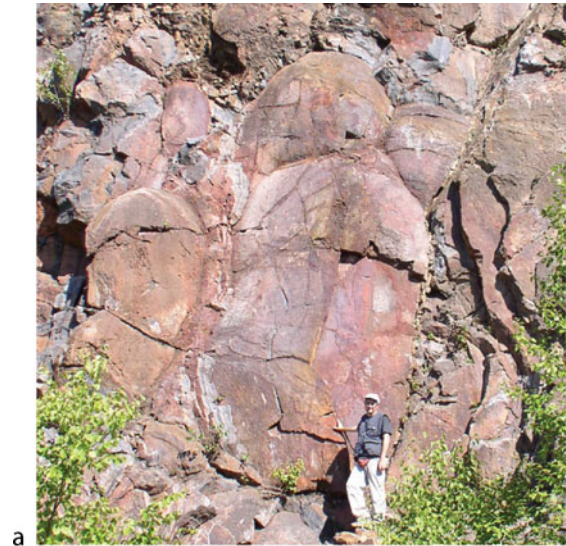


Biosignatures in Rocks, Figure 2 Macrometer-scale biosignatures of in rocks. Devonian cold seep mound, Hollard Mound (Morocco), with a core mound facies dominated by microbial hydrocarbon-derived carbonate. The mound on the right is 30 m high.

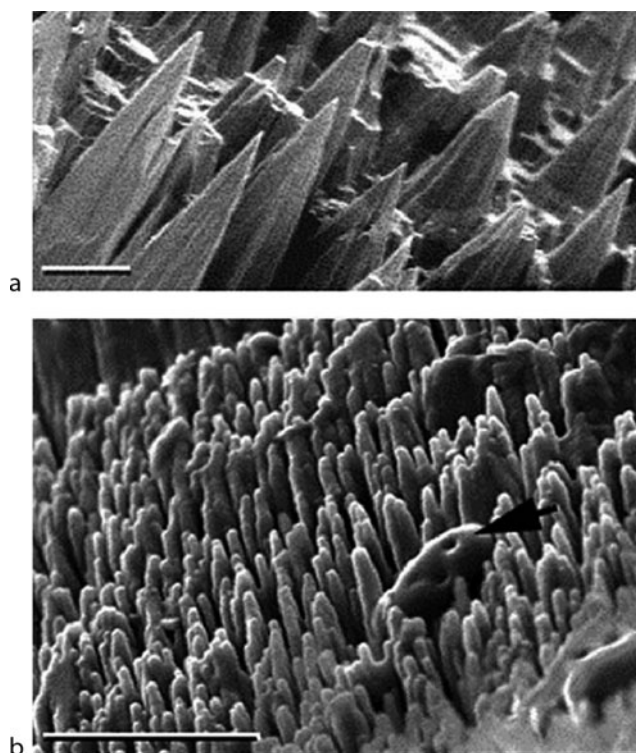
surfaces of minerals colonized by microorganisms may be different to that of the bulk mineral because the microorganisms leach out those elements that are important for their metabolic activities. For instance, sulfur can be leached from sulfides by sulfur-oxidizing bacteria (McGuire et al., 2001) and phosphorus from P-containing minerals is leached in P-poor environments (Taunton et al., 2000a, b). Also, unusual concentrations of trace elements may be associated with mineral-entombed microorganisms since the microbes preferentially concentrate elements that are necessary for their metabolic functions, such as Co, Ni, Cu, Mn, Mo, Se, and V, (Banfield et al., 2001).

The incorporation of organic compounds within a mineral structure may be indicative of biologically induced precipitation as, for instance, when microorganisms are permeated and entombed by minerals (Westall et al., 1995, 2000). Indeed, association of organic molecules with microfossils is a key complementary biosignature for traces of early life on Earth (Westall and Southam, 2006).

Whereas biologically controlled mineral growth produces specific crystalline shapes, biologically induced mineralization produces minerals with non-perfect habits (Banfield et al., 2001) due to alteration of the stability of the crystal surface in the presence of organics (Mann et al., 1993; Albeck et al., 1996). However, the presence of organic matter does not always have an influence on the morphology of a mineral precipitate. For instance, Orange et al. (2009) note that silica precipitated in nutrient media with and without cells had exactly the same structure, the only difference being the visible incorporation of EPS in the medium with cells.



Biosignatures in Rocks, Figure 3 Biosignatures of macro- and mesometric scale in rocks. (a) Huge 2.8-billion-year old stromatolites in the Steep Rock Group, Ontario, Canada. (Person as scale is 1.90 m high). (b) Lithified active lacustrine stromatolites, Lake Thetis, Western Australia. (c) Polished slab from (a) showing internal thrombotic to stromatolitic columnar structures. Arrow indicates growth direction. (a) Photograph by F. Westall; (b, c) photographs by B. Cavalazzi and A. D. George.



Biosignatures in Rocks, Figure 4 Experimental dissolution of apatite (a) in an inorganic acid and (b) in the presence of actively metabolizing cells. Arrow in (b) indicates a cellular body. Scale bars = 2 μm . (Images reprinted with permission from Banfield et al., 2001).

A number of studies have noted a distinct difference in the size of minerals precipitated in the presence of microorganisms and those precipitated abiologically with the former being smaller than the latter. Douglas et al. (2008), for example, document smaller biofilm-induced calcite and Mn-oxyhydroxide mineral precipitates compared to abiogenic gypsum precipitates in an unusual microbial mat from a Death Valley pool.

Isotopic fractionation: Until recently, the preference of microorganisms for the lighter stable isotope of carbon ^{12}C over the heavier one, ^{13}C was considered to be one of the most important biosignatures in rocks. Indeed, Schidlowski (1988) had shown that these signatures can be traced back to rocks 3.8 Ga-old from the Isua province in Greenland. Recent studies, however, have demonstrated that $^{12}\text{C}/^{13}\text{C}$ ratios similar to those produced by living organisms occur in carbon produced by abiotic hydrothermal/metasomatic Fischer–Tropsch synthesis (e.g., van Zuilen et al., 2002). Contamination of the rock by younger organisms can also produce an erroneous biosignature. Westall and Folk (2003), for instance, identified Holocene fossilized endolithic microorganisms in the Isua banded iron formation that was used for Schidlowski's (1988) isotopic studies.

Biosignatures of structural components

Cells, colonies, biofilms, mats, EPS

Microorganisms generally occur as assemblages (colonies) of cells that may be monospecific, but, more often than not are plurispecies. Colonies are often linked by a film of polymers known as extracellular polymeric substances (EPS). Colonies coating a substrate in a two-dimensional manner are termed biofilms. Microbial mats are complex structures formed by layers of biofilms and may be microscopic to macroscopic in size, sometimes reaching dimensions of several square kilometers, for instance, on the floors of lagoons (e.g., Margulis et al., 1980). Although typically associated with the activity of photosynthetic microorganisms in shallow-water environments, microbial mats may also be constructed by non-photosynthetic microorganisms, such as the sulfur-oxidizers *Beggiatoa*, in deep-water habitats on the flanks of deep-sea hydrothermal vents and at hydrocarbon seeps (e.g., Kelley et al., 2005).

The structural components, cells, colonies, biofilms, mats, and EPS, may be preserved in rocks in a number of ways. Cells may leave organic walled impressions, mineral-coated or impregnated structures, or empty casts in a mineral precipitate. Biofilms and mats may also be preserved as organic impressions in sediments or mineralized structures.

Preservation of microbial structures

Organic walled structures: Cells walls can be preserved as organic impressions in fine-grained, anaerobic sediments (Javaux et al., 2001). The fixation of metals, such as Fe, on cell envelopes may retard lysis, thus contributing to the potential for preservation of cell walls (Ferris et al., 1988). While structures such as pollen spores are large enough to be visible in the rocks using light microscopy, individual microbial cells are often difficult to identify *in situ* in sediments because of their small size. Study of these organic remains is derived from palynological methods in which the detrital silicates encasing the cells are eliminated by acid-digestion of the rock. A major problem with this method is that microorganisms commonly colonize fractures in rocks, thus introducing younger contamination (e.g., Westall and Folk, 2003; Walsh and Westall, 2008). Such contamination led Pflug (1979) to identify eukaryotic yeast cells in a 3.8 Ga-old rock from Isua as *in situ* microfossils. This type of problem can be overcome by verifying the maturity of the kerogen by high-resolution TEM and Raman spectroscopy and/or by demonstrating chemical bonding between the organic molecules and the mineral matrix that indicates the syngenecity of the kerogen (Marshall et al., 2005; Derenne et al., 2008).

Mineralized microbial structures: The most common form of preservation of microbial structures is mineral fossilization. In this process, minerals bind to the organic surfaces of the cells/EPS in a passive reaction. The surfaces of microorganisms and EPS are very reactive, containing

ionized ligands to which mineral nuclei can attach themselves. The sorption reactions may take place via cation bridging, hydrogen bonding with functional groups such as carboxyls or hydroxyls, or by direct electrostatic fixation of the mineral to the functional groups (Lalonde et al., 2005). The attached nuclei then attract further nuclei, resulting in encrustation or permeation of the organic structure. The microbial surfaces and exopolymers therefore act as “mineralizing templates” (Konhauser, 2007). Depending upon the availability of the minerals in solution, the microorganisms may be completely entombed in a mineral precipitate. The type of mineral precipitated depends on the composition of the fluids and the redox conditions in the immediate environment of the microorganism. Many mineral phases can bind to microbial cell walls (Konhauser, 2007), including silica (Figure 1), carbonates (Ca, MgCa, Fe, Mn), metal oxides/hydroxides (Fe/Mn and magnetite), sulfates (Ca, Sr, Ba, Fe), sulfides (Fe, Ni, Pb, Zn, CuFe), phosphates (Ca), clays, and zeolites.

As noted above, cell wall composition and structure plays an important role in the fixation of metals because of the differential distribution of functional groups in different cell components. Mineral nucleation is similarly affected. Thus, Gram positive bacteria with their thick outer layer of peptidoglycan and the abundance of functional groups can be encased by a thick mineral crust, whereas the Gram negative bacteria with their thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer only fixed a thin, delicate crust (Westall, 1997). The latter author concluded that Gram negative cells therefore had less chance of being preserved in the rock record than Gram positive ones.

The cell walls of Archaea differ in their composition and structure to those of Bacteria and are also more variable. *Crenarchaeota* are characterized by a layer of paracrystalline proteins, the S-layer, which is loosely associated with the plasma membrane (NB: S-layers are also common around many species of Bacteria). The *Euryarchaeota* exhibit great variability in the composition of their cell walls, including a peptidoglycan-like component, pseudomurein. Experimental silicification of an Archaea having an S-layer, the hyperthermophile *Pyrococcus abyssi* showed that it produced a delicate silica crust, similar to that forming around Gram negative bacteria (Orange et al., 2009).

What is the fate of the organic components in a mineralized cell? Experimental fossilization shows that the macromolecules of the cell envelope may remain trapped in the mineral crust (Westall et al., 1995). The cytoplasmic space, on the other hand, may be empty after lysis, or, if lysis did not occur, it may contain balls of degraded macromolecules that can also nucleate minerals (in the process producing false nuclei, Westall et al., 1995). As noted above, the organic components of fossilized microbial structures may be preserved in anaerobic environments but they can be completely oxidized in

oxidizing environments. For example, the silicified remains of cyanobacteria in hot spring deposits from Yellowstone no longer contain any organic molecules (Cady and Farmer, 1996).

In order to be preserved in the rock record, the mineral-coated/permeated microbial structure needs to become encased in a mineral cement or by fine-grained sediments. Here, further diagenetic changes may take place, including changes in mineralogy (e.g., transformation of oxyhydroxides to oxides), replacement (complete or partial) of one mineral by another (e.g., silicification of carbonate mineralized remains), or dissolution. The final mineral or sediment-encased microbial fossils may exhibit different morphological preservation modes.

Artifacts: Abiological mineral precipitates can be notoriously confused with fossilized microorganisms. Many minerals may form simple spherical, oval, elongated, and even twisted morphologies. Silica spheres may occur in aggregates that look surprisingly like dividing microorganisms (e.g., Monty et al., 1991). Jones (2004) found that spherical silica aggregates nucleated onto a mucus thread in a siliceous hot spring forming filamentous structures that could possibly be mistaken for silica encrusted filaments. García-Ruiz et al. (2003) even showed that artificial mixed silica/organic precipitates could produce microbial-like morphologies (bacteriomorphs). However, these precipitates were not produced from naturally occurring solutions.

Examples of mineralized microbial structures: Microbial fossils occur throughout almost the whole of the rock record. The oldest known microfossils are found in silicified shallow-water volcanic sediments from the 3.5–3.3 Ga Barberton and Pilbara greenstone belts in which filaments, coccoidal and rod-shaped silicified microorganisms and associated EPS have been documented (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b). [Older sediments from the 3.8 Ga-old Isua greenstone belt in Greenland are too highly metamorphosed to contain identifiable, syngenetic microfossils.] The very small size of the early Archaean microbial structures means that sophisticated instrumentation having high spatial and analytical resolution is necessary to observe and analyze them (Westall and Southam, 2006). Moreover, given the problem of establishing the biogenicity and syngenicity of microbial remains in these ancient sediments, multiple lines of evidence are required, including morphological, chemical, and isotopic biosignatures. In this way, Westall et al. (2006a, b) documented multispecies colonies of probable chemolithotrophic microorganisms within 3.446 Ga-old mudflat sediments from the Pilbara and microbial mats on the surfaces of littoral sediments in a 3.334 Ga-old formation from Barberton. The small crystal lattice of silica results in excellent preservation of fine textural detail, such as cell surface wrinkles (Westall et al., 2006a). Silicified (permineralized) microfossils occur throughout geological time (Knoll, 1985). Proterozoic formations contain extremely well-preserved cyanobacterial

fossils, for instance (Green et al., 1989), and silicified microfossils have been documented from numerous Phanerozoic hot spring environments (Jones et al., 2001; Jones, 2004).

The oldest calcified microfossils are relatively poorly preserved benthic cyanobacteria from the 2.6 Ga Campbellrand Subgroup in South Africa (Kazmierczak and Altermann, 2002). Well-preserved calcified microfossils occur in younger Proterozoic formations, such as the Draken Formation, Svalbard (Knoll et al., 1993).

Microfossils preserved by other minerals, such as pyrite, occur through much of the geological record, the oldest such fossils occurring in the above-mentioned 3.2 Ga-old massive sulfide deposits from the Pilbara (Rasmussen, 2000) and younger fossils occurring in the Proterozoic, e.g., pyritized filaments in stromatolites from the Altyn Formation of Montana (White, 1974), or pyritized biofilms coating soft body parts (Canfeld and Raiswell, 1991). Phosphatized microfossils are known from the Phanerozoic, for example, phosphate coatings on cyanobacteria in Cambrian sediments from Australia (Southgate, 1986) or phosphatized microbial biofilms on the soft body parts of Eocene eucaryotes (Liebig et al., 1996).

Microbialites and microbial fabrics in rocks

The physical expressions of microorganisms and the products of their enzymatic and metabolic activities result in microbialite structures and textures that are characterized by a particular internal structure or texture. They include a wide range of macroscopic to microscopic phenomena, such as stromatolites, thrombolites, oncolites, biolaminites, and crusts (Figure 3). Macro- to micro-scale fabrics and textures related to the presence of microorganisms also include MISS (the MISS of Noffke et al., 2003), and microfossils (Burne and Moore, 1987; Monty et al., 1995; Riding and Awramik, 2000). Microbialites and microbial fabrics are found in rocks throughout the geological record with the oldest stromatolites occurring in 3.5–3.3 Ga-old formations in the Pilbara of Australia and in Barberton, South Africa (Lowe, 1980; Walter, 1983; Byerly et al., 1986; Hofmann et al., 1999; Allwood et al., 2006). The biogenic origin of the early Archaean stromatolites has been challenged by Lowe (1994) and Lindsay et al. (2005) but Riding (2008) suggests that some of the Precambrian stromatolites may be mixtures of microbial and abiogenic, stromatolite-like fabrics. The large and diverse stromatolites characteristic of the Proterozoic declined in importance throughout the latest Precambrian to Cambrian times and, from the early Cambrian onwards, microbialite structures are dominated by microbial mounds formed by non-stromatolitic calcifying microbes (e.g., cyanobacteria, Monty, 1995). Microbial mounds are well-developed throughout the geological record spanning from the Proterozoic to the Recent in both shallow- and deep-water settings and are considered to be a significant expression of the history of Earth's microbial life (Figure 2) (Monty, 1995). Both stromatolites and

microbial mounds are characterized by the activity of microbes which calcify, trap, and bind sediments, forming delicate and persistent microstructures rich in micrite (microcrystalline calcite) and organomicrite. Microbially generated mounds and microbialites formed in shallow-water environments mainly involve photosynthetic/phototrophic and heterotrophic microbes. In deeper waters, however, chemosynthetic microbes are associated with hydrocarbon seeps and hydrothermal-related mound deposits (Peckmann and Thiel, 2004). Deep-water stromatolitic fabrics and crusts have been described from hydrocarbon seeps on the Aleutian accretionary margin as a product of methane-consuming Archaea (Greinert et al., 2002).

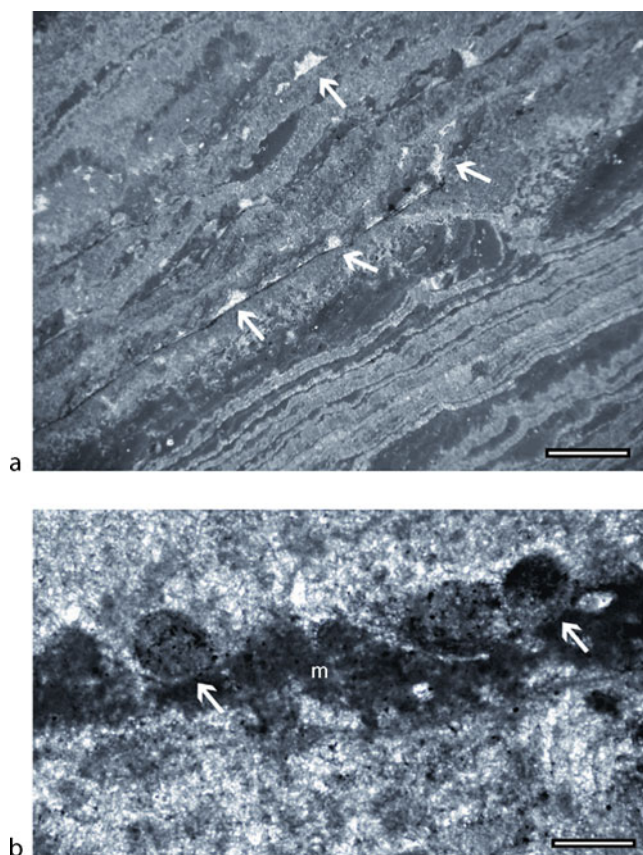
The most common microbially induced fabrics from carbonate rocks are clotted and peloidal fabrics (Burne and Moore, 1987; Shapiro, 2000; Flugel, 2004). These fabrics consist of spherical-to-elliptical microscopic aggregates of microcrystalline clots or peloids (Figure 5a) (e.g., calcite, aragonite), cemented by carbonate and/or sediments (MacIntyre, 1985; Shapiro, 2000). Fenestral and stromatactis fabrics from organic-rich sedimentary deposits (Figure 5b), such as microbial mounds or geothermal springs (e.g., travertine), are microbiological in origin (Flugel, 2004). Encrustations, concretions, and coatings are generally microbial products that contain a record (morphological, geochemical, and/or isotopic) in their sedimentary phases of the biochemical processes involved in their genesis (Raiswell and Fisher, 2000; Kiriakoulakis et al., 2000). For instance, Fe-Mn coatings and pyritiferous carbonate nodules are considered to be microbial fabrics (Peckmann and Thiel, 2004).

Microbially induced sedimentary structures (MISS) are formed by the rhythmic alternation of photosynthetic microbial mats and sediments in mudflat environments (Noffke, 2008). MISS are widespread in the geological record and have also been identified in sediments 3.2 Ga-old from South Africa (Noffke et al., 2003).

Banded iron formations (BIFs) occur throughout the rock record from 3.8 Ga onwards (BIFs occur in the Isua greenstone belt in Greenland). The influence of microorganisms on the formation of these laminated accumulations of iron oxides and chert is the subject of much controversy. Photochemical oxidation of ferric iron of hydrothermal origin is one explanation (Cairns-Smith, 1978) but other biological controls have also been suggested, such as oxidation by oxygenic photosynthesizers (Cloud, 1965), or simple sorption of Fe^{2+} onto microbial cell surfaces to catalyze a basically abiological precipitation process (Konhauser, 2000), or the precipitation of Fe oxides by Fe oxidizing microorganisms (Posth et al., 2008).

Relevance of biosignatures and their astrobiological implications

We have seen above that a number of the biosignatures described can be imitated by abiological processes



Biosignatures in Rocks, Figure 5 Micrometer-scale biosignatures in rocks. Transmitted light photomicrographs of petrographic thin sections. (a) Continuous and planar laminations of stromatolite structures from the Devonian Hollard Mound (Morocco). The arrows indicate the spar-filled cavities generating fenestral fabrics. (b) Detail from (a) showing a lamina of fine gray micrite (m) that locally consists of microbially produced peloids (arrows). Scale bars: (a) = 5 mm; (b) = 200 μ m. (Images reprinted with permission from Cavalazzi, 2007, and Cavalazzi et al., 2007).

resulting in significant controversy. These are serious limitations when searching for the first traces of life on Earth or for biosignatures on other planets. How can nonliving molecules be distinguished from very primitive living cells – and in the fossilized form? A carbon isotope signature on its own is not sufficient because it could be either abiogenic or biogenic in origin. A collection of simple-shaped minerals, even joined together with compromised boundaries, could represent abiogenic precipitates or possibly a fossilized microbial colony. Identification of biosignatures in ancient terrestrial or extraterrestrial materials therefore needs to be based on a combination of structural, chemical, and isotopic traces that are related to the three main characteristics of living organisms: their physical structures, the chemical components of the structures, and their metabolic or living processes (Westall and Southam, 2006; Westall, 2008). Another aspect to take

into account is the fact that living organisms by their very existence and metabolism interact with and influence their immediate environment.

On a biotic world where the context of structure, fabric, and composition are well-constrained, certain characteristics, such as element enrichment, mineral composition, shape, and size can be used as complementary evidence for the existence of living organisms. Can these characteristics be used on a world where the existence of life forms is uncertain, for instance on the primitive Earth or Mars? Prebiotic molecules of mostly extraterrestrial origin, characterized by a wide range in composition and structure, including simple amino acids, polycyclic acidic hydrocarbons (PAHs), thiophenes, fullerenes etc. (Cronin, 1998), abounded on these worlds (Maurette et al., 2000). Could prebiotic organic macromolecules scavenge elements from the environment in the same way as biomolecules and, if so, would some elements be preferentially fixed? These macromolecules have very few of the functional groups that characterize biogenic molecules, and it is therefore unlikely that they will be able to induce significant metal fixation. Thus, element concentration and distribution in potentially biogenic materials (organic or mineralized organics) could be considered as a useful complementary biosignature when associated with other types of biosignatures. Could prebiotic organic molecules induce mineral precipitation? Again this is unlikely since biominerals precipitate because of changes in the environment caused by the excretion of metabolic waste products, or enzymatic mediated changes in the redox state, or the presence of a charged cell surface that attracts mineral nucleation, conditions that are not met by prebiotic molecules. On the other hand, the physical presence of prebiotic molecular matter in an environment where minerals are being precipitated abiotically from supersaturated solutions (saline ponds, hot springs, the early, silica-saturated oceans) could eventually have an influence on the size and shape of minerals because of alteration in the stability of the crystal surfaces (cf. Mann et al., 1993; Albeck et al., 1996).

Once the biogenicity and syngenicity of the different biosignatures in a rock have been established, how are they to be interpreted? What do they tell us about the community of microorganisms that lived in a particular environment? Are all the original microorganisms in the community preserved? This is particularly relevant because not all microorganisms can be fossilized. For instance, Orange et al. (2009) noted that, although two species of thermophilic Archaea, *Pyrococcus abyssi* and *Methanocaldococcus janaschii* had similar cell wall structures and compositions, the former could be experimentally silicified whereas the latter naturally lysed before mineral nuclei could fix to the cell walls.

Another aspect of the study of biosignatures is the importance of observation and analysis at the scale of the microorganisms (Westall and Southam, 2006; Westall, 2008, 2009). Many fossilized microorganisms are very small and cannot readily be distinguished from abiogenic

mineral accumulations at the optical microscope scale. They need to be studied using higher-resolution electronic microscopes. The question of scale is also pertinent to analysis of chemical or isotopic signatures. What information about the microbial community is provided by isotope measurements made on kerogen extracted from the bulk rock when the rock may contain traces of different microbial communities at the micrometer scale? The microbial communities (colonies, biofilms, and mats) may, themselves, consist of multiple species, thus, resulting in additional mixture of the isotopic signature. To complicate matters even further, specific components of an individual cell may have different isotopic signatures. For example, van de Meer et al. (2000) note that the carbon isotope composition of the lipids of *Chloroflexus aurantiacus* differ from the bulk isotopic composition of the cells. This means that isotope measurements made even *in situ* (e.g., with a spot size of ~50 μm , van Zuilen et al., 2007) need to be interpreted with caution.

Conclusion

While it is clear that the study of biosignatures in rocks is not without difficulties, these can be overcome by combining signatures of the different aspects of living organisms, i.e., structural, chemical, and isotopic traces, as well as evidence for the interaction of living organisms with the environment. Further investigation of the microbial communities that produced the biosignatures needs to be undertaken at the microbial scale in order to obtain relevant information about the types of organisms and their metabolic activities. This is a challenge for spatial and analytical resolution but it is this level of detail that is necessary for disentangling the bulk biosignatures from those produced by the individual microorganisms and for better understanding the complexity of past communities.

Bibliography

- Albeck, S., Addadi, L., and Weiner, S., 1996. Regulation of calcite crystal morphology by intracrystalline acidic proteins and glycoproteins. *Connective Tissue Research*, **35**, 419–424.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–718.
- Banerjee, N. R., Furnes, H., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2006. Preservation of 3.4–3.5 Ga microbial biomarkers in pillow lavas and hyaloclastites from the Barberton greenstone belt, South Africa. *Earth and Planetary Science Letters*, **241**, 707–722.
- Banfield, J. F., Moreau, J. W., Chan, C. S., Welch, S. A., and Little, B., 2001. Mineralogical biosignatures in search for life on Mars. *Astrobiology*, **1**, 447–465.
- Bazylinski, D. A., Frankel, R. B., Heywood, B. R., Mann, S., King, J. W., Donaghay, P. L., and Hanson, A. K., 1995. Controlled biomineralisation of magnetite (Fe_3O_4) and greigite (Fe_3S_4) in a magnetotactic bacterium. *Applied and Environmental Microbiology*, **61**, 3232–3239.
- Beveridge, T. J., Forsberg, C. W., and Doyle, R. J., 1982. Major sites of metal binding in *Bacillus licheniformis* walls. *Journal of Bacteriology*, **150**, 1438–1448.
- Brack, A., 2002. Water, the spring of life. In Horneck G., and Baumstark-Khan, C. (eds.), *Astrobiology. The Quest for the Conditions of Life*. Berlin: Springer, pp. 79–88.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1036.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaios*, **2**, 241–254.
- Byerly, G. R., Walsh, M. M., and Lowe, D. L., 1986. Stromatolites from the 3300–3500 Myr Swaziland Supergroup, Barberton Mountain Land, South Africa. *Nature*, **319**, 489–491.
- Cady, S. L., and Farmer, J. D., 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In Bock, G. R., and Goode, J. A. (eds.), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*, Ciba Foundation Symposium 202. Chichester: Wiley, pp. 150–173.
- Cairns-Smith, A. G., 1978. Precambrian solution photochemistry, inverse segregation, and banded iron formations. *Nature*, **276**, 807–808.
- Canfield, D. E., and Raiswell, R., 1991. Pyrite formation and fossil preservation. In Allison, P. A., and Briggs, D. E. G. (eds.), *Taphonomy: Releasing the Data Locked in the Fossil Record*. New York: Plenum, pp. 337–387.
- Cavalazzi, B., 2007. Chemotrophic filamentous microfossils from the Hollard Mound (Devonian, Morocco) as investigated by focus ion beam. *Astrobiology*, **7**, 402–415.
- Cavalazzi, B., Barbieri, R., and Ori, G. G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Research*, **54**, 289–316.
- Chang, S.-B. R., and Kirschvink, J. L., 1989. Magnetofossils, the magnetization of sediments, and the evolution of magnetite biomineralization. *Annual Review of Earth and Planetary Sciences*, **17**, 169–195.
- Cloud, P. E., 1965. Significance of the Gunflint (Precambrian) microflora. *Science*, **148**, 27–35.
- Cronin, J. R., 1998. Clues from the origin of the Solar System: meteorites. In Brack, A. (ed.), *The Molecular Origins of Life: Assembling Pieces of the Puzzle*. Cambridge: Cambridge University Press, pp. 119–146.
- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J. -N., 2008. Molecular evidence for life in the 3.5 billion-year old Warreawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Douglas, S., Abbey, W., Mielke, R., Conrad, P., and Kanik, I., 2008. Textural and mineralogical biosignatures in an unusual microbialite from Death Valley, California. *Icarus*, **193**, 620–636.
- Eigenbrode, J., and Freeman, K. H., 2006. Late Archaean rise of aerobic microbial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15759–15764.
- Ferris, G., Fyfe, W. S., and Beveridge, T. J., 1988. Metallic ion binding by *Bacillus subtilis*: implications for the fossilization of microorganisms. *Geology*, **16**, 149–152.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks. Analysis, Interpretation and Application*. Berlin, Heidelberg: Springer.
- Friedmann, E. I., Wierzbos, J., Ascaso, C., and Winklhofer, M., 2001. Chains of magnetite crystals in the meteorite ALH84001: evidence of biological origin. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2176–2181.

- Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- García-Ruiz, J. M., Hyde, S. T., Camerup, A. M., Christy, A. G., van Kranendonk, M. J., and Welham, N. J., 2003. Self-assembled silica-carbonate structures and detection of ancient microfossils. *Science*, **302**, 1194–1197.
- Geesey, G. G., and Jang, L., 1989. Interactions between metal ions and capsular polymer. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley, pp. 325–357.
- Golden, D. C., Ming, D. W., Morris, R. V., Brearley, A. J., Lauer, Jr. H. V., Treiman, A. H., Zolensky, M. E., Schwandt, C. S., Lofgren, G. E., and McKay, G. A., 2004. Evidence for exclusively inorganic formation of magnetite in Martian meteorite ALH84001. *American Mineralogist*, **89**, 681–695.
- Green, J. W., Knoll, A. H., and Swett, K., 1989. Microfossils from silicified stromatolitic carbonates of the Upper Proterozoic Limestone-Dolomite 'Series', central East Greenland. *Geological Magazine*, **126**, 567–585.
- Greinert, J., Bohrmann, G., and Elvert, M., 2002. Stromatolitic fabric of authigenic carbonate crusts in 4850 m water depth, Aleutian accretionary margin: result of anaerobic methane oxidation by archaea at cold seeps. *International Journal of Earth Sciences*, **91**, 698–711.
- Hofmann, H. J., Grey, K., Hickman, A. H., and Thorpe, R. I., 1999. Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. *Geological Society of America Bulletin*, **111**, 1256–1262.
- Javaux, E. J., Knoll, A. H., and Walter, M. R., 2001. Morphological and ecological complexity in early eukaryotic ecosystems. *Nature*, **412**, 66–69.
- Jones, B., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Geological Society, London*, **161**, 983–993.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2001. Taphonomy of Silicified Filamentous Microbes in Modern Geothermal Sinters-Implications for Identification. *Palaios*, **16**, 580–592.
- Kazmierczak, J., and Altermann, W., 2002. Neoproterozoic biomineralization by benthic cyanobacteria. *Science*, **298**, 2351.
- Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted submarine ecosystem: The Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- Kiriakoulakis, K., Marshall, J. D., and Wolff, G. A., 2000. Biomarkers in a Lower Jurassic concretion from Dorset (UK). *Journal of the Geological Society*, **157**, 207–220.
- Knoll, A. H., 1985. Exceptional preservation of photosynthetic organisms in silicified carbonates and silicified peats. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **311**, 111–122.
- Knoll, A. H., Fairchild, I. J., and Swett, K., 1993. Calcified microbes in Neoproterozoic carbonates: implications for our understanding of the Proterozoic/Cambrian transition. *Palaios*, **8**, 512–525.
- Konhauser, K. O., 2000. Hydrothermal bacterial biomineralisation: potential modern-day analogues for Precambrian banded iron formation. In Glenn, C. R., Prévôt-Lucas, L., and Lucas, J. (eds.), *Marine Authigenesis: from Global to Microbial*. SEPM Special Publications No 66, pp. 133–145.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Science.
- Konhauser, K. O., Fyfe, W. S., Schultze-Lam, S., Ferris, F. G., and Beveridge, T. J., 1994. Iron phosphate precipitation by epilithic microbial biofilms in Arctic Canada. *Canadian Journal of Earth Sciences*, **31**, 1320–1324.
- Kucha, H., Schroll, E., and Strumpf, E. F., 2005. Fossil sulphate-reducing bacteria in the Bleiberg lead-zinc deposit, Austria. *Mineralium Deposita*, **40**, 123–126.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A. -L., and Ferris, F. G., 2005. The experimental silicification of Aquificales and their role in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Laval, B., Cady, S. L., Pollack, J. C., McKay, C. P., Bird, J. S., Grotzinger, J. P., Ford, D. C., and Bohm, H. R., 2000. Modern freshwater microbialite analogues for ancient dendritic reef structures. *Nature*, **407**, 626–629.
- Ledin, M., Krantz-Rülker, C., and Allard, B., 1999. Microorganisms as metal sorbants: comparison with other soil constituents in multi-compartmental systems. *Soil Biology and Biochemistry*, **31**, 1639–1648.
- Lemelle, L., Labrot, P., Salomé, M., Simionovici, A., Viso, M., and Westall, F., 2008. In situ imaging of organic sulfur in 700–800 My-old Neoproterozoic microfossils by X-ray spectromicroscopy at the S K-edge. *Organic Geochemistry*, **39**, 188–202.
- Lepot, K., Benzerara, K., Brown, G., and Philippot, P., 2008. Microbially influenced formation of 2,724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Liebig, K., Westall, F., and Schmitz, M., 1996. A study of fossil microstructures from the Eocene Messel formation using transmission electron microscopy. *Neues Jahrbuch für Geologie und Paläontologie-Monatshefte*, **4**, 218–231.
- Lindsay, J. F., Brasier, M. D., McLoughlin, N., Green, O. R., Fogel, M., Steele, A., and Mertzman, S. A., 2005. The problem of deep carbon - an Archean paradox. *Precambrian Research*, **143**, 19–22.
- Lowe, D. R., 1980. Stromatolites 3,400-Myr old from the Archean of Western Australia. *Nature*, **284**, 441–443.
- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 287–390.
- MacIntyre, I. G., 1985. Submarine cements - the peloidal question. In Schneidermann, N., and Harris, P. M. (eds.), *Carbonate Cements*. SEPM Special Publications 36, pp. 109–116.
- Mann, S., 1988. Molecular recognition in biomineralisation. *Nature*, **332**, 119–124.
- Mann, S., Archibald, D. D., Didymus, J. M., Douglas, T., Heywood, B. R., Meldrum, F. C., and Reeves, N. J., 1993. Crystallisation at inorganic-organic interfaces-biominerals and biomimetic synthesis. *Science*, **261**, 1286–1292.
- Margulis, L., Barghoorn, E. S., Ashendorf, D., Banerjee, S., Chase, D., Francis, S., Giovannoni, S., and Stolz, J., 1980. The microbial community in the layered sediments at Laguna Figueroa, Baja California, Mexico: does it have Precambrian analogues. *Precambrian Research*, **11**, 93–123.
- Marshall, C. P., Javaux, E. J., Knoll, A. H., and Walter, M. R., 2005. Combined micro-Fourier transform infrared (FTIR) spectroscopy and micro-Raman spectroscopy of Proterozoic acritarchs: a new approach to Palaeobiology. *Precambrian Research*, **138**, 208–224.
- Maurette, M., Duprat, J., Engrand, C., Gounelle, M., Kurat, G., Matrajt, G., and Toppani, A., 2000. Accretion of neon, organics, CO₂, nitrogen and water from large interplanetary dust particles on the early Earth. *Planetary and Space Science*, **48**, 1117–1137.
- McGuire, M. M., Edwards, K. J., Banfield, J. F., and Hammers, R. J., 2001. Kinetics, surface chemistry, and structural evolution of microbially mediated sulphide mineral dissolution. *Geochimica et Cosmochimica Acta*, **65**, 57–72.

- McKay, D. S., Gibson, E. K., Thomas-Keptra, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chillier, X. D. F., Maechling, C. R., and Zare, R. N., 1996. Search for past life on Mars: possible relic biogenic activity in martian meteorite ALH8400. *Science*, **273**, 924–930.
- Mero, J. L., 1962. Ocean-floor manganese nodules. *Economic Geology*, **57**, 747–767.
- Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P., and Friend, C. R. L., 1996. Evidence for life on Earth before 3,800 million years ago. *Nature*, **384**, 55–59.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-Mounds: Their Origin and Evolution*. Oxford: Blackwell, International Association of Sedimentologists, Special Publication 23, pp. 11–48.
- Monty, C. L. V., Westall, F., and Van Der Gaast, S., 1991. The diagenesis of siliceous particles in Subantarctic sediments, ODP Leg 114, Hole 699: possible microbial mediation. In Ciesielski, P. F., Kristoffersen, Y. et al. (eds.), *Proceedings ODP Scientific Results, 114*. College Station, TX (Ocean Drilling Program), pp. 685–710.
- Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R., 1995. *Carbonate Mud-Mounds: Their Origin And Evolution*. Oxford: Blackwell, International Association of Sedimentologists, Special Publication 23.
- Noffke, N., 2008. Turbulent lifestyle: microbial mats on Earth's sandy beaches-today and 3 billion years ago. *GSA Today*, **18**(10), 4–9.
- Noffke, N., Gerdes, G., and Klenke, Th., 2003. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty and evaporitic carbonatic). *Earth-Sciences Reviews*, **12**, 1–14.
- Orange, F., Westall, F., Prieur, D., Biennu, N., Le Romancer, M., Disnar, J.-R., and Défarge, C., 2009. Experimental silicification of the extremophilic Archaea *Pyrococcus abyssi* and *Methanocaldococcus jannaschii*. Applications in the search for evidence of life in early Earth and extraterrestrial rocks. *Geobiology*, **7**, 403–418.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Pflug, H. D., 1979. Archean fossil finds resembling yeasts. *Geologica et Palaeontologica*, **13**, 1–8.
- Pollmann, K., Raff, J., Merroun, M., Fahmy, K., and Selenska-Pobell, S., 2006. Metal binding by bacteria from uranium mining waste piles and its technological applications. *Biotechnology Advances*, **24**, 58–68.
- Posth, N. R., Hegler, F., Konhauser, K. O., and Kappler, A., 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. *Nature Geoscience*, **1**, 703–708.
- Raiswell, R., and Fisher, Q. J., 2000. Mudrock-hosted carbonate concretions: a review of growth mechanisms and their influence on chemical and isotopic composition. *Journal of the Geological Society*, **157**, 239–251.
- Rasmussen, B., 2000. Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature*, **405**, 676–679.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J., and Kilburn, M. R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, **455**, 1101–1104.
- Riding, E. R., and Awramik, S. M., 2000. *Microbial Sediments*. Berlin Heidelberg: Springer.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2008. Abiogenic, microbial and hybrid authigenic carbonate crusts: components of Precambrian stromatolites. *Geologica Croata*, **61**, 73–103.
- Schidlowski, M., 1988. A 3800 million-year isotopic record of life from carbon in sedimentary rocks. *Nature*, **333**, 313–318.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaio*, **15**, 166–169.
- Southgate, P. N., 1986. Cambrian phoscrete profiles, coated grains, and microbial processes in phosphogenesis: Georgina Basin, Australia. *Journal of Sedimentary Petrology*, **56**, 429–441.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, J. H., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Summons, R. E., Albrecht, P., McDonald, G., and Moldowan, J. M., 2008. Molecular biosignatures. *Space Science Reviews*, **135**, 115–132.
- Taunton, A. E., Welch, S. A., and Bandfield, J. F., 2000a. Geomicrobial controls on light rare earth element, Y, and Ba distributions during granite weathering and soil formation. *Journal of Alloys and Compounds*, **303**, 30–36.
- Taunton, A. E., Welch, S. A., and Bandfield, J. F., 2000b. Microbial controls on phosphate and lanthanide distributions during granite weathering. *Chemical Geology*, **169**, 371–382.
- Thomas-Keptra, K. L., Bazylinski, D. A., Kirschvink, J. L., Clemett, S. J., McKay, D. S., Wentworth, S. J., Vali, H., Gibson, E. K., and Romanek, C. S., 2001. Truncated hexa-octahedral magnetite crystals in ALH84001: presumptive biosignatures. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2164–2169.
- Thomas-Keptra, K. L., Clemett, S. J., Bazylinski, D. A., Kirschvink, J. L., McKay, D. S., Wentworth, S. J., Vali, H., Gibson, E. K., and Romanek, C. S., 2002. Magnetofossils from ancient Mars: a robust biosignature in the martian meteorite ALH84001. *Applied and Environmental Microbiology*, **68**, 3663–3672.
- Thorseth, I. H., Furnes, H., and Tumyr, O., 1995. Textural and chemical effects of bacterial activity on basaltic glass: an experimental approach. *Chemical Geology*, **119**, 139–160.
- van de Meer, M. T. J., Schouten, S., De Leeuw, J. W., and Ward, D. M., 2000. Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. *Environmental Microbiology*, **2**, 428–435.
- van Zuilen, M., Lepland, A., and Arrhenius, G., 2002. Reassessing the evidence for the earliest traces of life. *Nature*, **418**, 627–630.
- van Zuilen, M. A., Chaussidon, M., Rollion-Bard, C., and Marty, B., 2007. Carbonaceous cherts of the Barberton greenstone belt, South Africa; isotopic, chemical, and structural characteristics of individual microstructures. *Geochimica et Cosmochimica Acta*, **71**, 655–669.
- Walsh, M. M., 1992. Microfossils and possible microfossils from the Early Archean Onverwacht Group, Barberton Mountain Land, South Africa. *Precambrian Research*, **54**, 271–293.
- Walsh, M., and Westall, F., 2008. Disentangling the microbial fossil record in the Barberton greenstone belt: a cautionary tale. In Seckbach, J., and Walsh, M. (eds.), *From Fossil to Astrobiology*. New York: Springer, pp. 25–37.
- Walter, M. R., 1983. Archean stromatolites: evidence of the Earth's earliest benthos. In Schopf, J. W. (ed.), *Earth's Earliest Biosphere*. Princeton: Princeton University Press, pp. 187–213.
- Welch, S. A., Taunton, A. E., and Banfield, J. F., 2002. Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiology Journal*, **19**, 343–367.
- Westall, F., 1994. Silicified bacteria and associated biofilm from the deep-sea sedimentary environment. *Kaupia-Darmstädter Beiträge zur Naturgeschichte*, **4**, 29–43.

- Westall, F., 1997. The influence of cell wall composition on the fossilization of bacteria and the implications for the search for early life forms. In Cosmovici, C., Bowyer, S., and Werthimer, D. (eds.), *Astronomical and Biochemical Origins and the Search for Life in the Universe*. Bologna: Editori Compositrici, pp. 491–504.
- Westall, F., 2008. Morphological biosignatures in terrestrial and extraterrestrial materials. *Space Science Reviews*, **135**, 95–114.
- Westall, F., 2009. Life on an anaerobic planet. *Science*, **232**, 471–472.
- Westall, F., and Folk, R. L., 2003. Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua Greenstone Belt: implications for the search for life in ancient rocks. *Precambrian Research*, **126**, 313–330.
- Westall, F., and Southam, G., 2006. Early life on Earth. In Benn, K., Mareschal, J. C., and Condie, K. (eds.), *Archean Geodynamics and Environments*. AGU Geophysical Monographs No 164, pp. 283–304.
- Westall, F., Boni, L., and Guerzoni, E., 1995. The experimental silicification of microorganisms. *Palaeontology*, **38**, 495–528.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., Gibson, E., McKay, D., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24,511–524,527.
- Westall, F., De Wit, M. J., Dann, J., Van Der Gaast, S., De Ronde, C., and Gerneke, D., 2001. Early Archaean fossil bacteria and biofilms in hydrothermally influenced, shallow water sediments, Barberton greenstone belt, South Africa. *Precambrian Research*, **106**, 91–112.
- Westall, F., de Ronde, C. E. J., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006a. Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361**, 1857–1875.
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006b. The 3.466 Ga Kitty's Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications No 405, pp. 105–131.
- White, B., 1974. Microfossils from the Late Precambrian Altyn Formation of Montana. *Nature*, **247**, 452–453.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biofilms and Fossilization
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Cyanobacteria
 Deep Biosphere of Salt Deposits
 Dolomite, Microbial
 Geochronology
 Isotope Fractionation (Metal)
 Isotopes and Geobiology
 Mat-Related Sedimentary Structures
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites

Origins of the Metazoa
 Sediment Diagenesis - Biologically Controlled
 Stromatactis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOSILICIFICATION

The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges, molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants. See entries “*Silica Biomineralization, Sponges*”, “*Microbial Silicification – Bacteria (or Passive)*” and “*Microbial Biomineralization*” for further reading.

BLACK SHALES

Wolfgang Oschmann
 University of Frankfurt, Frankfurt, Germany

Definition

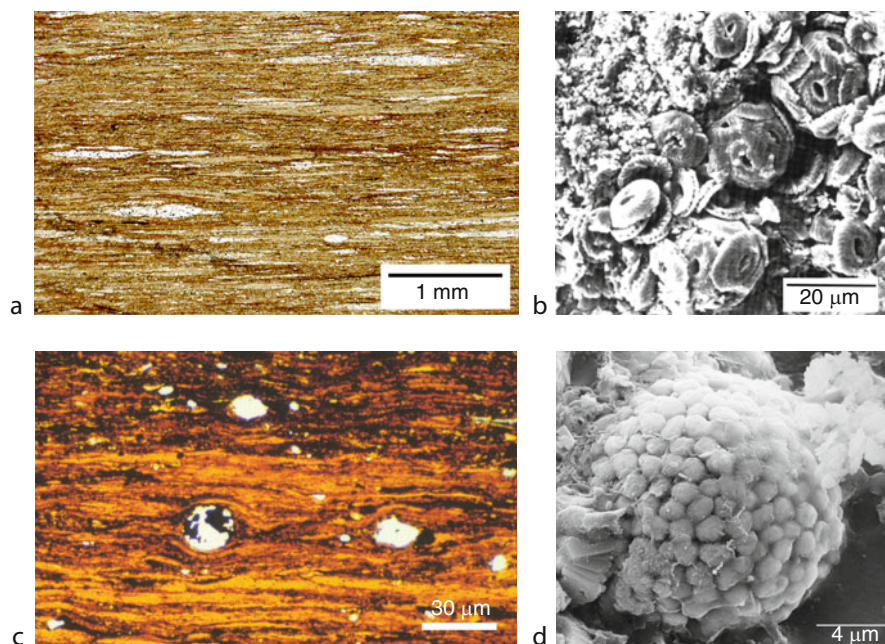
Most modern and ancient aquatic (marine, brackish, and lacustrine) environments were sufficiently oxygenated to permit colonization by numerous benthic, planktic, and nectic organisms. Due to excess oxygen the biologically produced organic matter is rapidly degraded. The sediments formed thereby exhibit only very low amounts of organic matter.

In contrast, very high rates of primary production and/or stagnant water bodies result in black shale sedimentation, which is associated with oxygen depletion or even lack of oxygen within the substrate and to some extent also within the water column. Degradation of organic matter is incomplete, partly by anaerobic bacteria, causing strong enrichment of organic matter in sediments. Black shales therefore commonly exhibit excellent preservation of organic molecules and mineralized soft tissues and hard parts of organisms.

The importance of black shales for scientists and the society are twofold. Black shales serve as source rocks for fossil fuels (oil and gas), which cover most of the energy needs of modern societies, and due to excellent preservation they provide particular sites for palaeontological studies.

Sedimentary characteristics of black shales

Black shales sediments in common are dark gray to black in color and are characterized by microlaminations, which may vary from distinct to blurred. The lamination is caused by quiet depositional conditions and the scarcity or lack of bioturbation. In many cases, the lamina were formed by alignments of fecal pellets ([Figure 1a](#)), which rained down in great numbers from surface waters. The



Black Shales, Figure 1 (a) Indistinct type of lamination formed by alignments of fecal pellets within bituminous mudstone facies (Kimmeridge Clay, Yorkshire, England, thin-section). (b) Most pellets are rich in carbonate and consist of coccoliths and coccospheres (Kimmeridge Clay, Yorkshire, England, SEM-view). (c) Organic rich filamentous mat-structures of bacterial origin (orange color) with dispersed silt-sized sedimentary particles (white) and pyritic aggregates (black) (thin-section, Kimmeridge Clay, Yorkshire). (d) SEM-view of a framboidal pyrite consisting of pyritohedral microcrystals precipitated during iron and sulfate reduction (Kimmeridge Clay, Yorkshire).

diameters of fecal pellets exceed the average annual sedimentation rate by far, and cause indistinct to blurred types of lamination. Commonly, it is assumed that seasonal variation in sedimentation (clastic input vs. primary production) may cause microlamination. However, the low sedimentation rates in general practically hamper a clear proof of varves.

Black shales typically consist of four main components (e.g., Röhl et al., 2001). *Siliciclasts*, mainly clay to silt sized particles form the major component of the rocks (50 to >90 wt.%). They derive from fluvial or aeolian input from hinterland areas. *Carbonates* originate either as skeletal remains of phytoplankton organisms (e.g., coccoliths, Figure 1b), or they are precipitated during early diagenesis at or close to the sediment surface (Frimmel et al., 2004). Their content varies remarkably. The proportion of *organic matter* may vary between 0.5 and 50 wt.% of the whole rock. It mainly derived from surface water primary production, but detritus from land plants and bacterial organic matter may also contribute. Black shales with a content of organic matter below 10% are termed as bituminous mudstones, and above 10% as oil shales. *Pyrite* (FeS_2) in black shales occurs as finely dispersed tiny framboids (Figure 1d), which were formed as syngenetic precipitates due to bacterially driven iron- and sulfate-reduction. In case of excess organic matter, larger pyrite aggregates may be formed during

diagenesis. The proportion of pyrite reaches 10–20 wt.% maximum.

History of organic matter preservation

During the early history of the Earth and life (Hadean and Archaean periods), virtually no free oxygen existed in the ocean and atmosphere. After oxygenic photosynthesis was established and the formerly reduced compounds had been oxidized, free oxygen started to accumulate in the ocean and the atmosphere ca. 2.2 billion years ago (e.g., Catling and Claire, 2005; Rouxel et al., 2005). The first eukaryotic organisms with highly resistant organic cell walls (acritarchs and/or primitive green algae) evolved during late early Proterozoic times (e.g., Samuelsson et al., 1999; Arouri et al., 2000). Their remains escaped rapid degradation and formed a major sink for organic carbon. Large quantities of organic matter had been embedded within marine sediments, leading to the first black shale formation (e.g., Banerjee et al., 2006). At least since Phanerozoic times, the atmosphere showed roughly the present-day oxygen levels (Catling and Claire, 2005; Berner, 2006) and aerated conditions persisted in the oceans. However, certain climatic and geographic factors promoted sluggish circulations with reduced turn over rates, thus preventing mixing of the water column. Related environments are commonly

controlled by a temporary or permanent density-stratification (thermo- and/or halocline) within the water column (e.g., Demaison and Moore, 1980; Oschmann, 1988; Wignall, 1994).

Oceanographic models for oxygen depletion and black shale formation

Certain combinations of physical and biotic factors cause oxygen depletion within marine areas, and have been described in various models from modern and ancient environments. In the following, a brief overview on the various models and fossil examples are given. Fossil examples in many cases however, were described in more than one of these models.

Silled basins

Land - locked silled basins with a positive water balance and a shallow and narrow connection to the open sea are characterized by a low salinity surface water outflow and a high salinity deeper water inflow (estuarine circulation). The difference in density between low and high salinity waters results in a permanent halocline and subsequently, thermocline. As convection and influx of oxygen to lower parts of the water column are prohibited, anoxic conditions develop below the halocline. The Baltic Sea and the Black Sea (e.g., Bahr et al., 2005, 2006) represent modern examples. Proposed fossil examples are e.g., the Permian Kupferschiefer (e.g., Bechtel and Püttmann, 1997) and Toarcian black shales in Central Europe (e.g., Röhl et al., 2001; van de Schootbrugge et al., 2005).

Upwelling systems

Today, upwelling is known from western continental margins in subtropical areas. Trade winds cause an offshore flow of surface waters and force oxygen-poor and nutrient-rich bottom waters to ascend. The mixing with oxygen-rich surface waters results in a very high plankton productivity and export production to the sea floor. Decomposition consumes all available oxygen, resulting in stagnant conditions and enrichment of organic matter in and on the sediment. Well-known modern to Pleistocene examples are the coastal areas off Peru and off Namibia (e.g., Wefer et al., 1996; Berger and Wefer, 2002; Hebbeln et al., 2002).

Oceanic anoxic events (OAEs)

Oceanic anoxic events (OAEs) were characterized by oxygen depletion in intermediate waters in major parts or even complete ocean basins. They were explained by a number of factors, namely (1) intensified deep water formation in high latitudes, (2) origin of warm and saline bottom waters at low latitudes, (3) enhanced fresh water runoff from continents associated with high rates of nutrient delivery, and (4) increased primary production associated with extreme greenhouse conditions. These factors all favor the development of low oxic to anoxic conditions with increased organic matter preservation. A modern to

Pleistocene example is the Arabian Sea in the low-latitude Indian Ocean (e.g., Schmiedl and Mackensen, 2006). Numerous OAEs occurred during the Earth's history, of which the Cretaceous OAEs are the most widely known. They are explained by high organic carbon burial related to global warming and greenhouse periods (e.g., Erbacher et al., 2001; Herrle et al., 2003). Many of these events were probably regional occurrences within restricted basins. However, there is increasing evidence for an open ocean and maybe global extension of the OAE 2 at the Cenomanian/Turonian boundary (e.g., Erbacher et al., 2005).

Eutrophic shelf

Many modern shelf environments (e.g., North Sea and Adriatic Sea) are characterized by man-made eutrophication that stimulates primary production with subsequent oxygen consumption and accumulation of organic matter (Dethlefsen and von Westernhagen, 1983; Kollmann and Stachowitsch, 2000). Fossil examples refer to a number of epicontinental seas particularly during the periods of greenhouse climate (e.g., Kimmeridge clay in western Europe (Oschmann, 1990; Miller, 1991), Toarcian black shales (van de Schootbrugge et al., 2005)).

Tidal mud flats and shallow lagunal environments

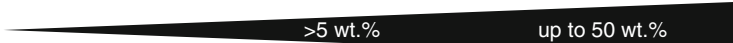
Most of the land-derived organic matter is deposited in near-shore environments, particularly in tidal mud flats. Additionally, land-derived nutrients stimulate primary production and subsequent oxygen depletion, thus promoting the enrichment of organic matter in the sediment (e.g., Wilms et al., 2006; Rink et al., 2007). Well-known fossil examples refer to the middle and late Triassic tidal and shallow subtidal mud flat areas from the northern Tethyan margin, e.g., at Mt. St. Giorgio, Switzerland (Röhl et al., 2002) and Seefeld, Austria (Hopf et al., 2001).

Black shales as ecosystems

Black shales and modern oxygen-depleted environments show various geochemical and ecological characteristics indicative of increasingly harsh life conditions (Table 1). Rhoads and Morse (1971) distinguished aerobic, dysaerobic, and anaerobic biofacies within marine environments. This classification has subsequently been used in many studies of modern and ancient oxygen-controlled environments (e.g., Tyson and Pearson, 1991; Wignall, 1994). Observations from modern environments show a remarkable seasonal oxygen fluctuation (Oschmann, 1994, 2000). Therefore, a modified scheme of classification was introduced, where the term dysaerobic was replaced by poikiloaerobic to address seasonal variation in oxygen supply.

The proportions of organic matter, pyrite, and biomarkers increase from the aerobic toward the anaerobic biofacies. In the same direction, the diversity of eukaryotes diminishes. Particularly, deep burrowing organisms

Black Shales, Table 1 A synoptic sketch of the geochemical and ecological characteristics of modern and fossil aerobic, dysaerobic/poikiloaerobic, and anaerobic biofacies

Biofacies			Aerobic	Dysaerobic/poikiloaerobic			Anaerobic	
Oxygen content	(Tyson and Pearson, 1991)	$\frac{\text{ml O}_2}{\text{l H}_2\text{O}}$	Aerobic >2	Moderate 2–1	Dysaerobic Severe 1–0.5	Extreme 0.5–0	Anaerobic 0 ml	
	(Oschmann, 2000)	$\frac{\text{mmol O}_2}{\text{l H}_2\text{O}}$	Aerobic >0.1	Moderate summer < 0.1	Poikiloaerobic Severe winter : <0.1 summer : <0.05	Extreme winter : <0.1 summer : <0.02	Anaerobic < 0.01	
Sediment:			Bioturbated				Laminated	
Organic matter content:		>0.5 wt. %						up to 50 wt. %
Content of pyrite:								
Content of biomarkers:								
Diversity of eukaryota:								
Epifauna:								
Shallow infauna (shallow bioturbation):								
Deep infauna (deep bioturbation):								
Microbial mat preservation:								
Larval shell preservation:								
Juveniles and dwarfed species:								
Reproduction via:								
Lecithotrophic larvae:								
Planktotrophic larvae:								
Position of redox boundary:		Deep in substrate	Close to sediment surface			Within the water column		

are affected by the rise of the redox boundary and try to settle close to the sediment surface (Oschmann, 1994; Schmiedl and Mackensen, 2006). Additionally, the proportion of organisms with benthic (lecithotrophic) larval development decreases with increasing oxygen depletion. If the redox boundary finally migrates out of the substrate, eukaryotic life is excluded. Dwarfs with body size remarkably smaller than normal and juveniles occur within the dysaerobic/poikiloaerobic facies, whereas microbial mats and partly also larval shells can be found within the anaerobic facies. The sporadic occurrence of the latter indicates failed larval spatfalls during unfavorable conditions.

Conclusions

1. Black shale sediments mainly form in marine to brackish waters under low oxic to anoxic conditions.
2. They are characterized by remarkably enriched proportions of organic matter and pyrite. The organic matter derives from marine primary production, terrestrial input, and bacterial derived compounds.
3. Black shales date back to late Proterozoic times and are widespread during Phanerozoic age.
4. Origination of black shales is explained by a number of models. A common aspect of most of these models is the formation of a density stratified water column, which prevents oxygenation of deeper waters.

5. The degree of oxygen depletion in benthic ecosystems during black shale deposition can be reconstructed by a number of geochemical and ecological features.

Bibliography

- Arouri, K. R., Greenwood, P. F., and Walter, M. R., 2000. Biological affinities of Neoproterozoic acritarchs from Australia: microscopic and chemical characterisation. *Organic Geochemistry*, **31**, 75–89.
- Bahr, A., Lamy, F., Arz, H. W., and Wefer, G., 2005. Late glacial to Holocene climate and sedimentation history in the NW Black Sea. *Marine Geology*, **214**, 309–322.
- Bahr, A., Arz, H. W., Lamy, F., and Wefer, G., 2006. Late glacial to Holocene paleoenvironmental evolution of the Black Sea, reconstructed with stable oxygen isotope records obtained on ostracod shells. *Earth and Planetary Science Letters*, **241**, 863–875.
- Banerjee, S., Dutta, S., Paikaray, S., and Mann, U., 2006. Stratigraphy, sedimentology and bulk organic geochemistry of black shales from the Proterozoic Vindhyan Supergroup (central India). *Journal of Earth Systems Science*, **115**, 37–47.
- Bechtel, A., and Püttmann, W., 1997. Palaeoceanography of the early Zechstein Sea during Kupferschiefer deposition in the Lower Rhine Basin (Germany): A reappraisal from stable isotope and organic geochemical investigations. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **136**, 331–358.
- Berger, W. H., and Wefer, G., 2002. On the reconstruction of upwelling history: Namibia upwelling in context. *Marine Geology*, **180**, 3–28.
- Berner, R. A., 2006. GEOCARBSULF: A combined model for Phanerozoic atmospheric O₂ and CO₂. *Geochimica et Cosmochimica Acta*, **70**, 5653–5664.
- Catling, C. D., and Claire, M. W., 2005. How Earth's atmosphere evolved to an oxic state: A status report. *Earth and Planetary Science Letters*, **237**, 1–20.
- Demaison, G. J., and Moore, G. T., 1980. Anoxic environments and oil source bed genesis. *Bulletin American Association Petroleum Geologists*, **64**, 1179–1209.
- Dethlefsen, V., and von Westernhagen, H., 1983. Oxygen deficiency and effects on bottom fauna in the eastern German Bight 1982. *Meeresforschung*, **30**, 42–53.
- Erbacher, J., Huber, B. T., Norris, R. D., and Markey, M., 2001. Increased thermohaline stratification as a possible cause for an ocean anoxic event in the Cretaceous period. *Nature*, **409**, 325–329.
- Erbacher, J., Friedrich, O., Wilson, P. A., Birch, H., and Mutterlose, J., 2005. Stable organic carbon isotope stratigraphy across oceanic anoxic event 2 of Dmerara Rise, western tropical Atlantic. *Geochemistry Geophysics Geosystems*, **6**, Q06010, doi:10.1029/2004GC000850.
- Frimmel, A., Oschmann, W., and Schwark, L., 2004. Chemostratigraphy of the Posidonia Black Shale, SW Germany. I. Influence of sea-level variation on organic facies evolution. *Chemical Geology*, **206**, 199–230.
- Hebbeln, D., Marchant, M., and Wefer, G., 2002. Paleoproductivity in the southern Peru-Chile current through the last 33,000 yr. *Marine Geology*, **186**, 487–504.
- Herrle, J. O., Pross, J., Friedrich, O., and Hemleben, C., 2003. Short-term environmental changes in the Cretaceous Tethyan Ocean: Micropaleontological evidence from the Early Albian Oceanic Anoxic Event 1b. *Terra Nova*, **15**, 14–19.
- Hopf, H., Thiel, V., and Reitner, J., 2001. An example for black shale development on a carbonate platform (late Triassic, Seefeld Austria). *Facies*, **45**, 203–210.
- Kollmann, H., and Stachowitsch, M., 2000. Long-term changes in the benthos of the Northern Adriatic Sea: a phototranssect approach. *Marine Ecology*, **22**, 135–154.
- Miller, R. G., 1991. A paleoceanographic approach to the Kimmeridge clay formation. In Huc, A. Y. (ed.), *Deposition of Organic Facies*. American Association of Petroleum Geologists Studies in Geology, **30**, pp. 13–26.
- Oschmann, W., 1988. Kimmeridge clay sedimentation - a new cyclic model. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **65**, 217–251.
- Oschmann, W., 1990. Environmental cycles in the late Jurassic northwest European epeiric basin: interaction with atmospheric and hydrospheric circulations. In Aigner, T., and Dott, R. H. (eds.), *Processes and Patterns in Epeiric Basins*. Sedimentary Geology, **69**, pp. 313–332.
- Oschmann, W., 1994. Adaptive pathways of marine benthic organisms in oxygen-controlled environments. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **191**, 393–444.
- Oschmann, W., 2000. Oxygen in the ocean. In Briggs, D. E. G., and Crowther, P. R. (eds.), *Palaeobiology: A Synthesis*. Oxford: Blackwell, pp. 474–477.
- Rhoads, D. C., and Morse, I. W., 1971. Evolutionary and ecological significance of oxygen-deficient marine basins. *Lethaia*, **4**, 413–428.
- Rink, B., Seeberger, S., Martens, T., Dürselen, C., Simon, M., and Brinkhoff, T., 2007. Effects of a phytoplankton bloom in a coastal ecosystem on the composition of bacterial communities. *Aquatic Microbial Ecology*, **48**, 47–60.
- Röhl, H. J., Schmid-Röhl, A., Oschmann, W., Frimmel, A., and Schwark, L., 2001. The Posidonia Shale (Lower Toarcian) of SW-Germany: an oxygen depleted ecosystem controlled by sea level and palaeoclimate. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **165**, 27–52.
- Röhl, H. J., Schmid-Röhl, A., Frimmel, A., Oschmann, W., Furrer, H., and Schwark, L., (2002). Microfacies, geochemistry and palaeoecology of the middle Triassic Grenzbitumenzone from Monte San Giorgio (Tessin). *Geologia Insubrica*, **6**, 1–13.
- Rouxel, O. J., Bekker, A., and Edwards, K. J., 2005. Iron isotope constraints on the Archean and Paleoproterozoic ocean redox state. *Science*, **307**, 1088–1091.
- Samuelsson, J., Dawes, P. R., and Vidal, G., 1999. Organic-walled microfossils from Proterozoic Thule Supergroup, Northwest Greenland. *Precambrian Research*, **96**, 1–23.
- Schmiedl, G., and Mackensen, A., 2006. Multi-species stable isotopes of benthic foraminifers reveal past changes of organic matter decomposition and deep-water oxygenation in the Arabian Sea. *Paleoceanography*, **21**, PA4213, doi:10.1029/2006PA001284.
- Tyson, R. V., and Pearson, T. H., 1991. Modern and ancient continental shelf anoxia: an overview. In Tyson, R. V., and Pearson, T. H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. Tulsa, OK: Geological Society Special Publication **58**, pp. 1–24.
- van de Schootbrugge, B., McArthur, J. M., Bailey, T. R., Rosenthal, Y., Wright, J. D., and Miller, K. G., 2005. Toarcian oceanic anoxic event: an assessment of global causes using belemnite C isotope records. *Paleoceanography*, **20**, PA3008, doi:10.1029/2004PA001102.
- Wefer, G., Berger, W. H., Bickert, T., Donner, B., Fischer, G., Kemle-von Mücke, S., Meinecke, G., Müller, P. J., Mulitza, S., Niebler, H. S., Pätzold, J., Schmidt, H., Schneider, R. R., and Segl, M., 1996. Late Quaternary surface circulation of the South Atlantic: the stable isotope record and implications for heat transport and productivity. In Wefer, G., Berger, W. H., Siedler, G., and Webb, D. (eds.), *The South Atlantic: Present and Past Circulation*. Berlin: Springer, pp. 461–502.

- Wignall, P., 1994. Black shales. *Oxford Monographs in Geology and Geophysics*, **30**, 1–127.
- Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., and Engelen, B., 2006. Specific bacterial archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. *Applied and Environmental Microbiology*, **72**, 2756–2764.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Biosignatures in Rocks](#)
[Carbon \(Organic, Cycling\)](#)
[Carbon \(Organic, Degradation\)](#)
[Critical Intervals in Earth History](#)
[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Microbial Degradation](#)
[Sediment Diagenesis - Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)
[Sulfur Isotopes](#)

BREAKUP OF RODINIA

Zheng-Xiang Li
 Curtin University of Technology, Perth, WA, Australia

Synonyms

Break-up of the supercontinent Rodinia

Definition

Supercontinent Rodinia. The earth's geodynamic system periodically pushes almost all continents together to form a single landmass – a supercontinent. The best known supercontinent is Pangaea (or Pangea) that existed between 320 and 170 Ma. A less known supercontinent Rodinia is believed to have existed between 900 Ma (some believe 1,000 Ma) and 750 Ma. The name “Rodinia” was coined by McMenamin and McMenamin (1990) from the Russian word “rodit” meaning “to beget” or “to give birth.” McMenamin and McMenamin (1990, p. 95) considered Rodinia to have been the supercontinent that spawned all subsequent continents, while “the edges (continental shelves) of Rodinia were the cradle of the earliest animals.” Three landmark paper in 1991 (Moores, 1991; Dalziel, 1991; Hoffman, 1991) triggered a global debate on this supercontinent hypothesis. The configuration of Rodinia is still controversial today, with some alternative reconstructions given in Hoffman (1991), Pisarevsky et al. (2003), and Li et al. (2008) (Figure 1).

The breakup of Rodinia: introduction

Although it is widely accepted that Rodinia broke apart during the latter half of the Neoproterozoic era (1,000–542 Ma) and that some fragments reassembled by the early Cambrian (*ca.* 530 Ma) to form Gondwanaland (Hoffman, 1991), our knowledge of the timing of the breakup events and the processes involved is still sketchy.

Record of Rodinia breakup

The breakup of Rodinia was recorded both geologically and geophysically. The most important geological records of the breakup process are globally widespread continental rift deposits and related magmatism, and records of the geological transition from continental rifting to passive margin deposits where rifting succeeded to form new oceans. One of the best matches of rifting records between continents is that between southeast Australia and South China, where episodes of rift-related magmatism at *ca.* 825 Ma, 800 Ma, 780 Ma, and 750 Ma have been well documented (Preiss, 2000; Li et al., 2003) (Figure 2). Similar records are emerging from other continents.

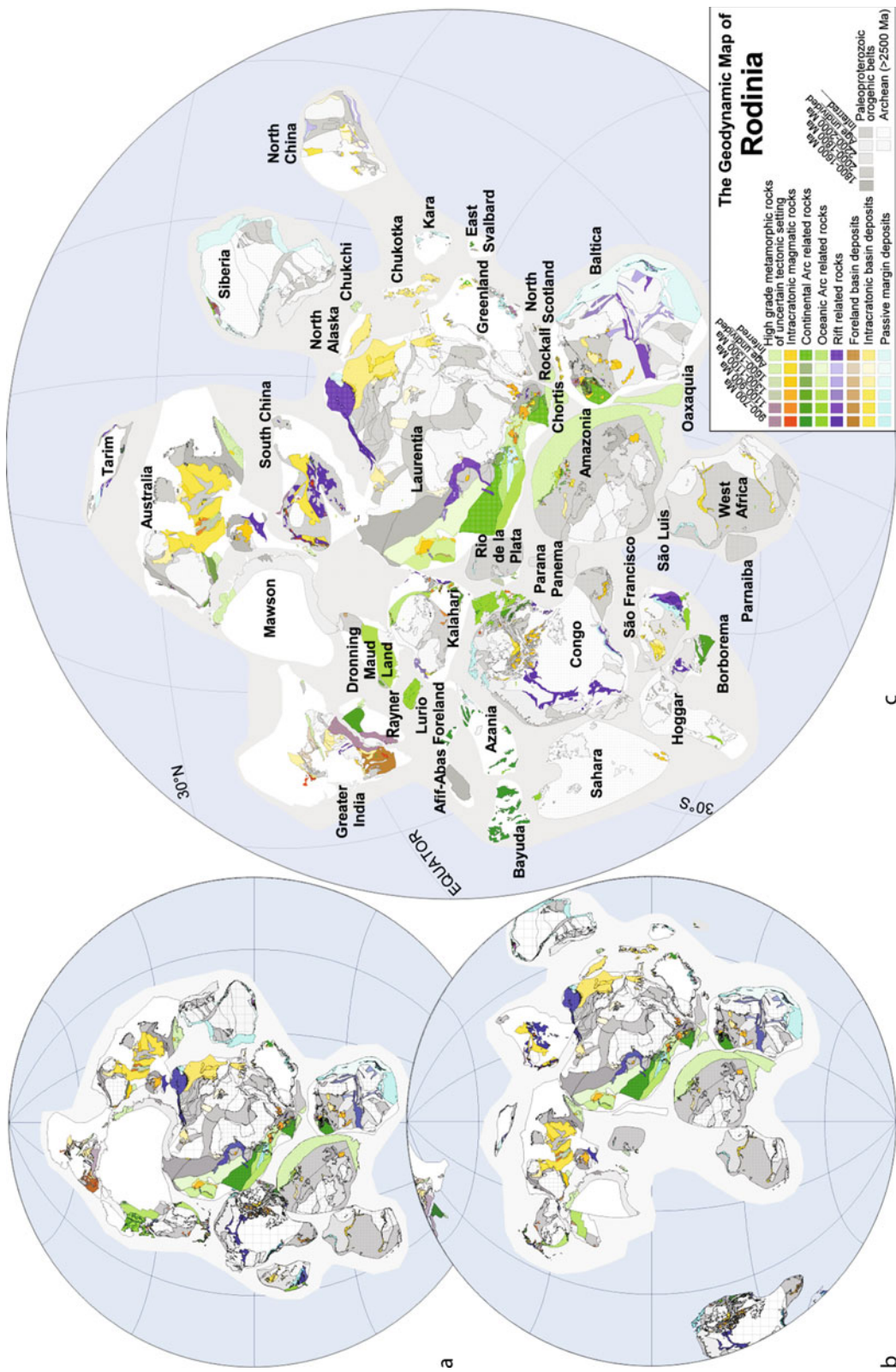
Radiating dyke swarms and remnants of large igneous provinces (LIPs) have also been regarded as important geological records of the breakup of Rodinia (Figure 2c and d; see discussion below under *Mechanism for the breakup*).

The most important geophysical constraint on the breakup of Rodinia is provided by paleomagnetism. Whereas, an individual pole of a given age for a continent constrains its paleolatitude at that time but not its paleolongitude, comparable apparent polar wander paths (APWPs) for different continents can indicate a unique paleogeographic fit between them. In the latter case, when two matched APWPs start to diverge, it implies that the two continents started to move independently (*i.e.*, a breakup between them occurred). Unfortunately, such an ideal situation has rarely been identified for the Rodinia time because of the limited data currently available.

Timing of events

The ages of continental rifting events can be easily dated because of the availability of datable minerals such as zircon in the rift-related magmatic rocks, and it is now widely accepted that continental rifting in the lead-up to Rodinia breakup started at *ca.* 825 Ma or earlier (see summary in Li et al., 2008; Figure 2). However, the timing of the breakup events is still controversial, and different parts of the supercontinent seem to have broken apart at different times.

The stratigraphic record in southeast Australia indicates a rift-drift transition between the Sturtian glacial deposits (with a poor age constraint of *ca.* 750–690 Ma) and the overlying sag-phase deposits (Powell et al., 1994) that were recently dated at between 657 ± 5 and 643 ± 2 Ma (Re-Os ages, see Kendall et al., 2006). These age



Breakup of Rodinia, Figure 1 Three of the alternative Rodinia reconstructions: (a) Hoffman (1991), (b) Pisarevsky et al. (2003), and (c) model adapted by IGCP440 for the Geodynamic Map of Rodinia. (Li et al., 2008.)

constraints seem to agree with those for south China and western Laurentia. Although rifting occurred at *ca.* 750 Ma along the eastern margins of Laurentia, the breakup there appears to have occurred during *ca.* 615–570 Ma (Cawood and Pisarevsky, 2006). These younger events are possibly recognizable in global tectonic subsidence curves (Bond et al., 1984).

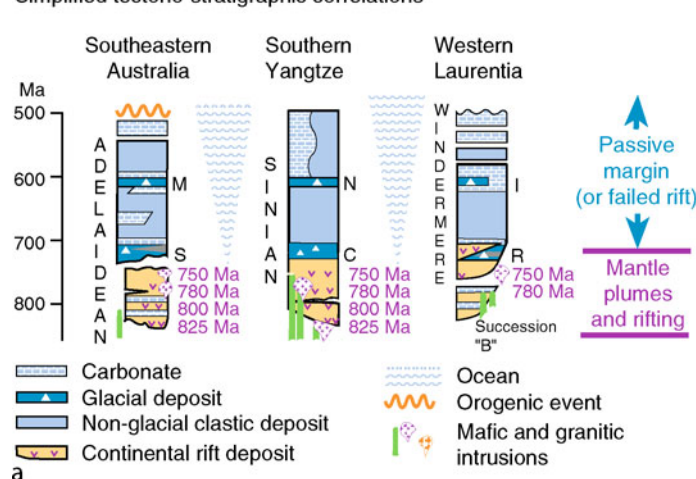
Such geological evidence for the breakup of Rodinia is sometimes at variance with paleomagnetic constraints. Paleomagnetic data suggests that India was either adjacent to western Australia (Torsvik et al., 2001) or had already broken away from Rodinia at 750 Ma (Figure 3b). South

China must also have broken away from Australia and Laurentia by 750 Ma if it occupied a central position in Rodinia as shown in Figures 1c and 3a (Wingate and Giddings, 2000; Li et al., 2008). Alternatively, Rodinia may have remained intact at *ca.* 750 Ma if a different configuration occurred (see Fig. 5 of Li et al., 2004).

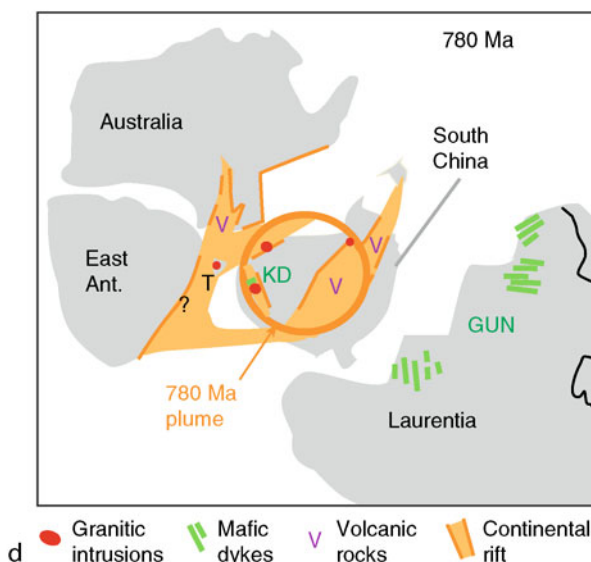
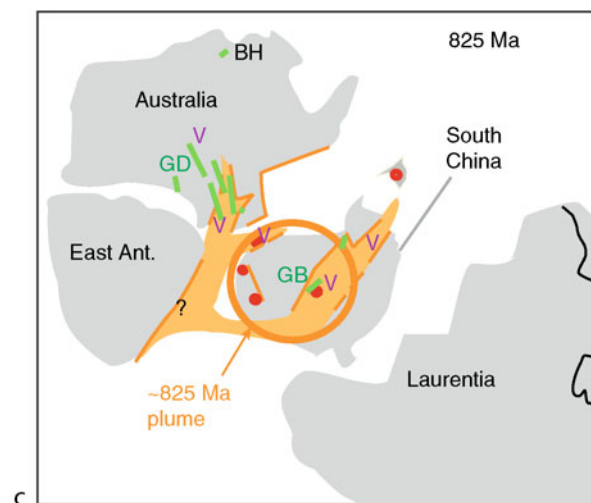
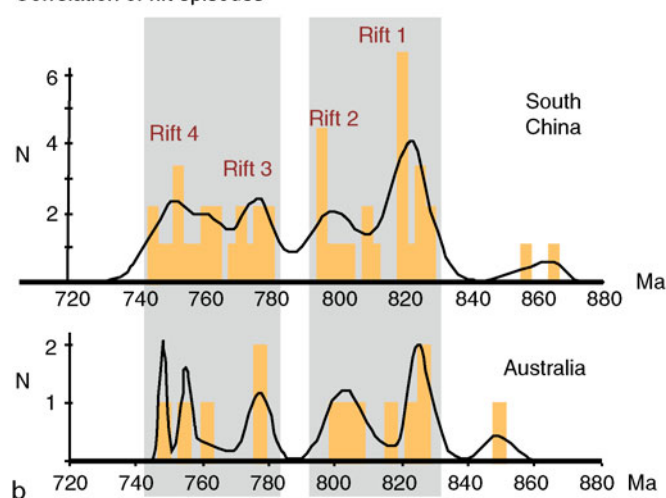
Mechanism for the breakup

The most commonly invoked mechanism for the breakup of Rodinia is the activity of mantle plumes or of a mantle superplume (superswell) beneath the supercontinent,

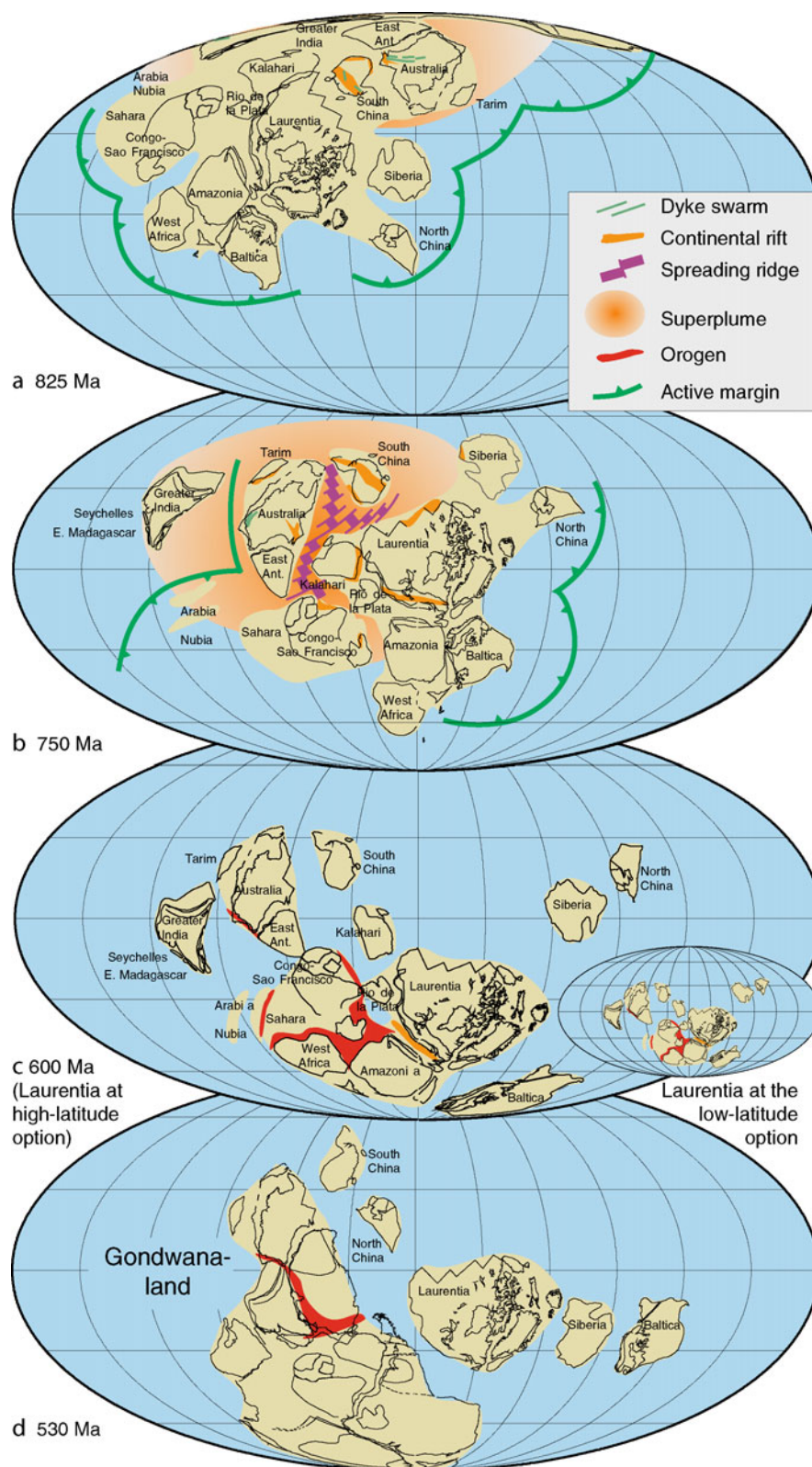
Simplified tectono-stratigraphic correlations



Correlation of rift episodes



Breakup of Rodinia, Figure 2 (a) Tectonostratigraphic correlations between Australia, South China, and Laurentia during the breakup of Rodinia. Major glaciations: M = Marinoan; S = Sturtian; N = Nantuo; C = Changan; I = Ice Brook; R = Rapitan. (b) Correlatable episodes of intra-continental magmatism and continental rifting between Australia and South China. *N* = number of magmatic ages. (c, d) Cartoon figures illustrating possible plume breakouts in central Rodinia during the Neoproterozoic time. (Modified after Li et al., 2003, 2008.)



Breakup of Rodinia, Figure 3 Cartoons illustrating the breakup process of Rodinia. (After Li et al., 2008.)

which thinned and thermally weakened the lithosphere resulting in widespread continental rifting and eventually breakup (Figure 3a and b).

Evidence for plume/superplume activity during the time of Rodinia include remnants of LIP and radiating dyke swarms (Figure 2c), syn-magmatic, continental-scale doming indicating the arrival of a plume-head (Li et al., 1999), large-scale anorogenic but often dominantly felsic melting that requires a pan-Rodinia introcontinental heat source (Li et al., 2003), and abnormally high-temperature ($>1,500^{\circ}\text{C}$) mantle melting (Wang et al., 2007). Mechanisms proposed for the formation of such a superplume (or superswell) include thermal shielding by the supercontinent (e.g., Anderson, 1994), gravitational push-up effects on the hotter lower mantle by circum-Rodinia avalanching slabs (Li et al., 2004, 2008), return flow of whole-mantle convection (Zhong et al., 2007), and partial melt of recycled mid-ocean ridge basalts near the core-mantle boundary under Rodinia (Maruyama et al., 2007).

Summary

The breakup of Rodinia was a diachronous and protracted process. Widespread continental rifting started at *ca.* 825 Ma when Rodinia spanned from the equator to high latitudes (Figure 3a), but the breakup, initially between Australia/East Antarctica, Laurentia, and possibly South China did not occur until *ca.* 750 Ma (Figure 3b) or later when the supercontinent was centered on the equator. Gondwanaland was already assembling before some of the West Gondwanan continents broke away from Laurentia at *ca.* 600 Ma (Figure 3c), and by the early Cambrian (530 Ma) the assembly was complete (Figure 3d). The breakup of Rodinia is believed by some to have been related to the formation of a mantle superplume beneath the supercontinent.

Bibliography

- Anderson, D. L., 1994. Superplume or supercontinents? *Geology*, **22**, 39–42.
- Bond, G. C., Nickeson, P. A., and Kominz, M. A., 1984. Breakup of a supercontinent between 625 Ma and 555 Ma: new evidence and implications for continental histories. *Earth and Planetary Science Letters*, **70**, 325–345.
- Cawood, P. A., and Pisarevsky, S. A., 2006. Was Baltica right-way-up or upside-down in the Neoproterozoic? *Journal of the Geological Society*, **163**, 753–759.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica - Australia as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside-out? *Science*, **252**, 1409–1412.
- Kendall, B., Creaser, R. A., and Selby, D., 2006. Re-Os geochronology of postglacial black shales in Australia: constraints on the timing of “Sturtian” glaciation. *Geology*, **34**, 729–732.
- Li, Z. X., Li, X. H., Kinny, P. D., and Wang, J., 1999. The breakup of Rodinia: did it start with a mantle plume beneath South China? *Earth and Planetary Science Letters*, **173**(3), 171–181.
- Li, Z. X., Li, X. H., Kinny, P. D., Wang, J., Zhang, S., and Zhou, H., 2003. Geochronology of Neoproterozoic syn-rift magmatism in the Yangtze Craton, South China and correlations with other continents: evidence for a mantle superplume that broke up Rodinia. *Precambrian Research*, **122**(1–4), 85–109.
- Li, Z. X., Evans, D. A. D., and Zhang, S., 2004. A 90° Spin on Rodinia: possible causal links between the Neoproterozoic supercontinent, superplume, true polar wander and low-latitude glaciation. *Earth and Planetary Science Letters*, **220**, 409–421.
- Li, Z. X., Bogdanova, S. V., Collins, A. S., Davidson, A., De Waele, B., Ernst, R. E., Fitzsimons, I. C. W., Fuck, R. A., Gladkochub, D. P., Jacobs, J., Karlstrom, K. E., Lu, S., Natapov, L. M., Pease, V., Pisarevsky, S. A., Thrane, K., and Vernikovsky, V., 2008. Assembly, configuration, and break-up history of Rodinia: a synthesis. *Precambrian Research*, **160**(1–2), 179–210.
- Maruyama, S., Santosh, M., and Zhao, D., 2007. Superplume, supercontinent, and post-perovskite: Mantle dynamics and anti-plate tectonics on the Core-Mantle Boundary. *Gondwana Research*, **11**(1–2), 7–37.
- McMenamin, M. A. S., and McMenamin, D. L. S., 1990. *The Emergence of Animals: The Cambrian Breakthrough*. New York: Colombia University Press, 217 pp.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Pisarevsky, S. A., Wingate, M. T. D., Powell, C. M., Johnson, S., and Evans, D. A. D., 2003. Models of Rodinia assembly and fragmentation. In Yoshida, M., Windley, B. F., and Dasgupta, S. (eds.), *Proterozoic East Gondwana: Supercontinent Assembly and Breakup*. Geological Society of London Special Publications 206, pp. 35–55.
- Powell, C. M., Preiss, W. V., Gatehouse, C. G., Krapez, B., and Li, Z. X., 1994. South Australian record of a Rodinian epicontinental basin and its mid-Neoproterozoic breakup (~ 700 Ma) to form the palaeo-Pacific Ocean. *Tectonophysics*, **237**(3–4), 113–140.
- Preiss, W. V., 2000. The Adelaide Geosyncline of South Australia and its significance in Neoproterozoic continental reconstruction. *Precambrian Research*, **100**, 21–63.
- Torsvik, T. H. et al., 2001. Rodinia refined or obscured: palaeomagnetism of the Malani Igneous Suite (NW India). *Precambrian Research*, **108**(3–4), 319–333.
- Wang, X. C., Li, X. H., Li, W. X., and Li, Z. X., 2007. Ca. 825 Ma komatiitic basalts in South China: First evidence for $>1500^{\circ}\text{C}$ mantle melts by a Rodinian mantle plume. *Geology*, **35**(12), 1103–1106.
- Wingate, M. T. D., and Giddings, J. W., 2000. Age and palaeomagnetism of the Mundine Well dyke swarm, Western Australia: implications for an Australia-Laurentia connection at 755 Ma. *Precambrian Research*, **100**, 335–357.
- Zhong, S., Zhang, N., Li, Z. X., and Roberts, J. H., 2007. Supercontinent cycles, true polar wander, and very long-wavelength mantle convection. *Earth and Planetary Science Letters*, **261**, 551–564.

Cross-references

Gondwanaland, Formation
Snowball Earth

BIOFILMS AND FOSSILIZATION

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Carbonaceous bacterial biofilms; Hautschatten

Definition

Microbial biofilms cover normally in aquatic environments dead organisms and control partly the process of decay. The soft tissues of the body outline are preserved as a layered structure which comprises a carbonaceous microbial biofilm, in the literature often called “Hautschatten” (Figure 1). Fossils with soft part preservation are extremely rare, but under certain circumstances, like suboxic or anaerobic conditions, remains of their soft tissues with detailed histological fabrics are found. The evidence of non-biomineralized soft tissue normally degrading completely during decay is linked with special microbial processes. This exceptional preservation requires soft tissues to be replicated by authigenic minerals or pseudomorphed by bacterial biofilms (e.g., Wuttke, 1983; Toporski et al., 2002; Briggs, 2003). The different organs in many cases often exhibit different microbially controlled lithified biofilms with an unusual preservation of involved microbes.

Geobiological implications and examples

Understanding the relationship between decay and mineralization is of fundamental importance to palaeontologists

interested in the record of soft-bodied organisms. Soft-bodied fossils also provide insights into early diagenetic processes in sediments and give basic information on related organic geochemistry. The lack of biomineralized hard parts in many organisms is the normal condition in the fossil record. However, the mode of soft-tissue decay is far from uniform and the sum of various processes results in a preservation in so-called Konservat-Lagerstätten (Seilacher, 1970; Seilacher et al., 1985).

Derek Briggs and his group have made fundamental experiments on how the preservation of soft-tissues works (e.g., Briggs, 1999, 2003; Briggs and Kear, 1993; Briggs and Wilby, 1996; Grimes et al., 2001; Gupta et al., 2006). For example, they could demonstrate that initial pyritization of a plant tissue can be an extremely rapid process (within 80 days) and is driven by anaerobic bacterially mediated decay. They have made comparable experiments mainly with arthropods. Toporski's and coworkers' (Toporski et al., 2002) investigation on the Oligocene Enspel Maar black shale is a milestone in the research on soft-tissue preservation via microbial biofilms. The multitask biogeochemical analyses give the deepest insight into the taphonomy of soft tissue and microbial-related decay processes. The most famous locality with preserved soft tissues is the Eocene Messel locality. From this locality, soft tissues covered with lithified biofilms were first described by Wuttke (1983). Thick microbial biofilms are generally much darker than the surrounding black-shale and form the so-called “Hautschatten” and were for a long time believed to represent fossilized soft tissues. The biofilms trace back the decaying soft tissue in an anaerobic environment and “casting” of the former soft tissues. Often the digestive



Biofilms and Fossilization, Figure 1 A very well-preserved mouse (*Apodemus atavus*) from the late Pliocene fossil lagerstaette Willershausen (Lower Saxony, Germany) with perfectly preserved biofilm-related “Hautschatten” with visible remains of the former fur (Geoscience Museum University of Göttingen).

system of large fossils is excellently preserved (Franzen, 1994). Fundamental studies on biofilm soft-tissue relationship were carried out by Richter (1994) and Liebig (1997). Another intriguing example is the “soft-tissue” preservation in dinosaur bones (Kaye et al., 2008). Kaye and coworkers have found microbially altered soft tissue in a dinosaur bone and they give a critical explanation of this fact. Within their paper they discuss the processes of tissue preservation and also demonstrate the importance of recent microbial biofilm invasion and late diagenetic alteration of primarily formed pyrite framboids. The clou of this investigation is also that there is no real soft-tissue preservation – however, preserved biofilms cast the former tissue and give an idea about the original structures.

All “Hautschatten” soft-tissue preservations are probably preserved lithified biofilms and not real soft tissues.

Conclusion

Biofilms play a significant role in suboxic and anaerobic milieus preserving soft tissue remains. The body outline of dead organisms is exactly traced by the margins of the mineralised biofilms and give a perfect image of the entire fossil. These structures are often called after the German word “Hautschatten”-preservation and is commonly found under black shale condition.

Bibliography

- Briggs, D. E. G., 1999. Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis. *Philosophical Transactions of Royal Society Series B*, **354**, 7–16.
- Briggs, D. E. G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences*, **31**, 275–301.
- Briggs, D. E. G., and Kear, A. J., 1993. Fossilization of soft-tissue in the laboratory. *Science*, **259**, 1439–1442.
- Briggs, D. E. G., and Wilby, P. R., 1996. The role of calcium carbonate – calcium phosphate switch in the mineralization of soft bodied fossils. *Journal of the Geological Society, London*, **153**, 665–668.
- Franzen, J. L., 1994. Bacterial preservation of the digestive system in Messel Equoids. *Kaupia*, **4**, 3–11.
- Grimes, S. T., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Gupta, N. S., Michels, R., Briggs, D. E. G., Evershed, R. P., and Pancost, R. D., 2006. The organic preservation of fossil arthropods: an experimental study. *Proceedings of the Royal Society of London, Series B*, **273**, 2777–2783.
- Kaye, T. G., Gaugler, G., and Sawlowicz, Z., 2008. Dinosaur soft tissues interpreted as bacterial biofilms. *PLOS One*, **3**(7), e2808.
- Liebig, K., 1997. *Fossil Microorganisms from the Eocene Messel Oil Shale of Southern Hessen*. University Dissertation, Heidelberg, Germany.
- Richter, G., 1994. Bacteria and bacteria-like structures from the oil-shale of Messel. *Kaupia*, **4**, 21–28.
- Seilacher, A., 1970. Begriff und Bedeutung der Fossil-Lagerstätten. *Neues Jahrbuch Fur Geologie und Paläontologie, Monatshefte*, **1970**, 34–39.
- Seilacher, A., Reif, W. E., and Westphal, F., 1985. Sedimentological, ecological and temporal patterns of fossil lagerstätten.

Philosophical Transactions of the Royal Society of London, Series B, **311**, 5–23.

Toporski, J. K. W., Steele, A., Westall, F., Avet, R., Martill, D. M., and McKay, D. S., 2002. Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany. *Geochimica et Cosmochimica Acta*, **66**, 1773–1791.

Wuttke, M., 1983. “Weichteilerhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

[Bacteria](#)
[Beggiatoa](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Black Shales](#)
[Detachment](#)
[Ediacaran Biota](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Thiotrophic Bacteria](#)
[Whale and Wood Falls](#)

BIOGEOCHEMICAL CYCLES

A biogeochemical cycle defines the pathways by which chemical elements occurring in organisms are cycled between different living and nonliving compartments on the Earth (e.g., biosphere, atmosphere, hydrosphere, geosphere, etc.). Typical driving forces of a biogeochemical cycle are the metabolisms of living organisms, geological processes, or chemical reactions. For specific reading on major biogeochemical cycles, please refer to “[Carbon Cycle](#),” “[Nitrogen](#),” “[Sulfur Cycle](#),” “[Phosphorus](#),” “[Phosphorites](#).”

BIOLOGICAL CONTROL ON DIAGENESIS: INFLUENCE OF BACTERIA AND RELEVANCE TO OCEAN ACIDIFICATION

Fred T. Mackenzie¹, Andreas J. Andersson²

¹University of Hawaii, Honolulu, HI, USA

²Bermuda Institute of Ocean Sciences, Ferry Reach, St. George's, Bermuda

Introduction

Soon after carbonate mineral particles are deposited, a series of chemical, physical, and biological processes start that can significantly alter their mineralogy, isotopic composition and chemistry, and the fabric of the accumulating sediment. These processes include dissolution and precipitation reactions, abrasion, and transport, and

- Vooren, C. M., 1981. Photosynthetic rates of benthic algae from the deep coral reef of Curaçao. *Aquatic Botany*, **10**, 143–154.
- Walker, S. E., 2007. Traces of gastropod predation on molluscan prey in tropical reef environments. In Miller III, W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier, pp. 324–344.
- Wanders, J. B. W., 1977. The role of benthic algae in the shallow reef of Curaçao (Netherlands Antilles) III: the significance of grazing. *Aquatic Botany*, **3**, 357–390.
- Wielgus, J., Glassom, D., and Chadwick, N. E., 2006. Patterns of polychaete worm infestation of stony corals in the northern Red Sea and relationships to water chemistry. *Bulletin of Marine Science*, **78**, 377–388.
- Wilhelm, C., and Jakob, T., 2006. Uphill energy transfer from long-wavelength absorbing chlorophylls to PS II in *Ostreobium* sp. is functional in carbon assimilation. *Photosynthesis Research*, **87**, 323–329.
- Wilkinson, C., 2004. *Status of Coral Reefs of the World: 2004*. Townsville, Australia: Australian Institute of Marine Science, 557 pp.
- Wilson, M. A., 2007. Macroborings and the evolution of marine bioerosion. In Miller III, W. (ed.), *Trace Fossils: Concepts, Problems, Prospects*. Amsterdam: Elsevier Scientific Publishing Co., pp. 356–367.
- Wisshak, M., 2006. High-latitude bioerosion. In Bhattacharji, S., Neugebauer, H. J., Reitner, J., and Stüwe, K. (eds.), *Lecture Notes in Earth Sciences*, **109**, 1–202. Berlin-Heidelberg: Springer-Verlag.
- Wisshak, M., Gektidis, M., Freiwald, A., and Lundälv, T., 2005. Bioerosion along a bathymetric gradient in cold-temperate setting (Kosterfjord, SW Sweden): an experimental study. *Facies*, **51**, 93–117.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from Early Proterozoic stromatolites, Hebei, China, *Acta Micropaleontologica Sinica*, **4**, 1–12.
- Zubia, M., and Peyrot-Clausade, M., 2001. Internal bioerosion of *Acropora formosa* in Reunion (Indian Ocean): microborer and macroborer activities. *Oceanologica Acta*, **24**, 251–262.
- Zundeleich, A., Lazar, B., and Ilan, M., 2007. Chemical versus mechanical bioerosion of coral reefs by boring sponges – lessons from *Pione cf. vastifica*. *The Journal of Experimental Biology*, **210**, 91–96.

Cross-references

Algae (Eukaryotic)
 Animal Skeletons, Advent
 Basalt (Glass, Endoliths)
 Biodeterioration (of Stone)
 Biogeochemical Cycles
 Biomineral (Mineral Bioleaching, Mineral Biooxidation)
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Carbon Cycle
 Carbonate Environments
 Carbonates
 Cyanobacteria
 Endoliths
 Extracellular Polymeric Substances (EPS)
 Extreme Environments
 Foraminifera
 Fungi and Lichens
 Glass
 Geomycology
 Ichnology
 Karst Ecosystems
 Microbial Biomineralization

Microbiocorrosion

Reefs

Sediment Diagenesis – Biologically Controlled
 Sponges (Porifera) and Sponge Microbes
 Trace Fossils: Neoproterozoic

Additional references

- <http://www.sbg.ac.at/ipk/avstudio/pierofun/transcript/bioeros.pdf> (Bioerosion)
<http://bioweb.usc.edu/documents/bisc469-outlines.pdf>
<http://paleopolis.rediris.es/phoronida/ref/ref90.htm>
<http://www.wooster.edu/geology/Bioerosion/Bioerosion.html>
<http://www.wooster.edu/geology/Bioerosion/BioerosionBiblio.pdf> (Wilson, M.A.)
<http://en.wikipedia.org/wiki/Bioerosion> (Wilson, M.A.)
<http://www.diva-portal.org> (urn_nbn_se_uu_diva-5726-2_full-text.pdf)
<http://www.havforsker.dk/hjemmeside.php?id=4&forskning> (Benthic Foraminifera)

BIOFILMS

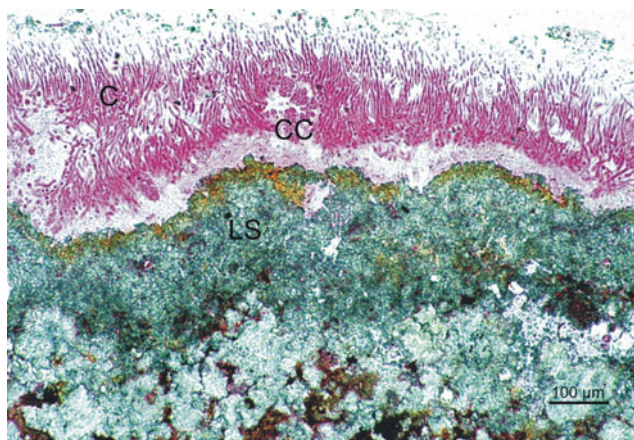
Joachim Reitner

University of Göttingen, Göttingen, Germany

Definition

Nearly all hard surfaces exhibit biofilms, organic coatings of microbial cells which are enclosed in extracellular polymeric substances (EPS). Biofilms interact between hard substrates such as minerals and rocks, water bodies, and the atmosphere. Metabolic exchange processes controlled by biofilms, globally influence bio-geochemical cycles.

Biofilms and microbial mats are benthic systems formed by different microbial communities which secrete hydrated mucus substances (EPS) (e.g., Characklis and Wilderer, 1989; Costerton et al., 1987, 1995) in various amounts. These EPS substances are mainly polysaccharides, glycoproteins, exoenzymes, and nucleic acids (Sutherland, 1977; Decho, 1990; Wingender et al., 1999). Biofilms and microbial mats display a continuous spectrum ranging from thin micrometer-sized coatings of mono-specific and poly-specific microbial populations up to centimeter thick communities of various microbes such as archaea, bacteria, eukaryotic algae, fungi, and protozoa. In many cases, these thick microbial mats exhibit a clearly visible ecological zoning owing to the presence of different microbial communities which are often characterized by colored pigments. The geobiological importance of microbial mats was recognized early by geoscientists (Walcott, 1914; Black, 1933; Pia, 1933) in contrast to the hardly visible biofilms which are globally much more widespread. Engineers and microbiologists, for example, Characklis and Wilderer (1989); Flemming (1991); Muyzer et al. (1993); Costerton et al. (1987), (1995); Neu and Lawrence (1999), have recognized the enormous importance and potential of biofilms in



Biofilms, Figure 1 Cyanobacterial biofilm forming a modern aragonitic stromatolite (Walker Lake, Nevada). Biofilm is dominated by filamentous cyanobacteria – *Calothrix* (C). Few coccoid cyanobacteria are also present (CC). Within the youngest lithified part, remains of cyanobacterial sheets are visible (LS).

many common processes. Geoscientists have increasingly investigated the rock- and mineral-forming potentials of biofilms in the 90s of the last century (e.g., Ferris, 1991; Pedley, 1992; Reitner, 1993). Biofilms were also found in deep subsurface environments and, significantly for the Deep Biosphere, up to the upper known temperature limit of life (110–120°C) (Pedersen, 1993; Fyfe, 1996; Gold, 1999; Stetter, 1994, 1996).

Organo-films are special organic coatings which are formed by various macromolecules and do not contain living cells. Organo-films are taphonomic remains of biofilms or newly formed from biogenic degradation products (geopolymers sensu Killops and Killops, 1997; Gold, 1999). Adsorption on mineral surfaces and self organizing catalytic processes play a central role during formation (Ferris et al., 1996; Reitner, 1993).

Stromatolites, thrombolites, and generally microbialites, are lithified products of bio- and organofilms, as well as thick microbial mats (Figure 1).

Bibliography

- Black, M., 1933. *The Algal Sediments of Andros Island, Bahamas*. London: Philosophical Transactions of the Royal Society of London, Vol. B222, pp. 165–192.
- Characklis, W. G., and Wilderer, P. A., (eds.), 1989. *Structure and Function of Biofilms*. Life sciences research reports, 46: 387 S. Chichester: Wiley.
- Costerton, J. W., Cheng, K. J., Geesey, G. G., Ladd, T. J., Nickel, J. C., Dasgupta, M., and Marrie, T., 1987. Bacterial biofilms in nature and disease. *Annual Reviews in Microbiology*, **41**, 435–464.
- Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., and Lappin-Scott, H. M., 1995. Microbial biofilms. *Annual Reviews in Microbiology*, **49**, 711–745.
- Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review*, **28**, 73–153 (Aberdeen).

- Ferris, F. G., 1991. Biogeochemical importance of microbial biofilms. *Geological Association of Canada Program with Abstracts*, **37**, Waterloo.
- Ferris, J. P., Hill, A. R., Liu, R., and Orgel, L. E., 1996. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature*, **381**, 59–61.
- Flemming, H. C., 1991. Biofilms as a particular form of microbial life. In Flemming, H. C., and Geesey, G. G. (eds.), *Biofouling and Biocorrosion in Industrial Water Systems*. Heidelberg: Springer, pp. 3–9.
- Fyfe, W. S., 1996. The biosphere is going deep. *Science*, **273**, 448 (Washington).
- Godderis, Y., and Veizer, J., 2000. Tectonic control of chemical and isotopic composition of ancient oceans; the impact of continental growth. *American Journal of Science*, **300**(5), 434–461.
- Gold, T., 1999. *The Deep Hot Biosphere*. New York: Copernicus, p. 235 S.
- Killops, S. D., and Killops, V. J., 1997. *Einführung in die organische Geochemie*. Stuttgart: Enke.
- Muyzer, G., de Waal, E. C., and Uitterlinden, A. G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology*, **59**, 695–700.
- Neu, T. R., and Lawrence, J. R., 1999. Lectin-binding analysis in biofilm systems. *Methods in Enzymology*, **310**, 145–152.
- Pedersen, K., 1993. The deep subterranean biosphere. *Earth Science Reviews*, **34**, 243–260 (Amsterdam).
- Pedley, M., 1992. Freshwater (phytoherm) reefs. The role of biofilms and their bearing on marine reef cementation. *Sedimentary Geology*, **79**, 255–274 (Amsterdam).
- Pia, J. v., (1933): *Die rezenten Kalksteine*. - 420 S., Leipzig (Akademische Verlagsgesellschaft).
- Reitner, J., 1993. Modern cryptic microbialite/Metazoan facies from lizard island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40 (Erlangen).
- Stetter, K. O., 1994. The lesson of archaeobacteria. In Bengtson, S. (ed.), *Early Life on Earth*. Nobel Symposium, 84, New York: Columbia University Press, pp. 143–151.
- Stetter, K. O., 1996. Hyperthermophilic procaryotes. *FEMS Microbiology Reviews*, **18**, 149–158 (Amsterdam).
- Sutherland, I. W. (ed.), 1977. *Surface Carbohydrates of the Prokaryotic Cell*. London: Academic Press, p. 472 S.
- Walcott, C. D., 1914. Prekambrian algonkian flora. *Smithsonian Miscellaneous Collections*, **64**, 77–156.
- Wingender, J., Neu, T. R., and Flemming, H. C. (eds.), 1999. *Microbial Extracellular Polymeric Substances. Characterization, Structure and Function*. Berlin: Springer, p. 258 S.

Cross-references

[Biofilms and Fossilization](#)
[Calcified Cyanobacteria](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Desert Varnish](#)
[Detachment](#)
[Ediacaran Biota](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Frutexites](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Moonmilk](#)
[Organomineralization](#)
[Stromatolites](#)
[Thrombolites](#)

system of large fossils is excellently preserved (Franzen, 1994). Fundamental studies on biofilm soft-tissue relationship were carried out by Richter (1994) and Liebig (1997). Another intriguing example is the “soft-tissue” preservation in dinosaur bones (Kaye et al., 2008). Kaye and coworkers have found microbially altered soft tissue in a dinosaur bone and they give a critical explanation of this fact. Within their paper they discuss the processes of tissue preservation and also demonstrate the importance of recent microbial biofilm invasion and late diagenetic alteration of primarily formed pyrite framboids. The clou of this investigation is also that there is no real soft-tissue preservation – however, preserved biofilms cast the former tissue and give an idea about the original structures.

All “Hautschatten” soft-tissue preservations are probably preserved lithified biofilms and not real soft tissues.

Conclusion

Biofilms play a significant role in suboxic and anaerobic milieus preserving soft tissue remains. The body outline of dead organisms is exactly traced by the margins of the mineralised biofilms and give a perfect image of the entire fossil. These structures are often called after the German word “Hautschatten”-perservation and is commonly found under black shale condition.

Bibliography

- Briggs, D. E. G., 1999. Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis. *Philosophical Transactions of Royal Society Series B*, **354**, 7–16.
- Briggs, D. E. G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences*, **31**, 275–301.
- Briggs, D. E. G., and Kear, A. J., 1993. Fossilization of soft-tissue in the laboratory. *Science*, **259**, 1439–1442.
- Briggs, D. E. G., and Wilby, P. R., 1996. The role of calcium carbonate – calcium phosphate switch in the mineralization of soft bodied fossils. *Journal of the Geological Society, London*, **153**, 665–668.
- Franzen, J. L., 1994. Bacterial preservation of the digestive system in Messel Equoids. *Kaupia*, **4**, 3–11.
- Grimes, S. T., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Gupta, N. S., Michels, R., Briggs, D. E. G., Evershed, R. P., and Pancost, R. D., 2006. The organic preservation of fossil arthropods: an experimental study. *Proceedings of the Royal Society of London, Series B*, **273**, 2777–2783.
- Kaye, T. G., Gaugler, G., and Sawlowicz, Z., 2008. Dinosaur soft tissues interpreted as bacterial biofilms. *PLOS One*, **3**(7), e2808.
- Liebig, K., 1997. *Fossil Microorganisms from the Eocene Messel Oil Shale of Southern Hessen*. University Dissertation, Heidelberg, Germany.
- Richter, G., 1994. Bacteria and bacteria-like structures from the oil-shale of Messel. *Kaupia*, **4**, 21–28.
- Seilacher, A., 1970. Begriff und Bedeutung der Fossil-Lagerstätten. *Neues Jahrbuch Fur Geologie und Paläontologie, Monatshefte*, **1970**, 34–39.
- Seilacher, A., Reif, W. E., and Westphal, F., 1985. Sedimentological, ecological and temporal patterns of fossil lagerstätten.

Philosophical Transactions of the Royal Society of London, Series B, **311**, 5–23.

Toporski, J. K. W., Steele, A., Westall, F., Avet, R., Martill, D. M., and McKay, D. S., 2002. Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany. *Geochimica et Cosmochimica Acta*, **66**, 1773–1791.

Wuttke, M., 1983. “Weichteilerhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

[Bacteria](#)
[Beggiatoa](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Black Shales](#)
[Detachment](#)
[Ediacaran Biota](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Thiotrophic Bacteria](#)
[Whale and Wood Falls](#)

BIOGEOCHEMICAL CYCLES

A biogeochemical cycle defines the pathways by which chemical elements occurring in organisms are cycled between different living and nonliving compartments on the Earth (e.g., biosphere, atmosphere, hydrosphere, geosphere, etc.). Typical driving forces of a biogeochemical cycle are the metabolisms of living organisms, geological processes, or chemical reactions. For specific reading on major biogeochemical cycles, please refer to “[Carbon Cycle](#),” “[Nitrogen](#),” “[Sulfur Cycle](#),” “[Phosphorus](#),” “[Phosphorites](#).”

BIOLOGICAL CONTROL ON DIAGENESIS: INFLUENCE OF BACTERIA AND RELEVANCE TO OCEAN ACIDIFICATION

Fred T. Mackenzie¹, Andreas J. Andersson²

¹University of Hawaii, Honolulu, HI, USA

²Bermuda Institute of Ocean Sciences, Ferry Reach, St. George's, Bermuda

Introduction

Soon after carbonate mineral particles are deposited, a series of chemical, physical, and biological processes start that can significantly alter their mineralogy, isotopic composition and chemistry, and the fabric of the accumulating sediment. These processes include dissolution and precipitation reactions, abrasion, and transport, and

Bibliography

- Andrews, S. C., Robinson, A. K., and Rodriguez-Quinones, F., 2003. Bacterial iron homeostasis. *FEMS Microbiology Ecology*, **27**, 215–237.
- Barbeau, K., 2006. Photochemistry of organic iron(III) complexing ligands in oceanic systems. *Photochemistry and Photobiology*, **82**, 1505–1516.
- Bellenger, J. P., Wichard, T., Kustka, A. B., and Kraepiel, A. M. L., 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience*, **1**, 243–246.
- Borer, P., Sulzberger, B., Reichard, P. U., and Kraemer, S. M., 2005. Effect of siderophores on the light-induced dissolution of colloidal iron(III)(hydr)oxides. *Marine Chemistry*, **93**, 179–193.
- Boukhalfa, H., Reilly, S. D., and Neu, M. P., 2007. Complexation of Pu(IV) with the natural siderophore Desferrioxamine B and the redox properties of Pu(IV)(siderophore) complexes. *Inorganic Chemistry*, **46**, 1018–1026.
- Cheah, S. F., Kraemer, S. M., Cervini-Silva, J., and Sposito, G., 2003. Steady-state dissolution kinetics of goethite in the presence of desferrioxamine B and oxalate ligands: implications for the microbial acquisition of iron. *Chemical Geology*, **198**, 63–75.
- Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S. L., Briat, J. -F., and Walker, E. L., 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature*, **409**, 346–349.
- Duckworth, O. W., and Sposito, G., 2005. Siderophore-manganese (III) interactions. I. Air-oxidation of manganese(II) promoted by desferrioxamine B. *Environmental Science Technology*, **39**, 6037–6044.
- Essen, S. A., Bylund, D., Holmstrom, S. J. M., Moberg, M., and Lundstrom, U. S., 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *Biometals*, **19**, 269–282.
- Hantke, K., 2001. Iron and metal regulation in bacteria. *Current Opinion in Microbiology*, **4**, 172–177.
- John, S. G., Ruggiero, C. E., Hersman, L. E., Tung, C. S., and Neu, M. P., 2001. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). *Environmental Science Technology*, **35**, 2942–2948.
- Kim, H. J., Graham, D. W., DiSpirito, A. A., Alterman, M. A., Galeva, N., Larive, C. K., Asunskis, D., and Sherwood, P. M. A., 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science*, **305**, 1612–1615.
- Kraemer, S. M., 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science*, **66**, 3–18.
- Kraemer, S. M., Cheah, S. F., Zapf, R., Xu, J. D., Raymond, K. N., and Sposito, G., 1999. Effect of hydroxamate siderophores on Fe release and Pb(II) adsorption by goethite. *Geochimica et Cosmochimica Acta*, **63**, 3003–3008.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Siderophores and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry*, **59**, 53–76.
- Kraemer, S. M., Crowley, D., and Kretzschmar, R., 2006. Siderophores in plant iron acquisition: geochemical aspects. *Advances in Agronomy*, **91**, 1–46.
- Liermann, L. J., Kalinowski, B. E., Brantley, S. L., and Ferry, J. G., 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta*, **64**, 587–602.
- Liu, X. W., and Millero, F. J., 2002. The solubility of iron in seawater. *Marine Chemistry*, **77**, 43–54.
- Martell, A. E., Smith, R. M., and Motekaitis, R. J., 2001. NIST critically selected stability constants of metal complexes database. Gaithersburg MD: NIST. http://www.nist.gov/srd/WebGuide/Critical/46_8.htm.
- Martinez, J. S., and Butler, A., 2007. Marine amphiphilic siderophores: marinobactin structure, uptake, and microbial partitioning. *Journal of Inorganic Biochemistry*, **101**, 1692–1698.
- Miethke, M., and Marahiel, M. A., 2007. Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews*, **71**, 413–451.
- Powell, P. E., Cline, G. R., Reid, C. P. P., and Szaniszlo, P. J., 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature*, **287**, 833–834.
- Reichard, P. U., Kraemer, S. M., Frazier, S. W., and Kretzschmar, R., 2005. Goethite dissolution in the presence of phytosiderophores: rates, mechanisms, and the synergistic effect of oxalate. *Plant and Soil*, **276**, 115–132.
- Reichard, P. U., Kretzschmar, R., and Kraemer, S. M., 2007. Dissolution mechanisms of goethite in the presence of siderophores and organic acids. *Geochimica et Cosmochimica Acta*, **71**, 5635–5650.
- Römheld, V., 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in gramineous species – an ecological approach. *Plant Soil*, **130**, 127–134.
- Rosenberg, D. R., and Maurice, P. A., 2003. Siderophore adsorption to and dissolution of kaolinite at pH 3 to 7 and 22 degrees C. *Geochimica et Cosmochimica Acta*, **67**, 223–229.
- Rue, E. L., and Bruland, K. W., 1995. Complexation of iron(III) by natural organic-ligands in the central North Pacific as determined by a new competitive ligand equilibration adsorptive cathodic stripping voltammetric method. *Marine Chemistry*, **50**, 117–138.
- Schaaf, G., Erenoglu, B., and von Wiren, N., 2004. Physiological and biochemical characterization of metal-phytosiderophore transport in graminaceous species. *Soil Science and Plant Nutrition*, **50**, 989–995.
- Takagi, S. I., 1976. Naturally occurring iron-chelating compounds in oat-root and rice-root washings. I. Activity measurement and preliminary characterization. *Soil Science and Plant Nutrition*, **22**, 423–433.
- Takagi, S., Nomoto, K., and Takemoto, T., 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *Journal Of Plant Nutrition*, **7**, 469–477.
- Völker, C., and Wolf-Gladrow, D. A., 1999. Physical limits on iron uptake mediated by siderophores or surface reductases. *Marine Chemistry*, **65**, 227–244.

Cross-references

Gallionella
 Leptothrix
 Metals, Acquisition by Marine Bacteria
 Microbial Biomineralization
 Microbial-Metal Binding

SILICA BIOMINERALIZATION, SPONGES

Hermann Ehrlich
 Dresden University of Technology, Dresden, Germany

Synonyms

Biosilicification, Silication

Definition

Biosilicification: The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges,

molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants.

Biom mineralization and biosilicification

Introduction

The growth in geobiology and biogeochemistry has led to a number of new existing research areas where the distinctions between the biological, chemical, and earth science disciplines melt away (Weiner and Dove, 2003). Of the intriguing topics that are receiving renewed attention, the study of biomineral formation based on organic templates is one of the most fascinating topics today. During the processes of biomineralization, the organic material acts variously as nucleator, cooperative modifier, and matrix or mold for the mineral ions with respect to biominerals formation which are characterized by a hierarchical order (Bäuerlein, 2007).

Biominerals may be deposited within the organism, and within its immediate surroundings or environment, as a result of the metabolism of the living creature (Skinner, 2005). The physiological pathways by which organisms precipitate skeletal minerals and the forms and functions of the skeletons they fashion have been shaped by natural selection through geologic time, and all have constrained continuing evolution in skeleton forming clades (Knoll, 2003). The variety of biomineralizers recently reviewed by Ehrlich et al. (2008a) can best be expressed by the fact that, approximately 128,000 species of molluscs, about 800 species of corals, 15,000 species of sponges including 550 species of glass sponges, 700 species of calcareous green, red and brown algae, more than 300 species of deep-sea benthic foraminifera, and 200,000 diatom species (Mann and Droop, 1996) exist.

Precipitation of silica on organic templates could have been abiotic, in which organic matter merely serves as a template for nucleation, or biogenic, i.e., induced by biological activities or a combination of both (Mukhopadhyay et al., 2004). Silicon, in the form of silicic acid, is a fundamental nutrient for diatoms, silicoflagellates, radiolaria, and many sponges, all of which polymerize it to build skeletons of biogenic silica (Maldonado et al., 2005).

Sponges

Sponges (Porifera) are the most simple and ancient multicellular animals on earth and live attached to the seabed or another substratum. Sponges diverged from other animals earlier in evolutionary history than any other known animal group, extant or extinct (Reitner and Mehl, 1996), with the first sponge-related record in earth history found in 1.8 billion year old sediments (Nichols and Wörheide, 2005). The huge diversity with respect to their natural habitat is probably the reason for the estimated number of approximately 15,000 different sponge species (Hooper and van Soest, 2002). To support life, sponges pump huge amounts of seawater (170–72,000 × their own body volume per day) through their bodies (Simpson,

1984) and filtrate it to capture food particles, such as bacteria, micro algae, other unicellular organisms, or dead organic particles. The whole sponge body is designed for efficient filtration of the surrounding seawater, which is essential because of the low nutrient availability at the sea floor.

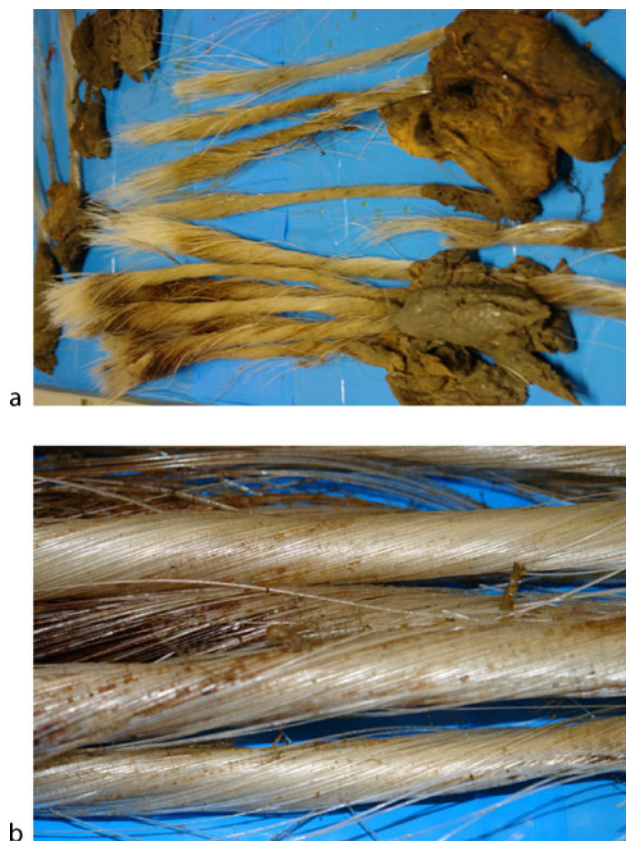
The phylum Porifera (sponges) is divided into three classes, Hexactinellida and Demospongiae that comprise a siliceous skeleton and the Calcarea with a calcareous skeletal network (Bergquist, 1978). Significant separation of the Calcarea from the Hexactinellida/Demospongiae (Silicispongia) clade is also implied by fundamental differences in morphology and development: unlike Silicispongia, calcareans lack morphologically distinct microscleres, and their calcitic spicules are secreted intercellularly within an organic sheath (vs. formation of silica spicules onto an intracellularly secreted axial organic filament in Silicispongia (Botting and Butterfield, 2005)). Sponges bear only a few different cell types of which the sclerocytes produce the spicules, i.e., siliceous structures, which are often needle-shaped. These spicules often form a distinct skeleton, but occasionally they are loosely distributed throughout the sponge body without identifiable order or they are lacking entirely. Size, type, shape, combination of spicules, and their skeletal arrangements are the fundamentals of current sponge systematics (Erpenbeck et al., 2006; Boury-Esnault and Rutzler, 1997).

Among the siliceous sponges most important are the monophyletic class Hexactinellida, and the polyphyletic “Lithistida,” which includes taxa belonging to different groups within the class Demospongiae. Both classes are characterized by rigid skeletons of fused spicules.

Among the class Hexactinellida, the order Hexactinosida (subclass Hexasterophora) (Schulze, 1886), known since the late Devonian (Rigby et al., 1981, 2001), developed a rigid and, to a certain extent, firm skeleton that consists of fused hexactins. This fused, three-dimensional, generally right-angled skeletal network stays intact even after the death of the sponge.

Hexactinellida

Hexactinellida Schmidt (Porifera) are deep-water marine sponges defined by their production of siliceous spicules of hexactinic, triaxonic (cubic) symmetry, or shapes clearly derived from such forms by reduction of primary rays or terminal branches added to the ends of primary rays. They lack calcareous minerals and sclerified organic spongin as skeletal components. Siliceous spicules may be entirely loose, or partially fused to form a rigid basal and choanosomal framework. Their living tissues are mainly syncytial, with distinctive porous plugs joining differentiated regions of the syncytium to each other or to discrete cellular components. Flagellated-collar units are anucleate. Hexactinellids are viviparous and, from detailed study of a single species, produce distinctive trichimella larvae. Two subclasses are recognized by different microsclere forms – amphidiscs and hexasters.



Silica Biomineralization, Sponges, Figure 1 Specimens of glass sponge *Hyalonema* (*Corynonema*) *populiferum* Schulze, 1899 recently collected in Pacific (Oregon, USA) (a) possess high flexible anchoring siliceous spicules (b).

Hexactinellids include more than 500 described species, 7% of all Porifera, distributed in five orders, 17 families, and 118 genera (Schulze 1886, 1904; Sollas, 1888, Okada, 1928; Reischwig, 1971, 2002; Lévi et al., 1989; Mehl, 1992; Leys, 2003).

The taxonomy of the class Hexactinellida is very incomplete in the sense that probably not even half of its species are known to science. This is obvious from the fact that many of the hexactinellids species collected during recent deep-sea expeditions are new. The spicules of most hexactinellids are larger (Figure 1) and more luxuriously architected than those in demosponges (Tabachnick, 2002).

Demospongiae

In demosponges (Demospongiae Sollas, 1885) we have much the largest group, with 95% of all extant sponges. They are also the most diverse group. Some are freshwater but predominantly they are marine species living from the intertidal to the deepest seas, with around 15 orders, 88 families, and about 500 valid genera (Hooper and van Soest, 2002). The skeleton is composed of monaxononic or

tetragononic siliceous spicules (never triaxononic) bound together with collagen-like protein spongin in discrete fibers or loosely aggregated and ubiquitous collagenous filaments forming the ground substance of the intercellular matrix.

Because the morphology of the spicules differs species-specifically, they are used as a major taxonomic character. However, very little is known about the molecular mechanism(s) that determine the position and arrangement of the spicules within the demosponge skeleton (Uriz et al., 2003; Uriz, 2006).

Lithistid demosponges

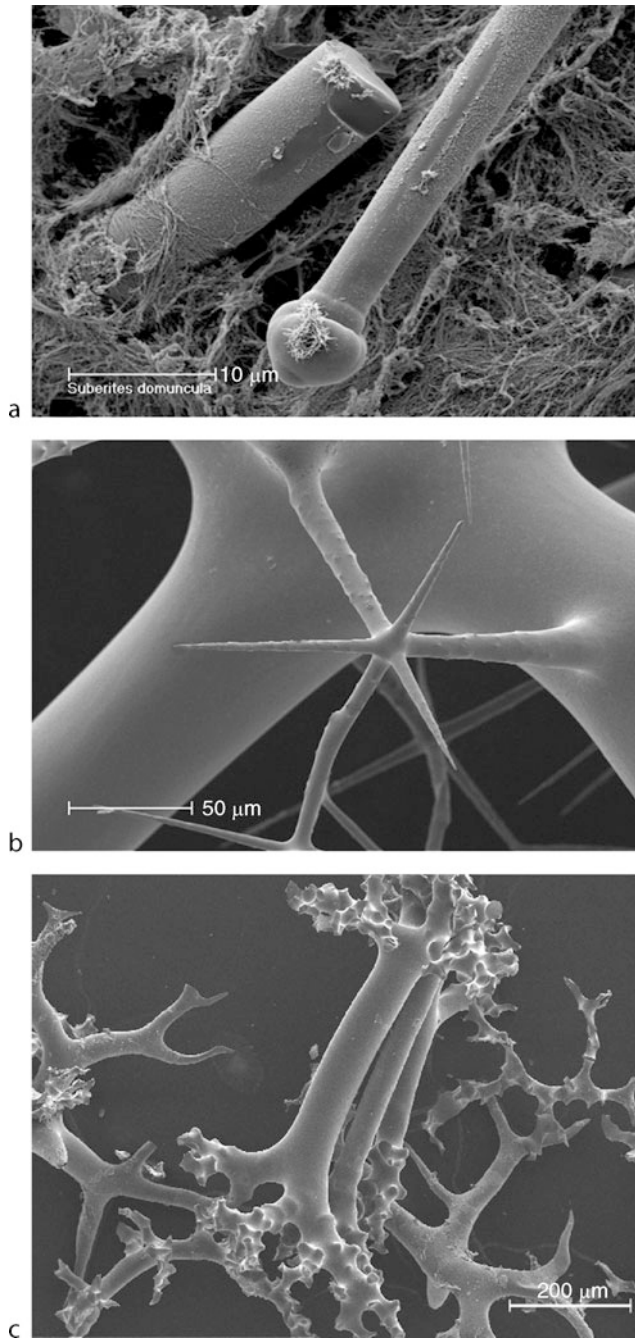
Like hexactinellid sponges, lithistid sponges also contain siliceous skeletons comprising spicules. These spicules (desmas) interlock, rendering the skeleton rigid and often stony (Pisera, 2000, 2003; Kelly, 2000, 2003). At present little is known about the deposition of silica within this taxonomic order.

Lithistid sponges are commonly found on tropical and temperate seamounts and continental margins down to depths of about 1,000 m (Kelly, 2000). Several Southwest Pacific species, form large cups, bowls, and plates that extend linearly from the sponge margin. Close inspection of the lamellae show irregular or concentric ridges, indicating that silica deposition is not continuous but rather variable in nature (Ellwood et al., 2007) presented detailed records of trace metals and carbon isotopes to understand siliceous spicule formation in the deep-sea lithistid sponge *Corallistes undulatus* Lévi and Lévi, 1983 (Demospongiae: Corallistidae). X-ray analysis of two longitudinal sections removed from the lamellae of the cup-shaped sponge revealed 144 and 137 light and dark density band-pairs, respectively, within the siliceous skeleton. Although there was some variability in the ^{32}Si data, the overall age established using these data indicated that the sponge was between 135- and 160-year old.

Forms of the silica deposition in sponges

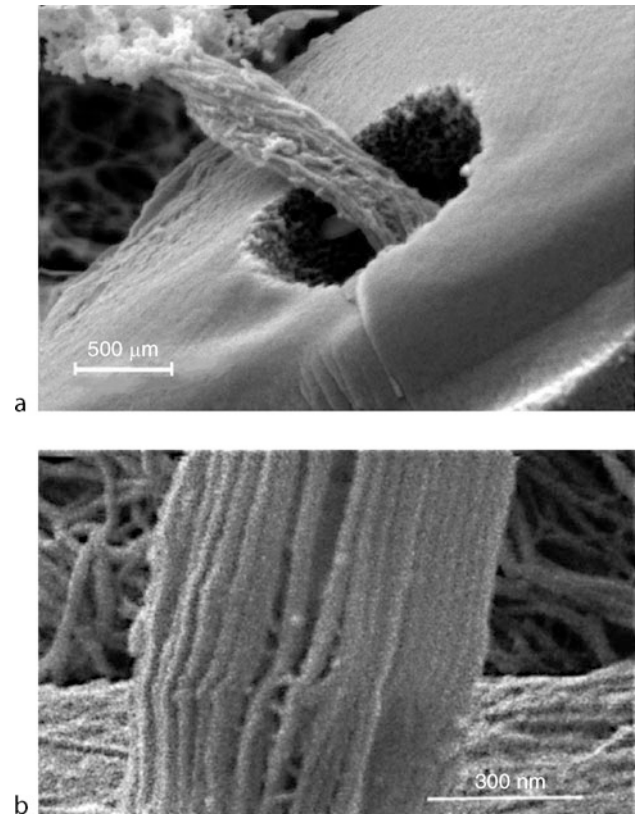
Spicules, skeletal frameworks and desmas are the main forms of the silica deposition in sponges as shown in Figure 2.

The history of the discoveries related to sponge spicules is well reviewed by Vosmaer and Wijsman (1905). According to these authors, after Schweigger in 1819 had demonstrated that the spicules of sponges in some cases do not consist of calcium carbonate, Grant in 1826 found them to contain silica, and Bowerbank in 1841 showed that, in addition to the silica some organic matter is present. Sollas in 1885 likewise finds that the sponge silica resembles opal. He states in general that the refractive index of sponge silica is that of opal or colloidal silica, and not of quartz. This kind of opal was proposed by Vosmaer and Wijsman to call *spicopal*, however, the organic matter of the spicule was called by F.E. Schulze *spiculine*. As to the structure of the spicules, Gray in 1835 had found them in *Hyalonema* glass sponge to



Silica Biomineralization, Sponges, Figure 2 SEM images of three main forms of the silica deposition in sponges: (a) spicules of demosponge *Suberites domuncula*; (b) skeletal silica-chitin-based framework of *Farrea occa* glass sponge (both images courtesy C. Ekkert), (c) siliceous desmas of *Neopelta* sp. lithistid demosponge. (Image courtesy A. Pisera.)

consist of layers, which became conspicuous by heating. These layers concentrically surround a “central canal,” which is filled out, as Kölliker in 1864 has shown, by an organic mass, the axial rod. Claus in 1868 found that the



Silica Biomineralization, Sponges, Silica Biomineralization, Sponges, Figure 3 SEM images of the axial rod (axial filament) of demosponge *S. domuncula* (a) show interconnections between individual proteinaceous nanofibrils (b) visible only at high magnification.

silica which directly surrounds this central rod, is homogenous. He called this homogenous cylinder the axial cylinder. The sponge spicule thus consists of a central organic axis, surrounded by concentric layers of opal, the outermost of which is invested in a spicule sheath of organic matter or rather of organic matter in intimate association with silica (Vosmaer and Wijsman, 1905) (Figure 3).

Using the freshwater sponge *Ephydatia fluviatilis*, Weissenfels and Landschoff (1977) and Weissenfels (1989) demonstrated that the formation of spicules starts in sclerocytes within a specific vesicle. After the production of an axial organic filament, silicon is deposited around it and the whole process of forming a spicule (190 µm in length and 6–8 µm in diameter) is completed after 40 h, at 21°C.

Siliceous sponge spicules have traditionally been separated into two categories termed, according to their size, megascleres and microscleres (Lévi, 1973). They are highly diverse in sponges and the selection pressures responsible are difficult to envisage. There are over 12 basic types of megasclere and 25 types of microsclere reported in Demospongiae, 20 basic types of megasclere, and 24 types of microsclere in

Hexactinellida, besides a long list of variations of the basic types (Lévi, 1973, 1993; Garrone et al., 1981; Simpson 1984, 1990; De Vos et al., 1991; Bavestrello et al., 1993; Boury-Esnault and Rutzler, 1997; Tabachnick and Reiswig, 2002).

Desmas differ from typical demosponge spicules: first they are joined by articulation; second, they always display irregularity and often complex morphology and sculpture. The most basic difference, perhaps, is in the fact that the crepis or axial filament is usually very short (or even invisible) and extends only a short distance from the spicule center. Growth ceases very rapidly, and an important part of a desma, including secondary branches as well as elements of sculptures, does not depend on the axial filament (Sollas, 1888; Lévi, 1991).

In contrast to the high diversity of spicules, there are relatively few basic types of skeletal framework in demosponges. Six elemental types of skeletons with intermediate forms can be differentiated: hymedesmoid, plumose, axial, radiate, reticulate, and arranged in strength confusion (Boury-Esnault and Rutzler, 1997)

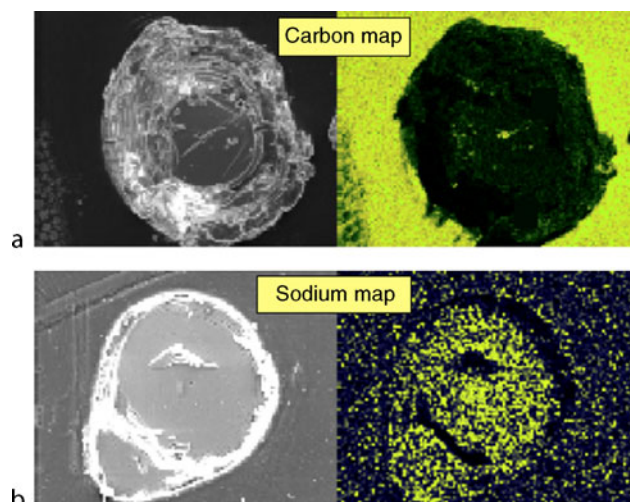
Chemistry of sponge silica

It is established that siliceous sponge spicules of both the Hexactinellida and Demospongiae consist of hydrated amorphous silica (Simpson, 1989; Uriz et al., 2003; Uriz, 2003, 2006). Chemically, they differ only slightly in their $\text{SiO}_2\text{:H}_2\text{O}$ proportions. Desma bearing demosponges ("Lithistida") have a ratio of 5:1 whereas Hexactinellida have 4:1 (Hartman, 1981). Holzhüter et al. (2005) reported that spicules of demosponge *Suberites domuncula* consist of more than 90% silicon and oxygen. They found that at the molecular level silica of sponge spicules has following amorphous structure: SiO_4 tetrahedra are connected to form polyhedra, which are comparable with those found in silica gels. These polyhedra form silica particles up to 3 nm in size which are interconnected forming a dense packing containing pores. However, recently Mugnaioli et al. (2009) reported for the first time crystalline nanorod-like structures which were localized within the organic axial filament of the primordial spicules of demosponge *S. domuncula*. These nanorods have a layered structure that matches smectitic phyllosilicates. It was suggested that this kind of crystalline structure acts as an inorganic template and gives shape and orientation to the newly forming spicule, although its function is admittedly not yet fully understood.

The major trace elements found in the spicules and siliceous sponge skeletons (i.e., S, Ca, K, Na, and Cl) are also the most common in seawater (Sandford, 2003). It was suggested (Aizenberg et al., 2004) that sodium can play an important role in physicochemical properties of glass sponge spicular formations (Figure 4).

Mechanisms of silicification in sponges

Silica deposition is a fundamental process in sponges. According to the modern point of view, two different

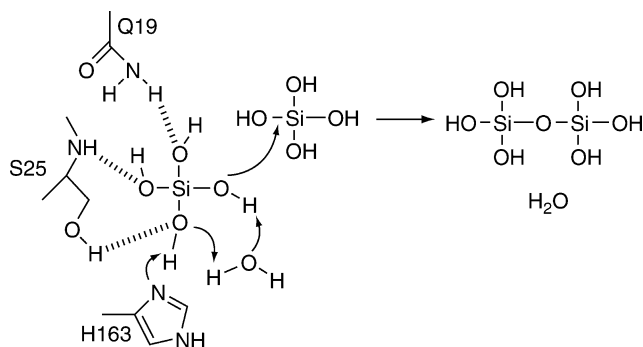


Silica Biomineralization, Sponges, Figure 4 SEM image and EDX carbon map (a) of microtome-cut spicule of *Euplectella aspergillum* glass sponge cross section highlights the presence of an organic filament. (b) EDX Na map indicates an enhanced concentration of Na ions within the spicule cross section, with the highest density of Na occurring in the 2- μm core. (Images courtesy J. Aizenberg.)

mechanisms of silicification in sponges are proposed: enzymatic (silicatein-based) and nonenzymatic, or self-assembling (chitin- and collagen-based).

Silicatein-based silicification

The group of Morse (Shimizu et al., 1998; Cha et al., 1999; Shimizu and Morse, 2000; Weaver and Morse, 2003; Weaver et al., 2003) discovered that organic filament in demosponge *Tethya aurantium* is composed of a cathepsin L related enzyme, termed silicatein. They cloned two of the proposed three isoforms of silicateins, the α - and β -forms, from this demosponge (Cha et al., 1999). In subsequent years these molecules were cloned also from other sponges, among them *S. domuncula* and *Lubomirskia baicalensis* (as reviewed in Müller, 2003; Müller et al., 2007). The spicules are produced in special cells, the sclerocytes; that formation starts intracellularly around a silicatein filament and is completed extracellularly by apposition of silicatein onto the growing spicules (Müller et al., 2005, 2006). Cathepsin L is an endopeptidase which cleaves peptide bonds with hydrophobic amino acid residues in P2 and P3 positions and occurs in lysosomes as well as extracellularly as secreted enzymes. The silicateins are distinguished from the cathepsins by the replacement of the first amino acid residue in the catalytic triad, cysteine by serine (Shimizu et al., 1998). It has been proposed that serine increases the nucleophilicity during the nucleophilic attack at the silicon atom. The polymerization-promoting activity of the silicateins has been shown to be catalytic and not stoichiometric. It is possible that the controlled punctuated secretion of low



Silica Biomineralization, Sponges, Figure 5 The chemical mechanism proposed by Fairhead et al. (2008) to operate for the polymerization of silicic acid using silicatein molecules. The authors proposed H163 deprotonates Si(OH)_4 ; the extensive water network could permit proton shuttling. They illustrated one possibility.

concentrations of monomeric silicateins, pulses in the transitory flux of silica-precursor molecules, oscillations in the pH, ionic, or other conditions of the condensation environment, or a combination of these factors may be responsible for the annular patterning and continued deposition of silica in spicule biosynthesis in vivo once the axial filament has become completely covered during the early stages of silica deposition around this proteinaceous core. Alternatively, the continued growth of silica may be a result of dangling bonds at the growth surface of the newly deposited silica (Saito et al., 1995).

There are two possible mechanisms for enzyme catalysis (Fairhead et al., 2008): (1) stabilize at the active site one molecule of deprotonated silicic acid (the nucleophile) which will then react with another molecule of silicic acid; or (2) stabilize a protonated silicic acid (the electrophile) which will then react with another molecule of silicic acid. In proposed mechanism (Figure 5) (Fairhead et al., 2008), the roles of C25S location and flanking mutations are simply to create a sufficiently sized pocket that will allow recognition of the tetrahedral Si(OH)_4 molecule in such a way that H163 location can deprotonate it. The deprotonated Si(OH)_4 protein complex can be thought of as a template for condensation reaction. In this proposal, there is no need to involve a high energy covalent intermediate. The presence of a specific Si(OH)_4 transporter in sponge suggests the true substrate in vivo is indeed silicic acid, not high energy silicon alkoxides. This being so, the simple acid–base activation mechanism proposed by authors seems a good model for the biological process.

According to Müller et al., formation of spicules is a biologically controlled extracellular process (Schröder et al., 2006; Müller et al., 2007). The following model of silicification in demosponges is derived from observations and propositions put forth by Morse's and Müller's groups (reviewed in Leys et al., 2007). Sclerocytes secrete axial filaments composed mainly of silicateins but other organic

molecules are included. Silicatein acts as a structural template for formation of the highly organized mesoporous axial filament. Silicate is used or is actively taken up into the sclerocyte and complexed with specific proteins to form an as yet unidentified organic-silica substrate. The organo-silica complex is transported across silicalemma to the "silica deposition space." Silica from organo-silica substrate is polymerized on the outer surface of the mesoporous axial filaments as nanospheres, perhaps at serine and histidine catalytic centre sites, and the complexing protein is released and possibly recycled to the sclerocyte cytoplasm.

Growth of the axial filament continues at spicule tips, providing primary patterning of the spicule, while at the same time transport and deposition of silica continue on lateral spicule surfaces between the tips. Once the first few layers of silica nanospheres encase the axial filament, enzymatic activity can no longer be expressed by silicateins of the axial filament. Continuing deposition of silica on the outer surface of spicules is facilitated and controlled by silicatein at active sites on the silicalemma, resulting in production of specific patterned morphologies of spicules. Several elements of this model remain unverified. Other unknowns include the roles of the minor non-silicatein organic compounds incorporated in axial filaments, whether silicateins associated with the silicalemma are the same forms as those in the axial filaments and how the final high-fidelity submicroscopic external patterning on spicule surfaces is genetically controlled. However, recently Kozhemyako et al. (2009) identified silicatein genes (*AcSilA* and *AcSilB*) in nonspicule-forming marine sponge *Acanthodendrilla* spp. The authors suggest that silicateins could participate also in the functions unrelated to spiculogenesis.

Chitin- and collagen-based silicification

Aminopolysaccharide chitin has a nanofibrous structure and the chain of pyranose ring arranges almost parallel to the (100) plane and extends along the fiber axis (Iijima and Moriwaki, 1990). The chitin molecule has C=O, O–H, and N–H groups and oxygen atoms, which have affinity to the calcium, phosphate, carbonate, and hydroxyl ions of corresponding calcium phases. However, the same functional groups possess affinity to silicate ions. Because there is a possibility that such an oriented organic matrix acts as a template, or an ordered structural framework, it was hypothesized the existence of naturally occurring silica-chitin composites (Ehrlich et al., 2007). Moreover, silicon was found associated with glycosaminoglycans bound as an ether or ester-like silicate with C–O–Si or C–O–Si–O–Si–O–C bonds, in amounts of one Si atom/130–280 repeating units of the organic (Schwartz, 1973). The role of chitin in biosilicification is still not completely understood. Although chitin is one of the most important biopolymers in nature, knowledge of its interaction with silicon in vivo was absent up to now. Only recently, Ehrlich et al., isolated and identified

chitin from skeletal formations of some marine glass sponges for the first time. The presence of chitin within the framework skeleton of *Farrea occa* (Ehrlich et al., 2007) and *Euplectella aspergillum* (Ehrlich and Worch, 2007a) as well as separate spicules *Rossella fibulata* (Ehrlich et al., 2008c) could also be revealed by gentle NaOH-based desilicification technique established by Ehrlich et al. (2006) and Ehrlich and Worch, (2007b). The structure of the chitin extracted from these sponges turned out to be similar to alpha-chitin from invertebrates.

It was suggested that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the chitin surface, presumably by polar and H-bonding interactions (Ehrlich et al., 2008b). On the basis of the obtained results, authors proposed a model for the nanostructure of the naturally occurring silica-chitin composite unit, including interaction between poly-N-acetylglucosamine fragment of the chitin nanofibril and silica nanoparticles, which can be seen in Figure 6.

Because chitin could play a crucial role also in biosilicification in fungi (Kolb et al., 2004), it was hypothesized (Ehrlich et al., 2007) that chitin molecules are probably part of a very old organic template system involved in a biosilicification phenomenon, which was established a long time before the origin of first metazoan (e.g., glass sponges).

Recently, Ehrlich et al. reported for the first time on example of *Hyalonema sieboldi* glass sponge that its spicules are a biocomposite containing a silicified collagen matrix, and that high collagen content is the origin of the unique mechanical flexibility of the spicules (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). Especially, interest is paid now to newly discovered highly hydroxylated collagen isolated from *H. sieboldi* spicules (Prof. M. Collins personal communication). Glass sponges are largely restricted to deep, cold-water (between 0 and 4°C) habitats (Tabachnick, 1991; Janussen et al., 2004; Dohrmann et al., 2008). Therefore, from the ecological point of view collagen- (as well as chitin-) based silicification which occurs in spicules and other skeletal formations of these sponges is an example of unique cold-water biomineralization. There is no evidence in the literature about silicatein activities at this temperature level. Skeletal biomineralization requires energy and so imposes a metabolic cost on skeleton-forming organisms (Knoll, 2003). The hypothesis is that probably some phosphate moieties and ATPase-based mechanisms are in some way involved in biosilicification at temperatures near zero (Ehrlich, 2009).

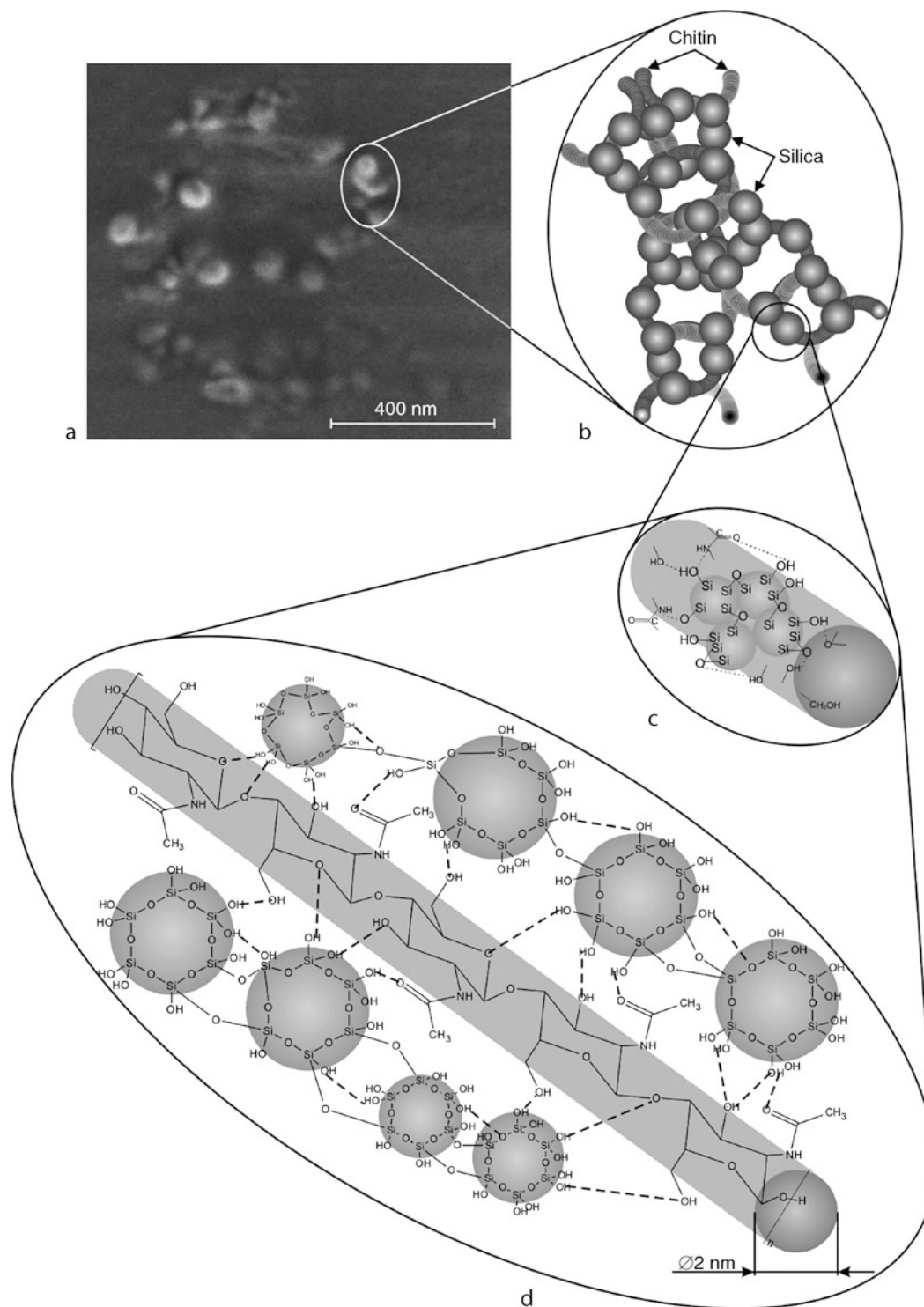
The procedure of alkali slow etching opens the possibility to observe the forms of collagen fibrils located within silica layers of sponge spicules and their distribution. For example, the results obtained by SEM observations of the desilicified spicular layers of *Monorhaphis chuni* provide strong evidence that collagen fibrils' orientation within spicules possesses twisted plywood architecture (Ehrlich et al., 2008b). Typical fibrillar formations were observed within the tubular silica structures of this sponge in all layers starting from the inner axial channel

containing axial filament (Figure 7c) up to the outermost surface layer of the spicules as shown in Figure 7b and Figure 7d. The fibrils in each cylinder form individual concentric 2D networks with the curvature of the corresponding silicate layers. These layers of about 1 µm in thickness are connected among each other by protein fibers (Figure 7a), which possess a characteristic nanofibrillar organization. Partially desilicified nanofibrillar organic matrix observed on the surface of silica-based inner layers of the demineralized spicule provides strong evidence that silica nanoparticles of diameter about 35 nm are localized on the surface of corresponding nanofibrils (Ehrlich et al., 2008b). This kind of silica nanodistribution is very similar to the silica distribution on the surface of collagen fibrils in the form of nanopearl necklets, firstly observed in the glass sponge *H. sieboldi* (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). It was suggested that the nanomorphology of silica on proteinous structures described above could be determined as an example of biodirected epitaxial nanodistribution of the amorphous silica phase on oriented collagenous fibrillar templates (Ehrlich et al., 2008b). From this point of view, basal spicules of *Monorhaphis* sponges could be also defined as natural plywood-like silica-ceramics organized similarly to the crossed-lamellar layers of seashells. The authors suggested also that the matrix of the *M. chuni* anchoring spicule is silicified fibrillar collagen rather than collagen-containing silica which is the reason for their remarkable mechanical flexibility.

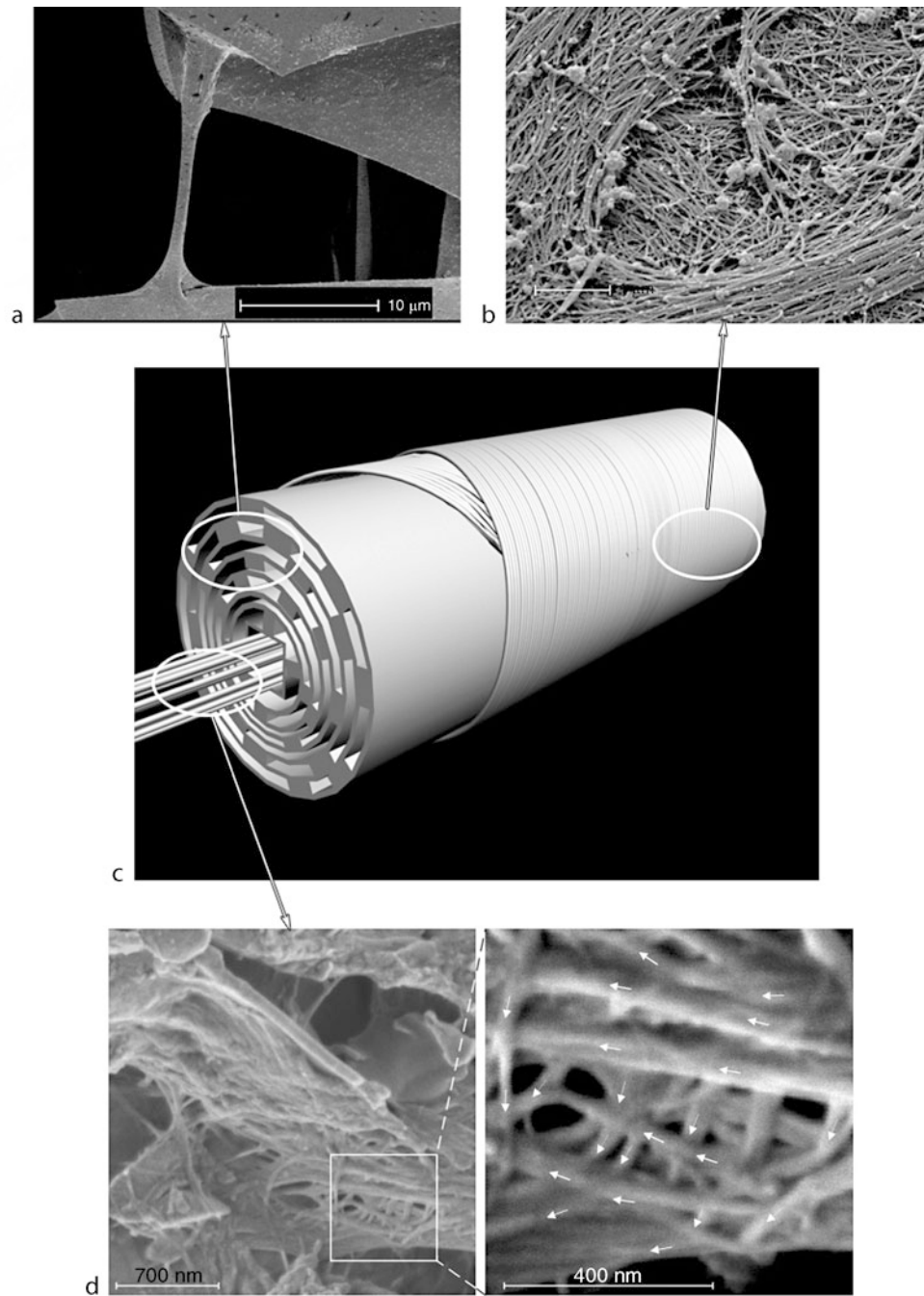
Biogeological aspects of silica deposition by sponges

Analysis of the biogeochemical cycle of silicon suggests that the biospheric environmental silicic acid concentration may have varied considerably during the time of life on Earth. Biochemical evolution today is proceeding within environments in which the silicic acid concentration is considerably lower than previous evolutionary periods (Exley, 1998).

Concomitant with the advent of dioxygen, about 2500 million years ago, and its subsequent gradual increase in atmospheric concentration from approximately 1% towards the level of 20% which is characteristic of today, there occurred an increasing number of organisms within which silicic acid was processed to silica. The most important of these, in the terms of their diversity, ubiquity (both freshwater and marine species) and biomass, were the diatoms, which occur however late in earth history (Mesozoic). The diatoms are characterized by a silica frustule which surrounds their cell wall. Interestingly that chitin-based organic networks are an integral part of cell wall biosilica from the diatom *Thalassiosira pseudonana* (Brunner et al., 2009). According to Maldonado et al. (2005), our current understanding of the silicon cycle in the ocean assumes that diatoms dominate not only the uptake of silicic acid, but also the production and



Silica Biomineralization, Sponges, Figure 6 Proposed model (adapted from Ehrlich et al., 2008c) of nanostructural organization of the naturally occurring silica-chitin composite unit (a) isolated from spicules of *Rossella fibulata* glass sponge. Silica nanoparticles tightly surround chitinous nanofibrils (b) Schematic view (c) shows a possible nanodistribution of silica on the surface of chitinous nanofibril. Image (d) represents the hypothetical scheme of interaction between silica and poly-N-acetylglucosamine-fragment of the chitin nanofibril and formation of the corresponding hydrogen bonds.



Silica Biomineralization, Sponges, Figure 7 Proposed model (c) of micro- and nanostructural organisation of the basal spicule of *Monorhaphis chuni* glass sponge with respect to organic matrix. Collagen nets, surrounding the spicules, showed a tight mat of nanofibrils (b, image courtesy C. Eckert). SEM image (a) shows a collagenous fibrillar matrix which could function as glue between concentric layers. Schematic image (c) represents the region of the axial canal and axial filament. The axial canal of *M. chuni* possesses a characteristic quadratic opening and contains oriented bundles of unsilicified collagenous nanofibrils. The base material of the walls of the axial canal and concentric layers distributed above it consists of silicified collagen fibrils with a twisted plywood orientation (d). This kind of fibrillar architecture could be responsible for the remarkable micromechanical properties of the spicule as a biocomposite made by silica and collagen.

recycling of biogenic silica, and that other organisms with siliceous skeletons, including sponges, radiolarians, and silicoflagellates, play a negligible role. The authors showed that the retention of Si by siliceous sponges in some sublittoral and bathyal environments is substantial and that sponge populations function as Si sinks. Therefore, sponges may affect Si cycling dynamics and Si availability for diatoms, particularly in Si-depleted environments. It was strongly suggested (Maldonado et al., 2005) that the role of sponges in the benthopelagic coupling of the Si cycle is significant. For example, Antarctic giant hexactinellids, such as *Rossella nuda* and *Scolymastra joubini*, which may be up to 2 m tall, 1.4 m in diameter, and up to 600 kg wet weight, containing up to 50 kg biogenic silica each (Maldonado et al., 2005). They are extremely slow-growing and seemingly reproduce only at long time intervals (Dayton et al., 1974) and become old, probably more than 1,500 years (Dayton, 1979; Gatti, 2002). While their living tissues represent only a modest biomass, the silicious spicules of hexactinellids become an important ecological factor. After the death of the sponges, the megascleres do not dissolve but accumulate on the bottom and over large areas to form spicule mats commonly about 50 cm thick but occasionally exceeding 2 m (Koltun, 1968; Dayton et al., 1974; Henrich et al., 1992). The mats structure the fauna living in and on them.

Marine sponges fractionate silicon isotopes during opal biomineralization to a greater degree than any other process on Earth thus far investigated (De La Rocha, 2003). This ability is in keeping with both their extremely negative silicon isotope composition and their inefficient uptake of silicic acid relative to other silicon-biomineralizing organisms.

Sponges seem to be important players in numerous geobiological processes (Reitner, 2004). For example, siliceous sponge reefs had a wide distribution in prehistoric times and once constructed the largest reefs known on earth, reaching an acme in the Upper Jurassic when a deep-water reef belt on the northern Tethys shelf existed that was 7,000-km long (Krautter et al., 2001). In present times hexactinellid sponges of the order Hexactinosida have constructed reefs at several localities off the coast of British Columbia, Canada. The reefs occur on relict glaciated seafloor areas with a low sedimentation rate and a high dissolved silica concentration in the Queen Charlotte Basin and in Georgia Basin (Conway et al., 2004, 2005) and the reefs represent stable communities that have been growing for up to 9,000 years. The main framebuilders include the hexactinosidan species *Aphrocallistes vastus*, *F. occa* and *Heterochone calyx* in Queen Charlotte Basin, and *A. vastus* and *H. calyx* in Georgia Basin (Conway et al., 2004). The framework of these reefs is constructed through several processes of framebuilding, and the reef matrix is derived from trapping of suspended sediments (Krautter et al., 2001). Similar framebuilding processes are thought to have contributed to the formation of the ancient reefs (Krautter

et al., 2006). Upwelling and downwelling oceanographic processes in a biologically productive coastal sea have contributed to the development of large reef complexes. The reefs form as bioherms (mounds) and biostromes (beds or sheets) that may rise up to 21 m above the seafloor and they cover around 1000 km² on the continental shelf. Individual reef complexes may cover areas of more than 300 km² and occur in 90–240 m depth range (Conway et al., 2001, 2005; Lehnert et al., 2005). Trapping fine-grained siliciclastic sediments in suspension in near-bottom currents is an important part of the mound-forming process, as it prevents the reef framework from collapsing under its own increasing weight.

Summary

Sponges are exclusively aquatic, sedentary, filter-feeding invertebrates, producing silica-made skeletons consisting of individualized elements (spicules) of lengths ranging from micrometers to centimeters, which can subsequently fuse or interlock with each other. The high diversity of spicule shapes and sizes in both fossil and living sponges has been repeatedly reported in literature. However, the mechanisms that determined such diversity remained elusive until recently. We are beginning to understand the mechanisms of spicule secretion and formation and the role of spicules and skeletal frameworks in the biogeology.

Molecular techniques and ecological experiments have demonstrated the genetic control of the process and the contribution of environmental factors to the expression of a sponge spicule, respectively. Silicatein proteins govern the enzymatic and structurally controlled synthesis of silica in marine demosponges. However, new biomineralization topics such as the role of chitin and/or collagen as self-assembling and nanofibrillar organized templates in sponge related biosilicification phenomena require further investigation.

Bibliography

- Aizenberg, J., Sundar, V. C., Yablon, A. D., Weaver, J. C., and Chen, G., 2004. Biological glass fibers: correlation between optical and structural properties. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 3358–3363.
- Bäuerlein, E., 2007. Handbook of biomineralization. In Bäuerlein, E., Behrens, P., and Epple, M., (eds.), *Growth and Form: What is the Aim of Biomineralization?* Weinheim: Wiley, pp. 1–20.
- Bavestrello, G., Bonito, M., and Sarà, M., 1993. Influence of depth on the size of sponge spicules. In Uriz, M. J., and Rützler, K. (eds.), *Recent Advances in Ecology and Systematics of Sponges*. Barcelona: Scientia Marina, Vol. 57(4), pp. 415–420.
- Bergquist, P. R., 1978. *Sponges*. London: Hutchinson & Co., Ltd.
- Botting, J., and Butterfield, N. J., 2005. Reconstructing early sponge relationships by using the Burgess Shale fossil *Eiffelia globosa*, Walcott. *Proceedings of National Academy of Sciences of the United States of America*, **102**, 1554–1559.
- Boury-Esnault, N., and Rützler, K., 1997. Thesaurus of sponge morphology. *Smithsonian Contributions to Zoology*, **596**, 1–55.
- Brunner, E., Richthammer, P., Ehrlich, H., Paasch, S., Simon, P., Ueberlein, S., and van Pee, K.-H., 2009. Chitin-based organic networks – an integral part of cell wall biosilica from the diatom

- Thalassiosira pseudonana. *Angewandte Chemie International Edition*, doi:10.1002/anie200.
- Cha, J. N., Shimizu, K., Zhou, Y., Christiansen, S. C., Chmelka, B. F., Stucky, G. D., and Morse, D. E., 1999. Silicate in filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. *Proceedings of National Academy of Sciences of the United States of America*, **96**, 361–365.
- Conway, K. W., Krautter, M., Barrie, J. V., and Neuweiler, M., 2001. Hexactinellid sponge reefs on the Canadian continental shelf: a unique 'living fossil'. *Geoscience, Canada*, **28**(2), 71–78.
- Conway, K. W., Barrie, J. V., and Krautter, M., 2004. Modern siliceous sponge reefs in a turbid, siliciclastic setting: Fraser River delta, British Columbia, Canada. *Neues Jahrbuch für Geologie und Paläontologie Monatshefte*, **6**, 335–350.
- Conway, K. W., Krautter, M., Barrie, J. V., Whitney, F., Thomson, R. E., Reischwig, H., Lehnert, H., Mungov, G., and Bertram, M., 2005. Sponge reefs in the Queen Charlotte Basin, Canada: controls on distribution, growth and development. In Freiwald, A., and Roberts, J. M. (eds.), *Cold Water Corals and Ecosystems*. Berlin: Springer, pp. 601–617.
- Dayton, P. K., 1979. Observations of growth, dispersal and population dynamics of some sponges in McMurdo Sound, Antarctica. In Lévi, C., and Boury-Esnault, N. (eds.), *Colloques internationaux du C.N.R.S. 291. Biologie des spongiaires*. Paris: Editions du Centre National de la Recherche Scientifique, pp. 271–282.
- Dayton, P. K., Robilliard, G. A., Paine, R. T., and Dayton, L. B., 1974. Biological accommodation in the benthic community at the McMurdo Sound, Antarctica. *Ecological Monographs*, **44**, 105–128.
- De La Rocha, C. L., 2003. Silicon isotope fractionation by marine sponges and the reconstruction of the silicon isotope composition of ancient deep water. *Geology*, **31**, 423–426.
- De Vos, L., Rützel, K., Boury-Esnault, N., Donadey, C., and Vacelet, J., 1991. *Atlas of Sponge Morphology*. Washington: Smithsonian Institution Press.
- Dohrmann, M., Janussen, D., Reitner, J., Collins, A., and Wörheide, G., 2008. Phylogeny and evolution of glass sponges (Porifera: Hexactinellida). *Systematic Biology*, **57**(3), 388–405.
- Ehrlich, H., 2010. Chitin and collagen as universal and alternative templates in biomineralization. *International Geology Review* (in press).
- Ehrlich, H., and Worch, H., 2007a. Collagen, a huge matrix in glass-sponge flexible spicules of the meter-long *Hyalonema sieboldi*. In Bäurlein, E. (ed.), *Handbook of Biomineralization. The Biology of Biominerals Structure Formation*, Weinheim: Wiley, Vol. 1, Chap. 2, pp. 23–41.
- Ehrlich, H., and Worch, H., 2007b. Sponges as natural composites: from biomimetic potential to development of new biomaterials. In Custodio, M. R., Lobo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera Research: Biodiversity, Innovation and Sustainability*, pp. 217–223.
- Ehrlich, H., Ereskovsky, A., Drozdov, A., Krylova, D., Hanke, T., Meissner, H., Heinemann, S., and Worch, H., 2006. A modern approach to demineralisation of spicules in the glass sponges (Hexactinellida: Porifera) for the purpose of extraction and examination of the protein matrix. *Russian Journal of Marine Biology*, **32**, 186–193.
- Ehrlich, H., Krautter, M., Hanke, T., Simon, P., Knieb, C., Heinemann, S., and Worch, H., 2007. First evidence of the presence of chitin in skeletons of marine sponges. Part II. Glass sponges (Hexactinellida: Porifera): *Journal of Experimental Zoology (Molecular Development and Evolution)*, **308B**, 473–483.
- Ehrlich, H., Kotsoulos, P. G., Demadis, K. D., and Pokrovsky, O., 2008a. Principles of demineralization: modern strategies for the isolation of organic frameworks. Part I. Common definitions and history. *Micron*, **39**, 1062–1091.
- Ehrlich, H., Heinemann, S., Heinemann, C., Simon, P., Bazhenov, V., Shapkin, N., Born, R., Tabachnick, K., Hanke, T., and Worch, H., 2008b. Nanostructural organization of naturally occurring composites. Part I. Silica-collagen-based biocomposites. *Journal of Nanomaterials* (published online, doi:10.1155/2008/623838).
- Ehrlich, H., Janussen, D., Simon, P., Heinemann, S., Bazhenov, V. V., Shapkin, N. P., Mertig, M., Erler, C., Born, R., Worch, H., and Hanke, T., 2008c. Nanostructural organisation of naturally occurring composites: Part II. Silica-chitin-based biocomposites, *Journal of Nanomaterials* (published online, Article ID 670235, 8 pages).
- Ellwood, M. J., Kelly, M., and de Forges, B. R., 2007. Silica banding in the deep-sea lithistid sponge *Corallistes undulatus*: investigating the potential influence of diet and environment on growth. *Limnology and Oceanography*, **52**(5), 1865–1873.
- Erpenbeck, D., Breeuwer, J. A. J., Parra-Velandia, F. J., and van Soest, R. W. M., 2006. Speculation with spiculation? – three independent gene fragments and biochemical characters versus morphology in demosponge higher classification. *Molecular Phylogenetics and Evolution*, **38**, 293–305.
- Exley, C., 1998. Silicon in life: a bioinorganic solution to bioorganic essentiality. *Journal of Inorganic Biochemistry*, **69**, 139–153.
- Fairhead, M., Johnson, K. A., Kowatz, T., McMahon, S. A., Carter, L. G., Oke, M., Liu, H., Naismith, J. H., and van der Walle, C. F., 2008. Crystal structure and silica condensing activities of silicatein a–cathepsin L chimeras. *Chemical Communications*, **21**, 1765–1767.
- Garrone, R., Simpson, T. L., and Pottu, J., 1981. Ultrastructure and deposition of silica in sponges. In Simpson, T. L., and Volcani, B. E. (eds.), *Silicon and Siliceous Structures in Biological Systems*. New York: Springer, pp. 495–525.
- Gatti, S., 2002. High Antarctic carbon and silicon cycling – how much do sponges contribute? VI International Sponge Conference. In *Book of Abstracts, Bollettino dei Musei Istituti Biologici*. Genoa: University of Genoa, Vol. 66–67, p. 76.
- Hartman, W. D., 1981. Form and distribution of silica in sponges. In Simpson, T. S., and Volcani, B. E. (eds.), *Silicon and Siliceous Structures in Biological Systems*. New York: Springer, pp. 453–493.
- Henrich, R., Hartmann, M., Reitner, J., Schäfer, P., Steinmetz, S., Freiwald, A., Dietrich, P., and Thiede, J., 1992. Facies belts, bio-coenoses, volcanic structures and associated sediments, of the arctic seamount Vesterisbanken (Central Greenland Sea). *Facies*, **27**, 71–104 Erlangen.
- Holzhüter, G., Lakshminarayanan, K., and Gerber, T., 2005. Silica structure in the spicules of the sponge *Suberites domuncula*. *Analytical and Bioanalytical Chemistry*, **382**, 1121–1126.
- Hooper, J. A., and van Soest, R. W. M., 2002. *Systema Porifera: A guide to the Classification of Sponges*. New York: Kluwer.
- Iijima, M., and Moriwaki, Y., 1990. Orientation of apatite and organic matrix in *Lingula unguis* shell. *Calcified Tissue International*, **47**, 237–242.
- Janussen, D., Tabachnick, K. R., and Tendal, O. S., 2004. Deep-sea Hexactinellida (Porifera) of the Weddell Sea. *Deep-Sea Research II*, **51**, 1857–1882.
- Kelly, M., 2000. Description of a new lithistid sponge from north-eastern New Zealand and consideration of the phylogenetic affinities of families Corallistidae and Neopeltidae. *Zoosystema*, **22**, 265–283.
- Kelly, M., 2003. Revision of the sponge genus *Pleroma* Sollas (Lithistida: Megamorina: Pleromidae) from New Zealand and New Caledonia, and description of a new species. *NZ Journal of Marine and Freshwater Research*, **37**, 113–127.

- Knoll, A., 2003. Biomineralization and evolutionary history. *Reviews in Mineralogy and Geochemistry*, **54**, 329–356.
- Kolb, V. M., Philip, A. I., and Perry, R. S., 2004. Testing the role of silicic acid and biochemical materials in the formation of rock coatings. In Hoover, R. B., Levin, G. V., and Rozanov, A.Y. (eds.), *Instruments, Methods, and Missions for Astrobiology VIII*. Bellingham: SPIE, pp. 116–125.
- Koltun, V. M., 1968. Spicules of sponges as an element of bottom sediments in the Antarctic. *SCAR Symp. Antarctic Oceanography*. Cambridge: Scott Polar Research Institute.
- Kozhemyako, V. B., Veremeichik, G. N., Shkryl, Y. N., Kovalchuk, S. N., Krasokhin, V. B., Rasskazov, V. A., Zhuravlev, Y. N., Bulgakov, V. P., and Kulchin, Y. N., 2009. Silicatein genes in spicule-forming and nonspicule-forming Pacific Demosponges. *Marine Biotechnology*, doi: 10.1007/s10126-009-9225-y.
- Krautter, M., Conway, K. W., Barrie, J. V., and Neuweiler, M., 2001. Discovery of a 'living dinosaur': globally unique modern hexactinellid sponge reefs off British Columbia, Canada. *Facies*, **44**, 265–282.
- Krautter, M., Conway, K. W., and Barrie, J. V., 2006. Recent hexactinosidan sponge reefs (silicate mounds) off British Columbia, Canada: frame-building processes. *Journal of Paleontology*, **80**(1), 38–48.
- Lehnert, H., Conway, K. W., Barrie, J. V., and Krautter, M., 2005. *Desmacella austini* sp. nov. from sponge reefs off the Pacific coast of Canada. *Contributions to Zoology*, **74**(3/4), 265–270.
- Lévi, C., 1973. Systématique de la classe des Demospongiaria (Demosponges). In Grassé, P. (ed.), *Spongiaires. Traité de Zoologie* 3(1). Paris: Masson, pp. 577–632.
- Lévi, C., 1991. Lithistid sponges from the Norfolk rise. Recent and Mesozoic genera. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 72–82.
- Lévi, C., 1993. Porifera Demospongiae: spongiaires bathyaux de Nouvelle-Calédonie, récoltés par le 'Jean Charcot' Campagne BIOCAL, 1985. In Crosnier, A. (ed.), *Résultats des Campagnes MUSORSTROM, II*. Paris: Mémoire du Muséum National d'Histoire Naturelle, pp. 9–87.
- Lévi, C., Barton, J. L., Guillemet, C., Le Bras, E., and Lehuède, P., 1989. A remarkably strong natural glassy rod: the anchoring spicule of the *Monoraphis* sponge. *Journal of Materials Science Letters*, **8**, 337–339.
- Leys, S. P., 2003. Comparative study of spiculogenesis in demosponge and hexactinellid larvae. *Microscopical Research and Technology*, **62**, 300–311.
- Leys, S. P., Mackie, G. O., and Reisswig, H. M., 2007. The biology of glass sponges. *Advances in Marine Biology*, **52**, 1–145.
- Maldonado, M., Carmona, M. C., Vela'squez, Z., Puig, A., Cruzado, A., Lo'pez, A., and Craig, M., 2005. Young siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography*, **50**(3), 799–809.
- Mann, D. G., and Droop, S. J. M., 1996. Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia*, **336**, 19–32.
- Mehl, D., 1992. Die Entwicklung der Hexactinellida seit dem Mesozoikum: Paläobiologie, Phylogenie und Evolution-ökologie. *Berliner geowissenschaftliche Abhandlungen*, **E**, **2**, 1–164.
- Mugnaioli, E., Natalio, F., Schloßmacher, U., Wang, X., Müller, W. E. G., and Kolb, U., 2009. Crystalline nanorods as possible templates for the synthesis of amorphous biosilica during spicule formation in Demospongiae. *Chemistry and Biochemistry*, **10**, 683–689.
- Mukhopadhyay, J., Gutzmer, J., and Beukes, N. J., 2004. Organotemplate silica deposition in Neoproterozoic deep-marine environments: evidence from the Penganga Group, Adilabad, India. *Terra Nova*, **16**, 338–343.
- Müller, W. E. G., 2003. *Silicon Biomineralization: Biology-Biochemistry-Molecular Biology-Biotechnology*. Berlin: Springer.
- Müller, W. E. G., Krasko, A., Le Pennec, G., and Schröder, H. C., 2003a. Biochemistry and cell biology of silica formation in sponges. *Microscopical Research and Technology*, **62**, 368–377.
- Müller, W. E. G., Krasko, A., Le Pennec, G., Steffen, R., Wiens, M., Ammar, M. S. A., Müller, I. M., and Schröder, H. C., 2003b. Molecular mechanism of spicule formation in the demosponge *Suberites domuncula*: Silicatein – myotrophin – collagen. In Müller, W. E. G. (ed.), *Silicon Biomineralization*, Berlin: Springer, pp. 195–222.
- Müller, W. E. G., Rothenberger, M., Boreiko, A., Tremel, W., Reiber, A., and Schröder, H., 2005. Formation of siliceous spicules in the marine demosponge *Suberites domuncula*. *Cell and Tissue Research*, **321**, 285–297.
- Müller, W. E. G., Belikov, S. I., Tremel, W., Perry, C. C., Gieskes, W. W. C., Boreiko, A., and Schröder, H. C., 2006. Siliceous spicules in marine demossponges (example *Suberites domuncula*). *Micron*, **37**, 107–120.
- Müller, W. E. G., Boreiko, A., Wang, X., Belikov, S. I., Wiens, M., Grebenjuk, V. A., schloßmacher, U., and Schröder, H. C., 2007. Silicateins, the major biosilica forming enzymes present in demossponges: protein analysis and phylogenetic relationship. *Gene*, **395**, 62–71.
- Nichols, S., and Wörheide, G., 2005. Sponges: new views of old animals. *Intergrative and Comparative Biology*, **45**, 333–334.
- Okada, Y., 1928. On the development of a Hexactinellid sponge, *Farrea sollasi*. *Journal of the Faculty of Science, Imperial University of Tokyo, Sect. 4, Zoology*, **2**, 1–27.
- Pisera, A., 2000. New species of lithistid sponges from the Paleogene of the Ukraine. *Zoosystema*, **22**, 285–298.
- Pisera, A., 2003. Some aspects of silica deposition in lithistid demossponge desmas. *Microscopy Research and Technique*, **62**, 312–326.
- Reisswig, H. M., 1971. The axial symmetry of sponge spicules and its phylogenetic significance. *Cahiers de Biologie Marine*, **12**, 505–514.
- Reisswig, H. M., 2002. Class Hexactinellida Schmidt, 1870. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1201–1202.
- Reitner, J., 2004. Sponges – a geobiological approach. *Integrative and Comparative Biology* **43**(6), 989.
- Reitner, J., and Mehl, D., 1996. Monophyly of the Porifera. *Verhandlungen des naturwissenschaftlichen Vereins Hamburg*, **36**, 5–32.
- Rigby, J. K., Racki, G., and Wrzolek, T., 1981. Occurrence of dictyloid hexactinellid sponges in the Upper Devonian of the Holy Cross Mts. *Acta Geologica Polonica*, **31**, 163–168.
- Rigby, J. K., Pisera, A., Wrzolek, T., and Racki, G., 2001. Upper Devonian sponges from the Holy Cross Mountains, central Poland. *Palaeontology*, **44**, 447–488.
- Saito, Y., Isobe, T., and Senna, M., 1995. Incipient chemical reaction on the scratched silicon (1 1 1) surface with ethoxy and hydroxyl groups. *Journal of Solid State Chemistry*, **120**(1), 96–100.
- Sandford, F., 2003. Physical and chemical analysis of the siliceous skeletons in six sponges of the two groups (Demospongiae and Hexactinellida). *Microscopical Research and Technology*, **62**, 336–355.
- Schröder, H. C., Boreiko, A., Korzhev, M., Tahir, M. N., Tremel, W., Eckert, C., Ushijima, H., Müller, I. M., and Müller, W. E. G., 2006. Coexpression and functional interaction of silicatein with galectin: matrix-guided formation of siliceous

- spicules in the marine demosponge *Suberites domuncula*. *Journal of Biological Chemistry*, **281**(17), 12001–12009.
- Schulze, F. E., 1886. *Über den Bau und das System der Hexactinelliden*. Berlin: Royal Prussian Academy of Sciences, pp. 3–97.
- Schulze, F. E., 1904. Hexactinellida. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **4**, 1–266.
- Schwartz, K., 1973. A bound form of silicon in glucosaminoglycans and polyuronides. *Proceedings of National Academy of Sciences of the United States of America*, **70**, 1608–1612.
- Shimizu, K., and Morse, D. E., 2000. The biological and biomimetic synthesis of silica and other polysiloxanes. In Baeuerlein, E. (ed.), *Biomimetalization: From Biology to Biotechnology and Medical Application*. Wiley, Weinheim, pp. 207–219.
- Shimizu, K., Cha, J. N., Stucky, G. D., and Morse, D. E., 1998. Silicatein a: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6234–6238.
- Simpson, T. L., 1984. *The Cell Biology of Sponges*. New York: Springer, pp. 1–660.
- Simpson, T. L., 1989. Silicification processes in sponges: *Geodia asters* and the problem of morphogenesis of spicule shape. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomimetalization in Plants and Animals*. New York: Kluwer, pp. 125–136.
- Simpson, T. L., 1990. Recent data on pattern of silicification and the origin of monaxons from tetraxons. In Rützler, K. (ed.), *New Perspectives in Sponge Biology. 3rd International Conference on the Biology of Sponges*, 1985. Washington: Smithsonian Institution Press, pp. 264–272.
- Skinner, H. C. W., 2005. Biominerals. *Mineralogical Magazine*, **69**(5), 621–641.
- Sollas, W. J., 1888. Report on Tetractinellida collected by H.M.S. challenger during the years 1873–1876. *Report of the Scientific Results of the Voyage of the H.M.S. Challenger. Zoology*, **5**, 1–458.
- Tabachnick, K. R., 1991. Adaptation of the Hexactinellid sponges to deep-sea life. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 378–386.
- Tabachnick, K. R., 2002. Family Monorhaphididae Ijima, 1927. In Hooper, N. A. V., and Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1264–1266.
- Tabachnick, K. R., and Reischwig, H. M., 2002. Dictionary of Hexactinellida. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1224–1229.
- Uriz, M.-J., 2003. Biology of silica deposition of sponges. *Microscopical Research and Technology*, Wiley-Liss. California, **62**(4), 277–381.
- Uriz, M.-J., 2006. Mineral skeletogenesis in sponges. *Canadian Journal of Zoology*, **84**, 322–356.
- Uriz, M.-J., Turon, X., Becero, M. A., and Agell, G., 2003. Siliceous spicules and skeletal frameworks in sponges: origin, diversità, ultrastructural patterns, and biological functions. *Microscopical Research and Technology*, **62**, 279–299.
- Vosmaer, G. C. J., and Wijsman, H. P., 1905. On the structure of some Siliceous Spicules of Sponges. I. The styli of *Tethya lyncurium*. *Koninklijke Nederlandsche Akademie van Wetenschappen Proceedings*, **8**, 15–28.
- Weaver, J. C., and Morse, D. E., 2003. Molecular biology of demosponge axial filaments and their roles in biosilicification. *Microscopical Research and Technology*, **62**(4), 356–367.
- Weaver, J. C., Pietrasanta, I., Hedin, N., Chmelka, B. F., Hansma, P. K., and Morse, D. E., 2003. Nanostructural features of demosponge biosilica. *Journal of Structural Biology*, **144**, 271–281.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–27.
- Weissenfels, N., 1989. *Biologie und Mikroskopische Anatomie der Süßwasserschwämme (Spongillidae)*. Stuttgart: Fischer.
- Weissenfels, N., and Landschoff, H. W., 1977. Bau und Funktion des Süßwasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die Entwicklung der monaxialen SiO₂-Nadeln in Sandwich-Kulturen, *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenese der Tiere*, **98**, 355–371.

Cross-references

[Biogeochemical Cycles](#)
[Critical Intervals in Earth History](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Origins of the Metazoa](#)
[Reefs](#)
[Sponges \(Porifera\) and Sponge Microbes](#)

SILICOFLAGELLATES

Silicoflagellates are planktonic, microscopic algae with siliceous skeletons, characterized by their simple geometries and remarkable variability. Taxonomically, they belong to different classes of the Stramenopiles and possess complex plastids derived from secondary endosymbiosis (see entry [Symbiosis](#)). Silicoflagellate skeletons may have considerable potential as environmental indicators and are common in fossil deposits, dating back to the middle Cretaceous (ca. 120 Ma), with species diversity reaching a peak in the Miocene (ca. 23–25 Ma). Today, only a single genus *Dictyocha* with approximately five recognized species exists. For more details, please refer to entry ["Algae \(Eukaryotic\)"](#).

SINTER

Robin W. Renaut¹, Brian Jones²
¹University of Saskatchewan, Saskatoon, SK, Canada
²University of Alberta, Edmonton, Alberta, Canada

Synonyms

Silica sinter; Siliceous sinter

Definition

Sinter: A sedimentary rock primarily composed of silica that is precipitated from hot waters at the vents of high-temperature (high-enthalpy) hot springs and geysers, and from cooled waters on their surrounding discharge aprons.

- Thauer, R. K., Jungermann, K., and Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews*, **41**, 100.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Aßmus, B., and Schink B., 1993. Anaerobic ferrous iron oxidation by anoxygenic phototrophs. *Nature (London)*, **362**, 834.
- Wolfe, R. S., 1992. Biochemistry of methanogenesis. *Biochemical Society Symposia*, **58**, 41.
- Zehnder, A. J. B., and Stumm, W., 1988. Geochemistry and biogeochemistry of anaerobic habitats. In Zehnder, A. J. B. (ed.), *Biology of anaerobic microorganisms*. New York: John Wiley & Sons, pp. 1–38.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Hydrogen](#)
[Iron Sulfide Formation](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Nitrogen](#)
[Photosynthesis](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Terrestrial Deep Biosphere](#)

ANAMMOX

Anammox refers to the microbial **anaerobic oxidation** of **ammonium** by nitrite (NO_2^-), which forms N_2 . See entry “[Nitrogen](#)” for further reading.

ANIMAL BIOCALCIFICATION, EVOLUTION

Gert Wörheide¹, Daniel J. Jackson²

¹Ludwig-Maximilians-Universität München, München, Germany

²University of Göttingen, Göttingen, Germany

Definition

SOM. Soluble organic matrix

IOM. Insoluble organic matrix

GRN. Gene regulatory network

Introduction

One of the major events in the evolution of multicellular animals was the transition from soft-bodied organisms to those that possessed mineralized hard parts for protection

and support. This major evolutionary hallmark supported the rapid diversification of animals and their occupation of a diverse range of novel ecological niches at the dawn of the Phanerozoic, between 560 and 530 million years ago, when mineralized skeletons appeared relatively synchronous in a variety of Phyla during the so-called Cambrian Explosion (Knoll, 2003; Conway Morris, 2006). However, it is still unclear exactly what drove this sudden capacity to construct mineralized structures, be it changes in ocean chemistry (Brennan et al., 2004) or the evolution of more diverse ecologies (Cohen, 2005), including predators (Vermeij, 1989). Investigation of biomineralization processes will provide insight into the molecular and genetic mechanisms that accompanied the appearance of mineralized body parts during the early Cambrian, shedding light on the processes that generate the diversity of life we see around us today.

Until recently, studies of biomineralization processes were mainly restricted to describing the (ultra)structure of the minerals and their interaction with the organic material provided by the organism (Lowenstam and Weiner, 1989; Mann and Webb, 1989). However, the generation and elaboration of biominerals is ultimately controlled by genes (Jackson et al., 2006, 2007a, b), which must in turn be expressed in the appropriate tissue and at an appropriate stage of development. Understanding the evolution of such biomineralizing genes and the gene regulatory networks (GRNs) in which they act is, therefore, vital to the understanding of how animals evolved and will also provide clues for the fabrication of biomimetic materials.

Biomineralization

Biomineralization refers to the processes by which organisms form minerals (e.g., calcium carbonate, calcium phosphate, silica, iron oxides) under strict biological control. These processes are, therefore, defined as *biologically controlled mineralization*, with the skeletons formed being integral, functional parts of the organisms (Lowenstam and Weiner, 1989). There are, however, several other serendipitous processes linked to living organisms (e.g., microbes) or organic substances that result in mineral (mainly calcium carbonate) formation that are not under strict control of organisms. These processes are termed either *biologically induced mineralization*, where inorganic minerals are precipitated by adventitious precipitation resulting from secondary interactions between various metabolic processes and the surrounding environment (Mann, 2001), or *organomineralization*, a term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not (e.g., degrading organic matter) (Trichet and Defarge, 1995).

While both *biologically induced* and *organomineralization* play an important role in the formation of carbonate systems in the geological record (e.g., microbialites, stromatolites, ooids) (Reitner and Neuweiler, 1995; Arp,

1999; Reitner et al., 2000), it is *biologically controlled mineralization* that is responsible for the production of sophisticated biomaterials such as bones, shells, and teeth, some of which show exceptional strength and beauty, often of high commercial value (i.e., pearls).

Biologically controlled mineralization almost always has organic macromolecules associated with the process of mineral formation, which can comprise up to 5% of the total mass of a biomineral and fulfill important functions during initial crystal nucleation, crystal growth, and growth termination, as well as contributing to the biomechanical properties of the mature product (Simkiss, 1986; Simkiss and Wilbur, 1989). Following the removal of the mineral itself, these organic macromolecules are often subclassified as being either water soluble organic matrices (SOMs), or water insoluble organic matrices (IOMs). IOMs are largely neutrally charged, often highly polymerized, and act as frame building matrices akin to collagen, chitin, or silk proteins (Yan et al., 2007; Yano et al., 2007). SOMs are often highly acidic, due to negatively charged carboxylate groups, are typically rich in aspartic acid (asp) and/or glutamic acid (glu), and usually have mean molecular weight of 10–30 kDa (Bédouet et al., 2001, 2006).

While organic macromolecules appear ubiquitous and pivotal in *biologically controlled* biomineralization (with the focus here on biocalcification), an increasing number of studies now show that a transient amorphous (noncrystalline) phase of calcium carbonate (ACC) is frequently involved in the formation of the earliest stages of calcium carbonate biominerals (Aizenberg et al., 2003; Politi et al., 2004; Weiner and Dove, 2003). However, little is known about the ACC as well as the organic macromolecules involved in calcification in most animal taxa.

Several SOM proteins have been sequenced and characterized, mainly in sea urchins, mollusks, and vertebrates (Treccani et al., 2003; Wilt et al., 2003). About 45 soluble proteins were detected by 2D gel electrophoresis in demineralized spicules from the embryo of the sea urchin *Strongylocentrotus purpuratus* (Killian and Wilt, 1996). Several of these proteins were later cloned and sequenced (e.g., SM27, SM30, SM50) (Zhu et al., 2001) and their role and function in the biomineralization process confirmed by immunohistochemical methods (Ameye et al., 1999; Urry et al., 2000), in situ hybridization (Illies et al., 2002), or by using antisense oligonucleotides directed against spicule matrix proteins (Peled-Kamar et al., 2002). All of the cloned spicule matrix proteins of *Strongylocentrotus* share as common features a C-type lectin domain and proline rich repeat regions. Furthermore, the first 35 amino acids of SpSM32 and SM50 are identical, probably representing splicing variants of the same transcript, although this has yet to be confirmed (Wilt et al., 2003). The precise functions of any of these proteins are unknown (Wilt et al., 2003) and their evolutionary origin and relationships remain largely unresolved (but see Bottjer et al., 2006). An increasing number of reports are, however, elucidating the functional roles of various biomineralizing proteins. For example, the starmaker

protein is known to influence crystal size and lattice formation in otolith biomineralization (Söllner et al., 2003), while the nacre protein perlucin (Mann et al., 2000; Weiss et al., 2000) is known to nucleate the growth of aragonitic calcium carbonate crystals (Blank et al., 2003).

Identification and characterization of the genes directly involved in the process of biocalcification, and the ways in which these “structural genes” are regulated, is essential for our understanding of the evolution of metazoan biocalcification. One of the most advanced systems currently used to study the complexity of the GRNs involved in specifying biomineralizing cell types is the sea urchin. The manipulability of urchin embryos and the availability of a high-quality genome sequence have allowed researchers to identify specific transcription factors (genes that activate the expression of target genes) that activate biomineralizing genes and proteins (Ettensohn et al., 2003, 2007). Furthermore, recent work has identified the nodes in biomineralizing GRNs that are responsible for specifying cells involved in larval skeletogenesis and adult skeletogenesis (Gao and Davidson, 2008), providing insight into the evolution of the metazoan biphasic lifecycle. Such endeavors are also highly relevant to issues of global climate change and ocean acidification – by assessing how resilient marine calcifying organisms are to fluctuations in ocean chemistry, highly species-rich marine ecosystems such as coral reefs may be effectively managed.

The evolution of biocalcification

In the geological record, major animal phyla began biomineralizing shortly before, or during, the so-called Cambrian Explosion, about 530 million years ago, when a highly diverse group of animals appeared suddenly in the fossil record (Knoll, 2003). As most of these phyla are thought to have diverged well before this biomineralization event (Peterson et al., 2008), various causes for this relatively synchronous adoption of biomineralizing strategies have been postulated and remain an area of continuing debate. These include the evolutionary driving force of predation (Vermeij, 1989) or the result of a response to changes in ocean chemistry with greater than previous levels of calcium, requiring the need for effective cellular calcium-detoxification mechanisms (Degens et al., 1985; Kazmierczak et al., 1985).

The vast majority of newly appearing biomineral products in the Cambrian was based on calcium (either as calcium carbonate or calcium phosphate) (Lowenstam and Margulis, 1980). Precise control of intracellular calcium levels is necessary for the formation of microtubules which are needed by eukaryotic cells. Based on this, Lowenstam and Margulis suggested that calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization. CaCO_3 biomineral formation can be useful for the organism because this process allows the elimination of a cell-toxic surplus of Ca^{2+} through enzymes such as calmodulin and membrane

bound Ca^{2+} -ATPase, which directly transports calcium to loci where it is used as a physiological control factor (Degens, 1979).

The question of whether the ability to biocalcify evolved several times independently among various animal groups, possibly to produce armor in response to increased pressure from predators (Marshall, 2006), or whether preexisting calcium regulation and transport systems in the late Precambrian metazoans provided the evolutionary prerequisites for their eventual use in biomineralization (Lowenstam and Margulis, 1980), still remains open to debate. If calcium carbonate biomineralization evolved as a physiological response (mutual to many taxa) to increasing calcium levels in the late Precambrian ocean, then an evolutionary link between the proteins and the regulatory elements involved in skeletogenesis can be postulated among metazoan groups. This evolutionary connection could either be due to massive horizontal gene transfer in ancestral Precambrian organisms or as a result of the exaptation of a preexisting genetic makeup into a new role (biocalcification) in many disparate animal lineages (Kirschvink and Hagadorn, 2000). The latter leads to the hypothesis that the last common ancestor of the metazoa (LCAM) provided a “genetic biomineralization toolkit,” a core set of conserved biomineralization genes to regulate and initiate the cellular functions required for the assembly of calcium carbonate biominerals (Ca^{2+} , HCO_3^-). Subsequently, this core gene cassette and associated regulatory elements have been elaborated upon to produce the range of biomineralized structures we observe in the Earth’s history. One major process by which complex biological systems evolve is by taking an existing genetic pattern evolved for a certain function, duplicating it, and adapting it for a new role (e.g., Carroll et al., 2001). The nascent system is gradually debugged and improved through the process of random mutation and natural selection. This powerful hypothesis, in the context of biomineralization, still awaits an expanded program of comparative geobiological research (Knoll, 2003).

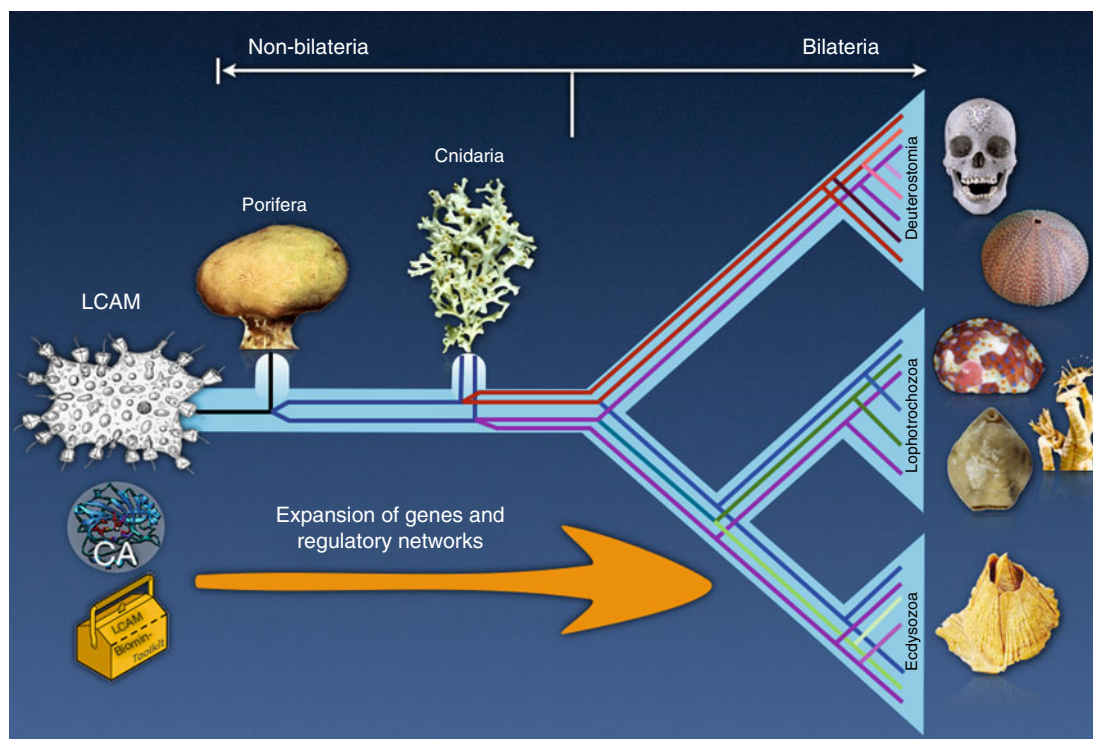
However, evidence for conserved biocalcification mechanisms is beginning to emerge. Some homologies between certain molluscan (Weiss et al., 2001) or sea urchin (Ettensohn et al., 2003) and vertebrate matrix proteins have already been postulated. Ettensohn et al. (2003), for example, found homologies of their sea urchin Sp/LvAlx1 protein, which is an essential component of the gene network that controls downstream genes required for biomineralization, with members of the vertebrate Cart1/Alx3/Alx4 protein family. Members of this gene family have been implicated to be instrumental in the formation of the limb skeleton and the neural crest-derived skeleton of the face and neck in vertebrates. Further, there seems to be some fundamental underlying immunological similarity between macromolecules involved in vertebrate hydroxyapatite and invertebrate aragonite formation (i.e., molluscan nacre), because freshly ground nacre did not result in an immune response when injected into human jaw bones, but in fact

stimulated bone regeneration (Marin and Westbroek, 1998; Lopez et al., 1992). Coral skeletons are already used as bone implants with a similar (albeit weaker) effect on bone regeneration (Demers et al., 2002). Although bone, nacre, and coral skeletons are not homologous structures as such, parts of the complex underlying physiological machinery that controls biomineral formation probably are – a hypothesis strongly supported by the above mentioned results. However, these homologous parts may have served a different function than biomineralization in the (nonskeletonized) common ancestor of corals, mollusks, and vertebrates, later being co-opted for producing biominerals when the necessity arose.

If, as outlined above, calcium carbonate biomineralization systems in the major metazoan phyla evolved from the core skeletogenic toolkit of the last common ancestor of animals, then ancestral metazoan systems should be used as an anchor for a paleogenomic approach to reconstruct this toolkit and explore the evolutionary road map of diversification by comparison with more derived animal taxa. Paleogenomics analyzes the genomes of contemporary taxa to reconstruct ancient genomes and genomic features and to infer the function of ancestral genes.

Recent paleogenomic studies go beyond the focus on classical matrix proteins. Expressed sequence tags (ESTs), cDNA libraries, and recently sequenced genomes have been utilized to examine the inheritance of ancestral toolkits that were involved in skeletogenesis. By using a calcifying coralline sponge of a “living fossil,” *Astrosclera willeyana*, a key component of the ancestral skeletogenic toolkit – α -carbonic anhydrase – has been identified (Jackson et al., 2007b). Duplication of this gene is likely to have allowed biocalcification to evolve in many clades of metazoans. In contrast, the recently sequenced genome of the sea urchin *Strongylocentrotus purpuratus* has revealed that genes essential for the construction of the echinoderm skeleton (the stereome) are echinoderm-specific and are likely to be the same as those in the earliest echinoderms and their direct ancestors more than 520 million years ago (Livingston et al., 2006). Similarly, in gastropods (snails; *Haliotis asinina* and *Lottia scutum*) an unexpected level of genetic novelty and complexity in the skeletogenic toolkit suggests that there are significant molecular differences in the ways in which gastropods synthesize their shells (Jackson et al., 2006). These features, along with the modular design of the molluscan mantle, contribute to the spectacular morphological and mineralogical variety we observe among contemporary mollusk shells (Jackson et al., 2007a).

From the limited data available, we can develop the following scenario as a working hypothesis (Figure 1): the last LCAM possessed a core biomineralization toolkit that at least consisted of an α -carbonic anhydrase, providing the core capacity to regulate the reversible hydration of CO_2 , necessary for biocalcification. Other, as yet undiscovered, parts of this toolkit most likely contained genes that regulate Ca^{2+} trafficking, scaffolding genes for organic matrix production, as well as regulatory



Animal Biocalcification, Evolution, Figure 1 Conceptual summary. The last common ancestor of all Metazoa (LCAM) possessed a core gene toolkit for the regulation of Ca_2^+ and an alpha-carbonic anhydrase to catalyse the reversible hydration of CO_2 . During subsequent metazoan evolution, parts of this toolkit (genes and regulatory elements) were exapted to new roles including calcium carbonate fabrication. Novel genes and biosynthetic pathways were added to the conserved biocalcification toolkit to produce the variety of skeletal forms we observe among extant animal lineages.

elements. During subsequent animal evolution, genes in this core set were duplicated in different lineages, resulting in a relatively simple genetic repertoire in basal metazoans, but in a highly diverse gene array in the various lineages of the Bilateria (Ecdysozoa, Lophotrochozoa, and Deuterostomia). A high degree of lineage-specific innovation on top of a conserved gene-set then allowed for the diversification of the numerous skeletal architectures we observe since the dawn of the Cambrian.

Conclusion

While the structural processes of biomineralization, and in particular biocalcification, are receiving vigorous attention from various international groups, a far more detailed characterization of the core ancestral skeletogenic toolkit as well as lineage specific innovations are needed before we understand how the impressive diversity and beauty of metazoan skeletal structures originated and evolved. As this knowledge is acquired, we can anticipate the construction of new high-performance biomimetic materials and the measurement and prediction of the resilience of marine calcifying organisms to rapidly changing environmental parameters, such as ocean acidification.

Bibliography

- Aizenberg, J., Weiner, S., and Addadi, L., 2003. Coexistence of amorphous and crystalline calcium carbonate in skeletal tissues. *Connective Tissue Research*, **44**, 20–25.
- Ameye, L., Hermann, R., Killian, C., Wilt, F., and Dubois, P., 1999. Ultrastructural localization of proteins involved in sea urchin biomineralization. *Journal of Histochemistry and Cytochemistry*, **47**, 1189–1200.
- Arp, G., 1999. *Calcification of Non-Marine Cyanobacterial Biofilms (USA, PR China, Indonesia, Germany) – Implications for the Interpretation of Fossil Microbialites*. PhD Thesis, Mathematisch-Naturwissenschaftliche Fakultät, Georg-August-Universität Göttingen, Göttingen, p. 118.
- Bédouet, L., Schuller, M. J., Marin, F., Milet, C., Lopez, E., and Giraud, M., 2001. Soluble proteins of the nacre of the giant oyster *Pinctada maxima* and of the abalone *Haliotis tuberculata*: extraction and partial analysis of nacre proteins. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, **128**, 389–400.
- Bédouet, L., Rusconi, F., Rousseau, M., Duplat, D., Marie, A., Dubost, L., Le Ny, K., Berland, S., Péduzzi, J., and Lopez, E., 2006. Identification of low molecular weight molecules as new components of the nacre organic matrix. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, **144**, 532–543.
- Blank, S., Arnoldi, M., Khoshnavaz, S., Treccani, L., Kuntz, M., Mann, K., Grathwohl, G., and Fritz, M., 2003. The nacre protein

- perlucin nucleates growth of calcium carbonate crystals. *Journal of Microscopy*, **212**, 280–291.
- Bottjer, D. J., Davidson, E. H., Peterson, K. J., and Cameron, R. A., 2006. Paleogenomics of echinoderms. *Science*, **314**, 956–960.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Carroll, S. B., Grenier, J. K., and Weatherbee, S. D., 2001. *From DNA to Diversity—Molecular Genetics and the Evolution of Animal Design*. Blackwell: Malden, MA.
- Cohen, B. L., 2005. Not armour, but biomechanics, ecological opportunity and increased fecundity as keys to the origin and expansion of the mineralized benthic metazoan fauna. *Biological Journal of the Linnean Society*, **85**, 483–490.
- Conway Morris, S., 2006. Darwin's dilemma: the realities of the Cambrian "explosion." *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **361**, 1069–1083.
- Degens, E., 1979. Why do organisms calcify. *Chemical Geology*, **25**, 257–269.
- Degens, E. T., Kazmierszak, J., and Ittekkot, V., 1985. Cellular response to Ca_2^+ stress and its geological implications. *Acta Palaeontologica Polonica*, **30**, 115–135.
- Demers, C., Hamdy, C. R., Corsi, K., Chellat, F., Tabrizian, M., and Yahia, L., 2002. Natural coral exoskeleton as a bone graft substitute: a review. *Bio-Medical Materials and Engineering*, **12**, 15–35.
- Ettensohn, C. A., Illies, M. R., Oliveri, P., and De Jong, D. L., 2003. Alx1, a member of the Cart1/Alx3/Alx4 subfamily of paired-class homeodomain proteins, is an essential component of the gene network controlling skeletogenic fate specification in the sea urchin embryo. *Development*, **130**, 2917–2928.
- Ettensohn, C. A., Kitazawa, C., Cheers, M. S., Leonard, J. D., and Sharma, T., 2007. Gene regulatory networks and developmental plasticity in the early sea urchin embryo: alternative deployment of the skeletogenic gene regulatory network. *Development*, **134**, 3077–3087.
- Gao, F., and Davidson, E., 2008. Transfer of a large gene regulatory apparatus to a new developmental address in echinoid evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6091–6096.
- Illies, M. R., Peeler, M. T., Dechtiaruk, A. M., and Ettensohn, C. A., 2002. Identification and developmental expression of new biomineralization proteins in the sea urchin *Strongylocentrotus purpuratus*. *Development Genes and Evolution*, **212**, 419–431.
- Jackson, D. J., McDougall, C., Green, K., Simpson, F., Wörheide, G., and Degnan, B. M., 2006. A rapidly evolving secretome builds and patterns a sea shell. *BMC Biology*, **4**, 40.
- Jackson, D., Wörheide, G., and Degnan, B. M., 2007a. Dynamic expression of ancient and novel molluscan shell genes during ecological transitions. *BMC Evolutionary Biology*, **7**, 160.
- Jackson, D. J., Macis, L., Reitner, J., Degnan, B. M., and Wörheide, G., 2007b. Sponge paleogenomics reveals an ancient role for carbonic anhydrase in skeletogenesis. *Science*, **316**, 1893–1895.
- Kazmierczak, J., Ittekkot, U., and Degens, E. T., 1985. Biocalcification through time: environmental challenge and cellular response. *Paläontologische Zeitschrift*, **59**, 15–33.
- Killian, C. E., and Wilt, F. H., 1996. Characterization of the proteins comprising the integral matrix of *Strongylocentrotus purpuratus* embryonic spicules. *Journal of Biological Chemistry*, **271**, 9150–9159.
- Kirschvink, J. L., and Hagadorn, J. W., 2000. A grand unifying theory of biomineralization. In Bäuerlein, E. (ed.), *The Biomineralization of Nano- and Micro-Structures*. Weinheim: Wiley, pp. 139–150.
- Knoll, A. H., 2003. Biomineralization and evolutionary history. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization*. Washington, DC: The Mineralogical Society of America/Geochemical Society, pp. 329–356.
- Livingston, B. T., Killian, C. E., Wilt, F., Cameron, A., Landrum, M. J., Ermolaeva, O., Sapojnikov, V., Maglott, D. R., Buchanan, A. M., and Ettensohn, C. A., 2006. A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus*. *Developmental Biology*, **300**, 335–348.
- Lopez, E., Vidal, B., Berland, S., Camprasse, S., Camprasse, G., and Silve, C., 1992. Demonstration of the capacity of nacre to induce bone-formation by human osteoblasts maintained invitro. *Tissue & Cell*, **24**, 667–679.
- Lowenstam, H. A., and Margulis, L., 1980. Evolutionary prerequisites for early phanerozoic calcareous skeletons. *Biosystems*, **12**, 27–41.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. Oxford: Oxford University Press.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. Oxford: Oxford University Press.
- Mann, S., and Webb, J., 1989. *Biomineralization*. Weinheim: VCH.
- Mann, K., Weiss, I. M., Andre, S., Gabius, H. J., and Fritz, M., 2000. The amino-acid sequence of the abalone (*Haliotis laevis*) nacre protein perlucin. *European Journal of Biochemistry*, **267**, 5257–5264.
- Marin, F., and Westbroek, P., 1998. A marriage of bone and nacre. *Nature*, **392**, 861–862.
- Marshall, C. R., 2006. Explaining the Cambrian "explosion" of animals. *Annual Review of Earth and Planetary Sciences*, **34**, 355–384.
- Peled-Kamar, M., Hamilton, P., and Wilt, F. H., 2002. Spicule matrix protein LSM34 is essential for biomineralization of the sea urchin spicule. *Experimental Cell Research*, **272**, 56–61.
- Peterson, K. J., Cotton, J. A., Gehling, J. G., and Pisani, D., 2008. The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philosophical Transactions of the Royal Society of London Series B, Biological Sciences*, **363**, 1435–1443.
- Politi, Y., Arad, T., Klein, E., Weiner, S., and Addadi, L., 2004. Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. *Science*, **306**, 1161–1164.
- Reitner, J., and Neuweiler, F. C., 1995. Mud mounds: a polygenetic spectrum of fine-grained carbonate buildups. *Facies*, **32**, 1–70.
- Reitner, J., Thiel, V., Zankl, H., Michaelis, W., Wörheide, G., and Gautret, P., 2000. Organic and biogeochemical patterns in cryptic microbialites. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 149–160.
- Simkiss, K., 1986. The process of biomineralization in lower plants and animals – an overview. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals. The Systematics Association Special Volume 30*. Oxford: Clarendon, pp. 19–38.
- Simkiss, K., and Wilbur, K. M., 1989. *Biomineralization – Cell biology and Mineral Deposition*. San Diego: Academic.
- Söllner, C., Burghammer, M., Busch-Nentwich, E., Berger, J., Schwarz, H., Riekel, C., and Nicolson, T., 2003. Control of crystal size and lattice formation by starmaker in otolith biomineralization. *Science*, **302**, 282–286.
- Treccani, L., Khoshnavaz, S., Blank, S., Roden, K. v., Schulz, U., Weiss, I. M., Mann, K., Radmacher, M., and Fritz, M., 2003. Biomineralizing proteins, with emphasis on invertebrate-mineralized structures. In Fahnestock, S. R., and Steinbüchel, A. (eds.), *Biopolymers*. Berlin: Wiley, Vol. 8, pp. 289–321.

- Trichet, J., and Defarge, C., 1995. Non-biologically supported organomineralization. *Bulletin de l'Institut Océanographique Monaco*, **14**, 203–236.
- Urry, L. A., Hamilton, P. C., Killian, C. E., and Wilt, F. H., 2000. Expression of spicule matrix proteins in the sea urchin embryo during normal and experimentally altered spiculogenesis. *Developmental Biology*, **225**, 201–213.
- Vermeij, G., 1989. The origin of skeletons. *PALAIOS*, **4**, 585–589.
- Weiner, S., and Dove, P., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–29.
- Weiss, I. M., Kaufmann, S., Mann, K., and Fritz, M., 2000. Purification and characterization of perlucin and perlustrin, two new proteins from the shell of the mollusc *Haliotis laevis*. *Biochemical and Biophysical Research Communications*, **267**, 17–21.
- Weiss, I. M., Gohring, W., Fritz, M., and Mann, K., 2001. Perlustrin, a *Haliotis laevis* (Abalone) nacre protein, is homologous to the insulin-like growth factor binding protein N-terminal module of vertebrates. *Biochemical and Biophysical Research Communications*, **285**, 244–249.
- Wilt, F. H., Killian, C. E., and Livingston, B. T., 2003. Development of calcareous skeletal elements in invertebrates. *Differentiation*, **71**, 237–250.
- Yan, Z., Jing, G., Gong, N., Li, C., Zhou, Y., Xie, L., and Zhang, R., 2007. N40, a novel nonacidic matrix protein from pearl oyster nacre, facilitates nucleation of aragonite in vitro. *Biomacromolecules*, **8**, 3597–3601.
- Yano, M., Nagai, K., Morimoto, K., and Miyamoto, H., 2007. A novel nacre protein N19 in the pearl oyster *Pinctada fucata*. *Biochemical and Biophysical Research Communications*, **362**, 158–163.
- Zhu, X., Mahairas, G., Illies, M., Cameron, R. A., Davidson, E. H., and Ettensohn, C. A., 2001. A large-scale analysis of mRNAs expressed by primary mesenchyme cells of the sea urchin embryo. *Development*, **128**, 2615–2627.

Cross-references

Animal Skeletons, Advent
 Calcium Biogeochemistry
 Carbonate Environments
 Critical Intervals in Earth History
 Ediacaran Biota
 Microbial Biomineralization
 Organomineralization
 Reefs
 Soda Ocean Hypothesis
 Sponges (Porifera) and Sponge Microbes
 Trace Fossils: Neoproterozoic

ANIMAL SKELETONS, ADVENT

Guoxiang Li, Maoyan Zhu, Zhe Chen
 Chinese Academy of Sciences, Nanjing, China

Synonyms

Early biomineralization event; Small shelly fossils

Definition

Animal skeleton. Biologically, an animal skeleton is a hard or rigid framework that could provide protection and

support in many types of animals. Most of the animal skeletons are mineralized, but some of them may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, excluding hydroskeletons. The “skeletons” of resistant organic biopolymers are not included herein.

Exoskeleton. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites.

Endoskeleton. Endoskeletons are internal hard parts (typical of many vertebrates). Most of the endoskeletons are generally surrounded by skin and musculature.

Small shelly fossils (SSF or SSFs). A non-taxonomic term was first used by Matthews and Missarzhevsky in the title of their review paper in 1975 to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites.

Introduction

Skeletal biomineralization (skeletonization) was part of the initial diversification of early animals. The fossil record provides an important clue to the origin of biomineralization in the animal phyla (Kirschvink and Hagadorn, 2000). The earliest occurrence of animal skeletons was in the late Ediacaran, e.g., *Cloudina*, *Sinotubulites*, and *Namapoikia*, although the generic diversity was quite low and the biomineralization of these calcareous skeletons was not well controlled biologically (Grant, 1990). The early Cambrian (542–525 Ma) saw the largest burst of diversified animal skeletons, represented in the fossil record by poriferans, cnidarians, many extant bilaterian phyla, and some problematic taxa (Figure 1) (Bengtson, 2004). In terms of structural and mineralogical diversity of animal skeletons, the early Cambrian represents a quantum jump (Bengtson and Conway Morris, 1992). The abrupt advent of diversified skeletal fossils not only has been taken as a major piece of evidence for the Cambrian radiation but also was envisioned the biomineralization event. Since most of these skeletal fossils are typically small in size, and lots of them represent disarticulated sclerites and are enigmatic with respect to their biological affinities, they are commonly referred to as “small shelly fossils (SSFs)” in the literature (Matthews and Missarzhevsky, 1975; Qian, 1999). But some of the early skeletal fossils, such as the sponge-like archaeocyaths, trilobites, and bivalved arthropods, are rarely described under this nontaxonomic term, while some fossils described under the term of SSFs, such as embryo fossils and cnidarian polyps, may have been originally composed of resistant organic membranes or tubes without biomineralization, and the skeleton-like appearance is mainly due to the secondary phosphatization.

- Hein, R., Crutzen, P. J., and Heimann, M., 1997. An inverse modeling approach to investigate the global atmospheric methane cycle. *Global Biogeochemical Cycles*, **11**(1), 43–76.
- Holzapfel-Pschorn, A., and Seiler, W., 1986. Methane emission during a cultivation period from an Italian rice paddy. *Journal of Geophysical Research-Atmospheres*, **91**(D11), 1803–1814.
- Hornafius, J. S., Quigley, D., and Luyendyk, B. P., 1999. The world's most spectacular marine hydrocarbon seeps (Coal Oil Point, Santa Barbara Channel, California): quantification of emissions. *Journal of Geophysical Research-Oceans*, **104**(C9), 20703–20711.
- Hovland, M., and Judd, A. G., 1988. *Seabed Pockmarks and Seepages: Impact on Geology, Biology and the Marine Environment*. London: Graham and Trotman.
- IPCC, 2001. Climate change 2001: the scientific basis. In Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C.A. (eds.), *Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 881 p.
- Judd, A. G., Hovland, M., Dimitrov, L. I., Garcia Gil, S., and Jukes, V., 2002. The geological methane budget at continental margins and its influence on climate change. *Geofluids*, **2**(2), 109–126.
- Kalff, J., 2001. *Limnology: Inland water ecosystems*. Prentice Hall, 592 p.
- Kelly, C. A. et al., 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. *Environmental Science & Technology*, **31**(5), 1334–1344.
- Khalil, M. A. K., 2000. Atmospheric methane: an introduction. In Khalil, M.A.K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 1–8.
- Kvenvolden, K. A., and Lorensen, T. D., 2001. The global occurrence of natural gas hydrate. In Paull, C. K., and Dillon, W. D. (eds), *Natural Gas Hydrates: Occurrence, Distribution and Detection*. Geophysical Monograph. Washington, DC: American Geophysical Union.
- Kvenvolden, K. A., and Rogers, B. W., 2005. Gaia's breath - global methane exhalations. *Marine and Petroleum Geology*, **22**(4), 579–590.
- McGinnis, D. F., Wüest, A., Schubert, C. J., Klauser, L., Lorke, A., and Kipfer, R., 2005. Upward flux of methane in the Black Sea: does it reach the atmosphere? In Lee, J. H. W., and Lam, K. M. (eds.), *Environmental Hydraulics and Sustainable Water Management*. London: Taylor & Francis Group, pp. 423–429.
- Matthews, E., and Fung, I., 1987. Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles*, **1**(1), 61–86.
- Megonigal, J. P., Hines, M. E., and Visscher, P. T., 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In Schlesinger, W. H. (ed.), *Biogeochemistry*. Oxford: Elsevier-Pergamon, pp. 317–424.
- Milkov, A. V., 2004. Global estimates of hydrate-bound gas in marine sediments: how much is really out there? *Earth-Science Reviews*, **66**(3–4), 183–97.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**(3–4), 443–467.
- Rasmussen, R. A., and Khalil, M. A. K., 1984. Atmospheric methane in the recent and ancient atmospheres: concentrations, trends, and interhemispheric gradient. *Journal of Geophysical Research*, **89**, 11599–11605.
- Schubert, C. J. et al., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**(10), 1844–1856.
- St. Louis, V. L., Kelly, C. A., Duchemin, E., Rudd, J. W. M., and Rosenberg, D. M., 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. *Bioscience*, **50**(9), 766–775.
- Sloan, E. D., 1998. *Clathrate Hydrates of Natural Gas*, New York: Marcel Dekker.
- Stevens, C. M., and Wahlen, M., 2000. The isotopic composition of atmospheric methane and its sources. In Khalil, M. A. K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 25–41.
- Tyler, S. C. et al., 1988. Measurements and interpretation of $\delta^{13}\text{C}$ of methane from termites, rice paddies, and wetlands in Kenya. *Global Biogeochemical Cycles*, **2**(4), 341–355.
- Tyler, S. C., 1991. The global methane budget. In Rogers, J. E., and Whitman, W. B. (eds.), *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*. Washington, DC: American Society for Microbiology, pp. 7–38.
- Whiticar, M. J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 219–314.
- Whiticar, M. J., Faber, E., and Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation - Isotope evidence. *Geochimica et Cosmochimica Acta*, **50**, 693–709.
- Wuebbles, D. J., and Hayhoe, K., 2002. Atmospheric methane and global change. *Earth-Science Reviews*, **57**(3–4), 177–210.
- Zinder, S. H., 1993. Physiological ecology of methanogens. In Jerry, J. G., (ed.), *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*, New York: Chapman Hall, pp. 128–206.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Cap Carbonates](#)
[Carbonates](#)
[Cold Seeps](#)
[Methane Oxidation \(Aerobic\)](#)

METHANOGENS

Please refer to "[Methane, Origin](#)" and "[Archaea](#)."

MICROBIAL BIOMINERALIZATION

Christine Heim
 University of Göttingen, Göttingen, Germany

Synonyms

Microbial biomineral formation

Definition

Microbial biomineralization describes the formation and deposition of minerals directly mediated or indirectly influenced by microorganisms (Mann, 2001; Weiner and Dove, 2003; Ehrlich, 1999). A huge variety of minerals results from individual biomineralization pathways linked to the phylogeny and metabolic activity of the microorganisms involved (Weiner and Dove, 2003;

Minsky et al., 2002). Moreover, microbial biominerals may differ distinctly from their inorganically formed equivalents in shape, size, crystallinity, isotopic, and trace element composition (Bazylinski et al., 2007; Haferburg and Kothe, 2007; Takahashi et al., 2007; Weiner and Dove, 2003). A compilation of microbial biominerals and their source organisms is given in Table 1.

Two principal modes of microbial biomineralization processes occur, namely biologically induced mineralization (BIM) and biologically controlled mineralization

(BCM, Lowenstam, 1981; Lowenstam and Weiner, 1989). These modes are introduced in the following.

Biologically induced mineralization (BIM)

In BIM, the nucleation and growth of biominerals are extracellular processes triggered by the metabolic activity of the microorganism. Biomineralization takes place because of the changes in the chemical equilibrium of the surrounding environment and may also be linked to

Microbial Biomineralization, Table 1 Overview of minerals formed by microbial biomineralization and the organism(s) involved

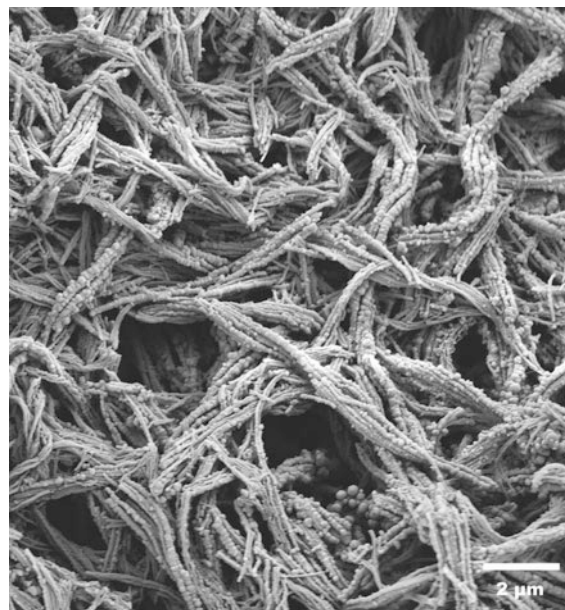
Chemical formula	Mineral name	Involved microorganism	References
Fe(OH) ₃ (approx.)	Ferric/iron oxyhydroxide	Fe-oxidizing bacteria	Chan et al. (2009) and Yoshida et al. (2008)
2Fe(OH) ₃ • Fe(OH) ₂ (approx.)	Green rust	<i>Shewanella putrefaciens</i>	Kukkadapu et al. (2004) and O'Loughlin (2008)
α-FeO(OH)	Goethite	<i>Gallionella ferruginea</i>	Hallberg and Ferris (2004)
γ-FeO(OH)	Lepidocrocite	Marine bacteriophage, <i>Bacillus subtilis</i>	Daughney et al. (2004) and Châtellier et al. (2001)
5Fe ₂ O ₃ • 9H ₂ O	Ferrihydrite	<i>Gallionella ferruginea</i> , <i>Leptothrix ochracea</i> , <i>Bacillus subtilis</i>	Hallberg and Ferris (2004) and Kennedy et al. (2004)
Fe ₂ O ₃	Hematite	<i>Gallionella ferruginea</i>	Hallberg and Ferris (2004)
Fe ₃ O ₄	Magnetite	<i>Shewanella putrefaciens</i> , (Fe(III)-reducing bacteria), magnetotactic bacteria (e.g., <i>Magnetospirillum magnetotacticum</i>), SRB (e.g., <i>Desulfovibrio magneticus</i>), thermophilic iron-reducing bacteria	Frankel et al. (1983), Lovley et al. (1987), Lovley (1991), Bazylinski et al. (1993), Zhang et al. (1997), Zhang et al. (1998), Kukkadapu et al. (2004), Bazylinski et al. (2007) and Faivre and Schüler (2008)
γ-Fe ₂ O ₃	Maghemite	<i>Actinobacter</i> sp., thermophilic iron-reducing bacteria	Zhang et al. (1997) and Bharde et al. (2008)
FeCO ₃	Siderite	<i>Shewanella alga</i> , thermophilic iron-reducing bacteria	Zhang et al. (1997) and Parmar et al. (2000)
FePO ₄ • nH ₂ O	Hydrous ferric phosphate	<i>Acidovorax</i> sp.	Miot et al. (2009)
Fe ₃ (PO ₄) ₂ • 2H ₂ O	Vivianite	<i>Shewanella putrefaciens</i> , <i>Desulfovibrio alaskensis</i> (SRB), <i>Alkaliphilus metalliredigens</i>	Kukkadapu et al. (2004), Zegeye et al. (2007), and Roh et al. (2007)
FeS	Cubic FeS (Sphalerite-type)	Magnetotactic bacteria	Pósfai et al. (1998a, b)
FeS	Mackinawite (tetragonal FeS)	Magnetotactic bacteria, <i>Desulfovibrio desulfuricans</i>	Pósfai et al. (1998a, b) and Ivarson and Hallberg (1976)
Fe ₃ S ₄	Greigite	Magnetotactic bacteria, <i>Actinobacter</i> sp., SRB	Bharde et al. (2008), Farina et al. (1990), Mann et al. (1990), Heywood et al. (1990), Reiter et al. (2005), and Faivre and Schüler (2008)
Fe _{1-x} S	Pyrrhotite	Magnetotactic bacteria	Farina et al. (1990)
FeS ₂	Pyrite	Magnetotactic bacteria, SRB	Mann et al. (1990), Bazylinski (1996), Folk (2005), Wilkin and Barnes (1997), and Donald and Southam (1999)
KFe ₃ (SO ₄) ₂ (OH) ₆	Jarosite	<i>Sulfobacillus thermosulfidooxidans</i> , <i>Acidithiobacillus ferrooxidans</i> , <i>Thiobacillus ferrooxidans</i>	Ding et al. (2007), Daoud and Karamanev (2006), and Ivarson and Hallberg (1976)
Fe ₈ O ₈ SO ₄ (OH) ₆	Schwertmanite	<i>Acidithiobacillus ferrooxidans</i>	Egal et al. (2009)
MnCO ₃	Rhodochrosite	<i>Leptothrix discophora</i>	Zhang et al. (2002)
MnO ₂	Manganese oxides	<i>Pseudomonas putida</i> , <i>Leptothrix discophora</i> , <i>Bacillus</i> sp.	Tebo et al. (2004), Villalobos et al. (2003), and Brouwers et al. (2000)
Na ₄ Mn ₁₄ O ₂₇ • 9H ₂ O	Birnessite	<i>Pseudomonas putida</i>	Villalobos et al. (2003)
S ⁰	Elemental sulfur	<i>Chromatiaceae</i> , <i>Beggiatoa</i> spp., <i>Thiothrix</i> , <i>Thiovulum</i> , <i>Thioploca</i>	Brune (1989), Brune (1995), Smith and Strohl (1991), Strohl et al. (1981) and Pasteris et al. (2001)

Microbial Biomineralization, Table 1 (Continued)

Chemical formula	Mineral name	Involved microorganism	References
Au ⁰	Elemental gold	<i>Bacillus</i> sp., <i>Rhodopseudomonas capsulate</i> , <i>Shewanella algae</i> , SRB	Reith et al. (2009), He et al. (2007), Konishi et al. (2007), Konishi et al. (2006), and Lengke and Southam (2006)
CaCO ₃	Calcite	Communities of SRB and archaea, cyanobacteria, soil bacteria (<i>Bacillus megaterium</i>), Algae (e.g., <i>Halimeda</i> , <i>Emiliana huxleyi</i>)	Boetius et al. (2000), Reitner et al. (2005), Thompson and Ferris (1990), Lian et al. (2006), and de Vrind-de Jong and de Vrind (1997)
	Aragonite	Cyanobacteria (<i>Synechococcus leopoliensis</i>), <i>Nesterenkonia halobia</i> , <i>Halomonas eurihalina</i>	Obst et al. (2009) and Rivadeneyra et al. (1998, 2000)
	Vaterite	<i>Kocuria</i> , <i>Myxococcus Xanthus</i> , <i>Bacillus sphaericus</i>	Zamarreño et al. (2009), and Rodriguez-Navarro et al. (2007)
CaMg(CO ₃) ₂	Dolomite	<i>Nesterenkonia halobia</i>	Rivadeneyra et al. (2000)
SiO ₂ • nH ₂ O	Amorphous silica	<i>Calothrix</i> , <i>Fischerella</i> sp., <i>Shewanella oneidensis</i>	Benning et al. (2004), Konhauser et al. (2001), and Furukawa and O'Reilly (2007)
SiO ₂	Silica	Diatoms, radiolarians, <i>Thiobacillus</i> , <i>Bacillus subtilis</i>	de Vrind-de Jong and de Vrind (1997), Fortin and Beveridge (1997), and Urrutia and Beveridge (1993)
Ca ₅ (PO ₄) ₃ (OH)	Hydroxyapatite/ calcium phosphate	<i>Ramlibacter tataouinensis</i> , <i>Corynebacterium matruchotii</i> , <i>Streptococcus mutans</i> , <i>Streptococcus sanguis</i>	Benzerara et al. (2004), Van Dijk et al. (1998), and Streckfuss et al. (1974)
MgNH ₄ PO ₄ • 6H ₂ O	Struvite	<i>Myxococcus xanthus</i> , <i>Pseudomonas</i> , <i>Flavobacterium</i> , <i>Acinetobacter</i> , <i>Yersinia</i> , <i>Corynebacterium</i> , <i>Azotobacter</i>	Da Silva et al. (2000) and Rivadeneyra et al. (1983)

For comprehensive reading, the following books are recommended: Sigel et al. (2008), Baeuerlein (2000), Banfield and Nealson (1997), Dove et al. (2003), Driessens and Verbeeck (1990), Lovely (2000), Lowenstam and Weiner (1989).

particular metabolic products. The resulting biominerals typically show a poor crystallinity, are chemically heterogeneous, and often closely associated with the cell wall (Frankel and Bazylinski, 2003). An active and a passive mineralization process can be distinguished (Fortin and Beveridge, 2000; Southam, 2000). Active mineralization refers to mineralization by (a) the direct redox conversion of specific metal ions bound to the bacterial surface or (b) the excretion of metabolically produced ions and thereby forming minerals. The term passive mineralization is used when *nonspecific* binding of cations and the involvement of surrounding anions causes nucleation and growth of minerals. Passive mineralization can even be mediated by dead cells, due to the exposure of negatively charged surfaces acting as nucleation sites for metal cations (Urrutia and Beveridge, 1993). Particularly in BIM, extracellular polymeric substances (EPS) are involved in the mineralization process (Chan et al., 2009; Ercole et al., 2007). As an example of BIM, the precipitation of iron oxyhydroxides by the iron oxidizing bacterium *Gallionella ferruginea* is displayed in Figure 1.



Biologically controlled mineralization (BCM)

BCM implies that the organism actively controls the nucleation site, growth, morphology, and final location of the mineral (Banfield and Nealson 1997; Bazylinski

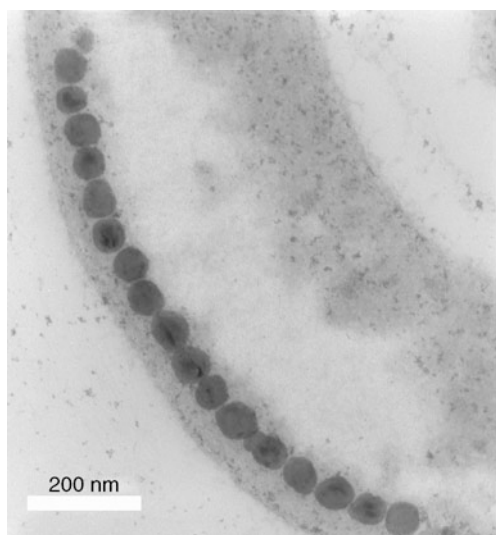
Microbial Biomineralization, Figure 1 Stalk-shaped extracellular polymeric substances (EPS) produced by the iron oxidizing bacterium *Gallionella ferruginea* are heavily encrusted with iron oxyhydroxide minerals formed by biologically induced mineralization (BIM).

and Frankel, 2003). Although the modes of exerting crystallochemical control over the mineralization process may greatly vary across species, the common characteristic of BCM is that mineral formation takes place in a closed, isolated environment. While Weiner and Dove (2003) categorized BCM in *extra*-, *inter*- or *intracellular* mineralization, Mann (2001) differentiated between two key modes of BCM, namely *matrix-mediated* mineralization and *boundary-organized* mineralization. Generally speaking, extracellular mineralization corresponds to matrix-mediated mineralization, whereas inter- and intracellular mineral formation are equivalent to boundary-organized mineralization.

Extracellular BCM implies the production of a macromolecular matrix outside the cell. This matrix is typically composed of proteins, polysaccharides, or glycoproteins, forming a three dimensional framework, and the cell actively supplies cations to the matrix for an “on-site” nucleation and growth of the biomineral (Weiner and Dove, 2003).

Intercellular BCM occurs mostly in single-celled organisms, existing in a community. This type of mineral formation is not common in bacteria, but is commonly found in calcareous algae, for example, *Halimeda* (de Vrind-de Jong and de Vrind, 1997; Borowitzka, 1982).

Intracellular BCM takes place inside specific compartments within the cell, for instance vesicles or vacuoles. Thus, the organism is able to exactly regulate the chemical composition, morphological structure, and particle size of the mineral. The only bacteria known to perform intracellular BCM are magnetotactic bacteria, which use a vacuole-based system for the crystallization of magnetic biominerals (Table 1). Similar systems of intracellular



Microbial Biomineralization, Figure 2 Intracellular iron sulfide (greigite, Fe_3S_4) crystals formed by biologically controlled mineralization (BCM) within a magnetotactic bacterium. (Image courtesy of Joachim Reitner.)

BCM are only known from higher eukaryotic organisms, controlling for example, the bone and teeth formation in mammals (Kirschvink and Hagadorn, 2000). An example of microbial biominerals formed by intracellular BCM is shown in Figure 2.

Bibliography

- Baeuerlein, E., 2000. *Biomineralization: From Biology to Biotechnology and Medical Application*. Weinheim: Wiley-VCH Verlag GmbH, 316 p.
- Banfield, J. F., and Nealson, K. H., (eds.), 1997. *Geomicrobiology: Interactions Between Microbes and Minerals. Reviews in Mineralogy*, Ribbe, P. H. (series ed.). Washington, DC: Mineralogical Society of America. Vol. 35, 448 p.
- Bazylinski, D. A., 1996. Controlled biomineralization of magnetic minerals by Magnetotactic bacteria. *Chemical Geology*, **132**, 191–198.
- Bazylinski, D. A., and Frankel, R. B., 2003. Biologically controlled mineralization in prokaryotes. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), Rosso, J. J. (series ed.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 217–247.
- Bazylinski, D. A., Frankel, R. B., and Konhauser, K. O., 2007. Modes of biomineralization of magnetite by microbes. *Geomicrobiology Journal*, **24**, 465–475.
- Bazylinski, D. A., Heywood, B. R., Mann, S., and Frankel, R. B., 1993. Fe_3O_4 and Fe_3S_4 in a bacterium. *Nature*, **366**, 218.
- Benning, L. G., Phoenix, V. R., Yee, N., and Tobin, M. J., 2004. Molecular characterization of cyanobacterial silification using synchrotron infrared micro-spectroscopy. *Geochimica et Cosmochimica Acta*, **68**(4), 729–741.
- Benzerara, K., Menguy, N., Guyot, F., Skouri, F., de Luca, G., Barakat, M., and Heulin, T., 2004. Biologically controlled precipitation of calcium phosphate by *Ramlibacter tataouinensis*. *Earth and Planetary Science Letters*, **228**, 439–449.
- Bharde, A. A., Paraikh, R. Y., Baidakova, M., Jouen, S., Hannoyer, B., Enoki, T., Prasad, B. L. V., Shouche, Y. S., Ogale, S., and Sastry, M., 2008. Bacteria-mediated precursor-dependent biosynthesis of superparamagnetic iron oxide and iron sulfide nanoparticles. *Langmuir*, **24**, 5787–5794.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Borowitzka, M. A., 1982. Morphological and cytological aspects of algal calcification. *International Review of Cytology*, **74**, 127–162.
- Brouwers, G. J., Vijgenboom, E., Corstjens, P. L. A. M., De Vrind, J. P. M., and De Vrind-De Jong, E. W., 2000. Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: an overview of mechanisms and functions. *Geomicrobiology Journal*, **17**, 1–24.
- Brune, D. C., 1989. Sulfur oxidation by phototrophic bacteria. *Biochimica et Biophysica Acta*, **975**, 189–221.
- Brune, D. C., 1995. Isolation and characterization of sulfur globule proteins from *Chromatium vinosum* and *Thiocapsa roseopersicina*. *Archives of Microbiology*, **163**, 391–399.
- Chan, C. S., Fakra, S. C., Edwards, D. C., Emerson, D., and Banfield, J. F., 2009. Iron oxyhydroxide mineralization of microbial extracellular polysaccharides. *Geochimica et Cosmochimica Acta*, **73**(13), 3807–3818.
- Châtellier, X., Fortin, D., West, M. M., Leppard, G. G., and Ferris, F. G., 2001. Effect of the presence of bacterial surfaces during the synthesis of Fe oxides by oxidation of ferrous ions. *European Journal of Mineralogy*, **13**(4), 705–714.

- Da Silva, S., Bernet, N., Delgenès, J. P., and Moletta, R., 2000. Effect of culture conditions on the formation of struvite by *Myxococcus Xanthus*. *Chemosphere*, **40**, 1289–1296.
- Daoud, J., and Karamanev, D., 2006. Formation of jarosite during Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans*. *Minerals Engineering*, **19**(9), 960–967.
- Daughney, C. D., Châtellier, X., Chan, A., Kenward, P., Fortin, D., Suttle, C. A., and Fowl, D., 2004. Adsorption and precipitation of iron from seawater by a marine bacteriophage (PWH3a-P1). *Marine Chemistry*, **91**, 101–115.
- de Vrind-de Jong, E. W., and de Vrind, J. P. M., 1997. Algal deposition of carbonates and silicates. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 267–307.
- Ding, J.-N., Gao, J., Wu, X.-l., Zhang, C.-G., and Qiu, G.-Z., 2007. Jarosite-type precipitates mediated by YN22, *Sulfobacillus thermosulfidooxidans*, and their influences on strain. *Transactions of Nonferrous Metals. Society of China*, **17**(5), 1038–1044.
- Donald, R., and Southam, G., 1999. Low temperature anaerobic bacterial diagenesis of ferrous monosulfide to pyrite. *Geochimica et Cosmochimica Acta*, **63**(13/14), 2019–2023.
- Dove, P. M., De Yoreo, J. J., and Weiner, S., (eds.), 2003. *Biomineralization. Reviews in Mineralogy and Geochemistry*, Rosso, J. J. (series ed.). Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, 381 p.
- Driessens, F. C. M., and Verbeeck, R. K., 1990. *Biominerals*. Boca Raton, Florida: CRC Press, 440 p.
- Egal, M., Casiot, C., Morin, G., Parmentier, M., Bruneel, O., Lebrun, S., and Elbaz-Poullichet, F., 2009. Kinetic control on the formation of tooeleite, schwertmannite and jarosite by *Acidithiobacillus ferrooxidans* stains in an As(III)-rich acid mine water. *Chemical Geology*, **265**(3–4), 432–441.
- Ehrlich, H. L., 1999. Microbes as geologic agents: their role in mineral formation. *Geomicrobiology Journal*, **16**, 135–153.
- Ercole, C., Cacchio, P., Botta, A. L., Centi, V., and Lepidi, A., 2007. Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides. *Microscopy and Microanalysis*, **13**, 42–50.
- Faivre, D., and Schüller, D., 2008. Magnetotactic bacteria and magnetosomes. *Chemical Reviews*, **108**, 4875–4898.
- Farina, M., Esquivel, D. M. S., and Lins de Barros, H. G. P., 1990. Magnetic iron-sulphur crystals from magnetotactic microorganism. *Nature*, **343**, 256–258.
- Folk, R. L., 2005. Nannobacteria and the formation of framboidal pyrite: textural evidence. *Journal of Earth System Science*, **114**(3), 369–374.
- Fortin, D., and Beveridge, T. J., 1997. Role of the bacterium *Thiobacillus* in the formation of silicates in acidic mine tailings. *Chemical Geology*, **141**, 235–250.
- Fortin, D., and Beveridge, T. J., 2000. Mechanistic routes to biomineral surface development. In Bäuerlein, E. (ed.), *Biomineralization: From Biology to Biotechnology and Medical Application*. Weinheim: Wiley-VCH GmbH, pp. 7–24.
- Frankel, R. B., and Bazylinski, D. A., 2003. Biologically induced mineralization by bacteria. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), Rosso, J. J. (series ed.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington, DC: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 95–114.
- Frankel, R. B., Papaefthymiou, G. C., Blakemore, R. P., and O'Brien, W., 1983. Fe_3O_4 precipitation in Magnetotactic bacteria. *Biochimica et Biophysica Acta*, **763**, 147–159.
- Furukawa, Y., and O'Reilly, S. E., 2007. Rapid precipitation of amorphous silica in experimental systems with nontronite (Nau-1) and *Shewanella oneidensis* MR-1. *Geochimica et Cosmochimica Acta*, **71**(2), 363–377.
- Haferburg, G., and Kothe, E., 2007. Microbes and metals: interactions in the environment. *Journal of Basic Microbiology*, **47**, 453–467.
- Hallberg, R., and Ferris, F. G., 2004. Biomineralization by Gallionella. *Geomicrobiology Journal*, **21**, 325–330.
- He, S., Gui, Z., Zhang, Y., Zhang, S., Wang, J., and Gu, N., 2007. Biosynthesis of gold nanoparticles using the bacteria *Rhodospseudomonas capsulata*. *Material Letters*, **61**(18), 3984–3987.
- Heywood, B. R., Bazylinski, D. A., Garratt-Reed, A., Mann, S., and Frankel, R. B., 1990. Controlled biosynthesis of greigite (Fe_3S_4) in magnetotactic bacteria. *Naturwissenschaften*, **77**, 536–538.
- Ivarson, K. C., and Hallberg, R. O., 1976. Formation of mackinawite by the microbial reduction of jarosite and its application to tidal sediments. *Geoderma*, **16**, 1–7.
- Kennedy, C. B., Scott, S. D., and Ferris, F. G., 2004. Hydrothermal phase stabilization of 2-line ferrihydrite by bacteria. *Chemical Geology*, **212**, 269–277.
- Kirschvink, J. L., and Hagadorn, J. W., 2000. 10 a Grand unified theory of Biomineralization. In Bäuerlein, E. (ed.), *The Biomineralisation of Nano- and Microstructures*. Weinheim: Wiley-VCH GmbH, pp. 139–150.
- Konhäuser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial-silica interactions in Icelandic hot spring sinter: possible analogues for some Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–433.
- Konishi, Y., Tsukiyama, T., Ohno, K., Saitoh, N., Nomura, T., and Nagamine, S., 2006. Intracellular recovery of gold by microbial reduction of AuCl_4^- ions using the anaerobic bacterium *Shewanella algae*. *Hydrometallurgy*, **81**(1), 24–29.
- Konishi, Y., Tsukiyama, T., Tachimi, T., Saitoh, N., Nomura, T., and Nagamine, S., 2007. Microbial deposition of gold nanoparticles by the metal-reducing bacterium *Shewanella algae*. *Electrochimica Acta*, **53**(1), 186–192.
- Kukkadapu, R. K., Zachara, J. M., Fredrickson, J. K., and Kennedy, D. W., 2004. Biotransformation of two-line silica-ferrihydrite by dissimilatory Fe(III)-reducing bacterium: formation of carbonate green rust in the presence of phosphate. *Geochimica et Cosmochimica Acta*, **68**(13), 2799–2814.
- Lengke, M., and Southam, G., 2006. Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold(I)-thiosulfate complex. *Geochimica et Cosmochimica Acta*, **70**, 3646–3661.
- Lian, B., Hu, Q., Chen, J., Ji, J., and Teng, H. H., 2006. Carbonate biomineralization induced by soil bacterium *Bacillus megaterium*. *Geochimica et Cosmochimica Acta*, **70**, 5522–5535.
- Lovley, D. (ed.), 2000. *Environmental Microbe-Metal Interactions*. Washington, DC: ASM Press, 395 p.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiology Reviews*, **55**, 259–287.
- Lovley, D. R., Stolz, J. F., Nord, Jr. G. L., and Phillips, E. J. P., 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature*, **330**, 252–254.
- Lowenstam, H. A., 1981. Minerals formed by organisms. *Science*, **211**, 1126–1131.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press, 324 p.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. In Compton, R. G., Davies, S. G., and Evans, J. (eds.), *Oxford Chemistry Masters Vol. 5*. New York: Oxford University Press, 216 p.
- Mann, S., Sparks, N. H. C., Frankel, R. B., Bazylinski, D. A., and Jannasch, H. W., 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a Magnetotactic bacterium. *Nature*, **343**, 258–261.

- Minsky, A., Shimoni, E., and Frenkel-Krispin, D., 2002. Stress, order and survival. *Nature Reviews Molecular Cell Biology*, **3**, 50–60.
- Miot, J., Benzerara, K., Morin, G., Kappler, A., Bernard, S., Obst, M., Férard, C., Skouri-Panet, F., Guigner, J.-M., Posth, N., Galvez, M., Brown, Jr. G. E., and Guyot, F., 2009. Iron biomineralization by anaerobic neutrophilic iron-oxidizing bacteria. *Geochimica et Cosmochimica Acta*, **73**(3), 696–711.
- Obst, M., Dynes, J. J., Lawrence, J. R., Swerhone, G. D. W., Benterara, K., Kaznatcheev, K., Tyliszczak, T., and Hitchcock, A. P., 2009. Precipitation of amorphous CaCO_3 (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. *Geochimica et Cosmochimica Acta*, **73**(14), 4180–4198.
- O'Loughlin, E. J., 2008. Effects of electron transfer mediators on the bioreduction of Lepidocrocite ($\gamma\text{-FeOOH}$) by *Shewanella putrefaciens* CN32. *Environmental Science and Technology*, **42**(18), 6876–6882.
- Parmar, N., Warren, L. A., Roden, E. E., and Ferris, F. G., 2000. Soil phase capture of strontium by the iron reducing bacteria *Shewanella alga* strain BRY. *Chemical Geology*, **169**, 281–288.
- Pasteris, J. D., Freeman, J. J., Goffredi, S. K., and Buck, K. R., 2001. *Chemical Geology*, **180**, 3–18.
- Pósfai, M., Buseck, P. R., Bazylinski, D. A., and Frankel, R. B., 1998a. Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. *Science*, **280**, 880–883.
- Pósfai, M., Buseck, P. R., Bazylinski, D. A., and Frankel, R. B., 1998b. Iron sulfides from magnetotactic bacteria: structure, compositions, and phase transitions. *American Mineralogist*, **83**, 1469–1481.
- Reith, F., Wakelin, S. A., Gregg, A. L., and Schmidt-Mumm, A., 2009. A microbial pathway for the formation of gold-anomalous calcrite. *Chemical Geology*, **258**(3–4), 315–326.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Rivadeneira, M. A., Delgado, G., Ramos-Cormenzana, A., and Delgado, R., 1998. Biomineralization of carbonates by *Halomonas eurihalina* in solid and liquid media with different salinities: crystal formation sequence. *Research in Microbiology*, **149**, 277–287.
- Rivadeneira, M. A., Delgado, G., Soriano, M., Ramos-Cormenzana, A., and Delgado, R., 2000. Precipitation of carbonates by *Nesterenkonia halobia* in liquid media. *Chemosphere*, **41**, 617–624.
- Rivadeneira, M. A., Ramos-Cormenzana, A., and García-Cervigón, A., 1983. Bacterial formation of struvite. *Geomicrobiology Journal*, **3**, 151–163.
- Rodríguez-Navarro, C., Jiménez-López, C., Rodríguez-Navarro, A., González-Muñoz, M. T., and Rodríguez-Gallego, M., 2007. Bacterially mediated mineralization of vaterite. *Geochimica et Cosmochimica Acta*, **71**(5), 1197–1213.
- Roh, Y., Chon, C.-M., and Moon, J.-W., 2007. Metal reduction and biomineralization by alkaliphilic metal reducing bacterium, *Alkaliphilus metalliredigens* (QYMF). *Geosciences Journal*, **11**(4), 415–423.
- Sigel, A., Sigel, H., and Sigel, R. O. (eds.), 2008. *Biomineralization: from Nature to Application. Metal Ions in Life Sciences*. West Sussex: Wiley and Sons, 4 Vols, 700 p.
- Smith, D. W., and Strohl, W. R., 1991. Sulfur oxidizing bacteria. In Shively, J. M., and Barton, L. L., (eds.), *Variations in Autotrophic Life*. London: Academic Press, pp. 121–146.
- Southam, G., 2000. Bacterial surface-mediated mineral formation. In Lovley, D. R. (ed.), *Environmental Microbe-Metal Interactions*. Washington DC: ASM Press, pp. 257–276.
- Streckfuss, J. L., Smith, W. N., Brown, L. R., and Campbell, M. M., 1974. Calcification of selected strains of *Streptococcus mutans* and *Streptococcus sanguis*. *Journal of Bacteriology*, **120**, 502–506.
- Strohl, W. R., Geffers, I., and Larkin, J. M., 1981. Structure of the sulfur inclusions envelopes from four beggiatoas. *Current Microbiology*, **6**, 75–79.
- Takahashi, Y., Hirata, T., Shimizu, H., Ozaki, T., and Fortin, D., 2007. A rare earth element signature of bacteria in natural waters. *Chemical Geology*, **244**, 569–583.
- Tebo, B. M., Bargar, J. R., Clement, B. G., Dick, G. J., Murray, K. J., Parker, D., Verity, R., and Webb, S. M., 2004. Biogenic manganese oxides: properties and mechanisms of formation. *Annual Review of Earth and Planetary Sciences*, **32**, 287–328.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Urrutia, M. M., and Beveridge, T. J., 1993. Mechanism of silicate binding to the bacteria cell wall in *Bacillus subtilis*. *Journal of Bacteriology*, **175**, 1936–1945.
- Van Dijk, S., Dean, D. D., Zhao, Y., Cirgwin, J. M., Schwartz, Z., and Boyan, B. D., 1998. Purification, amino acid sequence, and cDNA sequence of novel calcium-precipitating proteolipids involved in calcification of *Corynebacterium matruchotii*. *Calcified Tissue International*, **62**, 350–358.
- Villalobos, M., Toner, B., Bargar, J., and Sposito, G., 2003. Characterization of manganese oxide produced by *Pseudomonas putida*. *Geochimica et Cosmochimica Acta*, **67**(14), 2649–2662.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 1–29.
- Wilkin, R. T., and Barnes, H. K., 1997. Formation processes of framboidal pyrite. *Geochimica et Cosmochimica Acta*, **61**(2), 323–339.
- Yoshida, H., Yamamoto, K., Murakami, Y., Katsuta, N., Hayashi, T., and Naganuma, T., 2008. The development of Fe-nodules surrounding biological material mediated by microorganisms. *Environmental Geology*, **55**, 1363–1374.
- Zamarreño, D. V., Inkpen, R., and May, E., 2009. Studies on carbonate crystals precipitated by freshwater bacteria and their use as a limestone consolidant. *Applied and Environmental Microbiology*, **75**, 5981–5990.
- Zegeye, A., Huhuet, L., Abdelmoula, M., Carteret, C., Mullet, M., and Jorand, F., 2007. Biogenic hydroxysulfate green rust, a potential electron acceptor of SRB activity. *Geochimica et Cosmochimica Acta*, **71**, 5450–5462.
- Zhang, C., Liu, S., Phelps, T. J., Cole, D. R., Horita, J., Fortier, S. M., Elless, M., and Valley, J., 1997. Physicochemical, mineralogical, and isotopic characterization of magnetite-rich iron oxides formed by thermophilic iron-reducing bacteria. *Geochimica et Cosmochimica Acta*, **61**(21), 4621–4632.
- Zhang, C., Vali, H., Romanek, C. S., Phelps, T. J., and Liu, S. V., 1998. Formation of single-domain magnetite by a thermophilic bacterium. *American Mineralogist*, **83**, 1409–1418.
- Zhang, J., Lion, L. W., Nelson, Y. M., Shuler, M. L., and Ghiorse, W. C., 2002. Kinetics of Mn (II) oxidation by *Leptothrix discophora* SS1. *Geochimica et Cosmochimica Acta*, **65**(5), 773–781.

Cross-references

Banded Iron Formations
Biofilms and Fossilization

Biomining (Mineral Bioleaching, Mineral Biooxidation)
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Extracellular Polymeric Substances (EPS)
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Gallionella
 Gold
 Iron Sulfide Formation
 Leptothrix
 Magnetotactic Bacteria
 Manganese (Sedimentary Carbonates and Sulfides)
 Metallogenium
 Metals, Acquisition by Marine Bacteria
 Microbial-Metal Binding
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Nanocrystals, Microbially Induced
 Ores, Microbial Precipitation and Oxidation
 Organomineralization
 Shewanella
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Tufa, Freshwater

MICROBIAL COMMUNITIES, STRUCTURE, AND FUNCTION

Michael W. Friedrich
 University of Bremen, Bremen, Germany

Definition

Microbial species. Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization (Stackebrandt and Goebel, 1994); thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy.

Microbial population. Individual microorganisms (of the same “species”) form a population.

Guild. Metabolically related populations, e.g., sulfate-reducing microorganisms.

Microbial community. All microbial populations in a habitat.

Microbial community structure. The composition of a microbial community and the abundance of its members.

Microbial diversity. The number of different species in a habitat; sometimes (falsely) used as a synonym for community structure.

Habitat. The physical location or dwelling place of a particular organism, where an individual microbial eco-type can be found or isolated from.

Ecosystem. A community of microorganisms and their natural environment.

Ecological niche. Different from the habitat (spatial), the niche defines the function of an organism.

Introduction

Microorganisms are an indispensable part of the biogeochemical cycles on Earth, have the longest history in evolution, and represent the largest genetic reservoir. Mostly invisible to the naked eye, microbes represent the largest source of biomass on Earth, with an estimated number of $4\text{--}6 \times 10^{30}$ cells, and 350–550 Pg of C (1 Pg = 10^{15} g), and have colonized almost any niche available on Earth (Whitman et al., 1998). In their habitats, microorganisms usually do not exist as single, genetically identical population, but live together with other microbial populations as microbial communities carrying out important functions of an ecosystem such as primary production and remineralization of biomass.

A hallmark of microbial life is its diversity. For a long time, only little was known about the magnitude of microbial diversity, as this was based on cultivation. Only about ~7,000 noneukaryotic species have been formally described (Euzéby, 1997) (<http://www.bacterio.cict.fr/number.html>), and insects were regarded as much more diverse than microorganisms. In microbiology, the term “diversity” is often used to refer to the number of different species in a given habitat, or, when based on molecular data, refers to the number of different sequences present. Diversity reflects the types of microbes present in a habitat, but “community structure” encompasses quantitative information on the different taxa and guilds (Liesack et al., 1997). Eventually, the analysis of community structure will allow for assessing the role and importance of microbes in their habitats and ecosystems. Presently, it is still methodologically difficult to obtain a complete view of microbial diversity and community structure.

Methods of community structure analysis

Cultivation: Traditionally, the diversity of microbial communities has been studied by cultivation, i.e., by viable plate counts or most-probable-number (MPN) dilution techniques. However, the morphology of microorganisms is too simple for reliable identification and classification. Identification of microorganisms by cultivation requires pure cultures for testing physiological, biochemical, and cell biological characterization and suitable media for their growth, which is a major bottle neck. There are no cultivation media available that allow for capturing growth requirements of all microorganisms in a sample, and we often do not even know of their existence and presence. Direct microscopic counts can be of orders of magnitude higher than the counts of bacteria obtained on growth media (“plate counts”). Typically, only a small

- Volkman, J. K., Barrett, S. M., Dunstan, G. A., and Jeffrey, S. W., 1992. Advances in the biomarkers chemistry of marine algae. In *Third Conference on Petroleum Geochemistry and Exploration in the Afro-Asian Region*, Abstracts, February 1992, Melbourne.
- Werne, J. P., Baas, M., and Sinninghe Damsté, J. S., 2002. Molecular isotopic tracing of carbon flow and trophic relationships in a methane-supported benthic microbial community. *Limnology and Oceanography*, **47**, 1694–1701.
- Werne, J. P., and Hollander, D. J., 2004. Balancing supply and demand: Controls on carbon isotope fractionation in the Cariaco Basin (Venezuela) Younger Dryas to Present. *Marine Chemistry*, **92**, 1–4, 275–293.
- Werner, R. A., and Brand, W. A., 2001. Referencing strategies and techniques in stable isotope ratio analysis. *Rapid Communications in Mass Spectrometry*, **15**, 501–519.
- West, N., Alexander, R., and Kagi, R. I., 1990. The use of silicalite for the rapid isolation of branched and cyclic alkane fractions of petroleum. *Organic Geochemistry*, **15**, 499–501.
- Whiticar, M. J., Faber, E., and Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation-Isotope evidence. *Geochimica et Cosmochimica Acta*, **50**, 693–709.
- Wiesenberg, G. L. B., Schwarzbauer, J., Schmidt, M. W. I., and Schwark, L., 2004. Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. *Organic Geochemistry*, **35**, 1371–1393.
- Woltemate, I., Whiticar, M. J., and Schoell, M., 1984. Carbon and hydrogen isotopic composition of bacterial methane in a shallow freshwater lake. *Limnology and Oceanography*, **29**, 985–992.
- Xie, S., Nott, C. J., Avsejs, L. A., Maddy, D., Chambers, F. M., and Evershed, R. P., 2004. Molecular and isotopic stratigraphy in an ombrotrophic mire for paleoclimate reconstruction. *Geochimica et Cosmochimica Acta*, **68**, 2849–2862.
- Xie, S., Nott, C. J., Avsejs, L. A., Volders, F., Maddy, D., Chambers, F. M., Gledhill, A., Carter, J. F., and Evershed, R. P., 2000. Palaeoclimate records in compound-specific δD values of a lipid biomarker in ombrotrophic peat. *Organic Geochemistry*, **31**, 1053–1057.
- Xiong, Y., Geng, A., Pan, C., Liu, D., and Peng, P., 2005. Characterization of the hydrogen isotopic composition of individual *n*-alkanes in terrestrial source rocks. *Applied Geochemistry*, **20**, 455–464.
- Yang, H., and Huang, Y., 2003. Preservation of lipid hydrogen isotope ratios in Miocene lacustrine sediments and plant fossils at Clarkia northern Idaho, USA. *Organic Geochemistry*, **34**, 413–423.
- Zhang, Z., Metzger, P., and Sachs, J. P., 2007. Biomarker evidence for the co-occurrence of three races (A, B and L) of *Botryococcus braunii* in El Junco Lake, Galápagos. *Organic Geochemistry*, **38**, 1459–1478.
- Zhao, M., Dupont, L., Eglinton, G., and Teece, M., 2003. *n*-Alkane and pollen reconstruction of terrestrial climate and vegetation for N.W. Africa over the last 160 kyr. *Organic Geochemistry*, **34**, 131–143.
- Zhao, M., Eglinton, G., Haslett, S. K., Jordan, R. W., Sarnthein, M., and Zhang, Z., 2000. Marine and terrestrial biomarker records for the last 35,000 years at ODP site 658C off NW Africa. *Organic Geochemistry*, **31**, 919–930.
- Zhou, Y., Grice, K., Stuart-Williams, H., Farquhar, G. D., Hocart, C. H., Lu, H., and Liu, W., 2010. Biosynthetic origin of the saw-toothed profile in ¹³C and of *n*-alkanes and systematic isotopic differences between-, iso- & anteiso-alkanes in leaf waxes of land plants. *Phytochemistry*, **71**, 388–403.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Astrobiology
 Bacteria
 Biomarkers (Molecular Fossils)
 Cold Seeps
 Cyanobacteria
 Diatoms
 Isotopes and Geobiology
 Methane, Origin
 Methane Oxidation (Aerobic)
 Nitrogen
 Origins of the Metazoa
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOMINING (MINERAL BIOLEACHING, MINERAL BIOOXIDATION)

Douglas Eric Rawlings
 University of Stellenbosch, Matieland, South Africa

Summary

Biomining is the use of autotrophic, acidophilic, iron-, and sulfur-oxidizing microorganisms to produce ferric iron and sulfuric acid. These chemicals are capable of oxidizing a variety of minerals containing insoluble metal sulfides such as Fe₂S, CuS, NiS, and ZnS into their soluble sulfate forms e.g., Fe₂SO₄, CuSO₄, NiSO₄, and ZnSO₄, respectively, resulting in their extraction into water from which they may be more easily recovered. Metal leaching is carried out from mineral waste dumps, or more typically and especially from constructed heap or stirred tank reactors. The metals recovered commercially in the greatest quantities are copper and gold.

Description of the terms bioleaching, biooxidation, and biomining

The term *bioleaching* refers to the conversion of an insoluble metal (usually a metal sulfide, e.g., CuS, NiS, ZnS) into a soluble form (usually the metal sulfate, e.g., CuSO₄, NiSO₄, ZnSO₄). As the metal is extracted into water, this process is called bioleaching. Because this process involves oxidation, it may also be termed *biooxidation*. However, the term biooxidation usually refers to processes in which the recovery of a metal is enhanced by microbial decomposition of the mineral, but the metal being recovered is not solubilized. An example is the recovery of gold from arsenopyrite ores where the gold remains in the mineral after biooxidation and is extracted by cyanide in a subsequent step. The term bioleaching is clearly inappropriate when referring to gold recovery (although arsenic, iron, and sulfur are bioleached from

the mineral). *Biomining* is a general term that may be used to refer to both processes (Rawlings, 2002).

Definition

Biomining is a generic term that refers to a collection of biotechnological processes in which microorganisms are used in the recovery of metals from mineral ores and concentrates. The microbial processes involved are the same as those responsible for acid mine drainage, and where metals are recovered during the treatment of acid mine drainage such a process may be termed biomining. Although the bioadsorption of metals onto biological materials including microorganisms may be used in metal recovery, the term biomining has not been applied to bioadsorption processes.

Metals that are recovered in the greatest quantities in commercial processes are copper, gold, and cobalt. Illustrations of the number and scale of operations that employ microorganisms for metal recovery are to be found in Olsen et al. (2003) and Rawlings et al. (2003). Processes also exist for the treatment of nickel- and zinc-containing ores although recovery of these metals using "biomining" has not been commercialized. For several years uranium was recovered using related processes (McCready, 1988) and the technology has the potential for the recovery of several other types of metals. In general, to be amenable to biologically assisted leaching, the mineral should contain iron or a reduced form of sulfur.

Biomining processes use the action of microbes for one of two purposes (Rawlings, 2005). Either to convert insoluble metal sulfides (or oxides) to water soluble metal sulfates or as a pretreatment process to open up the structure of the mineral thereby permitting other chemicals to better penetrate the mineral and solubilize the desired metal. An example of the first type of process is the conversion of insoluble copper present in minerals such as covellite (CuS) or chalcocite (Cu_2S) to soluble copper sulfate. An example of the second is the removal of iron, arsenic, and sulfur from gold-bearing arsenopyrite so that the gold that remains in the mineral is more easily extracted by subsequent treatment with cyanide. Both are oxidation processes, but when the metal to be recovered is extracted into solution, the process is known as bioleaching, whereas when the metal remains in the mineral, bioleaching is an inappropriate term and the process should strictly be referred to as biooxidation. The term bioleaching is frequently used for both.

Mechanisms of biologically assisted leaching

Current understanding is that biooxidation is primarily a chemical process. Sand and coworkers have proposed that minerals can be divided into two broad categories, acid-insoluble minerals that are solubilized through oxidation by ferric iron (e.g., FeS_2 , MoS_2 , and WS_2) and acid-soluble minerals that are oxidized by the combined action of ferric iron and protons (e.g., ZnS , PbS , FeAsS , CuFeS_2 ,

and MnS_2). The role of the microorganisms is to provide the ferric iron and sulfuric acid leaching agents. Leaching reactions have been presented in Schippers and Sand (1999) and Rohwerder et al. (2003) and are reviewed in Rawlings (2005).

Because bioleaching processes are chemistry-driven, if a mineral (such as chalcopyrite) is recalcitrant to biooxidation at 40°C , the solution to the problem lies primarily in the realm of chemistry rather than biology. For example, an increase in the temperature of the biooxidation process to 80°C will allow the chemical reactions to take place at a much faster rate. However, the biology needs to follow the chemistry, and microorganisms that are capable of iron and sulfur oxidation at 80°C are required to generate or regenerate the lixiviants (leaching chemicals).

Although it is possible to separate the chemistry of metal solubilization from the biology into two different processes, separation is unlikely to be as efficient as when both processes are carried out together. The reason for this is that the role of the microorganisms is considered to be more than the generation of the ferric iron and acid required for the chemistry of mineral biooxidation. Contact between the microbes and the mineral is important (though not essential) because the microorganisms produce an exopolysaccharide layer when attached to a mineral (Sand et al., 1995). This layer serves as a reaction space where mineral dissolution reactions take place more efficiently than in the bulk solution. Increased efficiency is partly because conditions within the exopolysaccharide layer (e.g., concentration of ferric iron, redox potential, and pH) are substantially different and more favorable for leaching compared with conditions in the bulk solution (Rawlings, 2005).

Biomining processes

Heap reactors

Commercial mineral bioleaching can take place using a low technology process such the irrigation of waste ore dumps (Brierley, 1982). The efficiency of metal recovery may be improved by the construction and irrigation of specially designed heaps rather than by the irrigation of an existing, nonengineered dump (Brierley, 1982; Rawlings et al., 2003). During heap construction, agglomerated ore is piled onto an impermeable base and supplied with an efficient leach liquor distribution and collection system. Acidic leaching solution is percolated through the crushed ore, and microbes growing on the surface of the mineral in the heap produce the ferric iron and acid that carry out mineral dissolution and metal solubilization. Good aeration enhances the metal leaching reaction. Aeration can be passive, with air being drawn into the reactor as a result of the flow of liquid or may be active with air blown into the heap through piping installed near the bottom. Metal-containing leach solutions that drain from the heap are collected and sent for metal recovery. Heap reactors can be of enormous size (Rawlings et al., 2003), are

relatively cheap to construct and operate, and are therefore suited to the treatment of lower grade ores. Compared with tank reactors, the metal recovery efficiency of heap reactors tends to be low. Heap reactors are more difficult to aerate efficiently and undesirable gradients of pH, nutrients, and liquor channeling are difficult to manage. In addition, although initial rates of bioleaching can be improved by effective heap inoculation, it is difficult to achieve in practice and nondeliberate inoculation that occurs during irrigation may be the main method by which microorganisms are spread through a heap.

Tank reactors

Continuous-flow stirred tank reactors placed in series are used to treat high-value finely milled mineral concentrate or ore. Such tanks can be of considerable size, for example, the 1,300 m³ tanks used to process cobalt-containing pyrite at Kasese, Uganda. Mineral concentrate is added to the first tank, together with inexpensive inorganic nutrients in the form of ammonia- and phosphate-containing fertilizers. The mineral suspension flows through the series of highly aerated tanks that are pH- (typically pH 1.5–1.6) and temperature-controlled. Stirred tank reactors that operate at 40°C and 50°C have proven to be highly robust, and very little process adaptation is required for the treatment of different mineral types. Mineral solubilization takes place in days rather than the weeks or months required in heap reactors. A constraint on the operation of stirred tank reactors is the quantity of solids (pulp density) that can be maintained in suspension, which is limited to about 20%. At pulp densities >20%, physical mixing and microbial problems occur. The liquid becomes too thick for efficient gas transfer and the shear force induced by the impellers cause physical damage to the microbial cells. The limitation in solids concentration together with higher capital and running costs has restricted the use of stirred reactors to high value minerals or mineral concentrates. Stirred tanks are used as a pretreatment process for gold-containing arsenopyrite concentrates, with the first of these having been built at the Fairview mine, Barberton, South Africa in 1986. The largest stirred tank process is at Sansu in the Ashanti goldfields of Ghana, West Africa, where 960 tonnes of gold concentrate are processed per day.

Types of microorganisms

Both heap and tank biooxidation processes contain a variety of microorganisms, with two different organisms usually dominating: an iron-oxidizer and a sulfur-oxidizer. A wide variety of these microorganisms may be present (Rawlings and Johnson, 2007). Irrespective of the type of process or temperature, these microbes have several features in common that make them suitable for their role in mineral dissolution. They grow autotrophically by fixing CO₂ from the atmosphere which makes it unnecessary to provide them with a fixed carbon source. Their energy is obtained by using either ferrous iron or reduced

inorganic sulfur compounds (some use both) as electron donors. Oxygen is generally used as the electron acceptor. All are acidophiles and grow in low-pH environments (pH 1.4–1.6 is typical) and they tend to be tolerant of a wide range of metal ions (Dopson et al., 2003). These very modest nutritional requirements can generally be met by the aeration of the iron- and/or sulfur-containing mineral suspension in water or the irrigation of a heap. Small quantities of inorganic fertilizer may be added to ensure that nitrogen, phosphate, potassium, and trace element limitation does not occur.

The types of organisms found in heap and tank reactors that operate at different temperatures have been reviewed (Rawlings and Johnson, 2007). In general, bacteria belonging to the genera *Acidithiobacillus* and *Leptospirillum* tend to dominate the processes up to 40°C, with those belonging to the genera *Acidithiobacillus*, *Leptospirillum* and *Sulfobacillus* dominating the processes between 45 and 55°C. In high-temperature processes (60–80°C), archaea such as *Acidianus*, *Metalsphaera*, and *Sulfolobus* are responsible for iron- and sulfur-oxidation rather than bacteria.

Bibliography

- Brierley, C. L., 1982. Microbiological mining. *Scientific American*, **247**(2), 42–51.
- Dopson, M., Baker-Austin, C., Ram Kopponnedi, P., and Bond, P., 2003. Growth in sulfidic mineral environments: metal resistance mechanisms in acidophilic micro-organisms. *Microbiology*, **149**, 1959–1970.
- McCready, R. G. L., 1988. Progress in the bacterial leaching of metals in Canada. In Norris, P. R., and Kelly, D. P. (eds.), *Biohydrometallurgy-87*, Science and Technology Letters. UK: Kew, Surrey, pp. 177–195.
- Olsen, G. J., Brierley, J. A., and Brierley, C. L., 2003. Bioleaching review part B: progress in bioleaching: applications of the microbial processes by the mineral industries. *Applied Microbiology and Biotechnology*, **63**, 249–257.
- Rawlings, D. E., 2002. Heavy metal mining using microbes. *Annual Review of Microbiology*, **56**, 65–91.
- Rawlings, D. E., 2005. Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microbial Cell Factories*, **4**, 13.
- Rawlings, D. E., and Johnson, D. B., 2007. The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia. *Microbiology*, **153**, 315–324.
- Rawlings, D. E., Dew, D., and du Plessis, C., 2003. Biomineralization of metal-containing ores and concentrates. *Trends in Biotechnology*, **21**, 38–44.
- Rohwerder, T., Gehrke, T., Kinzler, K., and Sand, W., 2003. Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. *Applied Microbiology and Biotechnology*, **63**, 239–248.
- Sand, W., Gehrke, T., Hallmann, R., and Schippers, A., 1995. Sulfur chemistry, biofilm, and the (in)direct attack mechanism – critical evaluation of bacterial leaching. *Applied Microbiology and Biotechnology*, **43**, 961–966.
- Schippers, A., and Sand, W., 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. *Applied and Environmental Microbiology*, **65**, 319–321.

Cross-references

Acid Rock Drainage
Archaea
Bacteria
Copper
Extracellular Polymeric Substances (EPS)
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Gold
Microbial Biomineralization
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation
Sulfur Cycle

BIOPROTECTION

Carlos Rodriguez-Navarro, Maria T. González-Muñoz, Concepción Jimenez-Lopez, Manuel Rodriguez-Gallego
Universidad de Granada, Granada, Spain

Synonyms

Bioconservation; Bioconsolidation; Biopreservation; Biorestitution

Definition

Active or passive microbially induced or mediated consolidation, cleaning, and/or protection of (stone) artworks affected by chemical, physical, and/or biological weathering phenomena. Bioprotection includes the microbially mediated removal (cleaning) of deleterious compounds (sulfates, nitrates, and/or organics), and the in situ cementation (consolidation) of ornamental materials via microbially induced biomineralization. Bioprotection also occurs naturally on rock surfaces where microorganisms aid in the formation of cementing phases such as oxalates and carbonates, and biofilms.

Introduction

The term bioprotection has been applied to a range of processes and applications involving microorganisms. They include microbe-induced defense response in plant–soil interactions, plant/crop treatment, food processing, and toxic metal decontamination (Glazer and Nikaido, 2007), prevention of wood decay (Dawson-Andoh and Morrell, 1992), inhibition of steel corrosion (Volkland et al., 2000), natural protection of rock outcrops (Carter and Viles, 2005) and building stones (Di Bonaventura et al., 1999), as well as cultural heritage conservation (Ciferri et al., 2000; Rodriguez-Navarro et al., 2003; Ramirez et al., 2005; Webster and May, 2006). Here, the term bioprotection refers both to the use of microorganisms (mainly bacteria) for the conservation of stone artworks, and to the microbially mediated formation of natural protection layers on rock outcrops and stone surfaces. Such meanings are the most commonly used and accepted in

geobiology, as they involve the interaction between the biosphere and the geosphere.

Although “carved in stone” is meant to be durable or eternal, ornamental stones are subjected to a range of physical, chemical, and biological weathering phenomena that lead to their eventual crumbling and disintegration. Such weathering processes have been exacerbated by anthropogenic emission of pollutants since the Industrial Revolution (Winkler, 1994). As a consequence, stone monuments and statuary are at risk worldwide.

Granites, sandstones, and carbonate rocks – limestone, dolostone, and marble – are among the most common stones used in monuments and statuary. Carbonate stones, made up of calcite (CaCO_3) and/or dolomite ($\text{CaMg}(\text{CO}_3)_2$), are particularly prone to chemical weathering in polluted (urban) environments, as exemplified by the decay of the Parthenon or the Taj Mahal. Wet (acid rain) and dry deposition of atmospheric pollutants contribute to the leaching of these sparingly soluble carbonates and the formation of sulfate – gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) – crusts, also named “black crusts” (Figure 1). Carbonate stones are also prone to physical–mechanical weathering associated to salt crystallization, insolation weathering, frost damage, and hydric expansion/contraction (Winkler, 1994; Gauri and Bandyopadhyay, 1999; Rodriguez-Navarro et al., 2003). Biodeterioration is also a major



Bioprotection, Figure 1 Black crusts developed on a limestone gargoyle at the Royal Chapel, Granada (Spain).

Cross-references

Acid Rock Drainage
Archaea
Bacteria
Copper
Extracellular Polymeric Substances (EPS)
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Gold
Microbial Biomineralization
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation
Sulfur Cycle

BIOPROTECTION

Carlos Rodriguez-Navarro, Maria T. González-Muñoz, Concepción Jimenez-Lopez, Manuel Rodriguez-Gallego
Universidad de Granada, Granada, Spain

Synonyms

Bioconservation; Bioconsolidation; Biopreservation; Biorestoration

Definition

Active or passive microbially induced or mediated consolidation, cleaning, and/or protection of (stone) artworks affected by chemical, physical, and/or biological weathering phenomena. Bioprotection includes the microbially mediated removal (cleaning) of deleterious compounds (sulfates, nitrates, and/or organics), and the in situ cementation (consolidation) of ornamental materials via microbially induced biomineralization. Bioprotection also occurs naturally on rock surfaces where microorganisms aid in the formation of cementing phases such as oxalates and carbonates, and biofilms.

Introduction

The term bioprotection has been applied to a range of processes and applications involving microorganisms. They include microbe-induced defense response in plant–soil interactions, plant/crop treatment, food processing, and toxic metal decontamination (Glazer and Nikaido, 2007), prevention of wood decay (Dawson-Andoh and Morrell, 1992), inhibition of steel corrosion (Volkland et al., 2000), natural protection of rock outcrops (Carter and Viles, 2005) and building stones (Di Bonaventura et al., 1999), as well as cultural heritage conservation (Ciferri et al., 2000; Rodriguez-Navarro et al., 2003; Ramirez et al., 2005; Webster and May, 2006). Here, the term bioprotection refers both to the use of microorganisms (mainly bacteria) for the conservation of stone artworks, and to the microbially mediated formation of natural protection layers on rock outcrops and stone surfaces. Such meanings are the most commonly used and accepted in

geobiology, as they involve the interaction between the biosphere and the geosphere.

Although “carved in stone” is meant to be durable or eternal, ornamental stones are subjected to a range of physical, chemical, and biological weathering phenomena that lead to their eventual crumbling and disintegration. Such weathering processes have been exacerbated by anthropogenic emission of pollutants since the Industrial Revolution (Winkler, 1994). As a consequence, stone monuments and statuary are at risk worldwide.

Granites, sandstones, and carbonate rocks – limestone, dolostone, and marble – are among the most common stones used in monuments and statuary. Carbonate stones, made up of calcite (CaCO_3) and/or dolomite ($\text{CaMg}(\text{CO}_3)_2$), are particularly prone to chemical weathering in polluted (urban) environments, as exemplified by the decay of the Parthenon or the Taj Mahal. Wet (acid rain) and dry deposition of atmospheric pollutants contribute to the leaching of these sparingly soluble carbonates and the formation of sulfate – gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) – crusts, also named “black crusts” (Figure 1). Carbonate stones are also prone to physical–mechanical weathering associated to salt crystallization, insolation weathering, frost damage, and hydric expansion/contraction (Winkler, 1994; Gauri and Bandyopadhyay, 1999; Rodriguez-Navarro et al., 2003). Biodeterioration is also a major



Bioprotection, Figure 1 Black crusts developed on a limestone gargoyle at the Royal Chapel, Granada (Spain).

contributor to the decay of stones in general, and carbonate stones in particular (McNamara and Mitchell, 2005).

Conservation of decayed stoneworks typically involves cleaning (by mechanical or chemical means) and/or protection/consolidation. Organic (e.g., acrylic or epoxy resins), organo-inorganic (e.g., ethyl silicates), and inorganic (e.g., $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$ solutions) protection/consolidation treatments have been applied in the past to alter or mitigate stone decay (Lazzarini and Tabasso, 1986; Price, 1996). However, none of these treatments have been proven to be satisfactory (see Rodriguez-Navarro et al., 2003 and references therein). The limitations and drawbacks of these “classic” conservation treatments have prompted the search for new methods to clean, protect and consolidate weathered ornamental stones. One promising line of research has focused on microbial bioprotection as a new means for the restoration and consolidation of ornamental stone.

Microbial-stone interactions: biodeterioration versus bioprotection

It has been known for decades that microbes actively contribute to the degradation of ornamental stone (McNamara and Mitchell, 2005). For instance, the metabolic activity of nitrifying bacteria (*Nitrosomonas* sp. and *Nitrobacter* sp.) results in the production of nitric acid, which in turn causes dissolution of stone and formation of deleterious nitrate salts (Fernandez, 2006). Sulfur-oxidizing bacteria such as *Thiobacillus* sp. produce sulfuric acid and contribute to the development of sulfate crusts (Warscheid and Braams, 2000). Biofilms can exacerbate microbial biodeterioration as they provide a shelter for the aforementioned bacteria, and act as a reservoir for nutrient storage (McNamara and Mitchell, 2005). Wetting and drying of the biofilm matrix may lead to swelling/shrinking of the extracellular polymeric substances (EPS), causing mechanical damage to the host stone (Warscheid and Braams, 2000).

Although microbes are often the problem, they can also be the solution (Rinaldi, 2006). For instance, the anaerobic sulfate-reducing bacteria *Desulfovibrio desulfuricans* and *D. vulgaris* have been shown to be effective for the removal of gypsum crusts (Webster and May, 2006; Cappitelli et al., 2007). Similarly, nitrate salts can be eliminated through the application of nitrate-reducing bacterial cultures (Fernandez, 2006), while *Pseudomonas stutzeri* has been successfully applied to eliminate organic residues from frescoes (Ranalli et al., 2005).

There is growing evidence that several microorganisms contribute to the natural bioprotection of rock outcrops (Carter and Viles, 2005) as well as ornamental stone (Di Bonaventura et al., 1999) via the formation of biofilms and cemented surface layers. Some bacterial communities can promote the production of protective calcium oxalates on building stone (Di Bonaventura et al., 1999), or inhibit calcite dissolution (Rinaldi, 2006). Numerous strains of

bacteria isolated on different building stones are able to induce the precipitation of calcium carbonate (Urzi et al., 1999), an effect that may have aided in the natural protection of weathered ornamental stones. This latter ability has opened new ways for the effective protection and consolidation of decayed ornamental stones based on the in situ mineralization of bacterial carbonate cement (Rodriguez-Navarro et al., 2003; Webster and May, 2006; Jimenez-Lopez et al., 2007).

Bacterially mediated biomineralization of carbonates

The precipitation of carbonate minerals mediated or induced by both autotrophic and heterotrophic bacteria have attracted a substantial amount of research (see recent review by Wright and Oren, 2005). Bacterial precipitation of calcium carbonate has important implications in the past and present formation of carbonate rocks and sediments (Rodriguez-Navarro et al., 2003; Wright and Oren, 2005). It is known that cyanobacteria have aided in the formation of mineralized stromatolites since the Precambrian age (Konhauser, 2007), their photosynthetic activity thus contributing to atmosphere oxygen increase and CO_2 budgeting by carbonate precipitation. While early work (until the 1980s) examined the ability of photosynthetic bacteria to precipitate calcium carbonates in marine environments, precipitation of carbonates induced by heterotrophic bacteria in other environments such as caves, soils, and monuments has become the subject of much research in recent decades (Boquet et al., 1973; Urzi et al., 1999).

Bacteria play both passive and active roles in carbonate nucleation (Mitchell and Ferris, 2006). In the first case, the metabolic activity of heterotrophic bacteria (e.g., ammonification of amino acids, urea degradation, and dissimilatory reduction of nitrates and sulfates), or photosynthetic HCO_3^- consumption and OH^- production by cyanobacteria, increases the alkalinity (high pH, and elevated CO_3^{2-}) in the microenvironment surrounding the bacterial cells. This in turn increases supersaturation with respect to calcium carbonate if free Ca^{2+} is present, thus favoring calcification. Bacterial cell walls and/or sheets, as well as EPS, can actively aid in carbonate crystallization by providing heterogeneous nucleation sites (Ercole et al., 2007; Rodriguez-Navarro et al., 2007).

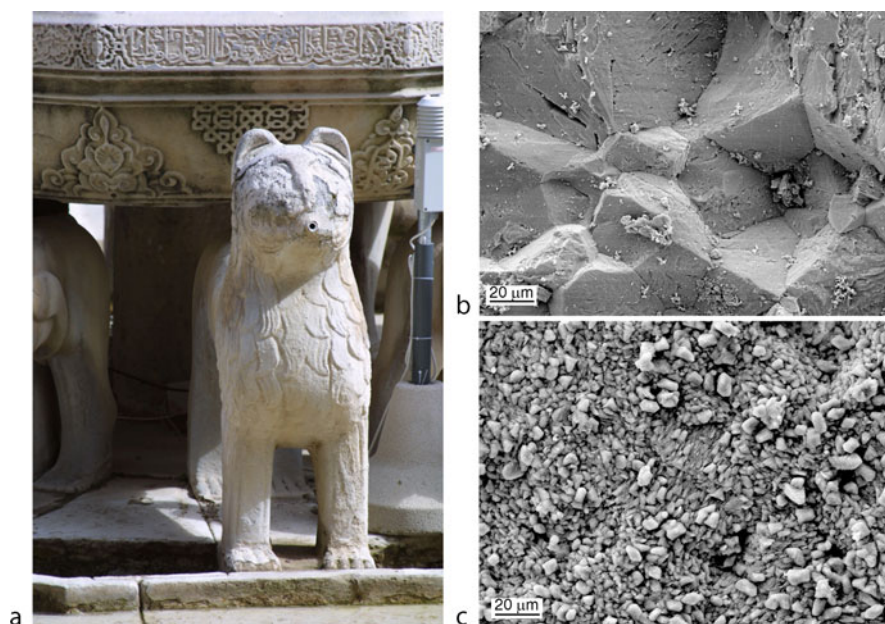
Bioprotection of stone by bacterial biomineralization of carbonates

Based on previous work on the natural ability of many heterotrophic bacteria to induce calcium carbonate precipitation, Adolphe et al. (1990) patented a method, CALCITE, for bacterial bioprotection of ornamental stones. The authors showed that calcinogenic bacteria, such as those typically found in soils (Boquet et al., 1973), were able to precipitate calcite on the surface of weathered limestone, thus contributing to their protection and consolidation. Le Métayer-Levrel et al. (1999) reported the

“successful” application of such a method on a sixteenth century limestone castle. Nonetheless, the authors used *Bacillus cereus* cultures, which may pose a health risk. In addition, potentially harmful biofilm formation and only a few micrometers thick consolidated layer were observed. Tiano et al. (1999) evaluated the effectiveness of such a treatment for bioprotection of a highly porous limestone (Pietra de Lecce) using *Micrococcus* sp. and *B. subtilis*. They observed detrimental obstruction of pores (presumably by biofilm formation), and called attention to other potential drawbacks e.g., reaction of the stone and by-products of bacterial activity, and the formation of stain patches. Rodriguez-Navarro et al. (2003) showed that such limitations could be overcome by using different culture media and a non-pathogenic carbonate-producing heterotrophic soil bacteria, *Myxococcus xanthus*. The treatment resulted in deep consolidation of a porous limestone (calcarenite) with new bacterial calcium carbonate cement, without detrimental pore plugging or extensive biofilm formation. *M. xanthus* metabolic activity results in the release of NH_3 , which leads to a pH rise in the medium surrounding the bacterial cells. This in turn favors carbonate supersaturation and results in CaCO_3 precipitation on different calcareous stones such as limestone and marble (Figure 2). Carbonates typically form on the bacterial cell walls, where heterogeneous nucleation is facilitated, and grow as a coherent cement (often epitaxially) on the stone support. Rodriguez-Navarro et al. (2003) also showed that the new bacterial calcite cement was tougher and more resistant to dissolution than the original calcite

in the stone. They interpreted these results considering that organic by-products of bacterial activity were incorporated into the bacterial calcite, which was in fact a (bio) composite material. Interestingly, Ben Chekroun et al. (2004) and Rodriguez-Navarro et al. (2007) showed that *M. xanthus* induced the precipitation of either calcite or vaterite just by modifying the culture medium. These results demonstrate that polymorph selection in carbonate precipitation is not strain specific. On the other hand, recent research using *B. subtilis* (Perito and Mastromei, 2003), *B. sphaericus* and *B. lentus* (Dick et al., 2006) shows that the range of microorganisms that can contribute to the bioprotection of ornamental stone via the precipitation of carbonates is very wide. In addition, it has been shown that bacteria such as *M. xanthus* can precipitate a range of minerals, other than carbonates, just by changing the composition of the culture medium (Ben Chekroun et al., 2004). These results imply that an accurate selection of growth media and bacterial strains could yield improved protection and consolidation of ornamental stones with disparate composition and textures.

Bioprotection treatments based on bacterial biomineralization are not restricted to ornamental stone. Castanier et al. (1996) used *Pseudomonas* sp. for calcite precipitation as a cementing phase in a biological mortar. Bang et al. (2001) have reported that polyurethane-immobilized *B. pasteurii* (a carbonatogenic ureolytic bacteria) can fill the cracks in concrete. Whiffin et al. (2007) have shown that carbonate precipitation induced by *Sporosarcina pasteurii* contributes to soil strengthening.



Bioprotection, Figure 2 Bacterially mediated calcite biomineralization on Macael marble: (a) Severe damage on a Macael marble lion sculpture at the Lions Court, The Alhambra, Granada, Spain; (b) scanning electron microscopy photomicrographs of a Macael marble sample before and (c) after bacterial conservation treatment resulting in *M. xanthus* mediated calcite cement formation.

Summary

Weathered ornamental stones typically contain a variety of endolithic and epilithic microbial communities (Urzi et al., 1999; McNamara and Mitchell, 2005). Application of bacterial treatments that include a nutritive medium may thus activate, among the microbial community contained in the stones, those bacteria that are able to induce the precipitation of calcium carbonate. In this respect, Jimenez-Lopez et al. (2007) have shown that the application of sterile nutritive media to porous limestone is able to activate carbonatogenic bacteria. As a consequence of the growth of these bacteria, an alkalization occurs that results in calcium carbonate precipitation. The new precipitate is compatible with the substrate and consolidates the stone without pore plugging. Such effects are reinforced by the presence of *M. xanthus*. Therefore, a good candidate to in situ consolidate decayed porous limestone is the application of a sterile culture medium with specific characteristics that favor the development of calcinogenic bacteria, while limiting the proliferation of detrimental microbiota (Jimenez-Lopez et al., 2007). Such an alternative to the direct application of life cells is highly promising. Finally, bacterial biomineralization of silicates as a means to consolidate non-carbonate stones such as granite and sandstone is another topic that should be further studied.

Bibliography

- Adolphe, J. M., Loubière, J. F., Paradas, J., and Soleilhavoup, F., 1990. Procédé de traitement biologique d'une surface artificielle. European Patent No. 90400G97.0 (after French Patent No. 8903517, 1989).
- Bang, S. S., Galinat, J. K., and Ramakrishnan, V., 2001. Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*. *Enzyme and Microbial Technology*, **28**, 404–409.
- Ben Chekroun, K., Gonzalez-Muñoz, M. T., Rodriguez-Navarro, C., Arias, J. M., and Rodriguez-Gallego, M., 2004. Precipitation and growth morphology of calcium carbonate induced by *Myxococcus xanthus*: implications for recognition of bacterial carbonates. *Journal of Sedimentary Research*, **74**, 868–876.
- Boquet, E., Boronat, A., and Ramos-Cormenzana, A., 1973. Production of calcite (calcium carbonate) crystals by soil bacteria is a common phenomenon. *Nature*, **246**, 527–529.
- Cappitelli, F., Toniolo, L., Sansonetti, A., Gulotta, D., Ranalli, G., Zanardini, E., and Sorlini, C., 2007. Advantages of using microbial technology over traditional chemical technology in removal of black crusts from stone surfaces of historical monuments. *Applied and Environmental Microbiology*, **73**, 5671–5675.
- Carter, N. E. A., and Viles, H. A., 2005. Bioprotection explored: the story of a little known earth surface process. *Geomorphology*, **67**, 273–281.
- Castanier, S., Le Métayer-Levrel, G., and Loubière, J. F., 1996. Biological mortar composition. French Patent FR2734261.
- Ciferri, O., Tiano, P., and Mastromei, G., 2000. *Of Microbes and Art: The Role of Microbial Communities in the Degradation and Protection of Cultural Heritage*. New York: Plenum.
- Dawson-Andoh, B. E., and Morrell, J. J., 1992. Enhancing the performance of bioprotection agents by pretreatment with waterborne salts. *Wood and Fiber Science*, **24**, 347–352.
- Di Bonaventura, M. P., Del Gallo, M., Cacchio, P., Ercole, C., and Lepidi, A., 1999. Microbial formation of oxalate films on monument surfaces: bioprotection or biodeterioration? *Geomicrobiology Journal*, **16**, 55–64.
- Dick, J., De Windt, W., De Graef, B., Saveyn, H., van der Meeren, P., De Belie, N., and Verstraete, W., 2006. Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species. *Biodegradation*, **17**, 357–367.
- Ercole, C., Cacchio, P., Botta, A. L., Centi, V., and Lepidi, A., 2007. Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides. *Microscopy and Microanalysis*, **13**, 42–50.
- Fernandez, P., 2006. Applied microbiology and biotechnology in the conservation of stone cultural heritage materials. *Applied Microbiology Biotechnology*, **73**, 291–296.
- Gauri, K. L., and Bandyopadhyay, J. K., 1999. *Carbonate Stone: Chemical Behavior, Durability, and Conservation*. New York: Wiley.
- Glazer, A. N., and Nikaido, H., 2007. *Microbial Biotechnology: Fundamentals of Applied Microbiology*, 2nd edn. Cambridge, MA: Cambridge University Press.
- Jimenez-Lopez, C., Rodriguez-Navarro, C., Piñar, G., Carrillo-Rosúa, F. J., Rodriguez-Gallego, M., and Gonzalez-Muñoz, M. T., 2007. Consolidation of degraded ornamental porous limestone stone by calcium carbonate precipitation induced by the microbiota inhabiting the stone. *Chemosphere*, **68**, 1929–1936.
- Konhauser, K., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Lazzarini, L., and Tabasso, M., 1986. *Il Restauro della Pietra*. Padua: Cedam.
- Le Métayer-Levrel, G., Castanier, S., Orial, G., Loubière, J. F., and Perthuisot, J. P., 1999. Applications of bacterial carbonatogenesis to the protection and regeneration of limestones in buildings and historic patrimony. *Sedimentary Geology*, **126**, 25–34.
- McNamara, C. J., and Mitchell, R., 2005. Microbial deterioration of historic stone. *Frontiers in Ecology and the Environment*, **3**, 445–451.
- Mitchell, A. C., and Ferris, F. G., 2006. The influence of *Bacillus pasteurii* on the nucleation and growth of calcium carbonate. *Geomicrobiology Journal*, **23**, 213–226.
- Perito, B., and Mastromei, G., 2003. Conservation of monumental stones by bacterial biomineralization. *Microbiology Today*, **30**, 113–114.
- Price, C. A., 1996. *Stone Conservation. An Overview of Current Research*. Los Angeles, CA: Getty Conservation Institute.
- Ramirez, J. L., Santana, M. A., Galindo-Castro, I., and Gonzalez, A., 2005. The role of biotechnology in art preservation. *TRENDS in Biotechnology*, **23**, 584–588.
- Ranalli, G., Alfano, G., Belli, C., Lustrato, G., Colombini, M. P., Bonaduce, I., Zanardini, E., Abbruscato, P., Cappitelli, F., and Sorlini, C., 2005. Biotechnology applied to cultural heritage: bioremediation of Frescoes using viable bacterial cells and enzymes. *Journal of Applied Microbiology*, **98**, 73–83.
- Rinaldi, A., 2006. Saving a Fragile Legacy. *EMBO Reports*, **7**, 1075–1079.
- Rodriguez-Navarro, C., Rodriguez-Gallego, M., Chekroun, K. B., and Gonzalez-Muñoz, M. T., 2003. Conservation of ornamental stone by *Myxococcus xanthus*-induced carbonate biomineralization. *Applied and Environmental Microbiology*, **69**, 2182–2193.
- Rodriguez-Navarro, C., Jimenez-Lopez, C., Rodriguez-Navarro, A., Gonzalez-Muñoz, M. T., and Rodriguez-Gallego, M., 2007. Bacterially mediated mineralization of vaterite. *Geochimica et Cosmochimica Acta*, **71**, 1197–1213.
- Tiano, P., Biagiotti, L., and Mastromei, G., 1999. Bacterial bi-mediated calcite precipitation for monumental stones conservation: methods of evaluation. *Journal of Microbiological Methods*, **36**, 139–145.

- Urzi, C., Garcia-Vallez, M., Vendrell, M., and Pernice, A., 1999. Biomineralization processes on rock and monument surfaces observed in field and in laboratory conditions. *Geomicrobiology Journal*, **16**, 39–54.
- Volkland, H. P., Harms, H., Knopf, K., Wanner, O., and Zehnder, A. J. B., 2000. Corrosion of mild steel by bacteria. *Biofouling*, **15**, 287–297.
- Warscheid, T., and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, **46**, 343–368.
- Webster, A., and May, E., 2006. Bioremediation of weathered-building stone surfaces. *Trends in Biotechnology*, **24**, 255–260.
- Whiffin, V. S., van Passen, L. A., and Harkes, M. P., 2007. Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal*, **24**, 417–423.
- Winkler, E. M., 1994. *Stone in Architecture*. Berlin: Springer.
- Wright, D. T., and Oren, A., 2005. Nonphotosynthetic bacteria and the formation of carbonates and evaporites through time. *Geomicrobiology Journal*, **22**, 27–53.

Cross-references

Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Calcite Precipitation, Microbially Induced
 Chemolithotrophy
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Sulfate-Reducing Bacteria

BIOSIGNATURES IN ROCKS

Frances Westall, Barbara Cavalazzi
 Université de Orléans, Orléans, France

Definition

Biosignatures are morphological, chemical (organic, elemental and/or mineral), and isotopic traces of organisms preserved in minerals, sediments, and rocks. They represent the physical presence of the organisms as well as evidence of their metabolic activities and metabolites.

The nature of biosignatures

In this entry, we review (1) the nature of biosignatures in rocks, (2) how they are produced, and (3) the relevance of biosignatures and their astrobiological implications. Examples of biosignatures from throughout the rock record are given. We will limit our discussion to microbial biosignatures.

Microorganisms are characterized by (1) structural components, such as cells, colonies, biofilms/mats, extracellular polymeric substances (EPS); (2) the chemical composition of the different structural elements, including organic molecules and other essential elements; and (3) their living processes, including metabolic activity leading to the fractionation of elements such as C, S, O, N, P, and Fe, as well as cell division. Indirectly, microorganisms are also characterized by (4) the effects of their

physical presence and metabolic products on their immediate environment. These effects may be preserved on a microscopic to macroscopic scale. They include the precipitation of minerals; element leaching from, and dissolution of, minerals and rocks (biocorrosion); influence on mineral grain size and habit; the formation of microbially induced sedimentary structures (MISS; cf. Noffke et al., 2003) and biostabilization of sedimentary surfaces; and the formation of microbialites (e.g., microbial mounds, stromatolites, thrombolites) and microbial fabrics (e.g., clots, peloids, fenestralae). All of these phenomena can leave morphological, chemical, or isotopic traces in rocks (Table 1), but do not always do so. The ultimate fate of biosignatures is dependent upon their immediate preservation and the history of the sediments and rocks during subsequent diagenesis, metamorphism, and weathering.

The various types of biosignatures are discussed in detail elsewhere in this volume. Our aim here is to provide a brief overview of the broad range of biosignatures in rocks and to discuss their relevance and astrobiological implications.

Biosignatures of cell components and metabolic activity

Fossil carbon molecules

All terrestrial life consists of assemblages of macromolecules based on carbon (with water as the universal solvent, Brack, 2002) and the different components of the cell, i.e., cytoplasm, chromosomes, chloroplast, and cell walls and envelopes, etc., are characterized by molecules of different composition and structure. Molecular degradation starts with the death of the organism and may also be aided by the activity of other microorganisms, e.g., the heterotrophs, who obtain their energy and carbon from the breakdown of organic substances. The chemical changes that take place during degradation (diagenesis) are dependent on the redox state and water chemistry of the environment. Degradation of macromolecules to form “fossil” molecules results in the gradual loss of the functional groups. Specific characteristics of fossil molecules that suggest a biological origin include (1) enantiomeric excess (chirality), (2) diastereoisomeric preference, (3) structural isomer preference, (4) repeating constitutional subunits or atomic ratios, (5) systematic isotopic ordering at molecular and intramolecular levels, and (6) uneven distribution patterns or clusters, such as C-number, concentration, or $\delta^{13}\text{C}$, of structurally related compounds (Summons et al., 2008).

Preservation of fossil molecules

Fossil organic molecules or biomarkers are best preserved in anaerobic environments where they are protected from abiotic oxidation or aerobic microbial degradation. Subsequent burial, metamorphism, and heat events (e.g., hydrothermal/volcanic activity) affect the “maturity” or degradation state of the organic matter. This results in the formation of different types of kerogen characterized

- Urzi, C., Garcia-Vallez, M., Vendrell, M., and Pernice, A., 1999. Biomineralization processes on rock and monument surfaces observed in field and in laboratory conditions. *Geomicrobiology Journal*, **16**, 39–54.
- Volkland, H. P., Harms, H., Knopf, K., Wanner, O., and Zehnder, A. J. B., 2000. Corrosion of mild steel by bacteria. *Biofouling*, **15**, 287–297.
- Warscheid, T., and Braams, J., 2000. Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, **46**, 343–368.
- Webster, A., and May, E., 2006. Bioremediation of weathered-building stone surfaces. *Trends in Biotechnology*, **24**, 255–260.
- Whiffin, V. S., van Passen, L. A., and Harkes, M. P., 2007. Microbial carbonate precipitation as a soil improvement technique. *Geomicrobiology Journal*, **24**, 417–423.
- Winkler, E. M., 1994. *Stone in Architecture*. Berlin: Springer.
- Wright, D. T., and Oren, A., 2005. Nonphotosynthetic bacteria and the formation of carbonates and evaporites through time. *Geomicrobiology Journal*, **22**, 27–53.

Cross-references

Bacteria
 Biodeterioration (of Stone)
 Bioerosion
 Biofilms
 Calcite Precipitation, Microbially Induced
 Chemolithotrophy
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Sulfate-Reducing Bacteria

BIOSIGNATURES IN ROCKS

Frances Westall, Barbara Cavalazzi
 Université de Orléans, Orléans, France

Definition

Biosignatures are morphological, chemical (organic, elemental and/or mineral), and isotopic traces of organisms preserved in minerals, sediments, and rocks. They represent the physical presence of the organisms as well as evidence of their metabolic activities and metabolites.

The nature of biosignatures

In this entry, we review (1) the nature of biosignatures in rocks, (2) how they are produced, and (3) the relevance of biosignatures and their astrobiological implications. Examples of biosignatures from throughout the rock record are given. We will limit our discussion to microbial biosignatures.

Microorganisms are characterized by (1) structural components, such as cells, colonies, biofilms/mats, extracellular polymeric substances (EPS); (2) the chemical composition of the different structural elements, including organic molecules and other essential elements; and (3) their living processes, including metabolic activity leading to the fractionation of elements such as C, S, O, N, P, and Fe, as well as cell division. Indirectly, microorganisms are also characterized by (4) the effects of their

physical presence and metabolic products on their immediate environment. These effects may be preserved on a microscopic to macroscopic scale. They include the precipitation of minerals; element leaching from, and dissolution of, minerals and rocks (biocorrosion); influence on mineral grain size and habit; the formation of microbially induced sedimentary structures (MISS; cf. Noffke et al., 2003) and biostabilization of sedimentary surfaces; and the formation of microbialites (e.g., microbial mounds, stromatolites, thrombolites) and microbial fabrics (e.g., clots, peloids, finestræ). All of these phenomena can leave morphological, chemical, or isotopic traces in rocks (Table 1), but do not always do so. The ultimate fate of biosignatures is dependent upon their immediate preservation and the history of the sediments and rocks during subsequent diagenesis, metamorphism, and weathering.

The various types of biosignatures are discussed in detail elsewhere in this volume. Our aim here is to provide a brief overview of the broad range of biosignatures in rocks and to discuss their relevance and astrobiological implications.

Biosignatures of cell components and metabolic activity

Fossil carbon molecules

All terrestrial life consists of assemblages of macromolecules based on carbon (with water as the universal solvent, Brack, 2002) and the different components of the cell, i.e., cytoplasm, chromosomes, chloroplast, and cell walls and envelopes, etc., are characterized by molecules of different composition and structure. Molecular degradation starts with the death of the organism and may also be aided by the activity of other microorganisms, e.g., the heterotrophs, who obtain their energy and carbon from the breakdown of organic substances. The chemical changes that take place during degradation (diagenesis) are dependent on the redox state and water chemistry of the environment. Degradation of macromolecules to form “fossil” molecules results in the gradual loss of the functional groups. Specific characteristics of fossil molecules that suggest a biological origin include (1) enantiomeric excess (chirality), (2) diastereoisomeric preference, (3) structural isomer preference, (4) repeating constitutional subunits or atomic ratios, (5) systematic isotopic ordering at molecular and intramolecular levels, and (6) uneven distribution patterns or clusters, such as C-number, concentration, or $\delta^{13}\text{C}$, of structurally related compounds (Summons et al., 2008).

Preservation of fossil molecules

Fossil organic molecules or biomarkers are best preserved in anaerobic environments where they are protected from abiotic oxidation or aerobic microbial degradation. Subsequent burial, metamorphism, and heat events (e.g., hydrothermal/volcanic activity) affect the “maturity” or degradation state of the organic matter. This results in the formation of different types of kerogen characterized

Biosignatures in Rocks, Table 1 Morphological, chemical, and isotopic biosignatures in rocks

Major microbial components	Biosignature	Specific component or structure	
Cell components	Organic molecules (biomarkers, kerogen)	Composition chemical structure	Odd/even C number μ -structure
Cell metabolic activity	Biominerals	Direct (e.g., magnetite) indirect (e.g., microcrystalline calcite, aragonite, dolomite)	
	Elements	Concentration Isotopic ratios	e.g., Ni, Cu, Mn, Co, Mo, Se, V, Fe e.g., C, O, S, N, Fe
	Microbial influence on:	Mineral composition, mineral habit, mineral dissolution, mineral size	
Cell colonies, biofilms, mats, EPS	Fossil cells, colonies, mats	Clotted fabrics, microbial mounds, biolaminites	Stromatolites MISS ^a

Source: From Noffke et al. (2003)

^aMicrobially Induced Sedimentary Structures

by H/C ratios ranging from >1.25 for Type I kerogen to <0.5 for type IV kerogen. The small molecular size of some of the degraded molecules means that they are readily mobile and can be transported into other formations with which they are not syngenetic, thus causing contamination. The oldest known biomarkers occur in carbonaceous oil shales from the 2.7 Ga-old Fortescue Group in Australia where molecules such as hopanes and steranes, the degradation products of prokaryotes (specifically aerobic photosynthesizers, cyanobacteria) and eukaryotes, respectively, were interpreted as evidence for oxygenic photosynthetic activity by 2.7 Ga (Brocks et al., 1999) and, more importantly, the early rise of eukaryotes (Summons et al., 1999). These biomarkers have, however, recently given rise to controversy regarding their syngeneticity (Eigenbrode and Freeman, 2006; Rasmussen et al., 2008).

Syngenetic organic molecules occur in even older sediments but the kerogens in the volcanic sediments in the 3.5–3.3 Ga-old Barberton (South Africa) and Pilbara (Australia) greenstone belts are so degraded by age and low-grade metamorphism (prehnite–pumpellyite to lowest greenschist facies) that they no longer contain recognizable compositional biomarkers. The kerogens do, however, retain structural characteristics that are indicative of a biological origin (Westall et al., 2006a; Derenne et al., 2008). Evidence of syngeneticity of the kerogens analyzed is provided by in situ Raman spectra showing D and G peaks indicative of a maturity consistent with the metamorphic grade of the rocks (Westall et al., 2006a), as well as covalent linking of the molecular structures in the kerogen matrix (Derenne et al., 2008).

Traces of cell metabolism and metabolites

The metabolic activity of microorganisms can result in elemental, isotopic, and mineral signatures that can be preserved in rocks.

Bioelements: Unusual concentrations of metals may be associated with microorganisms and their metabolic products. Adsorption of these elements may be either an active or a passive process. Active adsorption includes the chelation of metals to anionic functional groups in the cell walls to neutralize their negative charges, thus contributing to the stability of the cell wall. Microorganisms also require metals, such as Fe, Co, Ni, Cu, Mn, Mo, Se, and V, for certain enzymatic activities (Banfield et al., 2001). They are actively adsorbed onto the cell walls by ligands that are specific for a particular metal (Konhauser, 2007). Microorganisms can also concentrate toxic elements, such as U, Cu, Pb, Al, As, and Cd, as well as precious metals (U, Cu, Pd, Pt, and Au) that are bound to cells and S-layers (Pollmann et al., 2006).

Passive adsorption of metal ions to cell surfaces is an additional kind of metal sequestration (Ledin, 1999). The availability of functional molecules, such as carboxyl or phosphate groups, determines the amount of metals that can be sorbed to the cell surface. With their large quantities of peptidoglycan, Gram positive bacteria can readily scavenge metal ions. Gram negative bacteria, on the other hand, are characterized by only a thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer. The amount of metal that they can bind is an order of magnitude less than that of the Gram positive bacteria (e.g., Beveridge et al., 1982). Microbial EPS is also an important factor in the binding of metals owing to their highly hydrated character that allows the metal ions to freely migrate through the structure (Geesey and Jang, 1989). This characteristic means that microorganisms, such as encapsulated bacteria, are more likely to chelate metals than non-encapsulated varieties. Metal-replaced microorganisms are common and are among the oldest traces of life. Fossil microorganisms preserved as pyritized filaments occur in a 3.2 Ga-old volcanogenic

massive sulfide deposit (the equivalent of a fossilized black smoker) from the Pilbara in Australia (Rasmussen, 2000). A younger example consists of ZnS-replaced filaments in Triassic calcareous rocks in the southern Alps (Kucha et al., 2005).

Concentrations of other biologically important elements, such as P and S, are considered to be significant biosignatures. For example, the combination of graphite associated with apatite inclusions (apatite can be a biomineral, cf. Table 2) and a $\delta^{13}\text{C}$ of -26‰ in a highly metamorphosed rock from the 3.85 Ga-old Akilia province in W. Greenland was interpreted as a biosignature by Mojzsis et al. (1996). However, van Zuilen et al. (2002) demonstrated that abiological processes linked to alteration of the rocks by hydrothermal circulation could have produced carbon via the Fischer–Tropsch process with a similar isotopic signature. On the other hand, phosphatized microorganisms are well-known from the rock record. The spectacular preservation of soft body parts of animals, birds, amphibians, and fish from the Eocene Messel oil shales in Germany is due to phosphatized microbial biofilms that coated the dead organisms (Liebig et al., 1996).

High concentrations of sulfur can be found in kerogen owing to the process of sulfurization during which sulfur, liberated from degrading organic matter by the activity of sulfur-reducing bacteria, is bound to the organic macromolecules. Thus, the identification of the S-molecule thiophene in the organic cell walls of microfossils from the

800 My-old Draken Formation of Svalbard (Lemelle et al., 2008) and even in 2.7 Ga-old kerogen associated with stromatolites from the Tumbiana Formation in the Pilbara (Lepot et al., 2008) has been linked to the degradation of organic matter by sulfur-reducing bacteria.

Biominerals: Minerals of different phases can be formed both actively and passively by microorganisms in biologically controlled and biologically induced processes, respectively. Microbially induced mineralization occurs either as a result of changes in the environment of a microorganism caused by the excretion of metabolic waste products, enzymatic mediated changes in the redox state, or simply the presence of a charged cell surface that attracts mineral nucleation (Banfield et al., 2001; Konhauser, 2007). We have seen above that reactive functional group sites on microbial surfaces readily chelate metal ions. In the same way mineral nuclei in saturated solutions bind to the surfaces of cells and their extrapolymeric substances (EPS), often completely entombing the microorganisms. A non-exhaustive list of microbially induced mineral precipitates is given in Table 2.

Iron hydroxides (ferrihydrite) are among the most common microbially induced deposits and may be formed passively, or by the activities of chemoheterotrophic, photoautotrophic, or chemolithoautotrophic (e.g., *Gallionella*) microorganisms. The ferric hydroxides may act as precursors for Fe oxides, such as hematite. Manganese nodules are an example of microbially induced

Biosignatures in Rocks, Table 2 Non-exhaustive list of microbially induced mineral precipitates

Metal oxides	Fe/Mn hydroxides and oxyde-hydroxides (or oxyhydroxides), e.g., ferrihydrite (ferric hydroxide), $\text{Fe}^{3+}_2\text{O}_3 \cdot 0.5(\text{H}_2\text{O})$, goethite, $\text{Fe}^{3+}\text{O}(\text{OH})$, schwertmannite, $\text{Fe}^{3+}_{16}\text{O}_{16}(\text{OH})_{12}(\text{SO}_4)_2$
Amorphous silica	Fe/Mn/Ti oxides, e.g., magnetite, $\text{Fe}^{3+}_2\text{Fe}^{2+}\text{O}_4$, hematite, $\text{Fe}^{3+}_2\text{O}_3$, anatase, TiO_2
Carbonates	Opal, $\text{SiO}_2 \cdot n(\text{H}_2\text{O})$, e.g., opal-A and opal-A/CT
Carbonates	Ca-carbonates, e.g., calcite, $\text{Ca}(\text{CO}_3)$, magnesian calcite, High-/Low-Mg $\text{Ca}(\text{CO}_3)$, aragonite, CaCO_3 , dolomite, $\text{CaMg}(\text{CO}_3)_2$
Carbonates	Magnesite, $\text{Mg}(\text{CO}_3)$
Carbonates	Strontianite, $\text{Sr}(\text{CO}_3)$
Phosphates	Ca-phosphates, e.g., apatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH}, \text{F}, \text{Cl})$, carbonate-hydroxylapatite, $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, francolite, or carbonate fluoroapatite, $\text{Ca}_5(\text{PO}_4)_3\text{F}$
Phosphates	Fe-phosphates, e.g., vivianite, $\text{Fe}^{3+}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
Sulfates	Barite, BaSO_4
Sulfates	Celestine, SrSO_4
Sulfates	Gypsum, $\text{Ca}(\text{SO}_4) \cdot 2(\text{H}_2\text{O})$
Sulfates	Fe-sulfates, e.g., jarosite, $\text{KFe}^{3+}_3(\text{SO}_4)_2(\text{OH})_6$
Sulfides	Chalcopyrite, $\text{CuFe}^{2+}\text{S}_2$
Sulfides	Galena, PbS
Sulfides	Greigite, Fe_3S_4
Sulfides	Mackinawite, FeS
Sulfides	Millerite, NiS
Sulfides	Pearceite, $\text{Ag}_{16}\text{As}_2\text{S}_{11}$
Sulfides	Proustite, Ag_3AsS_3
Sulfides	Pyrite, Fe^{2+}S_2
Sulfides	Sphalerite, $(\text{Zn}, \text{Fe})\text{S}$
Silicates	Clay minerals, e.g., phillipsite, $(\text{Ca}, \text{Na}, \text{K})1-2(\text{Si}, \text{Al})_8\text{O}_{16} \cdot 6(\text{H}_2\text{O})$, chamosite, $(\text{Fe}^{2+}, \text{Mg}, \text{Fe}^{3+})_5\text{Al}(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH}, \text{O})_8$, illite, $(\text{K}, \text{H}_3\text{O})(\text{Al}, \text{Mg}, \text{Fe})_2(\text{Si}, \text{Al})_4\text{O}_{10}[(\text{OH})_2, (\text{H}_2\text{O})]$, smectite $(\text{Na}, \text{Ca})\text{Al}_4(\text{Si}, \text{Al})_8\text{O}_{20}(\text{OH})_4 \cdot 2(\text{H}_2\text{O})$

iron-manganese oxides that can form volumetrically important deposits in the deep-sea and lacustrine environments (Mero, 1962).

Amorphous silica commonly nucleates passively onto the surfaces of microorganisms in hot spring environments (Figure 1a) (e.g., Cady and Farmer, 1996) and precipitates from saturated solutions in other environments as well. For example, Monty et al. (1991) and Westall (1994) documented silicified microorganisms in the pore space of 15–10 My-old deep-sea sediments. At the other end of the geological record, silica-saturated seawaters on the early Earth led to the silicification of early Archaean microbes and the preservation of the oldest known traces of life (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b; Westall and Southam, 2006).

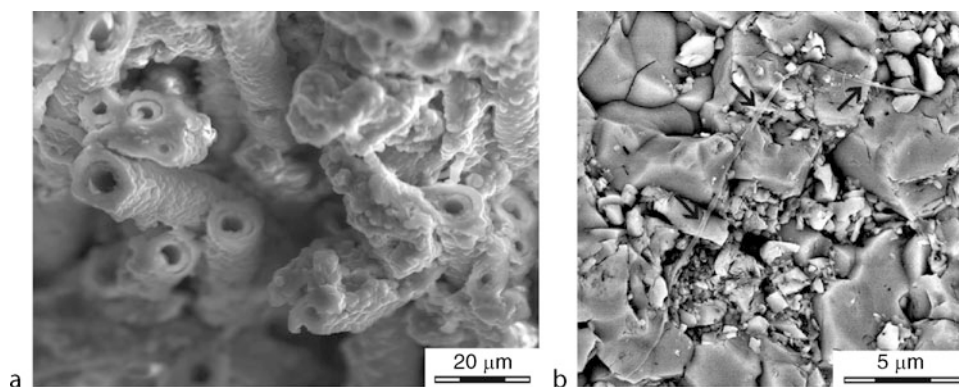
Perhaps the most geologically significant microbially induced mineral deposits are those related to carbonates. The carbonate minerals are produced by processes including mineralization of microbial surfaces, chemical precipitation from supersaturated solutions, and physical erosion of preexisting carbonates (Riding, 2000). Microbial carbonates occur in a wide variety of environments ranging from deep-water cold seeps (Figure 2) (e.g., Cavalazzi et al., 2007), shallow-water platforms (Figure 3a) (e.g., Monty, 1995), lacustrine environments (Figure 3b and c) (e.g., Laval et al., 2000) to carbonate (travertine) springs (e.g., Chafetz and Folk, 1984).

Biologically controlled mineralization occurs when microorganisms exert direct control on the nucleation and growth of the mineral (Mann, 1988). The resulting minerals are characterized by their highly ordered crystal-line structure, size, and morphology. Chains of single-domain magnetite crystals are formed in this way by magnetotactic bacteria (e.g., Bazylinski et al., 1995) and Chang and Kirschvink (1989) record chains of magnetite crystals in the 1.9 Ga-old Gunflint Formation (Canada) that are similar in size and shape to those produced by

recent bacteria. Indeed, the presence of chemically pure magnetite crystals with a specific size/shape range in an ancient Martian meteorite, ALH84001, is one of the key lines of evidence for life on Mars by 3.9 Ga (McKay et al., 1996; Thomas-Keprta et al., 2001, 2002; Friedmann et al., 2001). However, the supposedly biogenic magnetites form only a fraction (<30%) of a larger population that includes magnetites of clearly abiological origin and it is questioned whether this assemblage is purely fortuitous. Moreover, similar magnetites have been produced in the laboratory by abiogenic processes (Golden et al., 2004).

The presence of microbial metabolites plus the physical presence of the organic components can have an enormous effect on the elemental composition and dissolution of minerals, as well as the shape and size of the crystals. Microbial dissolution of nonorganic substrates is a common process, especially in oligotrophic environments where access to essential elements is necessary for survival. Microbial dissolution of silicate minerals, such as feldspars and mafic minerals, contributes strongly to crustal weathering. Microbial colonies and biofilms attach to a mineral surface and obtain their nutrients by physical disaggregation of the minerals, as well as by dissolution through the production of organic acids (Konhauser et al., 1994; Konhauser, 2007). Etching patterns produced in the presence of actively metabolizing cells show clear differences to those produced by inorganic acids (Figure 4) (Welch et al., 2002). Microbial etching of volcanic glass (e.g., Thorseth et al., 1995) has recently received much attention because certain tunnel-like structures in ~3.5 Ga-old pillow lava rims from the Barberton and Pilbara greenstone belts have been interpreted as microbial corrosion biosignatures (Furnes et al., 2004; Banerjee et al., 2006).

Compositional variability can occur in minerals and rocks as a result of microbial metabolic activity. The distribution of elements (major, minor, and trace) in the



Biosignatures in Rocks, Figure 1 Micrometer-scale biosignatures of in rocks. Micrographs of mineralized filamentous structures. (a) SEM image of (sub-)recent silicified microbial filaments from the El Tatio geysers area, northern Chile. (b) Carbonaceous filament (arrows) from the 3.44 Ga-old "Kitty's Gap Chert," Pilbara, Australia. SEM micrograph in backscatter mode.

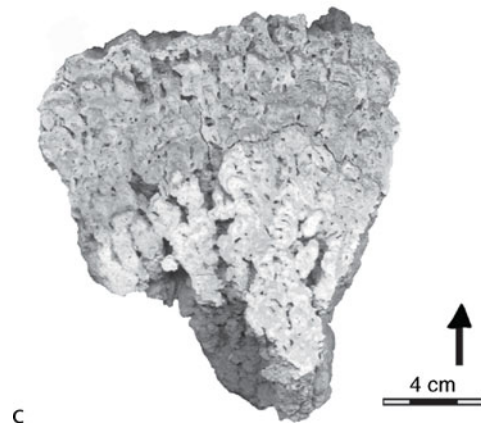
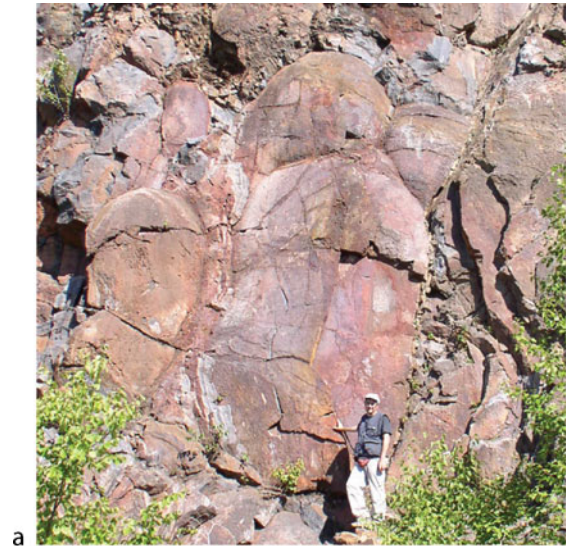


Biosignatures in Rocks, Figure 2 Macrometer-scale biosignatures of in rocks. Devonian cold seep mound, Hollard Mound (Morocco), with a core mound facies dominated by microbial hydrocarbon-derived carbonate. The mound on the right is 30 m high.

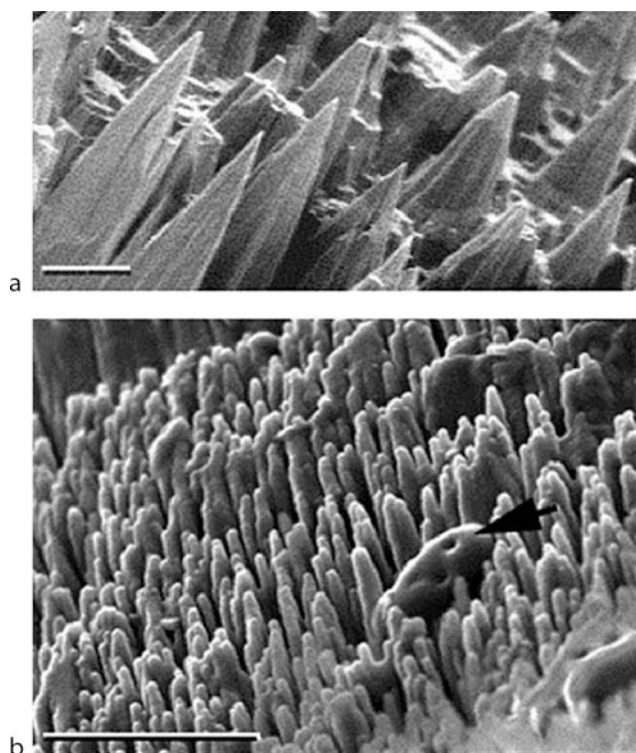
surfaces of minerals colonized by microorganisms may be different to that of the bulk mineral because the microorganisms leach out those elements that are important for their metabolic activities. For instance, sulfur can be leached from sulfides by sulfur-oxidizing bacteria (McGuire et al., 2001) and phosphorus from P-containing minerals is leached in P-poor environments (Taunton et al., 2000a, b). Also, unusual concentrations of trace elements may be associated with mineral-entombed microorganisms since the microbes preferentially concentrate elements that are necessary for their metabolic functions, such as Co, Ni, Cu, Mn, Mo, Se, and V, (Banfield et al., 2001).

The incorporation of organic compounds within a mineral structure may be indicative of biologically induced precipitation as, for instance, when microorganisms are permeated and entombed by minerals (Westall et al., 1995, 2000). Indeed, association of organic molecules with microfossils is a key complementary biosignature for traces of early life on Earth (Westall and Southam, 2006).

Whereas biologically controlled mineral growth produces specific crystalline shapes, biologically induced mineralization produces minerals with non-perfect habits (Banfield et al., 2001) due to alteration of the stability of the crystal surface in the presence of organics (Mann et al., 1993; Albeck et al., 1996). However, the presence of organic matter does not always have an influence on the morphology of a mineral precipitate. For instance, Orange et al. (2009) note that silica precipitated in nutrient media with and without cells had exactly the same structure, the only difference being the visible incorporation of EPS in the medium with cells.



Biosignatures in Rocks, Figure 3 Biosignatures of macro- and mesometric scale in rocks. (a) Huge 2.8-billion-year old stromatolites in the Steep Rock Group, Ontario, Canada. (Person as scale is 1.90 m high). (b) Lithified active lacustrine stromatolites, Lake Thetis, Western Australia. (c) Polished slab from (a) showing internal thrombotic to stromatolitic columnar structures. Arrow indicates growth direction. (a) Photograph by F. Westall; (b, c) photographs by B. Cavalazzi and A. D. George.



Biosignatures in Rocks, Figure 4 Experimental dissolution of apatite (a) in an inorganic acid and (b) in the presence of actively metabolizing cells. Arrow in (b) indicates a cellular body. Scale bars = 2 μm . (Images reprinted with permission from Banfield et al., 2001).

A number of studies have noted a distinct difference in the size of minerals precipitated in the presence of microorganisms and those precipitated abiologically with the former being smaller than the latter. Douglas et al. (2008), for example, document smaller biofilm-induced calcite and Mn-oxyhydroxide mineral precipitates compared to abiogenic gypsum precipitates in an unusual microbial mat from a Death Valley pool.

Isotopic fractionation: Until recently, the preference of microorganisms for the lighter stable isotope of carbon ^{12}C over the heavier one, ^{13}C was considered to be one of the most important biosignatures in rocks. Indeed, Schidlowski (1988) had shown that these signatures can be traced back to rocks 3.8 Ga-old from the Isua province in Greenland. Recent studies, however, have demonstrated that $^{12}\text{C}/^{13}\text{C}$ ratios similar to those produced by living organisms occur in carbon produced by abiotic hydrothermal/metasomatic Fischer–Tropsch synthesis (e.g., van Zuilen et al., 2002). Contamination of the rock by younger organisms can also produce an erroneous biosignature. Westall and Folk (2003), for instance, identified Holocene fossilized endolithic microorganisms in the Isua banded iron formation that was used for Schidlowski's (1988) isotopic studies.

Biosignatures of structural components

Cells, colonies, biofilms, mats, EPS

Microorganisms generally occur as assemblages (colonies) of cells that may be monospecific, but, more often than not are plurispecies. Colonies are often linked by a film of polymers known as extracellular polymeric substances (EPS). Colonies coating a substrate in a two-dimensional manner are termed biofilms. Microbial mats are complex structures formed by layers of biofilms and may be microscopic to macroscopic in size, sometimes reaching dimensions of several square kilometers, for instance, on the floors of lagoons (e.g., Margulis et al., 1980). Although typically associated with the activity of photosynthetic microorganisms in shallow-water environments, microbial mats may also be constructed by non-photosynthetic microorganisms, such as the sulfur-oxidizers *Beggiatoa*, in deep-water habitats on the flanks of deep-sea hydrothermal vents and at hydrocarbon seeps (e.g., Kelley et al., 2005).

The structural components, cells, colonies, biofilms, mats, and EPS, may be preserved in rocks in a number of ways. Cells may leave organic walled impressions, mineral-coated or impregnated structures, or empty casts in a mineral precipitate. Biofilms and mats may also be preserved as organic impressions in sediments or mineralized structures.

Preservation of microbial structures

Organic walled structures: Cells walls can be preserved as organic impressions in fine-grained, anaerobic sediments (Javaux et al., 2001). The fixation of metals, such as Fe, on cell envelopes may retard lysis, thus contributing to the potential for preservation of cell walls (Ferris et al., 1988). While structures such as pollen spores are large enough to be visible in the rocks using light microscopy, individual microbial cells are often difficult to identify *in situ* in sediments because of their small size. Study of these organic remains is derived from palynological methods in which the detrital silicates encasing the cells are eliminated by acid-digestion of the rock. A major problem with this method is that microorganisms commonly colonize fractures in rocks, thus introducing younger contamination (e.g., Westall and Folk, 2003; Walsh and Westall, 2008). Such contamination led Pflug (1979) to identify eukaryotic yeast cells in a 3.8 Ga-old rock from Isua as *in situ* microfossils. This type of problem can be overcome by verifying the maturity of the kerogen by high-resolution TEM and Raman spectroscopy and/or by demonstrating chemical bonding between the organic molecules and the mineral matrix that indicates the syngenicity of the kerogen (Marshall et al., 2005; Derenne et al., 2008).

Mineralized microbial structures: The most common form of preservation of microbial structures is mineral fossilization. In this process, minerals bind to the organic surfaces of the cells/EPS in a passive reaction. The surfaces of microorganisms and EPS are very reactive, containing

ionized ligands to which mineral nuclei can attach themselves. The sorption reactions may take place via cation bridging, hydrogen bonding with functional groups such as carboxyls or hydroxyls, or by direct electrostatic fixation of the mineral to the functional groups (Lalonde et al., 2005). The attached nuclei then attract further nuclei, resulting in encrustation or permeation of the organic structure. The microbial surfaces and exopolymers therefore act as “mineralizing templates” (Konhauser, 2007). Depending upon the availability of the minerals in solution, the microorganisms may be completely entombed in a mineral precipitate. The type of mineral precipitated depends on the composition of the fluids and the redox conditions in the immediate environment of the microorganism. Many mineral phases can bind to microbial cell walls (Konhauser, 2007), including silica (Figure 1), carbonates (Ca, MgCa, Fe, Mn), metal oxides/hydroxides (Fe/Mn and magnetite), sulfates (Ca, Sr, Ba, Fe), sulfides (Fe, Ni, Pb, Zn, CuFe), phosphates (Ca), clays, and zeolites.

As noted above, cell wall composition and structure plays an important role in the fixation of metals because of the differential distribution of functional groups in different cell components. Mineral nucleation is similarly affected. Thus, Gram positive bacteria with their thick outer layer of peptidoglycan and the abundance of functional groups can be encased by a thick mineral crust, whereas the Gram negative bacteria with their thin layer of peptidoglycan sandwiched between an outer lipopolysaccharide layer and an inner phospholipid layer only fixed a thin, delicate crust (Westall, 1997). The latter author concluded that Gram negative cells therefore had less chance of being preserved in the rock record than Gram positive ones.

The cell walls of Archaea differ in their composition and structure to those of Bacteria and are also more variable. *Crenarchaeota* are characterized by a layer of paracrystalline proteins, the S-layer, which is loosely associated with the plasma membrane (NB: S-layers are also common around many species of Bacteria). The *Euryarchaeota* exhibit great variability in the composition of their cell walls, including a peptidoglycan-like component, pseudomurein. Experimental silicification of an Archaea having an S-layer, the hyperthermophile *Pyrococcus abyssi* showed that it produced a delicate silica crust, similar to that forming around Gram negative bacteria (Orange et al., 2009).

What is the fate of the organic components in a mineralized cell? Experimental fossilization shows that the macromolecules of the cell envelope may remain trapped in the mineral crust (Westall et al., 1995). The cytoplasmic space, on the other hand, may be empty after lysis, or, if lysis did not occur, it may contain balls of degraded macromolecules that can also nucleate minerals (in the process producing false nuclei, Westall et al., 1995). As noted above, the organic components of fossilized microbial structures may be preserved in anaerobic environments but they can be completely oxidized in

oxidizing environments. For example, the silicified remains of cyanobacteria in hot spring deposits from Yellowstone no longer contain any organic molecules (Cady and Farmer, 1996).

In order to be preserved in the rock record, the mineral-coated/permeated microbial structure needs to become encased in a mineral cement or by fine-grained sediments. Here, further diagenetic changes may take place, including changes in mineralogy (e.g., transformation of oxyhydroxides to oxides), replacement (complete or partial) of one mineral by another (e.g., silicification of carbonate mineralized remains), or dissolution. The final mineral or sediment-encased microbial fossils may exhibit different morphological preservation modes.

Artifacts: Abiological mineral precipitates can be notoriously confused with fossilized microorganisms. Many minerals may form simple spherical, oval, elongated, and even twisted morphologies. Silica spheres may occur in aggregates that look surprisingly like dividing microorganisms (e.g., Monty et al., 1991). Jones (2004) found that spherical silica aggregates nucleated onto a mucus thread in a siliceous hot spring forming filamentous structures that could possibly be mistaken for silica encrusted filaments. García-Ruiz et al. (2003) even showed that artificial mixed silica/organic precipitates could produce microbial-like morphologies (bacteriomorphs). However, these precipitates were not produced from naturally occurring solutions.

Examples of mineralized microbial structures: Microbial fossils occur throughout almost the whole of the rock record. The oldest known microfossils are found in silicified shallow-water volcanic sediments from the 3.5–3.3 Ga Barberton and Pilbara greenstone belts in which filaments, coccoidal and rod-shaped silicified microorganisms and associated EPS have been documented (Figure 1b) (Walsh, 1992; Westall et al., 2001, 2006a, b). [Older sediments from the 3.8 Ga-old Isua greenstone belt in Greenland are too highly metamorphosed to contain identifiable, syngenetic microfossils.] The very small size of the early Archaean microbial structures means that sophisticated instrumentation having high spatial and analytical resolution is necessary to observe and analyze them (Westall and Southam, 2006). Moreover, given the problem of establishing the biogenicity and syngeneticity of microbial remains in these ancient sediments, multiple lines of evidence are required, including morphological, chemical, and isotopic biosignatures. In this way, Westall et al. (2006a, b) documented multispecies colonies of probable chemolithotrophic microorganisms within 3.446 Ga-old mudflat sediments from the Pilbara and microbial mats on the surfaces of littoral sediments in a 3.334 Ga-old formation from Barberton. The small crystal lattice of silica results in excellent preservation of fine textural detail, such as cell surface wrinkles (Westall et al., 2006a). Silicified (permineralized) microfossils occur throughout geological time (Knoll, 1985). Proterozoic formations contain extremely well-preserved cyanobacterial

fossils, for instance (Green et al., 1989), and silicified microfossils have been documented from numerous Phanerozoic hot spring environments (Jones et al., 2001; Jones, 2004).

The oldest calcified microfossils are relatively poorly preserved benthic cyanobacteria from the 2.6 Ga Campbellrand Subgroup in South Africa (Kazmierczak and Altermann, 2002). Well-preserved calcified microfossils occur in younger Proterozoic formations, such as the Draken Formation, Svalbard (Knoll et al., 1993).

Microfossils preserved by other minerals, such as pyrite, occur through much of the geological record, the oldest such fossils occurring in the above-mentioned 3.2 Ga-old massive sulfide deposits from the Pilbara (Rasmussen, 2000) and younger fossils occurring in the Proterozoic, e.g., pyritized filaments in stromatolites from the Altyn Formation of Montana (White, 1974), or pyritized biofilms coating soft body parts (Canfeld and Raiswell, 1991). Phosphatized microfossils are known from the Phanerozoic, for example, phosphate coatings on cyanobacteria in Cambrian sediments from Australia (Southgate, 1986) or phosphatized microbial biofilms on the soft body parts of Eocene eucaryotes (Liebig et al., 1996).

Microbialites and microbial fabrics in rocks

The physical expressions of microorganisms and the products of their enzymatic and metabolic activities result in microbialite structures and textures that are characterized by a particular internal structure or texture. They include a wide range of macroscopic to microscopic phenomena, such as stromatolites, thrombolites, oncolites, biolaminites, and crusts (Figure 3). Macro- to micro-scale fabrics and textures related to the presence of microorganisms also include MISS (the MISS of Noffke et al., 2003), and microfossils (Burne and Moore, 1987; Monty et al., 1995; Riding and Awramik, 2000). Microbialites and microbial fabrics are found in rocks throughout the geological record with the oldest stromatolites occurring in 3.5–3.3 Ga-old formations in the Pilbara of Australia and in Barberton, South Africa (Lowe, 1980; Walter, 1983; Byerly et al., 1986; Hofmann et al., 1999; Allwood et al., 2006). The biogenic origin of the early Archaean stromatolites has been challenged by Lowe (1994) and Lindsay et al. (2005) but Riding (2008) suggests that some of the Precambrian stromatolites may be mixtures of microbial and abiogenic, stromatolite-like fabrics. The large and diverse stromatolites characteristic of the Proterozoic declined in importance throughout the latest Precambrian to Cambrian times and, from the early Cambrian onwards, microbialite structures are dominated by microbial mounds formed by non-stromatolitic calcifying microbes (e.g., cyanobacteria, Monty, 1995). Microbial mounds are well-developed throughout the geological record spanning from the Proterozoic to the Recent in both shallow- and deep-water settings and are considered to be a significant expression of the history of Earth's microbial life (Figure 2) (Monty, 1995). Both stromatolites and

microbial mounds are characterized by the activity of microbes which calcify, trap, and bind sediments, forming delicate and persistent microstructures rich in micrite (microcrystalline calcite) and organomicrite. Microbially generated mounds and microbialites formed in shallow-water environments mainly involve photosynthetic/phototrophic and heterotrophic microbes. In deeper waters, however, chemosynthetic microbes are associated with hydrocarbon seeps and hydrothermal-related mound deposits (Peckmann and Thiel, 2004). Deep-water stromatolitic fabrics and crusts have been described from hydrocarbon seeps on the Aleutian accretionary margin as a product of methane-consuming Archaea (Greinert et al., 2002).

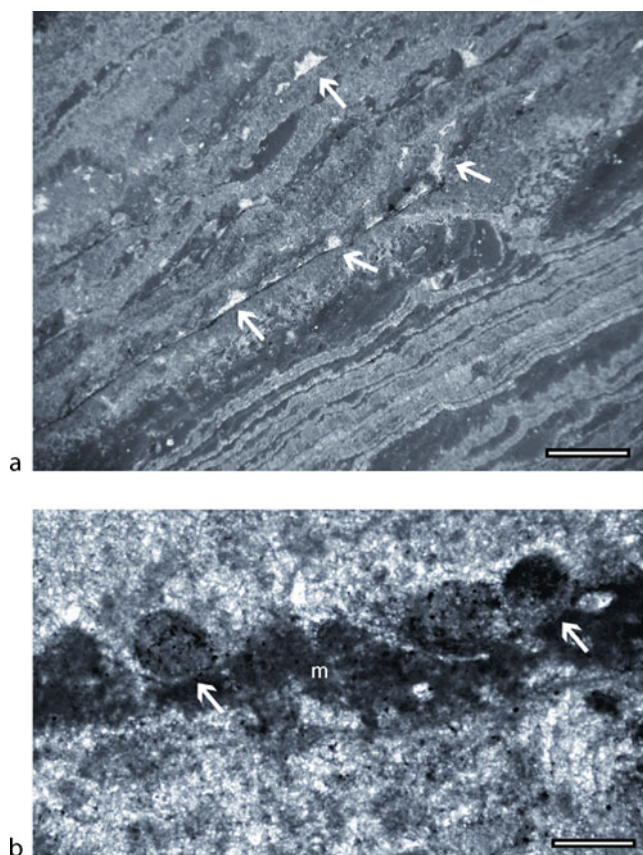
The most common microbially induced fabrics from carbonate rocks are clotted and peloidal fabrics (Burne and Moore, 1987; Shapiro, 2000; Flugel, 2004). These fabrics consist of spherical-to-elliptical microscopic aggregates of microcrystalline clots or peloids (Figure 5a) (e.g., calcite, aragonite), cemented by carbonate and/or sediments (MacIntyre, 1985; Shapiro, 2000). Fenestral and stromatactis fabrics from organic-rich sedimentary deposits (Figure 5b), such as microbial mounds or geothermal springs (e.g., travertine), are microbiological in origin (Flugel, 2004). Encrustations, concretions, and coatings are generally microbial products that contain a record (morphological, geochemical, and/or isotopic) in their sedimentary phases of the biochemical processes involved in their genesis (Raiswell and Fisher, 2000; Kiriakoulakis et al., 2000). For instance, Fe-Mn coatings and pyritiferous carbonate nodules are considered to be microbial fabrics (Peckmann and Thiel, 2004).

Microbially induced sedimentary structures (MISS) are formed by the rhythmic alternation of photosynthetic microbial mats and sediments in mudflat environments (Noffke, 2008). MISS are widespread in the geological record and have also been identified in sediments 3.2 Ga-old from South Africa (Noffke et al., 2003).

Banded iron formations (BIFs) occur throughout the rock record from 3.8 Ga onwards (BIFs occur in the Isua greenstone belt in Greenland). The influence of microorganisms on the formation of these laminated accumulations of iron oxides and chert is the subject of much controversy. Photochemical oxidation of ferric iron of hydrothermal origin is one explanation (Cairns-Smith, 1978) but other biological controls have also been suggested, such as oxidation by oxygenic photosynthesizers (Cloud, 1965), or simple sorption of Fe^{2+} onto microbial cell surfaces to catalyze a basically abiological precipitation process (Konhauser, 2000), or the precipitation of Fe oxides by Fe oxidizing microorganisms (Posth et al., 2008).

Relevance of biosignatures and their astrobiological implications

We have seen above that a number of the biosignatures described can be imitated by abiological processes



Biosignatures in Rocks, Figure 5 Micrometer-scale biosignatures in rocks. Transmitted light photomicrographs of petrographic thin sections. (a) Continuous and planar laminations of stromatolite structures from the Devonian Hollard Mound (Morocco). The arrows indicate the spar-filled cavities generating fenestral fabrics. (b) Detail from (a) showing a lamina of fine gray micrite (m) that locally consists of microbially produced peloids (arrows). Scale bars: (a) = 5 mm; (b) = 200 μ m. (Images reprinted with permission from Cavalazzi, 2007, and Cavalazzi et al., 2007).

resulting in significant controversy. These are serious limitations when searching for the first traces of life on Earth or for biosignatures on other planets. How can nonliving molecules be distinguished from very primitive living cells – and in the fossilized form? A carbon isotope signature on its own is not sufficient because it could be either abiogenic or biogenic in origin. A collection of simple-shaped minerals, even joined together with compromised boundaries, could represent abiogenic precipitates or possibly a fossilized microbial colony. Identification of biosignatures in ancient terrestrial or extraterrestrial materials therefore needs to be based on a combination of structural, chemical, and isotopic traces that are related to the three main characteristics of living organisms: their physical structures, the chemical components of the structures, and their metabolic or living processes (Westall and Southam, 2006; Westall, 2008). Another aspect to take

into account is the fact that living organisms by their very existence and metabolism interact with and influence their immediate environment.

On a biotic world where the context of structure, fabric, and composition are well-constrained, certain characteristics, such as element enrichment, mineral composition, shape, and size can be used as complementary evidence for the existence of living organisms. Can these characteristics be used on a world where the existence of life forms is uncertain, for instance on the primitive Earth or Mars? Prebiotic molecules of mostly extraterrestrial origin, characterized by a wide range in composition and structure, including simple amino acids, polycyclic acidic hydrocarbons (PAHs), thiophenes, fullerenes etc. (Cronin, 1998), abounded on these worlds (Maurette et al., 2000). Could prebiotic organic macromolecules scavenge elements from the environment in the same way as biomolecules and, if so, would some elements be preferentially fixed? These macromolecules have very few of the functional groups that characterize biogenic molecules, and it is therefore unlikely that they will be able to induce significant metal fixation. Thus, element concentration and distribution in potentially biogenic materials (organic or mineralized organics) could be considered as a useful complementary biosignature when associated with other types of biosignatures. Could prebiotic organic molecules induce mineral precipitation? Again this is unlikely since biominerals precipitate because of changes in the environment caused by the excretion of metabolic waste products, or enzymatic mediated changes in the redox state, or the presence of a charged cell surface that attracts mineral nucleation, conditions that are not met by prebiotic molecules. On the other hand, the physical presence of prebiotic molecular matter in an environment where minerals are being precipitated abiotically from supersaturated solutions (saline ponds, hot springs, the early, silica-saturated oceans) could eventually have an influence on the size and shape of minerals because of alteration in the stability of the crystal surfaces (cf. Mann et al., 1993; Albeck et al., 1996).

Once the biogenicity and syngenicity of the different biosignatures in a rock have been established, how are they to be interpreted? What do they tell us about the community of microorganisms that lived in a particular environment? Are all the original microorganisms in the community preserved? This is particularly relevant because not all microorganisms can be fossilized. For instance, Orange et al. (2009) noted that, although two species of thermophilic Archaea, *Pyrococcus abyssi* and *Methanocaldococcus janaschii* had similar cell wall structures and compositions, the former could be experimentally silicified whereas the latter naturally lysed before mineral nuclei could fix to the cell walls.

Another aspect of the study of biosignatures is the importance of observation and analysis at the scale of the microorganisms (Westall and Southam, 2006; Westall, 2008, 2009). Many fossilized microorganisms are very small and cannot readily be distinguished from abiogenic

mineral accumulations at the optical microscope scale. They need to be studied using higher-resolution electronic microscopes. The question of scale is also pertinent to analysis of chemical or isotopic signatures. What information about the microbial community is provided by isotope measurements made on kerogen extracted from the bulk rock when the rock may contain traces of different microbial communities at the micrometer scale? The microbial communities (colonies, biofilms, and mats) may, themselves, consist of multiple species, thus, resulting in additional mixture of the isotopic signature. To complicate matters even further, specific components of an individual cell may have different isotopic signatures. For example, van de Meer et al. (2000) note that the carbon isotope composition of the lipids of *Chloroflexus aurantiacus* differ from the bulk isotopic composition of the cells. This means that isotope measurements made even *in situ* (e.g., with a spot size of ~50 μm , van Zuilen et al., 2007) need to be interpreted with caution.

Conclusion

While it is clear that the study of biosignatures in rocks is not without difficulties, these can be overcome by combining signatures of the different aspects of living organisms, i.e., structural, chemical, and isotopic traces, as well as evidence for the interaction of living organisms with the environment. Further investigation of the microbial communities that produced the biosignatures needs to be undertaken at the microbial scale in order to obtain relevant information about the types of organisms and their metabolic activities. This is a challenge for spatial and analytical resolution but it is this level of detail that is necessary for disentangling the bulk biosignatures from those produced by the individual microorganisms and for better understanding the complexity of past communities.

Bibliography

- Albeck, S., Addadi, L., and Weiner, S., 1996. Regulation of calcite crystal morphology by intracrystalline acidic proteins and glycoproteins. *Connective Tissue Research*, **35**, 419–424.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–718.
- Banerjee, N. R., Furnes, H., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2006. Preservation of 3.4–3.5 Ga microbial biomarkers in pillow lavas and hyaloclastites from the Barberton greenstone belt, South Africa. *Earth and Planetary Science Letters*, **241**, 707–722.
- Banfield, J. F., Moreau, J. W., Chan, C. S., Welch, S. A., and Little, B., 2001. Mineralogical biosignatures in search for life on Mars. *Astrobiology*, **1**, 447–465.
- Bazylinski, D. A., Frankel, R. B., Heywood, B. R., Mann, S., King, J. W., Donaghay, P. L., and Hanson, A. K., 1995. Controlled biomineralisation of magnetite (Fe_3O_4) and greigite (Fe_3S_4) in a magnetotactic bacterium. *Applied and Environmental Microbiology*, **61**, 3232–3239.
- Beveridge, T. J., Forsberg, C. W., and Doyle, R. J., 1982. Major sites of metal binding in *Bacillus licheniformis* walls. *Journal of Bacteriology*, **150**, 1438–1448.
- Brack, A., 2002. Water, the spring of life. In Horneck G., and Baumstark-Khan, C. (eds.), *Astrobiology. The Quest for the Conditions of Life*. Berlin: Springer, pp. 79–88.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1036.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaios*, **2**, 241–254.
- Byerly, G. R., Walsh, M. M., and Lowe, D. L., 1986. Stromatolites from the 3300–3500 Myr Swaziland Supergroup, Barberton Mountain Land, South Africa. *Nature*, **319**, 489–491.
- Cady, S. L., and Farmer, J. D., 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In Bock, G. R., and Goode, J. A. (eds.), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*, Ciba Foundation Symposium 202. Chichester: Wiley, pp. 150–173.
- Cairns-Smith, A. G., 1978. Precambrian solution photochemistry, inverse segregation, and banded iron formations. *Nature*, **276**, 807–808.
- Canfield, D. E., and Raiswell, R., 1991. Pyrite formation and fossil preservation. In Allison, P. A., and Briggs, D. E. G. (eds.), *Taphonomy: Releasing the Data Locked in the Fossil Record*. New York: Plenum, pp. 337–387.
- Cavalazzi, B., 2007. Chemotrophic filamentous microfossils from the Hollard Mound (Devonian, Morocco) as investigated by focus ion beam. *Astrobiology*, **7**, 402–415.
- Cavalazzi, B., Barbieri, R., and Ori, G. G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Research*, **54**, 289–316.
- Chang, S.-B. R., and Kirschvink, J. L., 1989. Magnetofossils, the magnetization of sediments, and the evolution of magnetite biomineralization. *Annual Review of Earth and Planetary Sciences*, **17**, 169–195.
- Cloud, P. E., 1965. Significance of the Gunflint (Precambrian) microflora. *Science*, **148**, 27–35.
- Cronin, J. R., 1998. Clues from the origin of the Solar System: meteorites. In Brack, A. (ed.), *The Molecular Origins of Life: Assembling Pieces of the Puzzle*. Cambridge: Cambridge University Press, pp. 119–146.
- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J.-N., 2008. Molecular evidence for life in the 3.5 billion-year old Warreawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Douglas, S., Abbey, W., Mielke, R., Conrad, P., and Kanik, I., 2008. Textural and mineralogical biosignatures in an unusual microbialite from Death Valley, California. *Icarus*, **193**, 620–636.
- Eigenbrode, J., and Freeman, K. H., 2006. Late Archaean rise of aerobic microbial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15759–15764.
- Ferris, G., Fyfe, W. S., and Beveridge, T. J., 1988. Metallic ion binding by *Bacillus subtilis*: implications for the fossilization of microorganisms. *Geology*, **16**, 149–152.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks. Analysis, Interpretation and Application*. Berlin, Heidelberg: Springer.
- Friedmann, E. I., Wierzbos, J., Ascaso, C., and Winklhofer, M., 2001. Chains of magnetite crystals in the meteorite ALH84001: evidence of biological origin. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2176–2181.

- Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- García-Ruiz, J. M., Hyde, S. T., Camerup, A. M., Christy, A. G., van Kranendonk, M. J., and Welham, N. J., 2003. Self-assembled silica-carbonate structures and detection of ancient microfossils. *Science*, **302**, 1194–1197.
- Geesey, G. G., and Jang, L., 1989. Interactions between metal ions and capsular polymer. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley, pp. 325–357.
- Golden, D. C., Ming, D. W., Morris, R. V., Brearley, A. J., Lauer, Jr. H. V., Treiman, A. H., Zolensky, M. E., Schwandt, C. S., Lofgren, G. E., and McKay, G. A., 2004. Evidence for exclusively inorganic formation of magnetite in Martian meteorite ALH84001. *American Mineralogist*, **89**, 681–695.
- Green, J. W., Knoll, A. H., and Swett, K., 1989. Microfossils from silicified stromatolitic carbonates of the Upper Proterozoic Limestone-Dolomite 'Series', central East Greenland. *Geological Magazine*, **126**, 567–585.
- Greinert, J., Bohrmann, G., and Elvert, M., 2002. Stromatolitic fabric of authigenic carbonate crusts in 4850 m water depth, Aleutian accretionary margin: result of anaerobic methane oxidation by archaea at cold seeps. *International Journal of Earth Sciences*, **91**, 698–711.
- Hofmann, H. J., Grey, K., Hickman, A. H., and Thorpe, R. I., 1999. Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. *Geological Society of America Bulletin*, **111**, 1256–1262.
- Javaux, E. J., Knoll, A. H., and Walter, M. R., 2001. Morphological and ecological complexity in early eukaryotic ecosystems. *Nature*, **412**, 66–69.
- Jones, B., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Geological Society, London*, **161**, 983–993.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2001. Taphonomy of Silicified Filamentous Microbes in Modern Geothermal Sinters-Implications for Identification. *Palaios*, **16**, 580–592.
- Kazmierczak, J., and Altermann, W., 2002. Neoproterozoic biomineralization by benthic cyanobacteria. *Science*, **298**, 2351.
- Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Summons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted submarine ecosystem: The Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- Kiriakoulakis, K., Marshall, J. D., and Wolff, G. A., 2000. Biomarkers in a Lower Jurassic concretion from Dorset (UK). *Journal of the Geological Society*, **157**, 207–220.
- Knoll, A. H., 1985. Exceptional preservation of photosynthetic organisms in silicified carbonates and silicified peats. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **311**, 111–122.
- Knoll, A. H., Fairchild, I. J., and Swett, K., 1993. Calcified microbes in Neoproterozoic carbonates: implications for our understanding of the Proterozoic/Cambrian transition. *Palaios*, **8**, 512–525.
- Konhauser, K. O., 2000. Hydrothermal bacterial biomineralisation: potential modern-day analogues for Precambrian banded iron formation. In Glenn, C. R., Prévôt-Lucas, L., and Lucas, J. (eds.), *Marine Authigenesis: from Global to Microbial*. SEPM Special Publications No 66, pp. 133–145.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Science.
- Konhauser, K. O., Fyfe, W. S., Schultze-Lam, S., Ferris, F. G., and Beveridge, T. J., 1994. Iron phosphate precipitation by epilithic microbial biofilms in Arctic Canada. *Canadian Journal of Earth Sciences*, **31**, 1320–1324.
- Kucha, H., Schroll, E., and Strumpf, E. F., 2005. Fossil sulphate-reducing bacteria in the Bleiberg lead-zinc deposit, Austria. *Mineralium Deposita*, **40**, 123–126.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A. -L., and Ferris, F. G., 2005. The experimental silicification of Aquificales and their role in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Laval, B., Cady, S. L., Pollack, J. C., McKay, C. P., Bird, J. S., Grotzinger, J. P., Ford, D. C., and Bohm, H. R., 2000. Modern freshwater microbialite analogues for ancient dendritic reef structures. *Nature*, **407**, 626–629.
- Ledin, M., Krantz-Rülker, C., and Allard, B., 1999. Microorganisms as metal sorbants: comparison with other soil constituents in multi-compartmental systems. *Soil Biology and Biochemistry*, **31**, 1639–1648.
- Lemelle, L., Labrot, P., Salomé, M., Simionovici, A., Viso, M., and Westall, F., 2008. In situ imaging of organic sulfur in 700–800 My-old Neoproterozoic microfossils by X-ray spectromicroscopy at the S K-edge. *Organic Geochemistry*, **39**, 188–202.
- Lepot, K., Benzerara, K., Brown, G., and Philippot, P., 2008. Microbially influenced formation of 2,724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Liebig, K., Westall, F., and Schmitz, M., 1996. A study of fossil microstructures from the Eocene Messel formation using transmission electron microscopy. *Neues Jahrbuch für Geologie und Paläontologie-Monatshefte*, **4**, 218–231.
- Lindsay, J. F., Brasier, M. D., McLoughlin, N., Green, O. R., Fogel, M., Steele, A., and Mertzman, S. A., 2005. The problem of deep carbon - an Archean paradox. *Precambrian Research*, **143**, 19–22.
- Lowe, D. R., 1980. Stromatolites 3,400-Myr old from the Archean of Western Australia. *Nature*, **284**, 441–443.
- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 287–390.
- MacIntyre, I. G., 1985. Submarine cements - the peloidal question. In Schneidermann, N., and Harris, P. M. (eds.), *Carbonate Cements*. SEPM Special Publications 36, pp. 109–116.
- Mann, S., 1988. Molecular recognition in biomineralisation. *Nature*, **332**, 119–124.
- Mann, S., Archibald, D. D., Didymus, J. M., Douglas, T., Heywood, B. R., Meldrum, F. C., and Reeves, N. J., 1993. Crystallisation at inorganic-organic interfaces-biominerals and biomimetic synthesis. *Science*, **261**, 1286–1292.
- Margulis, L., Barghoorn, E. S., Ashendorf, D., Banerjee, S., Chase, D., Francis, S., Giovannoni, S., and Stolz, J., 1980. The microbial community in the layered sediments at Laguna Figueroa, Baja California, Mexico: does it have Precambrian analogues. *Precambrian Research*, **11**, 93–123.
- Marshall, C. P., Javaux, E. J., Knoll, A. H., and Walter, M. R., 2005. Combined micro-Fourier transform infrared (FTIR) spectroscopy and micro-Raman spectroscopy of Proterozoic acritarchs: a new approach to Palaeobiology. *Precambrian Research*, **138**, 208–224.
- Maurette, M., Duprat, J., Engrand, C., Gounelle, M., Kurat, G., Matrajt, G., and Toppani, A., 2000. Accretion of neon, organics, CO₂, nitrogen and water from large interplanetary dust particles on the early Earth. *Planetary and Space Science*, **48**, 1117–1137.
- McGuire, M. M., Edwards, K. J., Banfield, J. F., and Hammers, R. J., 2001. Kinetics, surface chemistry, and structural evolution of microbially mediated sulphide mineral dissolution. *Geochimica et Cosmochimica Acta*, **65**, 57–72.

- McKay, D. S., Gibson, E. K., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chillier, X. D. F., Maechling, C. R., and Zare, R. N., 1996. Search for past life on Mars: possible relic biogenic activity in martian meteorite ALH8400. *Science*, **273**, 924–930.
- Mero, J. L., 1962. Ocean-floor manganese nodules. *Economic Geology*, **57**, 747–767.
- Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P., and Friend, C. R. L., 1996. Evidence for life on Earth before 3,800 million years ago. *Nature*, **384**, 55–59.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-Mounds: Their Origin and Evolution*. Oxford: Blackwell, International Association of Sedimentologists, Special Publication 23, pp. 11–48.
- Monty, C. L. V., Westall, F., and Van Der Gaast, S., 1991. The diagenesis of siliceous particles in Subantarctic sediments, ODP Leg 114, Hole 699: possible microbial mediation. In Ciesielski, P. F., Kristoffersen, Y. et al. (eds.), *Proceedings ODP Scientific Results, 114*. College Station, TX (Ocean Drilling Program), pp. 685–710.
- Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R., 1995. *Carbonate Mud-Mounds: Their Origin And Evolution*. Oxford: Blackwell, International Association of Sedimentologists, Special Publication 23.
- Noffke, N., 2008. Turbulent lifestyle: microbial mats on Earth's sandy beaches-today and 3 billion years ago. *GSA Today*, **18**(10), 4–9.
- Noffke, N., Gerdes, G., and Klenke, Th., 2003. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty and evaporitic carbonatic). *Earth-Sciences Reviews*, **12**, 1–14.
- Orange, F., Westall, F., Prieur, D., Biennu, N., Le Romancer, M., Disnar, J.-R., and Défarge, C., 2009. Experimental silicification of the extremophilic Archaea *Pyrococcus abyssi* and *Methanocaldococcus jannaschii*. Applications in the search for evidence of life in early Earth and extraterrestrial rocks. *Geobiology*, **7**, 403–418.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Pflug, H. D., 1979. Archean fossil finds resembling yeasts. *Geologica et Palaeontologica*, **13**, 1–8.
- Pollmann, K., Raff, J., Merroun, M., Fahmy, K., and Selenska-Pobell, S., 2006. Metal binding by bacteria from uranium mining waste piles and its technological applications. *Biotechnology Advances*, **24**, 58–68.
- Posth, N. R., Hegler, F., Konhauser, K. O., and Kappler, A., 2008. Alternating Si and Fe deposition caused by temperature fluctuations in Precambrian oceans. *Nature Geoscience*, **1**, 703–708.
- Raiswell, R., and Fisher, Q. J., 2000. Mudrock-hosted carbonate concretions: a review of growth mechanisms and their influence on chemical and isotopic composition. *Journal of the Geological Society*, **157**, 239–251.
- Rasmussen, B., 2000. Filamentous microfossils in a 3,235-million-year-old volcanogenic massive sulphide deposit. *Nature*, **405**, 676–679.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J., and Kilburn, M. R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, **455**, 1101–1104.
- Riding, E. R., and Awramik, S. M., 2000. *Microbial Sediments*. Berlin Heidelberg: Springer.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2008. Abiogenic, microbial and hybrid authigenic carbonate crusts: components of Precambrian stromatolites. *Geologica Croata*, **61**, 73–103.
- Schidlowski, M., 1988. A 3800 million-year isotopic record of life from carbon in sedimentary rocks. *Nature*, **333**, 313–318.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaio*, **15**, 166–169.
- Southgate, P. N., 1986. Cambrian phoscrete profiles, coated grains, and microbial processes in phosphogenesis: Georgina Basin, Australia. *Journal of Sedimentary Petrology*, **56**, 429–441.
- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, J. H., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Summons, R. E., Albrecht, P., McDonald, G., and Moldowan, J. M., 2008. Molecular biosignatures. *Space Science Reviews*, **135**, 115–132.
- Taunton, A. E., Welch, S. A., and Bandfield, J. F., 2000a. Geomicrobial controls on light rare earth element, Y, and Ba distributions during granite weathering and soil formation. *Journal of Alloys and Compounds*, **303**, 30–36.
- Taunton, A. E., Welch, S. A., and Bandfield, J. F., 2000b. Microbial controls on phosphate and lanthanide distributions during granite weathering. *Chemical Geology*, **169**, 371–382.
- Thomas-Keprta, K. L., Bazylinski, D. A., Kirschvink, J. L., Clemett, S. J., McKay, D. S., Wentworth, S. J., Vali, H., Gibson, E. K., and Romanek, C. S., 2001. Truncated hexa-octahedral magnetite crystals in ALH84001: presumptive biosignatures. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 2164–2169.
- Thomas-Keprta, K. L., Clemett, S. J., Bazylinski, D. A., Kirschvink, J. L., McKay, D. S., Wentworth, S. J., Vali, H., Gibson, E. K., and Romanek, C. S., 2002. Magnetofossils from ancient Mars: a robust biosignature in the martian meteorite ALH84001. *Applied and Environmental Microbiology*, **68**, 3663–3672.
- Thorseth, I. H., Furnes, H., and Tumyr, O., 1995. Textural and chemical effects of bacterial activity on basaltic glass: an experimental approach. *Chemical Geology*, **119**, 139–160.
- van de Meer, M. T. J., Schouten, S., De Leeuw, J. W., and Ward, D. M., 2000. Autotrophy of green non-sulphur bacteria in hot spring microbial mats: biological explanations for isotopically heavy organic carbon in the geological record. *Environmental Microbiology*, **2**, 428–435.
- van Zuilen, M., Lepland, A., and Arrhenius, G., 2002. Reassessing the evidence for the earliest traces of life. *Nature*, **418**, 627–630.
- van Zuilen, M. A., Chaussidon, M., Rollion-Bard, C., and Marty, B., 2007. Carbonaceous cherts of the Barberton greenstone belt, South Africa; isotopic, chemical, and structural characteristics of individual microstructures. *Geochimica et Cosmochimica Acta*, **71**, 655–669.
- Walsh, M. M., 1992. Microfossils and possible microfossils from the Early Archean Onverwacht Group, Barberton Mountain Land, South Africa. *Precambrian Research*, **54**, 271–293.
- Walsh, M., and Westall, F., 2008. Disentangling the microbial fossil record in the Barberton greenstone belt: a cautionary tale. In Seckbach, J., and Walsh, M. (eds.), *From Fossil to Astrobiology*. New York: Springer, pp. 25–37.
- Walter, M. R., 1983. Archean stromatolites: evidence of the Earth's earliest benthos. In Schopf, J. W. (ed.), *Earth's Earliest Biosphere*. Princeton: Princeton University Press, pp. 187–213.
- Welch, S. A., Taunton, A. E., and Banfield, J. F., 2002. Effect of microorganisms and microbial metabolites on apatite dissolution. *Geomicrobiology Journal*, **19**, 343–367.
- Westall, F., 1994. Silicified bacteria and associated biofilm from the deep-sea sedimentary environment. *Kaupia-Darmstädter Beiträge zur Naturgeschichte*, **4**, 29–43.

- Westall, F., 1997. The influence of cell wall composition on the fossilization of bacteria and the implications for the search for early life forms. In Cosmovici, C., Bowyer, S., and Werthimer, D. (eds.), *Astronomical and Biochemical Origins and the Search for Life in the Universe*. Bologna: Editori Compositrici, pp. 491–504.
- Westall, F., 2008. Morphological biosignatures in terrestrial and extraterrestrial materials. *Space Science Reviews*, **135**, 95–114.
- Westall, F., 2009. Life on an anaerobic planet. *Science*, **232**, 471–472.
- Westall, F., and Folk, R. L., 2003. Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua Greenstone Belt: implications for the search for life in ancient rocks. *Precambrian Research*, **126**, 313–330.
- Westall, F., and Southam, G., 2006. Early life on Earth. In Benn, K., Mareschal, J. C., and Condie, K. (eds.), *Archean Geodynamics and Environments*. AGU Geophysical Monographs No 164, pp. 283–304.
- Westall, F., Boni, L., and Guerzoni, E., 1995. The experimental silicification of microorganisms. *Palaeontology*, **38**, 495–528.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., Gibson, E., McKay, D., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24,511–524,527.
- Westall, F., De Wit, M. J., Dann, J., Van Der Gaast, S., De Ronde, C., and Gerneke, D., 2001. Early Archaean fossil bacteria and biofilms in hydrothermally influenced, shallow water sediments, Barberton greenstone belt, South Africa. *Precambrian Research*, **106**, 91–112.
- Westall, F., de Ronde, C. E. J., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006a. Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361**, 1857–1875.
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006b. The 3.466 Ga Kitty's Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications No 405, pp. 105–131.
- White, B., 1974. Microfossils from the Late Precambrian Altyn Formation of Montana. *Nature*, **247**, 452–453.

Cross-references

[Archaea](#)
[Astrobiology](#)
[Bacteria](#)
[Biofilms and Fossilization](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Cold Seeps](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Deep Biosphere of Salt Deposits](#)
[Dolomite, Microbial](#)
[Geochronology](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Mat-Related Sedimentary Structures](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)

[Origins of the Metazoa](#)
[Sediment Diagenesis - Biologically Controlled](#)
[Stromatactis](#)
[Stromatolites](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

BIOSILICIFICATION

The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges, molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants. See entries “[Silica Biomineralization, Sponges](#)”, “[Microbial Silicification – Bacteria \(or Passive\)](#)” and “[Microbial Biomineralization](#)” for further reading.

BLACK SHALES

Wolfgang Oschmann
 University of Frankfurt, Frankfurt, Germany

Definition

Most modern and ancient aquatic (marine, brackish, and lacustrine) environments were sufficiently oxygenated to permit colonization by numerous benthic, planktic, and nectic organisms. Due to excess oxygen the biologically produced organic matter is rapidly degraded. The sediments formed thereby exhibit only very low amounts of organic matter.

In contrast, very high rates of primary production and/or stagnant water bodies result in black shale sedimentation, which is associated with oxygen depletion or even lack of oxygen within the substrate and to some extent also within the water column. Degradation of organic matter is incomplete, partly by anaerobic bacteria, causing strong enrichment of organic matter in sediments. Black shales therefore commonly exhibit excellent preservation of organic molecules and mineralized soft tissues and hard parts of organisms.

The importance of black shales for scientists and the society are twofold. Black shales serve as source rocks for fossil fuels (oil and gas), which cover most of the energy needs of modern societies, and due to excellent preservation they provide particular sites for palaeontological studies.

Sedimentary characteristics of black shales

Black shales sediments in common are dark gray to black in color and are characterized by microlaminations, which may vary from distinct to blurred. The lamination is caused by quiet depositional conditions and the scarcity or lack of bioturbation. In many cases, the lamina were formed by alignments of fecal pellets ([Figure 1a](#)), which rained down in great numbers from surface waters. The

- Westall, F., 1997. The influence of cell wall composition on the fossilization of bacteria and the implications for the search for early life forms. In Cosmovici, C., Bowyer, S., and Werthimer, D. (eds.), *Astronomical and Biochemical Origins and the Search for Life in the Universe*. Bologna: Editori Compositrici, pp. 491–504.
- Westall, F., 2008. Morphological biosignatures in terrestrial and extraterrestrial materials. *Space Science Reviews*, **135**, 95–114.
- Westall, F., 2009. Life on an anaerobic planet. *Science*, **232**, 471–472.
- Westall, F., and Folk, R. L., 2003. Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua Greenstone Belt: implications for the search for life in ancient rocks. *Precambrian Research*, **126**, 313–330.
- Westall, F., and Southam, G., 2006. Early life on Earth. In Benn, K., Mareschal, J. C., and Condie, K. (eds.), *Archean Geodynamics and Environments*. AGU Geophysical Monographs No 164, pp. 283–304.
- Westall, F., Boni, L., and Guerzoni, E., 1995. The experimental silicification of microorganisms. *Palaeontology*, **38**, 495–528.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., Gibson, E., McKay, D., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24,511–524,527.
- Westall, F., De Wit, M. J., Dann, J., Van Der Gaast, S., De Ronde, C., and Gerneke, D., 2001. Early Archaean fossil bacteria and biofilms in hydrothermally influenced, shallow water sediments, Barberton greenstone belt, South Africa. *Precambrian Research*, **106**, 91–112.
- Westall, F., de Ronde, C. E. J., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006a. Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361**, 1857–1875.
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006b. The 3.466 Ga Kitty's Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications No 405, pp. 105–131.
- White, B., 1974. Microfossils from the Late Precambrian Altyn Formation of Montana. *Nature*, **247**, 452–453.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biofilms and Fossilization
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Cyanobacteria
 Deep Biosphere of Salt Deposits
 Dolomite, Microbial
 Geochronology
 Isotope Fractionation (Metal)
 Isotopes and Geobiology
 Mat-Related Sedimentary Structures
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites

Origins of the Metazoa
 Sediment Diagenesis - Biologically Controlled
 Stromatactis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOSILICIFICATION

The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges, molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants. See entries “*Silica Biomineralization, Sponges*”, “*Microbial Silicification – Bacteria (or Passive)*” and “*Microbial Biomineralization*” for further reading.

BLACK SHALES

Wolfgang Oschmann
 University of Frankfurt, Frankfurt, Germany

Definition

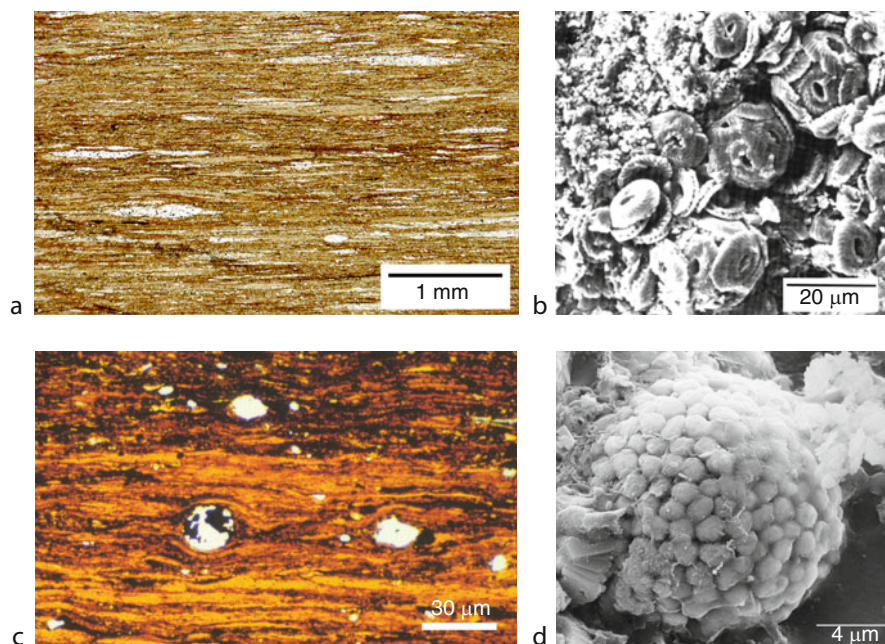
Most modern and ancient aquatic (marine, brackish, and lacustrine) environments were sufficiently oxygenated to permit colonization by numerous benthic, planktic, and nectic organisms. Due to excess oxygen the biologically produced organic matter is rapidly degraded. The sediments formed thereby exhibit only very low amounts of organic matter.

In contrast, very high rates of primary production and/or stagnant water bodies result in black shale sedimentation, which is associated with oxygen depletion or even lack of oxygen within the substrate and to some extent also within the water column. Degradation of organic matter is incomplete, partly by anaerobic bacteria, causing strong enrichment of organic matter in sediments. Black shales therefore commonly exhibit excellent preservation of organic molecules and mineralized soft tissues and hard parts of organisms.

The importance of black shales for scientists and the society are twofold. Black shales serve as source rocks for fossil fuels (oil and gas), which cover most of the energy needs of modern societies, and due to excellent preservation they provide particular sites for palaeontological studies.

Sedimentary characteristics of black shales

Black shales sediments in common are dark gray to black in color and are characterized by microlaminations, which may vary from distinct to blurred. The lamination is caused by quiet depositional conditions and the scarcity or lack of bioturbation. In many cases, the lamina were formed by alignments of fecal pellets ([Figure 1a](#)), which rained down in great numbers from surface waters. The



Black Shales, Figure 1 (a) Indistinct type of lamination formed by alignments of fecal pellets within bituminous mudstone facies (Kimmeridge Clay, Yorkshire, England, thin-section). (b) Most pellets are rich in carbonate and consist of coccoliths and coccospheres (Kimmeridge Clay, Yorkshire, England, SEM-view). (c) Organic rich filamentous mat-structures of bacterial origin (orange color) with dispersed silt-sized sedimentary particles (white) and pyritic aggregates (black) (thin-section, Kimmeridge Clay, Yorkshire). (d) SEM-view of a framboidal pyrite consisting of pyritohedral microcrystals precipitated during iron and sulfate reduction (Kimmeridge Clay, Yorkshire).

diameters of fecal pellets exceed the average annual sedimentation rate by far, and cause indistinct to blurred types of lamination. Commonly, it is assumed that seasonal variation in sedimentation (clastic input vs. primary production) may cause microlamination. However, the low sedimentation rates in general practically hamper a clear proof of varves.

Black shales typically consist of four main components (e.g., Röhl et al., 2001). *Siliciclasts*, mainly clay to silt sized particles form the major component of the rocks (50 to >90 wt.%). They derive from fluvial or aeolian input from hinterland areas. *Carbonates* originate either as skeletal remains of phytoplankton organisms (e.g., coccoliths, Figure 1b), or they are precipitated during early diagenesis at or close to the sediment surface (Frimmel et al., 2004). Their content varies remarkably. The proportion of *organic matter* may vary between 0.5 and 50 wt.% of the whole rock. It mainly derived from surface water primary production, but detritus from land plants and bacterial organic matter may also contribute. Black shales with a content of organic matter below 10% are termed as bituminous mudstones, and above 10% as oil shales. *Pyrite* (FeS_2) in black shales occurs as finely dispersed tiny framboids (Figure 1d), which were formed as syngenetic precipitates due to bacterially driven iron- and sulfate-reduction. In case of excess organic matter, larger pyrite aggregates may be formed during

diagenesis. The proportion of pyrite reaches 10–20 wt.% maximum.

History of organic matter preservation

During the early history of the Earth and life (Hadean and Archaean periods), virtually no free oxygen existed in the ocean and atmosphere. After oxygenic photosynthesis was established and the formerly reduced compounds had been oxidized, free oxygen started to accumulate in the ocean and the atmosphere ca. 2.2 billion years ago (e.g., Catling and Claire, 2005; Rouxel et al., 2005). The first eukaryotic organisms with highly resistant organic cell walls (acritarchs and/or primitive green algae) evolved during late early Proterozoic times (e.g., Samuelsson et al., 1999; Arouri et al., 2000). Their remains escaped rapid degradation and formed a major sink for organic carbon. Large quantities of organic matter had been embedded within marine sediments, leading to the first black shale formation (e.g., Banerjee et al., 2006). At least since Phanerozoic times, the atmosphere showed roughly the present-day oxygen levels (Catling and Claire, 2005; Berner, 2006) and aerated conditions persisted in the oceans. However, certain climatic and geographic factors promoted sluggish circulations with reduced turn over rates, thus preventing mixing of the water column. Related environments are commonly

controlled by a temporary or permanent density-stratification (thermo- and/or halocline) within the water column (e.g., Demaison and Moore, 1980; Oschmann, 1988; Wignall, 1994).

Oceanographic models for oxygen depletion and black shale formation

Certain combinations of physical and biotic factors cause oxygen depletion within marine areas, and have been described in various models from modern and ancient environments. In the following, a brief overview on the various models and fossil examples are given. Fossil examples in many cases however, were described in more than one of these models.

Silled basins

Land - locked silled basins with a positive water balance and a shallow and narrow connection to the open sea are characterized by a low salinity surface water outflow and a high salinity deeper water inflow (estuarine circulation). The difference in density between low and high salinity waters results in a permanent halocline and subsequently, thermocline. As convection and influx of oxygen to lower parts of the water column are prohibited, anoxic conditions develop below the halocline. The Baltic Sea and the Black Sea (e.g., Bahr et al., 2005, 2006) represent modern examples. Proposed fossil examples are e.g., the Permian Kupferschiefer (e.g., Bechtel and Püttmann, 1997) and Toarcian black shales in Central Europe (e.g., Röhl et al., 2001; van de Schootbrugge et al., 2005).

Upwelling systems

Today, upwelling is known from western continental margins in subtropical areas. Trade winds cause an offshore flow of surface waters and force oxygen-poor and nutrient-rich bottom waters to ascend. The mixing with oxygen-rich surface waters results in a very high plankton productivity and export production to the sea floor. Decomposition consumes all available oxygen, resulting in stagnant conditions and enrichment of organic matter in and on the sediment. Well-known modern to Pleistocene examples are the coastal areas off Peru and off Namibia (e.g., Wefer et al., 1996; Berger and Wefer, 2002; Hebbeln et al., 2002).

Oceanic anoxic events (OAEs)

Oceanic anoxic events (OAEs) were characterized by oxygen depletion in intermediate waters in major parts or even complete ocean basins. They were explained by a number of factors, namely (1) intensified deep water formation in high latitudes, (2) origin of warm and saline bottom waters at low latitudes, (3) enhanced fresh water runoff from continents associated with high rates of nutrient delivery, and (4) increased primary production associated with extreme greenhouse conditions. These factors all favor the development of low oxic to anoxic conditions with increased organic matter preservation. A modern to

Pleistocene example is the Arabian Sea in the low-latitude Indian Ocean (e.g., Schmiedl and Mackensen, 2006). Numerous OAEs occurred during the Earth's history, of which the Cretaceous OAEs are the most widely known. They are explained by high organic carbon burial related to global warming and greenhouse periods (e.g., Erbacher et al., 2001; Herrle et al., 2003). Many of these events were probably regional occurrences within restricted basins. However, there is increasing evidence for an open ocean and maybe global extension of the OAE 2 at the Cenomanian/Turonian boundary (e.g., Erbacher et al., 2005).

Eutrophic shelf

Many modern shelf environments (e.g., North Sea and Adriatic Sea) are characterized by man-made eutrophication that stimulates primary production with subsequent oxygen consumption and accumulation of organic matter (Dethlefsen and von Westernhagen, 1983; Kollmann and Stachowitsch, 2000). Fossil examples refer to a number of epicontinental seas particularly during the periods of greenhouse climate (e.g., Kimmeridge clay in western Europe (Oschmann, 1990; Miller, 1991), Toarcian black shales (van de Schootbrugge et al., 2005)).

Tidal mud flats and shallow lagunal environments

Most of the land-derived organic matter is deposited in near-shore environments, particularly in tidal mud flats. Additionally, land-derived nutrients stimulate primary production and subsequent oxygen depletion, thus promoting the enrichment of organic matter in the sediment (e.g., Wilms et al., 2006; Rink et al., 2007). Well-known fossil examples refer to the middle and late Triassic tidal and shallow subtidal mud flat areas from the northern Tethyan margin, e.g., at Mt. St. Giorgio, Switzerland (Röhl et al., 2002) and Seefeld, Austria (Hopf et al., 2001).

Black shales as ecosystems

Black shales and modern oxygen-depleted environments show various geochemical and ecological characteristics indicative of increasingly harsh life conditions (Table 1). Rhoads and Morse (1971) distinguished aerobic, dysaerobic, and anaerobic biofacies within marine environments. This classification has subsequently been used in many studies of modern and ancient oxygen-controlled environments (e.g., Tyson and Pearson, 1991; Wignall, 1994). Observations from modern environments show a remarkable seasonal oxygen fluctuation (Oschmann, 1994, 2000). Therefore, a modified scheme of classification was introduced, where the term dysaerobic was replaced by poikiloaerobic to address seasonal variation in oxygen supply.

The proportions of organic matter, pyrite, and biomarkers increase from the aerobic toward the anaerobic biofacies. In the same direction, the diversity of eukaryotes diminishes. Particularly, deep burrowing organisms

Black Shales, Table 1 A synoptic sketch of the geochemical and ecological characteristics of modern and fossil aerobic, dysaerobic/poikiloaerobic, and anaerobic biofacies

Biofacies			Aerobic	Dysaerobic/poikiloaerobic			Anaerobic
Oxygen content	(Tyson and Pearson, 1991)	$\frac{\text{ml O}_2}{\text{l H}_2\text{O}}$	Aerobic >2	Moderate 2–1	Dysaerobic Severe 1–0.5	Extreme 0.5–0	Anaerobic 0 ml
	(Oschmann, 2000)	$\frac{\text{mmol O}_2}{\text{l H}_2\text{O}}$	Aerobic >0.1	Moderate summer < 0.1	Poikiloaerobic Severe winter : <0.1 summer : <0.05	Extreme winter : <0.1 summer : <0.02	Anaerobic < 0.01
Sediment:			Bioturbated				Laminated
Organic matter content:		>0.5 wt. %					
Content of pyrite:							
Content of biomarkers:							
Diversity of eukaryota:							
Epifauna:							
Shallow infauna (shallow bioturbation):							
Deep infauna (deep bioturbation):							
Microbial mat preservation:							
Larval shell preservation:							
Juveniles and dwarfed species:							
Reproduction via:							
Lecithotrophic larvae:							
Planktotrophic larvae:							
Position of redox boundary:		Deep in substrate	Close to sediment surface			Within the water column	

are affected by the rise of the redox boundary and try to settle close to the sediment surface (Oschmann, 1994; Schmiedl and Mackensen, 2006). Additionally, the proportion of organisms with benthic (lecithotrophic) larval development decreases with increasing oxygen depletion. If the redox boundary finally migrates out of the substrate, eukaryotic life is excluded. Dwarfs with body size remarkably smaller than normal and juveniles occur within the dysaerobic/poikiloaerobic facies, whereas microbial mats and partly also larval shells can be found within the anaerobic facies. The sporadic occurrence of the latter indicates failed larval spatfalls during unfavorable conditions.

Conclusions

1. Black shale sediments mainly form in marine to brackish waters under low oxic to anoxic conditions.
2. They are characterized by remarkably enriched proportions of organic matter and pyrite. The organic matter derives from marine primary production, terrestrial input, and bacterial derived compounds.
3. Black shales date back to late Proterozoic times and are widespread during Phanerozoic age.
4. Origination of black shales is explained by a number of models. A common aspect of most of these models is the formation of a density stratified water column, which prevents oxygenation of deeper waters.

5. The degree of oxygen depletion in benthic ecosystems during black shale deposition can be reconstructed by a number of geochemical and ecological features.

Bibliography

- Arouri, K. R., Greenwood, P. F., and Walter, M. R., 2000. Biological affinities of Neoproterozoic acritarchs from Australia: microscopic and chemical characterisation. *Organic Geochemistry*, **31**, 75–89.
- Bahr, A., Lamy, F., Arz, H. W., and Wefer, G., 2005. Late glacial to Holocene climate and sedimentation history in the NW Black Sea. *Marine Geology*, **214**, 309–322.
- Bahr, A., Arz, H. W., Lamy, F., and Wefer, G., 2006. Late glacial to Holocene paleoenvironmental evolution of the Black Sea, reconstructed with stable oxygen isotope records obtained on ostracod shells. *Earth and Planetary Science Letters*, **241**, 863–875.
- Banerjee, S., Dutta, S., Paikaray, S., and Mann, U., 2006. Stratigraphy, sedimentology and bulk organic geochemistry of black shales from the Proterozoic Vindhyan Supergroup (central India). *Journal of Earth Systems Science*, **115**, 37–47.
- Bechtel, A., and Püttmann, W., 1997. Palaeoceanography of the early Zechstein Sea during Kupferschiefer deposition in the Lower Rhine Basin (Germany): A reappraisal from stable isotope and organic geochemical investigations. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **136**, 331–358.
- Berger, W. H., and Wefer, G., 2002. On the reconstruction of upwelling history: Namibia upwelling in context. *Marine Geology*, **180**, 3–28.
- Berner, R. A., 2006. GEOCARBSULF: A combined model for Phanerozoic atmospheric O₂ and CO₂. *Geochimica et Cosmochimica Acta*, **70**, 5653–5664.
- Catling, C. D., and Claire, M. W., 2005. How Earth's atmosphere evolved to an oxic state: A status report. *Earth and Planetary Science Letters*, **237**, 1–20.
- Demaison, G. J., and Moore, G. T., 1980. Anoxic environments and oil source bed genesis. *Bulletin American Association Petroleum Geologists*, **64**, 1179–1209.
- Dethlefsen, V., and von Westernhagen, H., 1983. Oxygen deficiency and effects on bottom fauna in the eastern German Bight 1982. *Meeresforschung*, **30**, 42–53.
- Erbacher, J., Huber, B. T., Norris, R. D., and Markey, M., 2001. Increased thermohaline stratification as a possible cause for an ocean anoxic event in the Cretaceous period. *Nature*, **409**, 325–329.
- Erbacher, J., Friedrich, O., Wilson, P. A., Birch, H., and Mutterlose, J., 2005. Stable organic carbon isotope stratigraphy across oceanic anoxic event 2 of Dmerara Rise, western tropical Atlantic. *Geochemistry Geophysics Geosystems*, **6**, Q06010, doi:10.1029/2004GC000850.
- Frimmel, A., Oschmann, W., and Schwark, L., 2004. Chemostratigraphy of the Posidonia Black Shale, SW Germany. I. Influence of sea-level variation on organic facies evolution. *Chemical Geology*, **206**, 199–230.
- Hebbeln, D., Marchant, M., and Wefer, G., 2002. Paleoproductivity in the southern Peru-Chile current through the last 33,000 yr. *Marine Geology*, **186**, 487–504.
- Herrle, J. O., Pross, J., Friedrich, O., and Hemleben, C., 2003. Short-term environmental changes in the Cretaceous Tethyan Ocean: Micropaleontological evidence from the Early Albian Oceanic Anoxic Event 1b. *Terra Nova*, **15**, 14–19.
- Hopf, H., Thiel, V., and Reitner, J., 2001. An example for black shale development on a carbonate platform (late Triassic, Seefeld Austria). *Facies*, **45**, 203–210.
- Kollmann, H., and Stachowitsch, M., 2000. Long-term changes in the benthos of the Northern Adriatic Sea: a phototranssect approach. *Marine Ecology*, **22**, 135–154.
- Miller, R. G., 1991. A paleoceanographic approach to the Kimmeridge clay formation. In Huc, A. Y. (ed.), *Deposition of Organic Facies*. American Association of Petroleum Geologists Studies in Geology, **30**, pp. 13–26.
- Oschmann, W., 1988. Kimmeridge clay sedimentation - a new cyclic model. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **65**, 217–251.
- Oschmann, W., 1990. Environmental cycles in the late Jurassic northwest European epeiric basin: interaction with atmospheric and hydrospheric circulations. In Aigner, T., and Dott, R. H. (eds.), *Processes and Patterns in Epeiric Basins*. Sedimentary Geology, **69**, pp. 313–332.
- Oschmann, W., 1994. Adaptive pathways of marine benthic organisms in oxygen-controlled environments. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **191**, 393–444.
- Oschmann, W., 2000. Oxygen in the ocean. In Briggs, D. E. G., and Crowther, P. R. (eds.), *Palaeobiology: A Synthesis*. Oxford: Blackwell, pp. 474–477.
- Rhoads, D. C., and Morse, I. W., 1971. Evolutionary and ecological significance of oxygen-deficient marine basins. *Lethaia*, **4**, 413–428.
- Rink, B., Seeberger, S., Martens, T., Dürselen, C., Simon, M., and Brinkhoff, T., 2007. Effects of a phytoplankton bloom in a coastal ecosystem on the composition of bacterial communities. *Aquatic Microbial Ecology*, **48**, 47–60.
- Röhl, H. J., Schmid-Röhl, A., Oschmann, W., Frimmel, A., and Schwark, L., 2001. The Posidonia Shale (Lower Toarcian) of SW-Germany: an oxygen depleted ecosystem controlled by sea level and palaeoclimate. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **165**, 27–52.
- Röhl, H. J., Schmid-Röhl, A., Frimmel, A., Oschmann, W., Furrer, H., and Schwark, L., (2002). Microfacies, geochemistry and palaeoecology of the middle Triassic Grenzbitumenzone from Monte San Giorgio (Tessin). *Geologia Insubrica*, **6**, 1–13.
- Rouxel, O. J., Bekker, A., and Edwards, K. J., 2005. Iron isotope constraints on the Archean and Paleoproterozoic ocean redox state. *Science*, **307**, 1088–1091.
- Samuelsson, J., Dawes, P. R., and Vidal, G., 1999. Organic-walled microfossils from Proterozoic Thule Supergroup, Northwest Greenland. *Precambrian Research*, **96**, 1–23.
- Schmiedl, G., and Mackensen, A., 2006. Multi-species stable isotopes of benthic foraminifers reveal past changes of organic matter decomposition and deep-water oxygenation in the Arabian Sea. *Paleoceanography*, **21**, PA4213, doi:10.1029/2006PA001284.
- Tyson, R. V., and Pearson, T. H., 1991. Modern and ancient continental shelf anoxia: an overview. In Tyson, R. V., and Pearson, T. H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. Tulsa, OK: Geological Society Special Publication **58**, pp. 1–24.
- van de Schootbrugge, B., McArthur, J. M., Bailey, T. R., Rosenthal, Y., Wright, J. D., and Miller, K. G., 2005. Toarcian oceanic anoxic event: an assessment of global causes using belemnite C isotope records. *Paleoceanography*, **20**, PA3008, doi:10.1029/2004PA001102.
- Wefer, G., Berger, W. H., Bickert, T., Donner, B., Fischer, G., Kemle-von Mücke, S., Meinecke, G., Müller, P. J., Mulitza, S., Niebler, H. S., Pätzold, J., Schmidt, H., Schneider, R. R., and Segl, M., 1996. Late Quaternary surface circulation of the South Atlantic: the stable isotope record and implications for heat transport and productivity. In Wefer, G., Berger, W. H., Siedler, G., and Webb, D. (eds.), *The South Atlantic: Present and Past Circulation*. Berlin: Springer, pp. 461–502.

- Wignall, P., 1994. Black shales. *Oxford Monographs in Geology and Geophysics*, **30**, 1–127.
- Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., and Engelen, B., 2006. Specific bacterial archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. *Applied and Environmental Microbiology*, **72**, 2756–2764.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Biosignatures in Rocks](#)
[Carbon \(Organic, Cycling\)](#)
[Carbon \(Organic, Degradation\)](#)
[Critical Intervals in Earth History](#)
[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Microbial Degradation](#)
[Sediment Diagenesis - Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)
[Sulfur Isotopes](#)

BREAKUP OF RODINIA

Zheng-Xiang Li
 Curtin University of Technology, Perth, WA, Australia

Synonyms

Break-up of the supercontinent Rodinia

Definition

Supercontinent Rodinia. The earth's geodynamic system periodically pushes almost all continents together to form a single landmass – a supercontinent. The best known supercontinent is Pangaea (or Pangea) that existed between 320 and 170 Ma. A less known supercontinent Rodinia is believed to have existed between 900 Ma (some believe 1,000 Ma) and 750 Ma. The name “Rodinia” was coined by McMenamin and McMenamin (1990) from the Russian word “rodit” meaning “to beget” or “to give birth.” McMenamin and McMenamin (1990, p. 95) considered Rodinia to have been the supercontinent that spawned all subsequent continents, while “the edges (continental shelves) of Rodinia were the cradle of the earliest animals.” Three landmark paper in 1991 (Moores, 1991; Dalziel, 1991; Hoffman, 1991) triggered a global debate on this supercontinent hypothesis. The configuration of Rodinia is still controversial today, with some alternative reconstructions given in Hoffman (1991), Pisarevsky et al. (2003), and Li et al. (2008) (Figure 1).

The breakup of Rodinia: introduction

Although it is widely accepted that Rodinia broke apart during the latter half of the Neoproterozoic era (1,000–542 Ma) and that some fragments reassembled by the early Cambrian (*ca.* 530 Ma) to form Gondwanaland (Hoffman, 1991), our knowledge of the timing of the breakup events and the processes involved is still sketchy.

Record of Rodinia breakup

The breakup of Rodinia was recorded both geologically and geophysically. The most important geological records of the breakup process are globally widespread continental rift deposits and related magmatism, and records of the geological transition from continental rifting to passive margin deposits where rifting succeeded to form new oceans. One of the best matches of rifting records between continents is that between southeast Australia and South China, where episodes of rift-related magmatism at *ca.* 825 Ma, 800 Ma, 780 Ma, and 750 Ma have been well documented (Preiss, 2000; Li et al., 2003) (Figure 2). Similar records are emerging from other continents.

Radiating dyke swarms and remnants of large igneous provinces (LIPs) have also been regarded as important geological records of the breakup of Rodinia (Figure 2c and d; see discussion below under *Mechanism for the breakup*).

The most important geophysical constraint on the breakup of Rodinia is provided by paleomagnetism. Whereas, an individual pole of a given age for a continent constrains its paleolatitude at that time but not its paleolongitude, comparable apparent polar wander paths (APWPs) for different continents can indicate a unique paleogeographic fit between them. In the latter case, when two matched APWPs start to diverge, it implies that the two continents started to move independently (*i.e.*, a breakup between them occurred). Unfortunately, such an ideal situation has rarely been identified for the Rodinia time because of the limited data currently available.

Timing of events

The ages of continental rifting events can be easily dated because of the availability of datable minerals such as zircon in the rift-related magmatic rocks, and it is now widely accepted that continental rifting in the lead-up to Rodinia breakup started at *ca.* 825 Ma or earlier (see summary in Li et al., 2008; Figure 2). However, the timing of the breakup events is still controversial, and different parts of the supercontinent seem to have broken apart at different times.

The stratigraphic record in southeast Australia indicates a rift-drift transition between the Sturtian glacial deposits (with a poor age constraint of *ca.* 750–690 Ma) and the overlying sag-phase deposits (Powell et al., 1994) that were recently dated at between 657 ± 5 and 643 ± 2 Ma (Re-Os ages, see Kendall et al., 2006). These age

- Wignall, P., 1994. Black shales. *Oxford Monographs in Geology and Geophysics*, **30**, 1–127.
- Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., and Engelen, B., 2006. Specific bacterial archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. *Applied and Environmental Microbiology*, **72**, 2756–2764.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biomarkers \(Molecular Fossils\)](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Biosignatures in Rocks](#)
[Carbon \(Organic, Cycling\)](#)
[Carbon \(Organic, Degradation\)](#)
[Critical Intervals in Earth History](#)
[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Microbial Degradation](#)
[Sediment Diagenesis - Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)
[Sulfur Isotopes](#)

BREAKUP OF RODINIA

Zheng-Xiang Li
 Curtin University of Technology, Perth, WA, Australia

Synonyms

Break-up of the supercontinent Rodinia

Definition

Supercontinent Rodinia. The earth's geodynamic system periodically pushes almost all continents together to form a single landmass – a supercontinent. The best known supercontinent is Pangaea (or Pangea) that existed between 320 and 170 Ma. A less known supercontinent Rodinia is believed to have existed between 900 Ma (some believe 1,000 Ma) and 750 Ma. The name “Rodinia” was coined by McMenamin and McMenamin (1990) from the Russian word “rodit” meaning “to beget” or “to give birth.” McMenamin and McMenamin (1990, p. 95) considered Rodinia to have been the supercontinent that spawned all subsequent continents, while “the edges (continental shelves) of Rodinia were the cradle of the earliest animals.” Three landmark paper in 1991 (Moores, 1991; Dalziel, 1991; Hoffman, 1991) triggered a global debate on this supercontinent hypothesis. The configuration of Rodinia is still controversial today, with some alternative reconstructions given in Hoffman (1991), Pisarevsky et al. (2003), and Li et al. (2008) (Figure 1).

The breakup of Rodinia: introduction

Although it is widely accepted that Rodinia broke apart during the latter half of the Neoproterozoic era (1,000–542 Ma) and that some fragments reassembled by the early Cambrian (*ca.* 530 Ma) to form Gondwanaland (Hoffman, 1991), our knowledge of the timing of the breakup events and the processes involved is still sketchy.

Record of Rodinia breakup

The breakup of Rodinia was recorded both geologically and geophysically. The most important geological records of the breakup process are globally widespread continental rift deposits and related magmatism, and records of the geological transition from continental rifting to passive margin deposits where rifting succeeded to form new oceans. One of the best matches of rifting records between continents is that between southeast Australia and South China, where episodes of rift-related magmatism at *ca.* 825 Ma, 800 Ma, 780 Ma, and 750 Ma have been well documented (Preiss, 2000; Li et al., 2003) (Figure 2). Similar records are emerging from other continents.

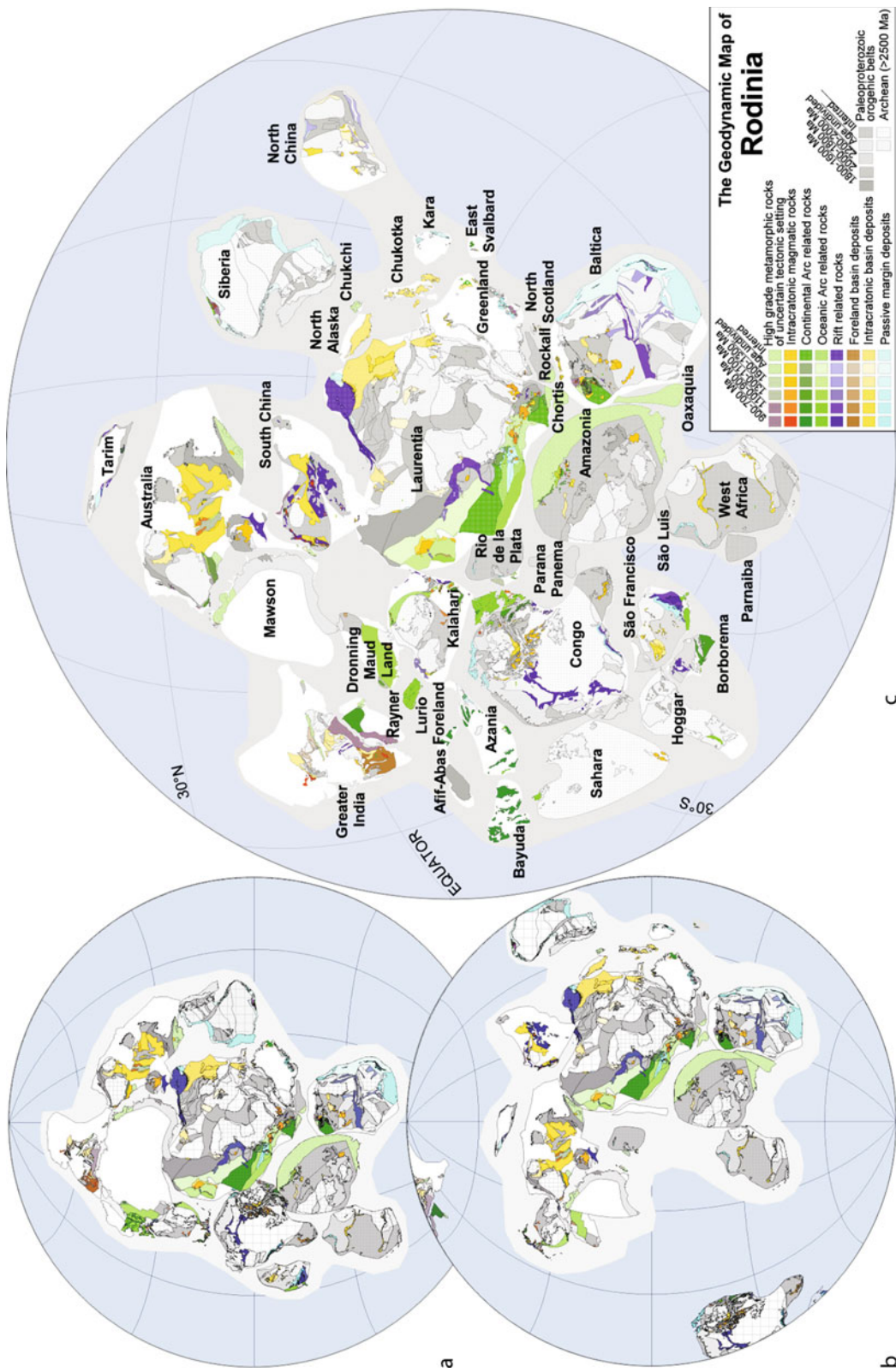
Radiating dyke swarms and remnants of large igneous provinces (LIPs) have also been regarded as important geological records of the breakup of Rodinia (Figure 2c and d; see discussion below under *Mechanism for the breakup*).

The most important geophysical constraint on the breakup of Rodinia is provided by paleomagnetism. Whereas, an individual pole of a given age for a continent constrains its paleolatitude at that time but not its paleolongitude, comparable apparent polar wander paths (APWPs) for different continents can indicate a unique paleogeographic fit between them. In the latter case, when two matched APWPs start to diverge, it implies that the two continents started to move independently (*i.e.*, a breakup between them occurred). Unfortunately, such an ideal situation has rarely been identified for the Rodinia time because of the limited data currently available.

Timing of events

The ages of continental rifting events can be easily dated because of the availability of datable minerals such as zircon in the rift-related magmatic rocks, and it is now widely accepted that continental rifting in the lead-up to Rodinia breakup started at *ca.* 825 Ma or earlier (see summary in Li et al., 2008; Figure 2). However, the timing of the breakup events is still controversial, and different parts of the supercontinent seem to have broken apart at different times.

The stratigraphic record in southeast Australia indicates a rift-drift transition between the Sturtian glacial deposits (with a poor age constraint of *ca.* 750–690 Ma) and the overlying sag-phase deposits (Powell et al., 1994) that were recently dated at between 657 ± 5 and 643 ± 2 Ma (Re-Os ages, see Kendall et al., 2006). These age



Breakup of Rodinia, Figure 1 Three of the alternative Rodinia reconstructions: (a) Hoffman (1991), (b) Pisarevsky et al. (2003), and (c) model adapted by IGCP440 for the Geodynamic Map of Rodinia. (Li et al., 2008.)

constraints seem to agree with those for south China and western Laurentia. Although rifting occurred at *ca.* 750 Ma along the eastern margins of Laurentia, the breakup there appears to have occurred during *ca.* 615–570 Ma (Cawood and Pisarevsky, 2006). These younger events are possibly recognizable in global tectonic subsidence curves (Bond et al., 1984).

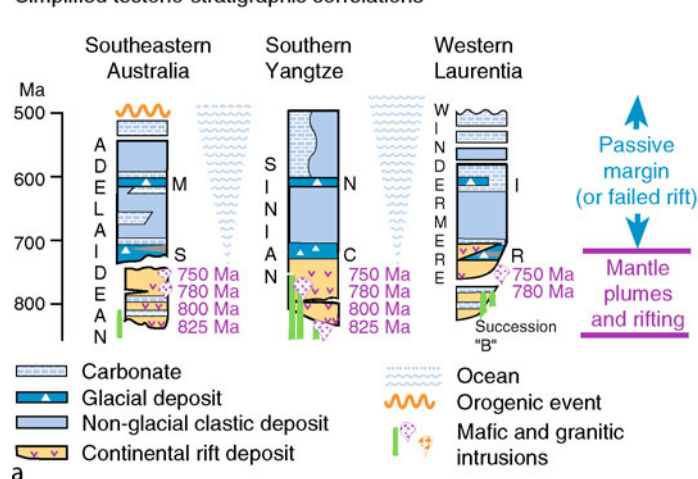
Such geological evidence for the breakup of Rodinia is sometimes at variance with paleomagnetic constraints. Paleomagnetic data suggests that India was either adjacent to western Australia (Torsvik et al., 2001) or had already broken away from Rodinia at 750 Ma (Figure 3b). South

China must also have broken away from Australia and Laurentia by 750 Ma if it occupied a central position in Rodinia as shown in Figures 1c and 3a (Wingate and Giddings, 2000; Li et al., 2008). Alternatively, Rodinia may have remained intact at *ca.* 750 Ma if a different configuration occurred (see Fig. 5 of Li et al., 2004).

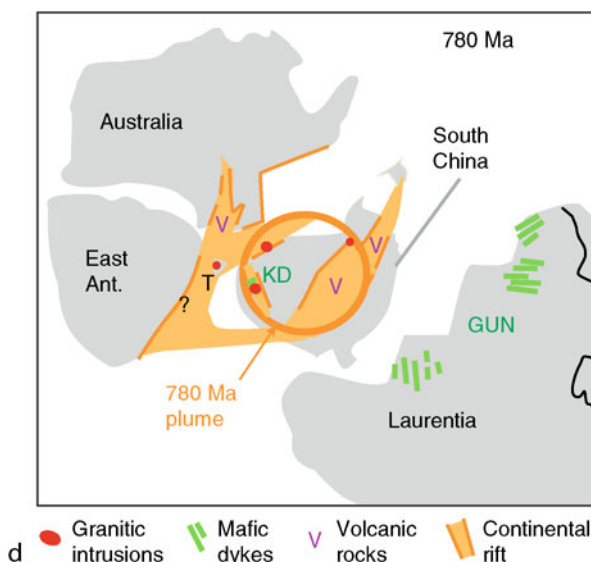
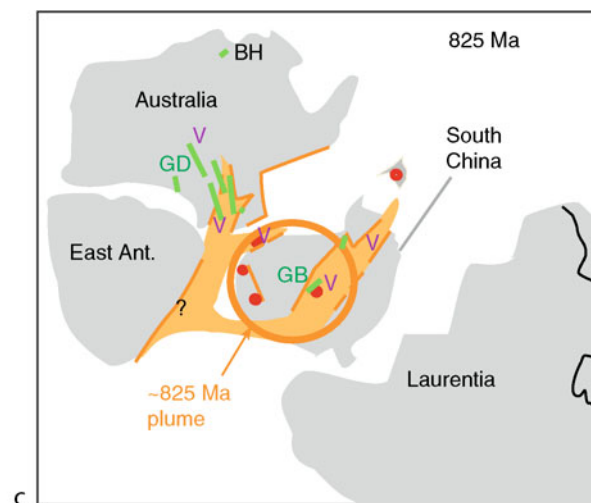
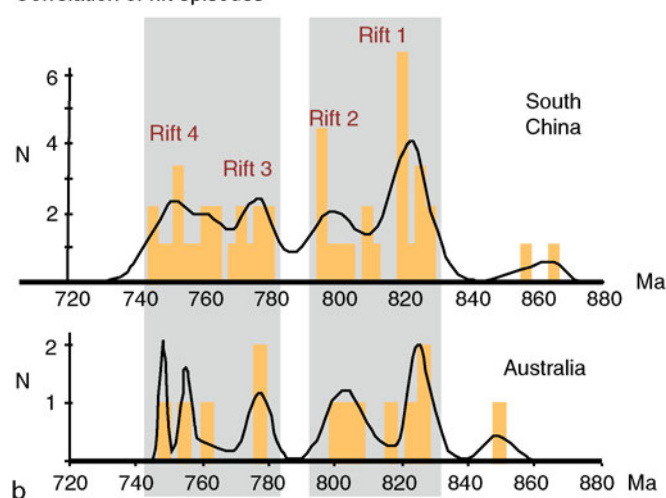
Mechanism for the breakup

The most commonly invoked mechanism for the breakup of Rodinia is the activity of mantle plumes or of a mantle superplume (superswell) beneath the supercontinent,

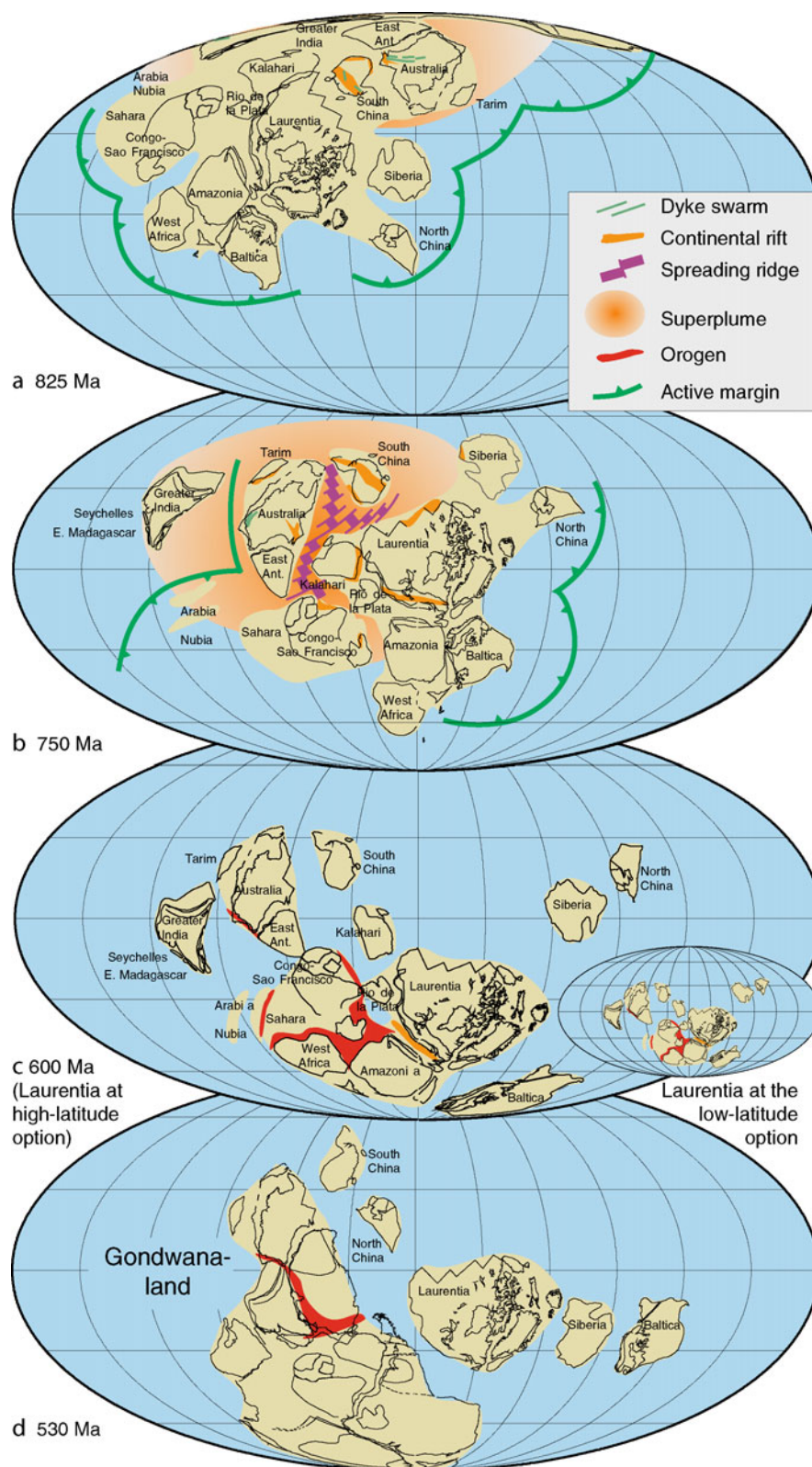
Simplified tectono-stratigraphic correlations



Correlation of rift episodes



Breakup of Rodinia, Figure 2 (a) Tectonostratigraphic correlations between Australia, South China, and Laurentia during the breakup of Rodinia. Major glaciations: M = Marinoan; S = Sturtian; N = Nantuo; C = Changan; I = Ice Brook; R = Rapitan. (b) Correlatable episodes of intra-continental magmatism and continental rifting between Australia and South China. *N* = number of magmatic ages. (c, d) Cartoon figures illustrating possible plume breakouts in central Rodinia during the Neoproterozoic time. (Modified after Li et al., 2003, 2008.)



Breakup of Rodinia, Figure 3 Cartoons illustrating the breakup process of Rodinia. (After Li et al., 2008.)

which thinned and thermally weakened the lithosphere resulting in widespread continental rifting and eventually breakup (Figure 3a and b).

Evidence for plume/superplume activity during the time of Rodinia include remnants of LIP and radiating dyke swarms (Figure 2c), syn-magmatic, continental-scale doming indicating the arrival of a plume-head (Li et al., 1999), large-scale anorogenic but often dominantly felsic melting that requires a pan-Rodinia introcontinental heat source (Li et al., 2003), and abnormally high-temperature ($>1,500^{\circ}\text{C}$) mantle melting (Wang et al., 2007). Mechanisms proposed for the formation of such a superplume (or superswell) include thermal shielding by the supercontinent (e.g., Anderson, 1994), gravitational push-up effects on the hotter lower mantle by circum-Rodinia avalanching slabs (Li et al., 2004, 2008), return flow of whole-mantle convection (Zhong et al., 2007), and partial melt of recycled mid-ocean ridge basalts near the core-mantle boundary under Rodinia (Maruyama et al., 2007).

Summary

The breakup of Rodinia was a diachronous and protracted process. Widespread continental rifting started at *ca.* 825 Ma when Rodinia spanned from the equator to high latitudes (Figure 3a), but the breakup, initially between Australia/East Antarctica, Laurentia, and possibly South China did not occur until *ca.* 750 Ma (Figure 3b) or later when the supercontinent was centered on the equator. Gondwanaland was already assembling before some of the West Gondwanan continents broke away from Laurentia at *ca.* 600 Ma (Figure 3c), and by the early Cambrian (530 Ma) the assembly was complete (Figure 3d). The breakup of Rodinia is believed by some to have been related to the formation of a mantle superplume beneath the supercontinent.

Bibliography

- Anderson, D. L., 1994. Superplume or supercontinents? *Geology*, **22**, 39–42.
- Bond, G. C., Nickeson, P. A., and Kominz, M. A., 1984. Breakup of a supercontinent between 625 Ma and 555 Ma: new evidence and implications for continental histories. *Earth and Planetary Science Letters*, **70**, 325–345.
- Cawood, P. A., and Pisarevsky, S. A., 2006. Was Baltica right-way-up or upside-down in the Neoproterozoic? *Journal of the Geological Society*, **163**, 753–759.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica - Australia as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside-out? *Science*, **252**, 1409–1412.
- Kendall, B., Creaser, R. A., and Selby, D., 2006. Re-Os geochronology of postglacial black shales in Australia: constraints on the timing of “Sturtian” glaciation. *Geology*, **34**, 729–732.
- Li, Z. X., Li, X. H., Kinny, P. D., and Wang, J., 1999. The breakup of Rodinia: did it start with a mantle plume beneath South China? *Earth and Planetary Science Letters*, **173**(3), 171–181.
- Li, Z. X., Li, X. H., Kinny, P. D., Wang, J., Zhang, S., and Zhou, H., 2003. Geochronology of Neoproterozoic syn-rift magmatism in the Yangtze Craton, South China and correlations with other continents: evidence for a mantle superplume that broke up Rodinia. *Precambrian Research*, **122**(1–4), 85–109.
- Li, Z. X., Evans, D. A. D., and Zhang, S., 2004. A 90° Spin on Rodinia: possible causal links between the Neoproterozoic supercontinent, superplume, true polar wander and low-latitude glaciation. *Earth and Planetary Science Letters*, **220**, 409–421.
- Li, Z. X., Bogdanova, S. V., Collins, A. S., Davidson, A., De Waele, B., Ernst, R. E., Fitzsimons, I. C. W., Fuck, R. A., Gladkochub, D. P., Jacobs, J., Karlstrom, K. E., Lu, S., Natapov, L. M., Pease, V., Pisarevsky, S. A., Thrane, K., and Vernikovsky, V., 2008. Assembly, configuration, and break-up history of Rodinia: a synthesis. *Precambrian Research*, **160**(1–2), 179–210.
- Maruyama, S., Santosh, M., and Zhao, D., 2007. Superplume, supercontinent, and post-perovskite: Mantle dynamics and anti-plate tectonics on the Core-Mantle Boundary. *Gondwana Research*, **11**(1–2), 7–37.
- McMenamin, M. A. S., and McMenamin, D. L. S., 1990. *The Emergence of Animals: The Cambrian Breakthrough*. New York: Colombia University Press, 217 pp.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Pisarevsky, S. A., Wingate, M. T. D., Powell, C. M., Johnson, S., and Evans, D. A. D., 2003. Models of Rodinia assembly and fragmentation. In Yoshida, M., Windley, B. F., and Dasgupta, S. (eds.), *Proterozoic East Gondwana: Supercontinent Assembly and Breakup*. Geological Society of London Special Publications 206, pp. 35–55.
- Powell, C. M., Preiss, W. V., Gatehouse, C. G., Krapez, B., and Li, Z. X., 1994. South Australian record of a Rodinian epicontinental basin and its mid-Neoproterozoic breakup (~ 700 Ma) to form the palaeo-Pacific Ocean. *Tectonophysics*, **237**(3–4), 113–140.
- Preiss, W. V., 2000. The Adelaide Geosyncline of South Australia and its significance in Neoproterozoic continental reconstruction. *Precambrian Research*, **100**, 21–63.
- Torsvik, T. H. et al., 2001. Rodinia refined or obscured: palaeomagnetism of the Malani Igneous Suite (NW India). *Precambrian Research*, **108**(3–4), 319–333.
- Wang, X. C., Li, X. H., Li, W. X., and Li, Z. X., 2007. Ca. 825 Ma komatiitic basalts in South China: First evidence for $>1500^{\circ}\text{C}$ mantle melts by a Rodinian mantle plume. *Geology*, **35**(12), 1103–1106.
- Wingate, M. T. D., and Giddings, J. W., 2000. Age and palaeomagnetism of the Mundine Well dyke swarm, Western Australia: implications for an Australia-Laurentia connection at 755 Ma. *Precambrian Research*, **100**, 335–357.
- Zhong, S., Zhang, N., Li, Z. X., and Roberts, J. H., 2007. Supercontinent cycles, true polar wander, and very long-wavelength mantle convection. *Earth and Planetary Science Letters*, **261**, 551–564.

Cross-references

Gondwanaland, Formation
Snowball Earth

C

CALCAREOUS ALGAE

The term “calcareous algae” refers to various kinds of benthic and planktonic algae whose thalli contain biochemically precipitated calcium carbonate (CaCO_3) as skeletal material (Wray, 1977; Braga and Riding, 2005). Precipitation of CaCO_3 (as calcite and/or aragonite) may occur within or on the algal bodies. The term may also include mechanically accreted deposits of calcium carbonate caused by algae, usually as an interaction of biological and physical processes. Calcareous algae are a highly artificial group that constitutes calcifying members of the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) and is sometimes also used for Cyanobacteria. At present, calcareous algae are one of the most important reef builders (see “[Carbonate Environments](#)”). For a detailed reading, please refer to “[Algae \(Eukaryotic\)](#).”

Bibliography

- Braga, J. C., and Riding, R., 2005. Calcareous algae. In Selley, R., Cocks, L. R. M., and Plimer, I. R. (eds.), *Encyclopedia of Geology*. Amsterdam: Elsevier, pp. 428–436.
- Wray, J. L., 1977. *Calcareous Algae. Developments in Palaeontology and Stratigraphy*. Amsterdam: Elsevier, Vol. 4, 185 pp.

CALCIFICATION

See entries “[Animal Biocalcification, Evolution](#),” “[Biofilms and Fossilization](#),” “[Calcified Cyanobacteria](#),” “[Calcite Precipitation, Microbially Induced](#),” “[Calcium Biogeochemistry](#),” “[Carbonate Environments](#),” “[Dolomite, Microbial](#),” “[Microbialites, Modern](#),” “[Microbialites, Stromatolites, and Thrombolites](#),” and “[Pedogenic Carbonates](#).”

CALCIFIED CYANOBACTERIA

Robert Riding
University of Tennessee, Knoxville, TN, USA

Definition

Cyanobacteria are alga-like bacteria that can perform oxygenic photosynthesis and nitrogen fixation (Whitton and Potts, 2000; Herrero and Flores, 2008). They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments at the present-day, where they are key primary producers in both microbial mat and planktic ecosystems. As the initiators of plastids, they have played a fundamental role in algal and plant evolution (Raven, 2002). Cyanobacteria occupy benthic substrates and can also drift in the water column. Their photosynthetic uptake of inorganic carbon can stimulate CaCO_3 precipitation. This calcification can produce filamentous microfossils in benthic mats that are preserved as stromatolites and thrombolites, and can also cause water column precipitation of carbonate mud that settles to lake and sea floors.

Introduction

However, cyanobacterial calcification is not obligatory and is directly dependent on environmental conditions. This accounts for apparent discrepancies between the geological ranges of organic-walled and calcified cyanobacterial fossils. Calcified cyanobacteria have not been recognized in marine sediments older than ~1200 Ma ago (Kah and Riding, 2007), whereas there is evidence that cyanobacteria appeared in the late Archaean or Palaeoproterozoic, in the range ~2900–2150 Ma ago (Cavalier-Smith et al., 2006; Hofmann, 1976). This mid-Proterozoic appearance of sheath-calcified cyanobacteria is thought to reflect the development of CO_2 -concentrating

- lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Sprachta, S., Camoin, G., Golubic, S., and Le Campion, T., 2001. Microbialites in a modern lagoonal environment: nature and distribution, Tikehau atoll (French Polynesia). *Palaeogeography Palaeoclimatology Palaeoecology*, **175**, 103–124.
- Suess, E., and Futterer, D., 1972. Aragonitic ooids 1: experimental precipitation from seawater in the presence of humic acid. *Sedimentology*, **19**, 129–139.
- Teng, H. H., Dove, P. M., Orme, C. A., and De Yoreo, J. J., 1998. Thermodynamics of calcite growth: baseline for understanding biomineral formation. *Science*, **282**, 724–727.
- Thompson, J. B., 2000. Microbial whittings, In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin Heidelberg: Springer, pp. 250–260.
- Thompson, J. B., Schultze-Lam, S., Beveridge, T. J., and DesMarais, D. J., 1997. Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial phytoplankton. *Limnology and Oceanography*, **42**, 133–141.
- Van Lith, Y., Warthmann, R., Vasconcelos, C., and McKenzie, J. A., 2003. Sulphate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology*, **1**, 71–79.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.

Cross-references

Alkalinity
 Algae (Eukaryotic)
 Animal Skeletons, Advent
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Carbonate Environments
 Carbonates
 Cold Seeps
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Hydrothermal Environments, Terrestrial
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Nan(n)obacteria
 Organomineralization
 Pedogenic Carbonates
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCIUM BIOGEOCHEMISTRY

Anton Eisenhauer
 IFM-GEOMAR, Christian Albrechts Universität zu Kiel,
 Kiel, Germany

Definition

Calcium (Ca) has the atomic number 20 and is an alkaline earth metal with an atomic mass of 40.978 amu. The

important role of Ca in biogeochemical processes is based on its chemical versatility, which is related to its highly adaptable coordination geometry, its divalent charge, modest binding energies, fast reaction kinetics, and its inertness in redox reactions (Williams, 1974). Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element in the Earth's crust and the seventh most abundant element in the ocean. It is essential for living organisms, particularly in cell physiology, shell formation, and calcification and hence, is usually the most common metal in many animals.

Calcium isotopes and their application in Biogeochemistry

There are seven Ca isotopes present in nature of which three isotopes (^{42}Ca (0.646%), ^{43}Ca (0.135%) through ^{44}Ca (2.086%)) are stable, one isotope (^{40}Ca (69.941%)) is radiogenic, one isotope (^{41}Ca) is cosmogenic, and two more isotopes (^{46}Ca (0.004%) and ^{48}Ca (0.187%)) are radioactive having extremely long half-lives (^{46}Ca : $2.8 \cdot 10^{15}$ years; ^{48}Ca : $> 4 \cdot 10^{19}$ years). The cosmogenic isotope ^{41}Ca is radioactive (half live of 103,000 years) and is produced by neutron activation of ^{40}Ca in the upper soil column. ^{41}Ca has received much attention in stellar studies because it decays to ^{41}K , a critical indicator of solar system anomalies. ^{40}Ca is the radiogenic daughter product of ^{40}K decay, along with ^{40}Ar . While K–Ar dating has been used extensively in the geological sciences, the prevalence of ^{40}Ca in nature has impeded its use in dating. Techniques using mass spectrometry and a double spike isotope dilution have been used for K–Ca age dating (Nägler and Villa, 2000).

Natural Ca isotope fractionation has, among other isotope systems such as oxygen (d^{18}O) and carbon (d^{13}C) fractionation, been applied for the study of calcification processes and the so-called vital effect (c.f. Gussone et al., 2003; Lemarchand et al., 2005). Latter effect describes the deviation of the expected inorganic isotope fractionation value or element partitioning coefficient (e.g., Sr/Ca, Mg/Ca, etc.) from the measured one in calcifying organisms such as coccolithophores, foraminifer, corals, and other calcifying organisms.

The more or less species-dependent Ca isotope fractionation of marine calcifying organisms as a function of the adjacent seawater temperature can be applied for the reconstruction of the dynamic of Ca concentrations throughout the Earth's history (c.f. Griffith et al., 2008; Farkas et al., 2007; Heuser et al., 2005; Fantle and DePaolo, 2005; De LaRocha and DePaolo, 2000) and for the reconstruction of past sea surface temperatures (c.f. Nägler et al., 2000; Hippler et al., 2006).

The dynamic of calcium in the ocean

Calcium is the second most abundant divalent cation (~ 10 mmol/l), besides magnesium (Mg, ~ 52 mmol/l). Calcium is considered to be a conservative element in the ocean water column, with a residence time of about

one million year. A number of scientific studies show that continental and oceanic weathering, mid-ocean ridge hydrothermal activity, and carbonate sedimentation are the major factors controlling the marine Ca cycle (Hart, 1973; Thompson, 1983; Zhu & Macdougall, 1998; Berner, 2004). Besides continental weathering and riverine influx, comprising about 70% of the Ca flux to the ocean, submarine magmatism and associated hydrothermal activity at the mid-ocean ridges as well as seafloor weathering are also recognized to be major sources for the marine Ca budget (Berner & Berner, 1996). A number of studies indicated that Ca is released from the oceanic crust during water–rock reactions ranging from high-temperature hydrothermal interactions to seafloor weathering at low temperatures below about 150°C (Berner, 2004; Amini et al., 2008). Ca-bearing mineral phases such as aragonite, calcite, anhydrite, and subordinately hydrous Ca-silicates (e.g., prehnite, zeolites) are formed during different stages of ocean crust alteration.

Marine calcification

The dynamic of Ca in the ocean is of particular interest because of the formation of biogenic and nonbiogenic calcium carbonate (CaCO_3), known as the process of calcification, and its interference and close linkage with the global carbon cycle (c.f. De LaRocha & DePaolo, 2000; Heuser et al., 2005). In particular, the formation of calcareous skeletons by marine planktonic organisms and their subsequent sinking to depth generate a continuous rain of CaCO_3 to the deep ocean and the underlying sediments. This is important in regulating marine carbon cycling and ocean–atmosphere CO_2 exchange. The present rise in atmospheric CO_2 levels causes a significant decrease (acidification) in surface ocean pH and a major change of carbonate chemistry. Laboratory experiments have shown that such changes slow down calcification in corals and coralline macroalgae. Over geological time scales, the Ca balance in the ocean is believed to be directly related to pCO_2 variations in the atmosphere, and it thus may interfere with climate changes on geological time scales (e.g., Demicco et al., 2003; Fantle and DePaolo, 2005).

Summary

Future progress in instrumental analytic will allow high-resolution measurement of Ca elemental and isotope partitioning in inorganic and biological systems, allowing to trace elemental pathways from a bulk solution to the site of calcification.

Bibliography

- Amini, M., Eisenhauer, A., Holmden, C., Boehm, F., Hauff, F., and Jochum, K. P., 2008. $\delta^{44/40}\text{Ca}$ variability in igneous rocks. *Geochimica et Cosmochimica Acta* **72**(12), A19–A19.
- Berner, R. A., 2004. A model for calcium, magnesium and sulfate in seawater over Phanerozoic time. *American Journal of Science*, **304**, 438–453.

- Berner, E. K., and Berner, R. A., 1996. *Global Environment: Water, Air, and Geochemical Cycles*. Upper Saddle River: Prentice Hall.
- De La Rocha, C. L., and DePaolo, D. J., 2000. Isotopic evidence for variations in the marine calcium cycle over the Cenozoic. *Science*, **289**, 1176–1178.
- Demicco, R. V., Lowenstein, T. K., and Hardie, L. A., 2003. Atmospheric pCO_2 since 60 Ma from records of seawater pH, calcium, and primary carbonate mineralogy. *Geology*, **31**(9), 793–796.
- Fantle, M. S., and DePaolo, D. J., 2005. Variations in the marine Ca cycle over the past 20 million years. *Earth and Planetary Science Letters*, **237**, 102–117.
- Farkas, J., Böhm, F., et al. 2007. Calcium isotope record of Phanerozoic oceans: Implications for chemical evolution of seawater and its causative mechanisms. *Geochimica et Cosmochimica Acta* **71**(21), 5117–5134.
- Griffith, E. M., Paytan, A., Caldeira, K., Bullen, T. D., and Thomas E., 2008. A dynamic marine calcium cycle during the past 28 million years. *Science*, **322**, 1671–1674.
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H., Lea, D. W., Bijma, J., and Nägler, T. F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochim Cosmochim Acta*, **67**(7), 1375–1382.
- Hart, S. R., and Zindler, A., 1989. Isotope fractionation laws: a test using calcium. *International Journal of Mass Spectrometry and Ion Physics*, **89**, 287–301.
- Hippler, D., Eisenhauer, A., and Thomas, F. N., 2006. Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140 ka. *Geochim Cosmochim Acta*, **70**, 90–100.
- Heuser, A., Eisenhauer, A., Böhm, F., Wallmann, K., Gussone, N., Pearson, P. N., Nägler, T. F., and Dullo, W.-C., 2005. Calcium isotope ($\delta^{44/40}\text{Ca}$) variations of Neogene planktonic foraminifera. *Paleoceanography*, **20**.
- Lemarchand, D., Wasserburg, G. J., and Papanastassiou, A., 2004. Rate-controlled calcium isotope fractionation in synthetic calcite. *Geochim Cosmochim Acta*, **68**(22), 4665–4678.
- Nägler, T. F., Villa, I. M., 2000. In pursuit of the ^{40}K branching ratios: K–Ca and ^{39}Ar – ^{40}Ar dating of gem silicates. *Chem Geol* **169**, 5–16.
- Nägler, T., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -isotopes: new powerful tool for reconstruction of past sea surface temperatures. *Geochemistry, Geophysics, Geosystems*, **1**, doi: 2000GC000091.
- Thompson, G., 1983. *Hydrothermal Fluxes in the Ocean*. New York: Academic Press.
- Williams, R. J. P., 1974. Calcium ions: their ligands and their function. *Biochemical Society Symposia*, **39**, 133–138.
- Zhu, P., and Macdougall, J. D., 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. *Geochim Cosmochim Acta*, **62**(10), 1691–1698.

Cross-references

[Animal Biocalcification, Evolution](#)
[Calcified Cyanobacteria](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbonate Environments](#)
[Carbonates](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Dolomite, Microbial](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

- Thompson, J. B., 2000. Microbial whittings. In Riding, R., and Agramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer-Verlag, pp. 250–260.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N. W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. SEPM special publication number, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaios*, **15**, 87–111.
- Vologdin, A. G., 1932. *Arkheotsiaty Sibiri. Vypusk 2: Fauna kembriyskikh izvestnykh Altaya*. Moscow; Leningrad: State Sci.Tech. Geol. Explor. Publishing House p. 106 (in Russian with English resume).
- Vologdin, A. G., 1962. *Drevneyshie vodorosli SSSR*. Moscow: U.S.S.R. Academy of Sciences Publishing House, 656 pp. (in Russian).
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research* **100**, 371–433.
- Westrop, S. R., and Adrain, J. M. 2001. Sampling at the species level: impact of spatial biases on diversity gradients. *Geology*, **29**, 903–906.
- Whitton, B. A., and Potts, M. (eds.), 2000. *The Ecology of Cyanobacteria, their Diversity in Time and Space*. Dordrecht: Kluwer, 669 pp.
- Woo, J., Chough, S. K., and Han, Z. 2008. Chambers of epiphyton thalli in microbial buildups, zhangxia formation (Middle Cambrian), Shandong Province, China. *Palaios* **23**, 55–64.
- Yee, N., Benning, L. G., Phoenix, V. R., and Ferris, F. G. 2004. Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environmental Science and Technology*, **38**, 775–782.
- Zhuravlev, A. Yu., 2001. Biotic diversity and structure during the Neoproterozoic-Ordovician transition. In Zhuravlev, A. Yu., and Riding, R. (eds.), *The Ecology of the Cambrian Radiation*, New York: Columbia University Press, pp.173–199.

Cross-references

Alkalinity
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Carbonate Environments
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Organomineralization
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCITE PRECIPITATION, MICROBIALY INDUCED

Tanja Bosak

Massachusetts Institute of Technology, Cambridge, MA, USA

Definition

Microbially induced calcite precipitation describes the formation of calcium carbonate minerals from a solution due to the presence of microbial cells, biosynthetic products, or metabolic activity.

Calcium carbonate precipitation

The most basic requirement for the precipitation of calcium carbonate (CaCO_3) minerals, calcite, and aragonite is that the product of the concentrations of calcium $[\text{Ca}^{2+}]$ and carbonate ions $[\text{CO}_3^{2-}]$ exceeds the solubility product of calcite (Equation 1) and aragonite, respectively.

$$[\text{Ca}^{2+}][\text{CO}_3^{2-}] > 10^{-8.35} \quad (1)$$

The solubility of carbonate minerals depends on the temperature and pressure, decreasing with increasing temperatures and increasing with the increasing pressure. When a solution is in equilibrium with carbon dioxide, $[\text{CO}_3^{2-}]$ is determined by pH. In solutions that are undersaturated or not highly saturated, such as modern seawater, the biological activity can strongly control the precipitation of CaCO_3 . Biological processes exert considerably less control on the precipitation of CaCO_3 in environments characterized by high temperature, high pH, and high evaporation and degassing rates such as soda lakes, hot springs, caves, and freshwater tufas.

Microbial processes that promote the precipitation of CaCO_3

Culture-dependent and culture-independent studies have shown that microbes (Bacteria and Archaea) can induce extracellular precipitation of calcium carbonate minerals by:

- Increasing the local pH and the concentration of carbonate ion
- Promoting the nucleation of calcium carbonate minerals and removing the kinetic inhibitors of CaCO_3 precipitation.

Increase in local pH and the concentration of carbonate ion

Because the concentration of carbonate ion increases with the increasing pH, the precipitation of calcium carbonate minerals will also increase with the increasing pH. Many microbial metabolic processes can increase the pH and/or the concentration of carbonate ions: sulfate reduction in marine sediments (Dupraz and Visscher, 2005; Van Lith et al., 2003; Visscher et al., 2000), oxygenic

photosynthesis in freshwater streams and lakes (Merz-Preiß and Riding, 1999; Thompson et al., 1997), anaerobic methane oxidation coupled with sulfate reduction in marine sediments (Reitner et al., 2005), denitrification, degradation of amino-acids and small organic acids (Chekroun et al., 2004), and degradation of calcium oxalate in soils (Braissant et al., 2002). Whether microbes are able to significantly increase local pH and $[\text{CO}_3^{2-}]$ will largely depend on the buffering of the medium by dissolved inorganic carbon. Microbial activity in poorly buffered solutions such as lakes and streams is more likely to influence the saturation state of CaCO_3 (Merz-Preiß and Riding, 1999) than in well-buffered solutions such as soda lakes, hot springs, and solutions analogous to the early soda oceans (Arp et al., 1999a, b; Bosak and Newman, 2005).

Precipitation of calcium carbonate minerals also can be inhibited by the adsorption of various metabolites such as phosphate, sulfate, and organic moieties onto the faces of the growing crystals (Bosak and Newman, 2005; Liu and DeYoreo, 2004; Plant and House, 2002; Teng et al., 1998). Microbial growth and metabolic processes that lower the concentration of these ions in the solution can promote the precipitation of minerals even when they do not significantly influence pH and $[\text{CO}_3^{2-}]$ (Bosak and Newman, 2005; Kawaguchi and Decho, 2002).

Nucleation of calcium carbonate minerals on organic surfaces

Minerals can nucleate either homogeneously on the surface of preexisting carbonate grains, or heterogeneously on organic or other surfaces. When the external calcium carbonate sediment is sparse or absent, nucleation is the most energetically expensive step in the precipitation of calcium carbonate minerals. Organic matter can lower the free energy barrier required to form the initial nuclei, thus promoting mineral formation. Nucleation of CaCO_3 on organic surfaces (i.e., heterogeneous nucleation) occurs when negatively charged surface groups such as carboxylate, phosphate, and sulfate complex bind calcium. Although this process can initially inhibit the precipitation of minerals, once the local concentrations of calcium and carbonate has exceeded the binding capacity of the organic matrix, or when the matrix is partially degraded, mineral nucleation occurs preferentially on the organic template (Arp et al., 2003; Braissant et al., 2003; Decho et al., 2005). Heterogeneous nucleation can increase the number of crystal nuclei even when the bulk medium is supersaturated with calcium carbonate by more than an order of magnitude (Arp et al., 1999b; Bosak and Newman, 2003).

Heterogeneous nucleation of CaCO_3 can take place on the cell surfaces and envelopes, or within the exopolymeric matrix surrounding microbial cells. Examples of the former are the calcification of the sheaths surrounding cyanobacterial cells (Merz-Preiß and Zankl, 1993) and the whitening events caused by the precipitation of calcite on the outer S-layer of some planktonic

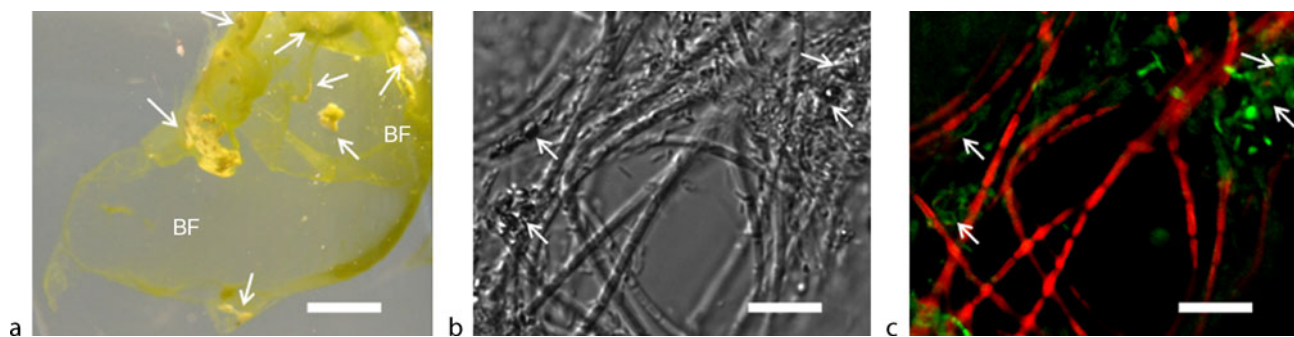
cyanobacteria (Thompson et al., 1997). These processes are most commonly observed in freshwater environments and require actively photosynthesizing cyanobacteria (Thompson et al., 1997). Cyanobacteria encrusted by CaCO_3 are considerably less abundant in modern marine calcium carbonates (Arp et al., 2001). Similarly, the relationship between marine whittings and cyanobacterial presence and activity has not been demonstrated conclusively (Thompson, 2000). Instead, in marine photic zones, carbonate minerals precipitate mainly within the extracellular exopolymeric matrix in areas of heterotrophic degradation (Chafetz and Buczynski, 1992; Dupraz and Visscher, 2005; Gautret et al., 2006; Van Lith et al., 2003; Visscher et al., 2000). In addition to the nucleation on exopolymeric substances or cell surfaces, CaCO_3 also preferentially nucleates around submicron vesicles that are secreted by sulfate-reducing bacteria (Aloisi et al., 2006). The nucleation of CaCO_3 on these vesicles may be a common mechanism in the environment, because outer-membrane vesicles are secreted by many Gram-negative bacteria (Beveridge, 1999; Kuehn and Kesty, 2005).

Recognition of microbially induced CaCO_3 precipitates in the rock record

Even when microbes do not induce measurable changes in the chemical composition of the bulk solution, they can alter the pH and carbonate chemistry, remove kinetic inhibitors, and promote mineral nucleation and precipitation at a microscale (Figure 1). When minerals precipitate, surrounded by microbial cells and microbial aggregates (biofilms) (Figure 1), they can preserve remnants of organic matter, such as acidic amino acids and microbial casts, long after the original cells have been degraded (Bosak et al., 2004a; Sprachta et al., 2001). Furthermore, CaCO_3 precipitated in the presence of organic compounds often has distinct shapes (Bosak and Newman, 2005; Braissant et al., 2003; Chafetz and Buczynski, 1992; Teng et al., 1998), although similar shapes can also be produced abiotically.

Biological influence on the morphology of carbonate crystals and structures is most prominent in skeleton-building organisms. Macroscopic and microscopic eukaryotic organisms such as calcareous algae, protists, corals, and mollusks precipitate hard body parts. Many of these organisms precipitate CaCO_3 by genetically encoded mechanisms that result in shells, skeletons, and carbonate grains with species-specific shapes. In contrast to these products of biologically controlled calcification, the shapes of microbially induced carbonate minerals can be distinguished from abiotically precipitated grains rarely (Golubic et al., 2000). Consequently, the potential identification of biosignatures of microbially induced carbonates in modern and past sedimentary carbonates can be controversial (Grotzinger and Knoll, 1999).

Recognition of microbially induced carbonates is also impeded by the difficulty in distinguishing between CaCO_3 structures that grew *in the presence* of microbes

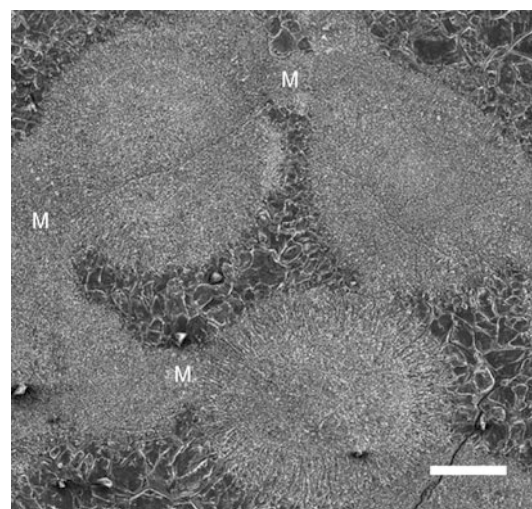


Calcite Precipitation, Microbially Induced, Figure 1 Aragonite precipitated on cyanobacterial biofilms (BF) floating in solution. (a) Photograph of a thin cyanobacterial biofilm with visible aragonite precipitates that preserve the biofilm. White arrows mark the densest mineral aggregates. (b) Transmitted-light micrograph of cyanobacterial filaments trapping small mineral grains (white arrows) in lightly mineralized areas. (c) Epifluorescence micrograph (deconvolved) of the same field of view as in (b). Cyanobacterial filaments fluoresce red and the nuclei of other microbes are stained green. Note that the mineral grains are surrounded by microbial aggregates that are not cyanobacteria (and are, presumably, heterotrophic). Scale bar: 5 mm in a, 5 μ m in b and c.

or organic matter from structures that grew *because* of microbial activity. Some examples of carbonate grains and structures with often ambiguous origin include peloids, ooids (Figure 2), whittings, fossil stromatolites, and nanobacteria. Recent developments in analytical techniques such as X-ray microscopy have enabled the detection of small quantities of fossil organic matrices in fossil carbonates at the microscale, thereby establishing that some fossil carbonates grew in the presence of microbes (Benzerara et al., 2006; Lepot et al., 2008). Despite the inclusion of organic matter, the role of biology in forming these structures remains unknown. As an alternative approach, experimental and quantitative models of the morphogenesis of sedimentary structures at the macroscale and microscale have been developed in an attempt to understand which fossil structures and shapes require biological processes (Batchelor et al., 2004; Bosak et al., 2004b; Dupraz et al., 2006; Grotzinger and Rothman, 1996; Reid et al., 2000; Suess and Futterer, 1972).

Summary

The potential of microbes to induce precipitation of calcium carbonate has sparked scientific interest for almost a century. The calcium carbonate (CaCO_3) minerals, calcite and aragonite, often precipitate in association with microbial cells and biofilms in marine and terrestrial sedimentary environments such as reefs, lakes, streams, beaches, soils, hot springs, and caves. Being ubiquitous and relatively insoluble, these minerals have helped preserve bacterial and archaeal cells and biofilms throughout geologic time (Figure 1). Microbes promote the precipitation of calcium carbonate metabolically, by increasing the pH and the alkalinity of their surroundings, or by the secretion of substances that facilitate the mineral nucleation. The recognition of microbially influenced calcium carbonate precipitates in the rock record is often challenging because abiotic carbonate precipitation can produce



Calcite Precipitation, Microbially Induced, Figure 2 An example of carbonate grains of controversial origin. Scanning electron micrograph of a thick polished section of late Jurassic ooids (rounded grains with layered interior structure) precipitated on the Adriatic Platform, Croatia, in low-energy, shallow water settings (Husinec and Reid, 2006). Ooids from low-energy environments were commonly interpreted as largely inorganic precipitates that adsorb organic molecules (Davies et al., 1978). More recently, cyanobacteria and other microbes have been shown to contribute to the formation of low-energy ooids and meniscus cements (M) (Brehm et al., 2006; Hillgartner et al., 2001; Plee et al., 2008). Although microbes may have contributed to the formation of ooids shown here, these grains do not preserve any textural evidence of the former presence of microorganisms. Scale bar: 100 μ m.

crystals with similar shapes and chemical signatures. Current research in the field of microbial carbonates tackles this challenge by conducting culture-dependent and culture-independent studies of microbial mineral precipitation, employing analytical techniques that can detect

small quantities of organic matter in fossil carbonates, and by developing theoretical models that explain the shape of macroscopic carbonate sedimentary structures. Microbially induced precipitation of carbonate minerals is likely to garner even more interest in the future with a primary focus on carbon sequestration, the production of biomimetic materials, and the conservation of ornamental stone.

Bibliography

- Aloisi, G., Gloter, A., Kruger, M., Wallmann, K., Guyot, F., and Zuddas, P., 2006. Nucleation of calcium carbonate on bacterial nanoglobules. *Geology*, **34**, 1017–1020.
- Arp, G., Reimer, A., and Reitner, J., 1999a. Calcification in cyanobacterial biofilms of alkaline salt lakes. *European Journal of Phycology*, **34**.
- Arp, G., Thiel, V., Reimer, A., Michaelis, W., and Reitner, J., 1999b. Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. *Sedimentary Geology*, **126**, 159–176.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Batchelor, M. T., Burne, R. V., Henry, B. I., and Jackson, M. J., 2004. A case for biotic morphogenesis of coniform stromatolites. *Physica A-Statistical Mechanics and Its Applications*, **337**, 319–326.
- Benzerara, K., Menguy, N., Lopez-Garcia, P., Yoon, T.-H., Kazmierczak, J., Tylliszczak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *Proceedings of the National Academy of Sciences*, **103**, 9440–9445.
- Beveridge, T. J., 1999. Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of Bacteriology*, **181**, 4725–4733.
- Bosak, T., and Newman, D. K., 2003. Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, **31**, 577–580.
- Bosak, T., and Newman, D. K., 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. *Journal of Sedimentary Research*, **75**, 190–199.
- Bosak, T., Souza-Egipsy, V., Corsetti, F. A., and Newman, D. K., 2004a. Micrometer-scale porosity as a biosignature in carbonate crusts. *Geology*, **32**, 781–784.
- Bosak, T., Souza-Egipsy, V., and Newman, D. K., 2004b. An abiotic model for peloid formation. *Geobiology*, **2**, 189–198.
- Braissant, O., Cailleau, G., Dupraz, C., and Verrecchia, E. P., 2003. Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids. *Journal of Sedimentary Research*, **73**, 485–490.
- Braissant, O., Verrecchia, E., and Aragno, M., 2002. Is the contribution of bacteria to terrestrial carbon budget greatly underestimated? *Naturwissenschaften*, **89**, 366–370.
- Brehm, U., Krumbein, W. E., and Palinska, K. A., 2006. Biomicrospheres generate ooids in the laboratory. *Geomicrobiology Journal*, **23**, 545–550.
- Chafetz, H. S., and Buczynski, C., 1992. Bacterially induced lithification of microbial mats. *Palaos*, **7**, 277–293.
- Chekroun, K. B., Rodriguez-Navarro, C., Gonzalez-Munoz, M. T., Arias, J. M., Cultrone, G., and Rodriguez-Gallego, M., 2004. Precipitation and growth morphology of calcium carbonate induced by *Myxococcus xanthus*: implications for recognition of bacterial carbonates. *Journal of Sedimentary Research*, **74**, 868–876.
- Davies, P. J., Bubela, B., and Ferguson, J., 1978. The formation of ooids. *Sedimentology*, **25**, 703–730.
- Decho, A. W., Visscher, P. T., and Reid, R. P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography Palaeoclimatology Palaeoecology*, **219**, 71–86.
- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Dupraz, C., Pattisina, R., and Verrecchia, E. P., 2006. Translation of energy into morphology: simulation of stromatolite morphospace using a stochastic model. *Sedimentary Geology*, **185**, 185–203.
- Gautret, P., De Wit, R., Camoin, G., and Golubic, S., 2006. Are environmental conditions recorded by the organic matrices associated with precipitated calcium carbonate in cyanobacterial microbialites? *Geobiology*, **4**, 93–107.
- Golubic, S., Seong-Joo, L., and Browne, K., 2000. Cyanobacteria: architects of sedimentary structures. In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth and Planetary Sciences*, **27**, 313–358.
- Grotzinger, J. P., and Rothman, D. H., 1996. An abiotic model for stromatolite morphogenesis. *Nature*, **383**, 423–425.
- Hillgartner, H., Dupraz, C., and Hug, W., 2001. Microbially induced cementation of carbonate sands: are micritic meniscus cements good indicators of vadose diagenesis? *Sedimentology*, **48**, 117–131.
- Husinec, A., and Reid, J. F., 2006. Transgressive oversized radial ooid facies in the Late Jurassic Adriatic Platform interior: low-energy precipitates from highly supersaturated hypersaline waters. *GSA Bulletin*, **118**, 550–556.
- Kawaguchi, T., and Decho, A. W., 2002. A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO₃ polymorphism. *Journal of Crystal Growth*, **240**, 230–235.
- Kuehn, M., and Kesty, N., 2005. Bacterial outer membrane vesicles and the host–pathogen interaction. *Genes and Development*, **19**, 2645–2655.
- Lepot, K., Benzerara, K., Brown, G. E., and Philippot, P., 2008. Microbially influenced formation of 2,724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Liu, X. Y., and DeYoreo, J. J., 2004. *Nanoscale Structure and Assembly at Solid-Fluid Interfaces*. Boston: Kluwer Academic Publishers.
- Merz-Pleiß, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams: ambient environment, chemical thresholds and biological processes. *Sedimentary Geology*, **126**, 103–124.
- Merz-Pleiß, M., and Zankl, H., 1993. The influence of the sheath on carbonate precipitation by cyanobacteria. *Bollettino della Società Paleontologica Italiana*, (Spec Vol), pp. 325–331.
- Plant, L. J., and House, W. A., 2002. Precipitation of calcite in the presence of inorganic phosphate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **203**, 143–153.
- Plee, K., Ariztegui, D., Martini, R., and Davaud, E., 2008. Unravelling the microbial role in ooid formation - results of an in situ experiment in modern freshwater Lake Geneva in Switzerland. *Geobiology*, **6**, 341–350.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., Macintyre, L. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early

- lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Sprachta, S., Camoin, G., Golubic, S., and Le Campion, T., 2001. Microbialites in a modern lagoonal environment: nature and distribution, Tikehau atoll (French Polynesia). *Palaeogeography Palaeoclimatology Palaeoecology*, **175**, 103–124.
- Suess, E., and Futterer, D., 1972. Aragonitic ooids 1: experimental precipitation from seawater in the presence of humic acid. *Sedimentology*, **19**, 129–139.
- Teng, H. H., Dove, P. M., Orme, C. A., and De Yoreo, J. J., 1998. Thermodynamics of calcite growth: baseline for understanding biomineral formation. *Science*, **282**, 724–727.
- Thompson, J. B., 2000. Microbial whittings, In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin Heidelberg: Springer, pp. 250–260.
- Thompson, J. B., Schultze-Lam, S., Beveridge, T. J., and DesMarais, D. J., 1997. Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial phytoplankton. *Limnology and Oceanography*, **42**, 133–141.
- Van Lith, Y., Warthmann, R., Vasconcelos, C., and McKenzie, J. A., 2003. Sulphate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology*, **1**, 71–79.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.

Cross-references

Alkalinity
 Algae (Eukaryotic)
 Animal Skeletons, Advent
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Carbonate Environments
 Carbonates
 Cold Seeps
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Hydrothermal Environments, Terrestrial
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Nan(n)obacteria
 Organomineralization
 Pedogenic Carbonates
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCIUM BIOGEOCHEMISTRY

Anton Eisenhauer
 IFM-GEOMAR, Christian Albrechts Universität zu Kiel,
 Kiel, Germany

Definition

Calcium (Ca) has the atomic number 20 and is an alkaline earth metal with an atomic mass of 40.978 amu. The

important role of Ca in biogeochemical processes is based on its chemical versatility, which is related to its highly adaptable coordination geometry, its divalent charge, modest binding energies, fast reaction kinetics, and its inertness in redox reactions (Williams, 1974). Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element in the Earth's crust and the seventh most abundant element in the ocean. It is essential for living organisms, particularly in cell physiology, shell formation, and calcification and hence, is usually the most common metal in many animals.

Calcium isotopes and their application in Biogeochemistry

There are seven Ca isotopes present in nature of which three isotopes (^{42}Ca (0.646%), ^{43}Ca (0.135%) through ^{44}Ca (2.086%)) are stable, one isotope (^{40}Ca (69.941%)) is radiogenic, one isotope (^{41}Ca) is cosmogenic, and two more isotopes (^{46}Ca (0.004%) and ^{48}Ca (0.187%)) are radioactive having extremely long half-lives (^{46}Ca : $2.8 \cdot 10^{15}$ years; ^{48}Ca : $> 4 \cdot 10^{19}$ years). The cosmogenic isotope ^{41}Ca is radioactive (half live of 103,000 years) and is produced by neutron activation of ^{40}Ca in the upper soil column. ^{41}Ca has received much attention in stellar studies because it decays to ^{41}K , a critical indicator of solar system anomalies. ^{40}Ca is the radiogenic daughter product of ^{40}K decay, along with ^{40}Ar . While K–Ar dating has been used extensively in the geological sciences, the prevalence of ^{40}Ca in nature has impeded its use in dating. Techniques using mass spectrometry and a double spike isotope dilution have been used for K–Ca age dating (Nägler and Villa, 2000).

Natural Ca isotope fractionation has, among other isotope systems such as oxygen (d^{18}O) and carbon (d^{13}C) fractionation, been applied for the study of calcification processes and the so-called vital effect (c.f. Gussone et al., 2003; Lemarchand et al., 2005). Latter effect describes the deviation of the expected inorganic isotope fractionation value or element partitioning coefficient (e.g., Sr/Ca, Mg/Ca, etc.) from the measured one in calcifying organisms such as coccolithophores, foraminifer, corals, and other calcifying organisms.

The more or less species-dependent Ca isotope fractionation of marine calcifying organisms as a function of the adjacent seawater temperature can be applied for the reconstruction of the dynamic of Ca concentrations throughout the Earth's history (c.f. Griffith et al., 2008; Farkas et al., 2007; Heuser et al., 2005; Fantle and DePaolo, 2005; De LaRocha and DePaolo, 2000) and for the reconstruction of past sea surface temperatures (c.f. Nägler et al., 2000; Hippler et al., 2006).

The dynamic of calcium in the ocean

Calcium is the second most abundant divalent cation (~ 10 mmol/l), besides magnesium (Mg, ~ 52 mmol/l). Calcium is considered to be a conservative element in the ocean water column, with a residence time of about

CAP CARBONATES

Joachim Reitner
University of Göttingen, Göttingen, Germany

Definition

Cap carbonates are unusual deposits of dolostones and limestones, often enriched in barite, that sharply overlie Neoproterozoic glacial deposits. They are from 2 to 50 m thick and occur on shallow platforms, shelves, and



Cap Carbonates, Figure 1 Marinoan ca. 635 My cap carbonate from Namibia (Fransfontein, Otavi Mountains). Characteristic features of this cap carbonate (mainly dolomite) are syndimentarily formed Zebra Limestone-type fabrics. The horizontal fractures were originally cemented with large aragonitic crystals. The remaining pore spaces are filled up with cherts.



Cap Carbonates, Figure 2 Sturtian ca. 750 My old cap carbonate from Namibia (Rasthof cap carbonate, Baesvalkte). These black laminated carbonates are lithified microbial mats. Very common are “roll up structures” which are known from many microbial mat systems, even from modern ones.

slopes worldwide, even in regions otherwise lacking in carbonate strata.

Glaciation events during the late Neoproterozoic (Cryogenian–Ediacaran) are widespread on Earth, reflecting up to four global ice ages (“Snowball Earth Hypothesis”) between 730 and 580 Ma. Numerous publications dealing with these events exist (for example, see Chapter by Hoffman P.F., Snowball Earth, this volume; Shields, 2005; Jiang et al., 2003). Some of them exhibit characteristic, microcrystalline cap dolostones, and also limestones, which is unique and reflects a climate paradox. Particularly the Marinoan glaciation ca. 635 Ma ago is peculiar, because it provides evidence for low altitude glaciation at equatorial latitudes. Three models have been proposed to explain an increase in alkalinity inducing these global carbonate formation events:

(1) Overturn of an anoxic deep ocean; (2) enormous rates of chemical weathering in the hinterland after glaciation (Snowball Earth Hypothesis); (3) destabilization of methane clathrates and increase of alkalinity caused by anaerobic oxidation of methane (AOM). However, none of the hypotheses sufficiently explains the consistent sequence of precipitation events observed within cap carbonate successions. The formation of massive dolostones is very problematic and hardly understood. Massive microbial sulfate reduction may explain this event. Barite precipitation is also very common. The source of sulfate is most probably the weathered hinterland. Caused by seawater oversaturated with aragonite, diagenetically very early large aragonite crystals were formed. It is suggested that cap carbonates are primarily formed by a microbially mediated precipitation. Possibly, the highly oxygenated shallow marine environments of the postglacial Neoproterozoic oceans provided excellent conditions for the evolutionary development of new microbial communities, phototrophic organisms, and of course, the rapid development of Metazoa (Figures 1 and 2).

Bibliography

- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopes evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Shields, G. A., 2005. Neoproterozoic cap carbonates: a critical appraisal of existing models and plume world hypothesis. *Terra Nova*, **17**, 299–310.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Animal Biocalcification, Evolution](#)
[Calcite Precipitation, Microbially Induced](#)
[Calcium Biogeochemistry](#)
[Carbonate Environments](#)
[Cold Seeps](#)
[Dolomite, Microbial](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Permafrost Microbiology](#)
[Snowball Earth](#)

CARBON (ORGANIC, CYCLING)

Amber K. Hardison, Elizabeth A. Canuel
Virginia Institute of Marine Science, Gloucester Point,
VA, USA

Definition

Organic carbon (OC) refers to any carbon that occurs in compounds of ultimately biological origin that can be oxidized.

Introduction

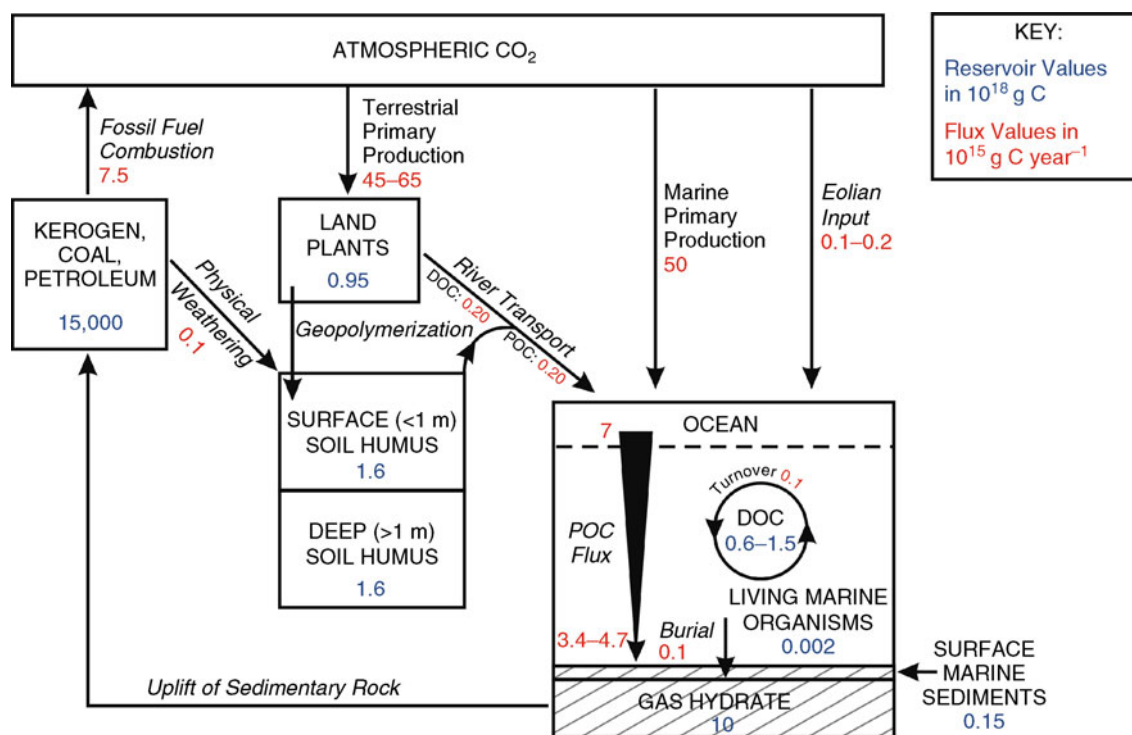
Carbon is made up of organic and inorganic fractions, and the biochemical and geochemical cycling of these forms through the Earth processes is referred to as the global carbon cycle. The global carbon cycle is closely linked to other global cycles such as oxygen, nitrogen, and sulfur because of the important role these elements play in biological processes. Organic carbon (OC) is present in both living and nonliving systems and ultimately fuels all of the Earth's biogeochemical processes (Summons, 1993); therefore, understanding the formation, cycling, and preservation of OC is essential to understanding the cycling of

all bioelements between living and nonliving reservoirs, the formation of economically important petroleum and coal deposits, and predicting how human activity may change the natural balance of these systems.

Much of the OC created during photosynthesis or by chemosynthetic autotrophs is decomposed by heterotrophic organisms. Heterotrophic processes occur in the water column of marine and aquatic environments, as well as in sediments, soils, and rocks. Much of the decomposition of organic matter (OM) occurs under low temperature (<50°C) and pressure during diagenesis. Diagenesis occurs primarily through biological processes (microbes and larger organisms that live in surface sediments) and over depths ranging from microns to hundreds of meters. The OC preserved following diagenesis is subject to further alteration by the processes of catagenesis and metagenesis, which occur at higher temperatures and pressures and lead to the formation of petroleum and natural gas.

Reservoirs

The cycling of OC involves fluxes to and from the major OC reservoirs (Figure 1). Sedimentary rocks are the largest OC reserve, making up approximately 99.5% of the



Carbon (Organic, Cycling), Figure 1 The global organic carbon (OC) cycle, adapted from Hedges (1992). The OC cycle involves fluxes to and from the major surficial and deep reservoirs. Reservoirs are denoted by capital letters, with values in blue. Fluxes due to physical processes (italicized) and primary production (not italicized) are denoted with *arrows*. Flux terms are presented in lower case font, with values given in red. Information presented in this figure is based on published values from Schlesinger (1991), Longhurst (1995), Hedges et al. (1997), Kvenvolden (2002), Richey (2004), IPCC (2007).

total global OC. Despite the relative size of this reservoir, OC only represents one-fifth of the total sedimentary carbon, since inorganic carbon is the dominant fraction (Hedges, 1992). Sedimentary OC occurs primarily as kerogen, coal beds, and petroleum reservoirs, which ultimately come from biogenic processes or sources. Kerogen is an amorphous, highly insoluble particulate OM that is thought to form by two pathways – selective preservation and geopolymerization, both of which occur under low temperature and pressure conditions (Killops and Killops, 2005). Selective preservation involves the formation of kerogen from biomacromolecules that are resistant to microbial degradation. Geopolymerization occurs through a random series of condensation and polymerization reactions involving humic substances. Petroleum is defined as naturally occurring fluid (liquid and gases) that evolves from kerogen. Petroleum is formed primarily from the remains of microbes (algae and bacteria), although some types of petroleum are formed from higher plants. In contrast, coal is a solid and is formed from the remains of higher plants. The most common type of coals, humic coals, is formed through the processes of peatification and coalification, which involve both biological and geochemical processes (Killops and Killops, 2005). The OC in ancient sedimentary rocks provides these economically important fossil fuels as well as a molecular record of life, which has allowed the construction of evolutionary trees and an understanding of the geologic timing of past events and the response of natural systems to tectonic, environmental, and biological change (Brocks and Pearson, 2005). Historically, sedimentary OC affects biologically mediated cycles on geologic time scales, although humans have recently accelerated this cycle through fossil fuel combustion (7.5×10^{15} g C year⁻¹; IPCC, 2007).

The active (surficial) pools of OC include soil humus, land and marine plant tissue, seawater dissolved OC (DOC, <0.5 μ m diameter), and surface marine sediments and gas hydrates (Kvenvolden, 2002; Hedges, 1992). Dead and living particulate OC (>0.5 μ m) are also present in freshwater and seawater pools; however, the DOC pool dominates. With the exception of plant tissue and gas hydrates, most of the active pools of OC are poorly characterized. Terrestrial plant tissue can be further classified as woody biomass (~75%), nonwoody biomass (~15%), and litter (~10%) (Olson et al., 1985). Gas hydrates contain mostly methane and some ethane molecules, and may serve as a large reservoir for OC, although global estimates of the size of this sink are still uncertain (see Kvenvolden, 2002). Soil OM is made up of plant remains (detritus), soil microbes, and humic substances.

Production and fluxes

The major fluxes between organic pools are driven by marine and terrestrial primary production. Photosynthesis, the harvesting of light energy to convert inorganic carbon

to reduced (organic) forms in the tissue of plants, is the primary mode of primary production. However, chemosynthesis by microbial organisms, which uses chemical energy rather than sunlight, is also important. Photosynthesis is carried out by prokaryotes such as cyanobacteria as well as eukaryotic algae and higher plants. The fixation of carbon by plants over the Earth's history accounts for the oxygen in our present atmosphere, thus the cycles of carbon and oxygen are closely linked over geologic time (Hedges, 2002; Hedges and Keil, 1995). In fact, to maintain current atmospheric oxygen levels, rapid cycling of carbon in the surficial pools is required. These pools exchange on relatively short timescales (10^2 – 10^4 years). In contrast, the OC that remains in the sedimentary system cycles slowly (~ 10^8 year; Schlesinger, 1991). Sedimentary reservoirs only become exchangeable through geologic uplift, followed by oxidative weathering of OC by exposure to the atmosphere.

Net global primary production (NPP) on land (45 – 65×10^{15} g C year⁻¹) is dominated by more labile nonwoody plant tissues such as leaves, grasses, and herbaceous annuals (Schlesinger, 1991). The highest gross terrestrial production (82×10^{15} g C year⁻¹) and storage as biomass (56%) occur in the tropics, while polar regions contribute the least (8×10^{15} g C year⁻¹, 0.5%; Taube, 1992). Within these regions, forests are the most productive continental biomes and deserts and tundra are the least (Taube, 1992), reflecting global patterns of temperature and precipitation. Marine primary productivity ($\sim 50 \times 10^{15}$ g C year⁻¹) is dominated by open water (pelagic) phytoplankton productivity because open water constitutes approximately 75% of the total ocean area (Ducklow and McCallister, 2005; Longhurst, 1995). However, when normalized to area, coastal and upwelling areas together account for nearly two thirds of the total oceanic productivity because a greater supply of nutrients is available to support phytoplankton in these areas (Longhurst, 1995). Other primary producers along the coasts (i.e., seagrass, marsh plants, macroalgae, mangroves) are locally important sources of OC.

Delivery of OC (as both dissolved and particulate fractions) from the land to coastal marine environments by rivers accounts for 0.4×10^{15} g C year⁻¹ (Meybeck, 1982; Ittekkot, 1988). However, there remains considerable uncertainty in our ability to adequately quantify carbon exchange from land to the coastal ocean and in our understanding of the processes influencing the fate of terrigenous carbon in coastal sediments (Berner, 1982; Sarmiento and Sundquist, 1992; Hedges and Keil, 1995; Schlünz and Schneider, 2000). Generally, the total annual riverine OC load to the ocean is proportional to the total annual river flow, which is dominated by a small set of rivers that transport the bulk of the fresh water from land to sea (Schlesinger, 1991). However, seasonal and annual/interannual variations in hydrology as well as large events such as storms and hurricanes influence the input, dispersion, and cycling of sediment OM in coastal environments, thus making it difficult to trace terrigenous OM.

In addition, recent molecular and isotopic evidence suggests that OC in seawater and marine sediments is marine, rather than terrestrial, in nature, which suggests that further remineralization of terrestrial material must occur in rivers prior to delivery to the coastal ocean (Schl nz and Schneider, 2000; Richey, 2004; Mayorga et al., 2005) or in the marine environment (Hedges et al., 1997). This is an area requiring further research. Eolian (air) flux from the land to the sea approximates 0.1×10^{15} g C year⁻¹, although this estimate remains uncertain due to methodological difficulties (Romankevich, 1984; Zafiriou et al., 1985).

Decomposition and preservation

Only ~0.1% of global NPP is ultimately preserved within sediments; however, it is this OC that will eventually become part of the sedimentary record and may be transformed into fossil fuels. The remaining 99.9% of global NPP is remineralized to inorganic carbon, nutrients, and water. Remineralization represents a sink for oxygen, linking the global cycles of OC and oxygen. Microfauna, bacteria and fungi (on land) are responsible for the breakdown of OM. Bacterial and fungal decomposition of OM involves a series of steps; macromolecules must first be broken down using extracellular enzymes into dissolved polymers, which are then broken down into low molecular weight monomers (Tissot and Welte, 1984).

The rates and extent of decomposition are influenced by environmental, biological, and physical factors. First, there must be a sufficient supply of labile OM to be degraded. The depth-integrated rate of OM remineralization in marine sediments correlates closely with carbon flux to the sediment surface (Henrichs, 1993). Interestingly, the fraction of OC that is *not* remineralized is also highly correlated with the carbon flux to the sediment surface. Thus, the efficiency of OM decomposition appears to be lower in systems that receive the highest input of OC compared to systems with lower accumulation rates (Henrichs and Reeburgh, 1983). Second, like most metabolic processes, decomposition rates are proportional to temperature, resulting in global patterns of decomposition rates (e.g., higher degradation rates in the tropics than in tundra systems) (Schlesinger, 1991). Oxygen is also a controlling factor for decomposition. Recent evidence suggests that oxygen exposure time, rather than oxygen concentration, may be an important determinant of the extent to which OM is decomposed (Hartnett et al., 1998). OM decomposition occurs under both oxic and anoxic conditions; however, the rates and bacterial communities involved in the processes differ. Oxygen is used preferentially as the terminal electron acceptor during decomposition and results in the most efficient mineralization (i.e., the highest energy yield). Once oxygen is depleted, bacteria utilize other electron acceptors such as sulfate and nitrate. Aerobic decomposition is facilitated

by microbes as well as higher organisms with a range in enzyme capabilities. Anaerobic decomposition is carried out exclusively by microorganisms, and complete remineralization is typically dependent on a consortium of cooperating organisms. Further, anaerobic decomposition processes differ in terrestrial and marine environments because sulfate is abundantly present in marine systems but limited in terrestrial systems. As a result, sulfate reduction is the dominant anaerobic pathway for oxidizing OM in marine systems, while fermentation and methanogenesis dominate in freshwater systems (Henrichs, 1993). The chemical and structural makeup of OM also plays a role in its susceptibility to decomposition (e.g., number of double bonds and functional group composition). For example, nitrogen-rich compounds such as proteins and polynucleotides are usually decomposed preferentially to carbon-rich structural compounds (Wakeham and Canuel, 2006). Additionally, the architecture of the OM may be an important control on its decomposition. Architecture refers to both physical structure (e.g., conformation) as well as OM associations with organic and inorganic matrices (see below).

Decomposition and preservation of OC are closely linked processes, since only OC that is not decomposed is available for preservation, but there are additional factors that may enhance preservation (Burdige, 2007; Wakeham and Canuel, 2006). For instance, the amount of OC that reaches the sediment surface and has the potential to be buried depends in part on productivity in the overlying photic zone and water depth. Sufficiently high productivity is essential for OC to reach the sediment with the potential for preservation (Killops and Killops, 2005). In addition, physical or chemical protection of OM plays a key role in controlling preservation. Protection by binding to inorganic ions, clays, and organic residues decreases the surface area of OM that is exposed to bacteria for degradation. This protection or encapsulation of OM explains why compounds that appear to be labile, or readily available for decomposition, have been found in sedimentary OC deposits. Lastly, the amount of time that OM is exposed to oxic conditions determines the extent to which oxygen-sensitive compounds, compounds that specifically require oxygen for degradation, are preserved (Burdige, 2007).

Preservation of OC occurs predominantly in anoxic marine sediments. More than 80% of preserved OC is located in deltaic-shelf environments associated with fine grain sediments (Bernier, 1982) that support the physical or chemical protection model, since these sediments provide the most potential surface area with which OM can bind. This has likely been true for millions of years, as current petroleum deposits are found in areas that were formerly coastal, fine grained sediments. Major depositional environments include lacustrine environments (open, closed lakes), peat swamps and coal, and continental margins, enclosed or silled basins, and the Black Sea (Killops and Killops, 2005).

Conclusion

The global OC cycle involves exchange of OC between surface and subsurface (buried) reservoirs on time scales of less than one year to millions of years. These fluxes are driven by the production and cycling of OC through biological, physical, and geological processes. The global OC cycle is intimately linked with other global cycles and is sensitive to human-induced perturbations. Humans may have reduced total NPP by up to 40% due to wildfires and pollution, which may be the largest sink for primary production to support a single species in the history of life on the Earth (Vitousek et al., 1986). Fossil fuel combustion and destruction of forests (especially tropical) have decreased the global pool of OC and increased atmospheric carbon dioxide, which has been linked to global climate change. Global atmospheric concentrations of CO₂, methane (CH₄), and nitrous oxide (N₂O) have increased markedly as a result of human activities since preindustrial times (i.e., 1,750). In 2005, concentrations of atmospheric CO₂ (379 ppm) and CH₄ (1,774 ppb) exceeded the natural range over the last 650,000 years (IPCC, 2007). Understanding the changes in the size and composition of the OC reservoir through space and time will help us to better predict the changes in its global cycle as well as the associated cycles of other biologically important elements.

Bibliography

- Berner, R. A., 1982. Burial of organic carbon and pyrite sulfur in the modern ocean: its geochemical and environmental significance. *American Journal of Science*, **282**, 451–473.
- Brocks, J., and Pearson, A., 2005. Building the biomarker tree of life. In Banfield, J. F., Cervinisilva, J., and Neelson, K. M. (eds.), *Molecular Geomicrobiology. Reviews in Mineralogy and Geochemistry*. Chantilly, VA: Mineralogical Society of America and Geochemical Society, Vol. 59, pp. 233–258.
- Burdige, D. J., 2007. Preservation of organic matter in marine sediments. *Chemical Reviews*, **107**, 467–485.
- Ducklow, H. W., and McCallister, S. L., 2005. The biogeochemistry of carbon dioxide in the coastal oceans. In Robinson, A. R., and Brink, K. (eds.), *The Sea, Volume 13, The Global Coastal Ocean – Multiscale Interdisciplinary Processes*. New York: Wiley, pp. 269–315.
- Ducklow, H. W., Purdie, D. A., LeB. Williams, P. J., and Davies, J. M., 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science*, **232**, 865–867.
- Hartnett, H. E., Keil, R. G., Hedges, J. I., and Devol, A. H., 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. *Nature*, **391**, 572–574.
- Harvey, H. R., Tuttle, J. H., and Bell, J. T., 1995. Kinetics of phytoplankton decay during simulated sedimentation - changes in biochemical-composition and microbial activity under oxic and anoxic conditions. *Geochimica et Cosmochimica Acta*, **59**, 3367–3377.
- Hedges, J. I., 1992. Global biogeochemical cycles: progress and problems. *Marine Chemistry*, **39**, 67–93.
- Hedges, J. I., 2002. Sedimentary organic matter preservation and atmospheric O₂ regulation. In Gianguzza, A., Pelizzetti, E., and Sammartano, S. (eds.), *Chemistry of Marine Water and Sediments*. Berlin: Springer, 508 pp.
- Hedges, J. I., and Keil, R. G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. *Marine Chemistry*, **49**, 81–115.
- Hedges, J. I., Keil, R. G., and Benner, R., 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry*, **27**, 195–212.
- Henrichs, S. M., 1993. Early diagenesis of organic matter: the dynamics (rates) of cycling of organic compounds. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 101–117.
- Henrichs, S. M., and Reeburgh, W. S., 1983. Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiology*, **5**, 191–237.
- IPCC, 2007. Climate Change 2007: The physical science basis. In Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L. (eds.), *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. New York: Cambridge University Press, 996 pp.
- Ittekkot, V., 1988. Global trends in the nature of organic matter in river suspensions. *Nature*, **332**, 436–438.
- Killops, S. D., and Killops, V. J., 2005. *An Introduction to Organic Geochemistry*. Oxford: Blackwell.
- Kvenvolden, K. A., 2002. Methane hydrate in the global organic carbon cycle. *Terra Nova*, **14**, 302–306.
- Longhurst, A., 1995. Seasonal cycles of pelagic production and consumption. *Progress in Oceanography*, **36**, 77–167.
- Mayorga, E., Aufdenkampe, A. K., Masiello, C. A., Krusche, A. V., Hedges, J. I., Quay, P. D., Richey, J. E., and Brown, T. A., 2005. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature*, **436**, 539–541.
- Meybeck, M., 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science*, **282**, 401–450.
- Olson, J. S., Garrels, R. M., Berner, R. A., Armentano, T. V., Dyer, M. I., and Yaalon, D. H., 1985. The natural carbon cycle. In Trabalka, J. R. (ed.), *Atmospheric Carbon Dioxide and the Global Carbon Cycle*. Washington, DC: U.S. Department of Energy, pp. 175–213.
- Richey, J. E., 2004. Pathways of atmospheric CO₂ through fluvial systems. In Fields, C. (ed.), *Toward CO₂ Stabilization: Issues, Strategies, and Consequences, A SCOPE/GCP Rapid Assessment Project*. Washington, DC: Island Press, pp. 329–340.
- Romankevich, E. A., 1984. *Geochemistry of Organic Matter in the Ocean*. Berlin: Springer.
- Sarmiento, J. L., and Sundquist, E. T., 1992. Revised budget for the oceanic uptake of anthropogenic carbon dioxide. *Nature*, **365**, 589–593.
- Schlesinger, W. H., 1991. *Biogeochemistry: An Analysis of Global Change*. San Diego, CA: Academic.
- Schlünz, B., and Schneider, R. R., 2000. Transport of terrestrial organic carbon to the oceans by rivers: re-estimating flux and burial rates. *International Journal of Earth Sciences*, **88**, 599–606.
- Summons, R. E., 1993. Biogeochemical cycles: a review of fundamental aspects of organic matter formation, preservation, and composition. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 3–21.
- Taube, M., 1992. Evolution of matter and energy. In Craig, P., and Hutzinger, O. (eds.), *The Natural Environment and the Biogeochemical Cycles*. New York: Springer, pp. 200.
- Tissot, B. P., and Welte, D. H., 1984. *Petroleum Formation and Occurrence*. Berlin: Springer, pp. 699.
- Vitousek, P., Ehrlich, P. R., Ehrlich, A. H., and Matson, P. A., 1986. Human appropriation of the products of photosynthesis. *BioScience*, **36**, 368–373.

- Wakeham, S. G., and Canuel, E. A., 2006. Degradation and preservation of organic matter in marine sediments. In Volkman, J. K. (ed.), *Handbook of Environmental Chemistry*. Berlin: Springer, Vol. 2, Part N, pp. 295–321.
- Zafiriou, O. C., Gagosian, R. B., Peltzer, E. T., Alford, J. B., and Loder, T., 1985. Air-to-sea fluxes of lipids at Enewetak Atoll. *Geophysical Research*, **90**(D1), 2409–2423.

Cross-references

[Aerobic Metabolism](#)
[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Methane Oxidation \(Aerobic\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

CARBON (ORGANIC, DEGRADATION)

Steven T. Petsch
 University of Massachusetts Amherst, Amherst, MA,
 USA

Synonyms

Carbon biodegradation; Carbon remineralization

Definition

Organic carbon (OC). In geochemistry, carbon that occurs in materials of ultimately biological origin and that can be oxidized. Organic matter (OM) is the material that contains organic carbon (OC).

Degradation. Transformations of OM within the range of temperatures, pressures and environmental conditions found in or near the Earth surface environments. If biological activity is confirmed to cause the transformations, it is termed biodegradation.

Remineralization. Degradation of OM that results in net conversion of OC to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- .

Introduction

The Earth exists in a near balance between photosynthesis and respiration. Photosynthesis, by which inorganic carbon is chemically reduced to biologically-available OC using light energy, generates an estimated 107 Pg C of net new production each year (1 Pg = 10^{15} g), nearly evenly distributed among terrestrial (57 Pg C year⁻¹) and marine (50 Pg C year⁻¹) environments (Field et al., 2007; Sabine et al., 2004). Of this biomass, over 90 Pg C is remineralized each year to inorganic carbon

through aerobic respiration on land and in the oceans, leaving 17 Pg year⁻¹ to be redistributed among different pools of nonliving OM (Field et al., 2007; Sabine et al., 2004). These pools include soils, aquatic and marine sediments, and nonliving OM in the world's oceans. Degradation of organic carbon in these pools balances the crustal carbon cycle, such that total global respiration plus net OC remineralization are believed to match global rates of photosynthesis (Bernier, 2003). Maintaining or slowly shifting this balance has been a critical component of the coupled atmosphere–ocean climate system throughout the Earth's history, and has served to maintain an equable amount of atmospheric oxygen (O_2) for the past 600 million years (Bernier, 1989, 2003).

Degradation of OC in soils

Soils contain complex mixtures of weathered rocks, new minerals formed within soils, and OM. The world's soils contain an estimated 3,200 Pg C (Sabine et al., 2004 and references therein). Much of this OM derives from terrestrial vegetation. As dead vegetation ages within soils, it is degraded by a combination of chemical reactions and biological processes. Soluble components of decaying vegetation may be leached out of soils and relocated by groundwater movement. Organic acids and chelating agents released by plant roots further facilitate the dissolution and hydrolysis of vegetation. Soil macrofauna, especially detritivores such as earthworms, serve to physically mix soil horizons from different depths, moving OM-rich surface leaf litter down to more water- and mineral-rich horizons and reducing the OC and nutrient content of surface soils (Bohlen et al., 2004). Detritivores also accelerate the formation and disintegration of soil carbon aggregates, resulting in overall larger aggregates of fresh (less-degraded) OM mixed to greater depth within soils (Blair et al., 1995; Cortez, 1998). This activity increases soil porosity and permeability, allowing access to oxygen to greater depth in soils to support aerobic respiration processes.

Throughout in the soils, microbial processes both positively and negatively influence soil OM degradation. Fungal hyphae and other microbial exopolymers bind soil carbon and mineral particles together into aggregates. These exopolymers add to the stock of soil OC contributed by terrestrial vegetation, and support mechanisms of physical protection of OM against further degradation; aggregate-bound OM is much less susceptible to degradation than unassociated OM (Baldock and Skjemstad, 2000). Other microorganisms participate in the degradation of soil OM as they obtain energy and cellular carbon through various metabolic processes. While aerobic respiration is confined to shallow depths of the soil where O_2 can penetrate, anaerobic processes extend throughout the soil profile, from O_2 -free microenvironments within surface soils to deeper O_2 -free soils and weathered regolith.

Soil OC degradation is limited by the availability and reactivity of both OM and electron acceptors that fuel anaerobic respiration. Sulfate is not an abundant

- Wakeham, S. G., and Canuel, E. A., 2006. Degradation and preservation of organic matter in marine sediments. In Volkman, J. K. (ed.), *Handbook of Environmental Chemistry*. Berlin: Springer, Vol. 2, Part N, pp. 295–321.
- Zafiriou, O. C., Gagosian, R. B., Peltzer, E. T., Alford, J. B., and Loder, T., 1985. Air-to-sea fluxes of lipids at Enewetak Atoll. *Geophysical Research*, **90**(D1), 2409–2423.

Cross-references

[Aerobic Metabolism](#)
[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Methane Oxidation \(Aerobic\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

CARBON (ORGANIC, DEGRADATION)

Steven T. Petsch
 University of Massachusetts Amherst, Amherst, MA,
 USA

Synonyms

Carbon biodegradation; Carbon remineralization

Definition

Organic carbon (OC). In geochemistry, carbon that occurs in materials of ultimately biological origin and that can be oxidized. Organic matter (OM) is the material that contains organic carbon (OC).

Degradation. Transformations of OM within the range of temperatures, pressures and environmental conditions found in or near the Earth surface environments. If biological activity is confirmed to cause the transformations, it is termed biodegradation.

Remineralization. Degradation of OM that results in net conversion of OC to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- .

Introduction

The Earth exists in a near balance between photosynthesis and respiration. Photosynthesis, by which inorganic carbon is chemically reduced to biologically-available OC using light energy, generates an estimated 107 Pg C of net new production each year (1 Pg = 10^{15} g), nearly evenly distributed among terrestrial (57 Pg C year⁻¹) and marine (50 Pg C year⁻¹) environments (Field et al., 2007; Sabine et al., 2004). Of this biomass, over 90 Pg C is remineralized each year to inorganic carbon

through aerobic respiration on land and in the oceans, leaving 17 Pg year⁻¹ to be redistributed among different pools of nonliving OM (Field et al., 2007; Sabine et al., 2004). These pools include soils, aquatic and marine sediments, and nonliving OM in the world's oceans. Degradation of organic carbon in these pools balances the crustal carbon cycle, such that total global respiration plus net OC remineralization are believed to match global rates of photosynthesis (Bernier, 2003). Maintaining or slowly shifting this balance has been a critical component of the coupled atmosphere–ocean climate system throughout the Earth's history, and has served to maintain an equable amount of atmospheric oxygen (O_2) for the past 600 million years (Bernier, 1989, 2003).

Degradation of OC in soils

Soils contain complex mixtures of weathered rocks, new minerals formed within soils, and OM. The world's soils contain an estimated 3,200 Pg C (Sabine et al., 2004 and references therein). Much of this OM derives from terrestrial vegetation. As dead vegetation ages within soils, it is degraded by a combination of chemical reactions and biological processes. Soluble components of decaying vegetation may be leached out of soils and relocated by groundwater movement. Organic acids and chelating agents released by plant roots further facilitate the dissolution and hydrolysis of vegetation. Soil macrofauna, especially detritivores such as earthworms, serve to physically mix soil horizons from different depths, moving OM-rich surface leaf litter down to more water- and mineral-rich horizons and reducing the OC and nutrient content of surface soils (Bohlen et al., 2004). Detritivores also accelerate the formation and disintegration of soil carbon aggregates, resulting in overall larger aggregates of fresh (less-degraded) OM mixed to greater depth within soils (Blair et al., 1995; Cortez, 1998). This activity increases soil porosity and permeability, allowing access to oxygen to greater depth in soils to support aerobic respiration processes.

Throughout in the soils, microbial processes both positively and negatively influence soil OM degradation. Fungal hyphae and other microbial exopolymers bind soil carbon and mineral particles together into aggregates. These exopolymers add to the stock of soil OC contributed by terrestrial vegetation, and support mechanisms of physical protection of OM against further degradation; aggregate-bound OM is much less susceptible to degradation than unassociated OM (Baldock and Skjemstad, 2000). Other microorganisms participate in the degradation of soil OM as they obtain energy and cellular carbon through various metabolic processes. While aerobic respiration is confined to shallow depths of the soil where O_2 can penetrate, anaerobic processes extend throughout the soil profile, from O_2 -free microenvironments within surface soils to deeper O_2 -free soils and weathered regolith.

Soil OC degradation is limited by the availability and reactivity of both OM and electron acceptors that fuel anaerobic respiration. Sulfate is not an abundant

electron acceptor in most soils, while ferric iron and nitrate can be. Soil OM is also susceptible to degradation other than respiration. Fermentative degradation of soil OM breaks down terrestrial inputs and biopolymers into small molecules including low molecular weight organic acids, alcohols, and sugar monomers. Methanogenesis too is an important mechanism of OC degradation in soils, consuming fermentation products of such as methanol, H_2 , and acetate, sustaining the fermentative breakdown of soil OM.

Many components of soil OM are fairly susceptible to degradation and do not persist in soil environments. These include carbohydrate biopolymers (e.g., cellulose, chitin, and other polysaccharides), proteins, and nucleic acids. Other components such as lipids, lignin, and black carbon are much more resistant to degradation, and can persist for millennia as intact compounds or transitional degradation products in soils (Derenne and Largeau, 2001). Lipids commonly found preserved in soils include highly aliphatic waxes, free hydrocarbons such as *n*-alkanes and isoprenoids, and biopolymers such as cutan and suberan. Lignin is a structural biopolymer in plant cell walls that derives from condensation of phenolic precursors. Black carbon comprises a broad suite of aromatic and graphitized organic materials that form during incomplete combustion of terrestrial biomass or fossil fuels; these materials are believed to be extremely resistant to degradation (Schmidt and Noack, 2000). During degradation in soils, soil OM components are remineralized at rates depending on a complex combination of intrinsic reactivity (polymeric material being more resistant than individual monomers), physical protection, (inside mineral aggregates or encapsulated within more resistant biopolymers) and lability (proteins, nucleic acids, and carbohydrates being more reactive than lipids and lignin).

Degradation of OC in the oceans

OC occurs in both dissolved and particulate forms in the oceans. Approximately $50 \text{ Pg C year}^{-1}$ derives from marine primary production driven by phytoplankton (Field et al., 2007; Sabine et al., 2004 and references therein). Rivers deliver an estimated 0.5 Pg of OC to the oceans each year. Primary production is confined to the photic zone of the world ocean, where light and nutrients combine in sufficient abundance to support photosynthetic activity. Within and immediately below the photic zone is a zone of intense heterotrophic activity, in which grazing zooplankton and aerobic heterotrophic bacteria consume particulate OM (Lee et al., 2004). The heterotrophic activity in near-surface waters has several impacts on OC degradation in the oceans. One is that $>90\%$ of primary production is effectively remineralized in the upper 200 m of the marine water column, leaving only a small fraction of surface particulate OM for export to the deep ocean (Suess, 1980; Honjo et al., 2008). Second is that mineral ballasting (association of OM with biogenic minerals produced by plankton or with minerals delivered

from continents to the surface ocean by winds and river) increases the sinking rate of OM through the water column, reducing the time available for degradation in the ocean (Lee et al., 2004; Armstrong et al., 2002). Aggregation of particulates in surface waters results in increased particle size and sinking velocity, further accelerating the export of less-degraded particulate OM to a depth (Sheridan et al., 2002). Lastly, heterotrophic grazing of surface particulates results in net evolution of the composition of sinking particulates, as more labile or easily degraded components are consumed (Lee et al., 2004; Wakeham et al., 1997).

In living biomass of the photic zone, OM that can be identified at the molecular level comprises $>80\%$ of total OC. Between 200 and 1,000 m water depth, molecularly uncharacterized material comes to dominate total OM, such that much of the material delivered to seafloor sediments cannot be assigned to typical compound classes such as lipids, carbohydrates, and amino acids (Lee et al., 2004). Some of this relative increase in uncharacterized material may result from selective preservation of photic zone precursors, while some may be added to sinking particulates as exopolymeric material generated by heterotrophic bacteria that feed on sinking particulate matter. There are also changes observed in the molecularly characterizable fraction of sinking particulate OM. Lipids resistant to degradation, such as long-chain saturated fatty acids and alkenones, are substantially enriched in relative abundance compared with initial photic zone biomass, while pigments, many carbohydrates, and polyunsaturated fatty acids are readily lost from particulates within or below the photic zone (Lee et al., 2004).

This background picture of OC degradation is confounded by the role of mineral surfaces. Minerals, whether delivered to surface waters from the continents by winds and rivers, or produced by plankton in the water column, make up the bulk of sinking particulate mass. OM can be sorbed to mineral particles, and with their greater density, OM-associated particulates can sink rapidly through the water column, helping to export relatively undegraded OC beyond the photic zone. In this way, terrestrially derived OM, which may be protected from degradation by association with detrital minerals or OM macromolecules, can be delivered to deep sea sediments. More generally, marine OM may be protected from degradation by adsorption in mesopores of sinking mineral particles, or OM mineral aggregates taken together may resist degradation, where OM is located in interstices between the mineral grains (Hedges et al., 2001). Clearly, degradation of OM within the marine water column reflects a complex interplay of inherent resistance of various organic materials across a continuum of lability, coupled with mechanisms of both speeding the rate of sinking through the water column and increasing protection from degradation by the association of OM with biogenic and terrigenous minerals.

This discussion of heterotrophic degradation of OC applies to an oxygenated water column. In several locations around the globe, large proportions of the water column are

anoxic. These include the Black Sea, the Cariaco Basin, and seasonally in the Arabian Sea, eastern tropical North Pacific and California Borderlands Basins, among others. Lack of oxygen effectively shuts down aerobic respiration in these regions, allowing the roles of anaerobic microbial processes to dominate OC degradation. In these anoxic basins, the extents of OM degradation as a fraction of primary production are lower than in open ocean sites, leading to higher sediment OC contents. Although the rates of degradation under oxic and anoxic conditions may be similar, the overall proportion of total OM that resists degradation may be greater under anoxic conditions (Van Mooy et al., 2002).

Degradation of OC in marine sediments

Efficient heterotrophic processes in the water column mean that only a small fraction ($<1\%$) of primary production reaches the sea floor. Of this, most is degraded within the upper meters of sediment, leading to OC burial of approximately $160 \text{ Tg C year}^{-1}$ (Berner, 1989; Hedges and Keil, 1995). Clearly, a large fraction of ocean carbon inputs ($\sim 50 \text{ Pg C year}^{-1}$) is degraded in both the water column and shallow sediments.

There is a positive relationship between sediment accumulation rate (either cm year^{-1} or $\text{g}_{\text{carbon}} \text{ cm}^{-2} \text{ year}^{-1}$) and OC burial efficiency, where burial efficiency is the fraction of OC ultimately preserved at depth in sediment relative to OC delivered to the sediment-water interface (Burdige, 2007; Aller, 1998; Canfield, 1994). At very low sedimentation rates, the sediment layers are buried very slowly, and aerobic processes have ample time to remineralize the OM that is delivered to these sediments, thereby explaining the low total OC contents of many low sedimentation rate marine settings. Closer to shore as sedimentation rates increase, rapid burial means that anoxic conditions dominate at shallower sediment depths, and anaerobic degradation processes become more important. Nonetheless, sediments underlying oxygen-deficient waters do not uniformly exhibit high carbon burial efficiency, indicating that oxygen alone is not the primary driver controlling OM degradation rates (Burdige, 2007). Burial efficiencies among a range of marine sediment types range between 10% and 20%. Thus, very little primary production escapes degradation processes to become incorporated into the sedimentary record.

Controls on OC degradation in sediment are similar to those operating in soils and in the marine water column: selective degradation of inherently resistant components, physical protection through association with mineral or organic polymeric surfaces, and availability of electron acceptors and/or appropriate substrates for nonrespiration degradation processes such as fermentation and methanogenesis (Burdige, 2007). Oxygen does not limit OC degradation. Most marine sediments are anoxic within centimeters of the sediment–water interface. Denitrification, sulfate-, iron- and manganese-reduction, fermentation, and methanogenesis are all active components of OM degradation. Oxygen exposure time is nonetheless

a useful proxy for understanding the extent of OC degradation from one environment to the other. Longer cumulative oxygen exposure times result in more OC degradation in sediment and overall lower OM burial efficiency (Aller, 1998; Hartnett et al., 1998).

Most OC is buried in shallow, continental margin sediments. These environments have been targets of numerous investigations over the years, and have provided insights into OC degradation processes in the uppermost few meters of buried sediments (see Martens et al., 1992). Deeply buried marine sediment such as from the Woodlark Basin and Peru Margin in turn demonstrate microbial activity hundreds of meters deep in sediments, indicating OC degradation can persist at slow rates for geologic spans of time (D'Hondt et al., 2004; Wellsbury et al., 2002).

Degradation of OC in ancient sedimentary rocks

The single largest pool of OC on the Earth (15 million Pg C) is represented by OM contained in ancient sedimentary rocks, a mass that dwarfs all other pools of OC combined (Field et al., 2007; Sabine et al., 2004). Traditionally, OC in sedimentary rocks has been viewed as resistant to degradation other than high temperature oil and gas generation or graphitization. Degradation can occur where sedimentary rocks are exposed at the Earth's surface through erosion and soil formation (Petsch et al., 2000, 2001). One unexpected theme emerging from this work is that OC degradation during rock weathering is not completely efficient. Highly aged OC is detected in river particulates from watersheds draining sedimentary lithologies (Longworth et al., 2007; Goni et al., 2005; Blair et al., 2003; Leithold et al., 2006).

However, very little of the total pool of sedimentary OM is exposed in the Earth surface environments; most remains deeply buried in the Earth's crust for millions of years. The persistence of OC in sedimentary rocks for long expanses of geologic time, and its preservation up to the present day, would suggest that degradation processes must largely cease after the burial of OM in sediments. Yet, microbial degradation of deeply buried OM has been described in a number of subsurface environments. These include clear signatures of hydrocarbon biodegradation in many petroleum systems (Head et al., 2003, 2006; Jones et al., 2008) and OM-rich sedimentary rocks (Formolo et al., 2008), coupled with molecular biological analyses that support an interpretation of an active subsurface biosphere involved in OC degradation (Waldron et al., 2007; Jones et al., 2008). These subsurface OC degradation processes are particularly valued for their role in natural gas generation in petroleum systems and other sedimentary basins.

Summary

OC degradation represents the suite of processes by which OM is transformed and remineralized, mainly through biological activity, within soils, aquatic systems,

sediments, and sedimentary rocks. The diverse forms and compositions of natural OM result in a wide range of rates of degradation, as some components are inherently resistant to degradation while others are labile and are thus quickly consumed following biosynthesis. The inherent resistance of some components of OM results in selective preservation in soils and sediments. This selective preservation is attenuated by mechanisms of physical protection, either by association with mineral surface or encapsulation of labile components within more recalcitrant organic matrices. In this way, nominally labile OC can be preserved for greater extents of time or transported across longer distances than would otherwise be the case. Future investigations of OC degradation will likely focus on (1) improved construction of carbon budgets for soils, the oceans, and sediments; (2) the mechanism(s) by which certain compound classes and organic materials remain resistant to biological degradation; (3) improved understanding of the association of organic materials with mineral surfaces, their selective adsorption/desorption behavior, and the physical scale of these relationships; (4) the roles that terrestrial inputs of OM to marine sediments and recycling of ancient OM during rock weathering play in controlling the overall reactivity and preservation of OC in sediments; and (5) improved understanding of couplings between OM composition and degradation processes active within deep subsurface sedimentary environments.

Bibliography

- Aller, R. C., 1998. Mobile deltaic and continental shelf muds as suboxic, fluidized bed reactors. *Marine Chemistry*, **61**, 143–155.
- Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S., and Wakeham, S. G., 2002. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. *Deep-Sea Research II*, **49**, 219–236.
- Baldock, J. A., and Skjemstad, J. O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry*, **31**, 697–710.
- Berner, R. A., 1989. Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **75**, 97–122.
- Berner, R. A., 2003. The long term carbon cycle, fossil fuels and atmospheric composition. *Nature*, **426**, 323–326.
- Blair, J. M., Parmelee, R. W., and Lavelle, P., 1995. Influences of earthworms on biogeochemistry. In Hendrix, P. F. (ed.), *Earthworm Ecology and Biogeography in North America*. Boca Raton, FL: Lewis, pp. 128–154.
- Blair, N. E., Leithold, E. L., Ford, S. T., Peeler, K. A., Holmes, J. C., and Perkey, D. W., 2003. The persistence of memory: the fate of ancient sedimentary organic carbon in a modern sedimentary system. *Geochimica et Cosmochimica Acta*, **67**, 63–73.
- Bohlen, P. J., Pelletier, D. M., Groffman, P. M., Fahey, T. J., and Fisk, M. C., 2004. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. *Ecosystems*, **7**, 13–27.
- Burdige, D. J., 2007. Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? *Chemistry Reviews*, **107**, 467–485.
- Canfield, D. E., 1994. Factors influencing organic carbon preservation in marine sediments. *Chemical Geology*, **114**, 315–329.
- Cortez, J., 1998. Field decomposition of leaf litters: relationships between decomposition rates and soil moisture, soil temperature and earthworm activity. *Soil Biology and Biochemistry*, **30**, 783–793.
- Derenne, S., and Largeau, C., 2001. A review of some important families of refractory macromolecules: composition, origin and fate in soils and sediments. *Soil Science*, **166**, 833–847.
- D'Hondt, S., Jorgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K. U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegel, J., Padilla, C. N., and Acosta, J. L. S., 2004. Distributions of microbial activities in deep seafloor sediments. *Science*, **306**, 2216–2221.
- Field, C. B., Sarmiento, J., and Hales, B., 2007. The carbon cycle of North America in a global context. In King, A. W., Dilling, L., Zimmerman, G. P., Fairman, D. M., Houghton, R. A., Marland, G., Rose, A. Z., and Wilbanks, T. J. (eds.), *The North American Carbon Budget and Implications for the Global Carbon Cycle. U.S. Climate Change Science Program, Synthesis and Assessment Product 2.2*. Asheville, NC: National Oceanic and Atmospheric Administration, National Climatic Data Center, pp. 21–29.
- Formolo, M. F., Martini, A. M., Petsch, S. T., Nüsslein, K., and Salacup, J., 2008. Hydrocarbon biodegradation in sedimentary rocks linked to atmospheric methane variation during deglaciation. *Geology*, **36**, 139–142.
- Goni, M. A., Yunker, M. B., Macdonald, R. W., and Eglinton, T. I., 2005. The supply and preservation of ancient and modern components of organic carbon in the Canadian Beaufort Shelf of the Arctic Ocean. *Marine Chemistry*, **93**, 53–73.
- Hartnett, H. E., Keil, R. G., Hedges, J. I., and Devol, A. H., 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. *Nature*, **391**, 572–574.
- Head, I., Jones, D. M., and Röling, W., 2006. Marine microorganisms make a meal of oil. *Nature Reviews Microbiology*, **4**, 173–182.
- Head, I. M., Jones, D. M., and Larter, S. R., 2003. Biological activity in the deep subsurface and the origin of heavy oil. *Nature*, **426**, 344–352.
- Hedges, J. I., and Keil, R. G., 1995. Sedimentary organic-matter preservation – an assessment and speculative synthesis. *Marine Chemistry*, **49**, 81–115.
- Hedges, J. I., Baldock, J. A., Gelinas, Y., Lee, C., Peterson, M. L., and Wakeham, S., 2001. Evidence for non-selective preservation of organic matter in sinking marine sediments. *Nature*, **409**, 801–804.
- Honjo, S., Manganini, S. J., Krishfield, R. A., and Francois, R., 2008. Particulate organic carbon fluxes to the ocean interior and factors controlling the biological pump: a synthesis of global sediment trap programs since 1983. *Progress in Oceanography*, **76**, 217–285.
- Jones, D. M., Head, I. M., Gray, N. D., Adams, J. J., Rowan, A. K., Aitken, C. M., Bennett, B., Huang, H., Brown, A., Bowler, B. F. J., Oldenburg, T., Erdmann, M., and Larter, S. R., 2008. Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature*, **451**, 176–181.
- Lee, C., Wakeham, S., and Arnosti, C., 2004. Particulate organic matter in the sea: the composition conundrum. *Ambio*, **33**, 565–575.
- Leithold, E. L., Blair, N. E., and Perkey, D. W., 2006. Geomorphologic controls on the age of particulate organic

- carbon from small mountainous and upland rivers. *Global Biogeochemical Cycles*, **20**, GB3022, doi:10.1029/2005GB002677.
- Longworth, B. E., Petsch, S. T., Raymond, P. A., and Bauer, J. E., 2007. Linking lithology and land use to sources of dissolved and particulate organic matter in headwaters of a temperate, passive-margin river system. *Geochimica et Cosmochimica Acta*, **71**, 4233–4250.
- Martens, C. S., Haddad, R. I., and Chanton, J. P., 1992. Organic matter accumulation, remineralization, and burial in an anoxic coastal sediment. In Whelan, J. K., and Farrington, J. W. (eds.), *Productivity, Accumulation and Preservation of Organic Matter in Recent and Ancient Sediments*. New York: Columbia University Press, p. 82.
- Petsch, S. T., Berner, R. A., and Eglinton, T. I., 2000. A field study of the chemical weathering of ancient sedimentary organic matter. *Organic Geochemistry*, **31**, 475–487.
- Petsch, S. T., Smernik, R. J., Eglinton, T. I., and Oades, J. M., 2001. A solid state ^{13}C -NMR study of kerogen degradation during black shale weathering. *Geochimica et Cosmochimica Acta*, **65**, 1867–1882.
- Sabine, C. L., Heiman, M., Artaxo, P., Bakker, D. C. E., Chen, C. T. A., Field, C. B., Gruber, N., LeQuéré, C., Prinn, R. G., Richey, J. E., Romero-Lankao, P., Sathaye, J. A., and Valentini, R., 2004. Current status and past trends of the carbon cycle. In Field, C. B., and Raupach, M. R. (eds.), *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World*. Washington, DC: Island Press, pp. 17–44.
- Schmidt, M. W. I., and Noack, A. G., 2000. Black carbon in soils and sediments: analysis, distribution, implications and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.
- Sheridan, C. C., Lee, C., Wakeham, S. G., and Bishop, J. K. B., 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific ocean. *Deep-Sea Research I*, **49**, 1983–2008.
- Suess, E., 1980. Particulate organic carbon fluxes in the oceans – surface productivity and oxygen utilization. *Nature*, **288**, 260–263.
- Van Mooy, B. A. S., Keil, R. G., and Devol, A. H., 2002. Impact of suboxia on sinking particulate organic carbon: enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta*, **66**, 457–465.
- Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. L., 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochimica et Cosmochimica Acta*, **61**, 5363–5369.
- Wakeham, S. G., Lee, C., and Hedges, J. I., 2002. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. *Deep-Sea Research I*, **47**, 1535–1568.
- Waldron, P., Petsch, S. T., Martini, A. M., and Nüsslein, K., 2007. Archaeal community structure and metabolic activity across a salinity gradient within a methane-producing sedimentary basin. *Applied and Environmental Microbiology*, **73**, 4171–4179.
- Wellsbury, P., Mather, I., and Parkes, R. J., 2002. Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. *FEMS Microbiology Ecology*, **42**, 59–70.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Black Shales](#)
[Carbon \(Organic, Cycling\)](#)

[Deep Biosphere of Sediments](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shales](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)

CARBON CYCLE

The carbon cycle is a biogeochemical cycle that describes the movement of carbon between its different compartments (reservoirs) on the Earth, namely biosphere, atmosphere, hydrosphere, pedosphere, and geosphere. Please refer to entries “*Carbon (Organic, Cycling)*” and “*Carbon (Organic, Degradation)*.”

CARBON ISOTOPES

Please refer to “*Isotopes and Geobiology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

CARBONATE ENVIRONMENTS

Eberhard Gischler

J.W. Goethe-Universität, Frankfurt am Main, Germany

Definition

Carbonate environments occur both in the terrestrial and marine realms as well as in transitional zones between the land and the sea. The variety of environments spans from high-elevation continental lakes to the deep sea, from the equator to latitudes of about 60°, and they include dry and wet climate realms. The majority of carbonates are produced in the sea, currently in more or less similar proportions in neritic and pelagic settings. Carbonate production is to a large part a consequence of biological activity, either directly as in shell and skeleton formation or indirectly as a result of metabolic reactions, which trigger precipitation. The highest rates of carbonate production per unit time and space are probably reached in tropical coral reefs where several kilograms of calcium carbonate form on 1 m² during 1 year's time.

Introduction

Modern carbonate sediments are largely a product of biologic activity, either enzymatically controlled like in calcium carbonate (CaCO₃) shell and skeleton formation of aquatic organisms or indirectly by the metabolic activity

- carbon from small mountainous and upland rivers. *Global Biogeochemical Cycles*, **20**, GB3022, doi:10.1029/2005GB002677.
- Longworth, B. E., Petsch, S. T., Raymond, P. A., and Bauer, J. E., 2007. Linking lithology and land use to sources of dissolved and particulate organic matter in headwaters of a temperate, passive-margin river system. *Geochimica et Cosmochimica Acta*, **71**, 4233–4250.
- Martens, C. S., Haddad, R. I., and Chanton, J. P., 1992. Organic matter accumulation, remineralization, and burial in an anoxic coastal sediment. In Whelan, J. K., and Farrington, J. W. (eds.), *Productivity, Accumulation and Preservation of Organic Matter in Recent and Ancient Sediments*. New York: Columbia University Press, p. 82.
- Petsch, S. T., Berner, R. A., and Eglinton, T. I., 2000. A field study of the chemical weathering of ancient sedimentary organic matter. *Organic Geochemistry*, **31**, 475–487.
- Petsch, S. T., Smernik, R. J., Eglinton, T. I., and Oades, J. M., 2001. A solid state ^{13}C -NMR study of kerogen degradation during black shale weathering. *Geochimica et Cosmochimica Acta*, **65**, 1867–1882.
- Sabine, C. L., Heiman, M., Artaxo, P., Bakker, D. C. E., Chen, C. T. A., Field, C. B., Gruber, N., LeQuéré, C., Prinn, R. G., Richey, J. E., Romero-Lankao, P., Sathaye, J. A., and Valentini, R., 2004. Current status and past trends of the carbon cycle. In Field, C. B., and Raupach, M. R. (eds.), *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World*. Washington, DC: Island Press, pp. 17–44.
- Schmidt, M. W. I., and Noack, A. G., 2000. Black carbon in soils and sediments: analysis, distribution, implications and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.
- Sheridan, C. C., Lee, C., Wakeham, S. G., and Bishop, J. K. B., 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific ocean. *Deep-Sea Research I*, **49**, 1983–2008.
- Suess, E., 1980. Particulate organic carbon fluxes in the oceans – surface productivity and oxygen utilization. *Nature*, **288**, 260–263.
- Van Mooy, B. A. S., Keil, R. G., and Devol, A. H., 2002. Impact of suboxia on sinking particulate organic carbon: enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta*, **66**, 457–465.
- Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. L., 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochimica et Cosmochimica Acta*, **61**, 5363–5369.
- Wakeham, S. G., Lee, C., and Hedges, J. I., 2002. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. *Deep-Sea Research I*, **47**, 1535–1568.
- Waldron, P., Petsch, S. T., Martini, A. M., and Nüsslein, K., 2007. Archaeal community structure and metabolic activity across a salinity gradient within a methane-producing sedimentary basin. *Applied and Environmental Microbiology*, **73**, 4171–4179.
- Wellsbury, P., Mather, I., and Parkes, R. J., 2002. Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. *FEMS Microbiology Ecology*, **42**, 59–70.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Black Shales](#)
[Carbon \(Organic, Cycling\)](#)

[Deep Biosphere of Sediments](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shales](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)

CARBON CYCLE

The carbon cycle is a biogeochemical cycle that describes the movement of carbon between its different compartments (reservoirs) on the Earth, namely biosphere, atmosphere, hydrosphere, pedosphere, and geosphere. Please refer to entries “*Carbon (Organic, Cycling)*” and “*Carbon (Organic, Degradation)*.”

CARBON ISOTOPES

Please refer to “*Isotopes and Geobiology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

CARBONATE ENVIRONMENTS

Eberhard Gischler

J.W. Goethe-Universität, Frankfurt am Main, Germany

Definition

Carbonate environments occur both in the terrestrial and marine realms as well as in transitional zones between the land and the sea. The variety of environments spans from high-elevation continental lakes to the deep sea, from the equator to latitudes of about 60°, and they include dry and wet climate realms. The majority of carbonates are produced in the sea, currently in more or less similar proportions in neritic and pelagic settings. Carbonate production is to a large part a consequence of biological activity, either directly as in shell and skeleton formation or indirectly as a result of metabolic reactions, which trigger precipitation. The highest rates of carbonate production per unit time and space are probably reached in tropical coral reefs where several kilograms of calcium carbonate form on 1 m² during 1 year's time.

Introduction

Modern carbonate sediments are largely a product of biologic activity, either enzymatically controlled like in calcium carbonate (CaCO₃) shell and skeleton formation of aquatic organisms or indirectly by the metabolic activity

C

CALCAREOUS ALGAE

The term “calcareous algae” refers to various kinds of benthic and planktonic algae whose thalli contain biochemically precipitated calcium carbonate (CaCO_3) as skeletal material (Wray, 1977; Braga and Riding, 2005). Precipitation of CaCO_3 (as calcite and/or aragonite) may occur within or on the algal bodies. The term may also include mechanically accreted deposits of calcium carbonate caused by algae, usually as an interaction of biological and physical processes. Calcareous algae are a highly artificial group that constitutes calcifying members of the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) and is sometimes also used for Cyanobacteria. At present, calcareous algae are one of the most important reef builders (see “[Carbonate Environments](#)”). For a detailed reading, please refer to “[Algae \(Eukaryotic\)](#).”

Bibliography

- Braga, J. C., and Riding, R., 2005. Calcareous algae. In Selley, R., Cocks, L. R. M., and Plimer, I. R. (eds.), *Encyclopedia of Geology*. Amsterdam: Elsevier, pp. 428–436.
- Wray, J. L., 1977. *Calcareous Algae. Developments in Palaeontology and Stratigraphy*. Amsterdam: Elsevier, Vol. 4, 185 pp.

CALCIFICATION

See entries “[Animal Biocalcification, Evolution](#),” “[Biofilms and Fossilization](#),” “[Calcified Cyanobacteria](#),” “[Calcite Precipitation, Microbially Induced](#),” “[Calcium Biogeochemistry](#),” “[Carbonate Environments](#),” “[Dolomite, Microbial](#),” “[Microbialites, Modern](#),” “[Microbialites, Stromatolites, and Thrombolites](#),” and “[Pedogenic Carbonates](#).”

CALCIFIED CYANOBACTERIA

Robert Riding
University of Tennessee, Knoxville, TN, USA

Definition

Cyanobacteria are alga-like bacteria that can perform oxygenic photosynthesis and nitrogen fixation (Whitton and Potts, 2000; Herrero and Flores, 2008). They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments at the present-day, where they are key primary producers in both microbial mat and planktic ecosystems. As the initiators of plastids, they have played a fundamental role in algal and plant evolution (Raven, 2002). Cyanobacteria occupy benthic substrates and can also drift in the water column. Their photosynthetic uptake of inorganic carbon can stimulate CaCO_3 precipitation. This calcification can produce filamentous microfossils in benthic mats that are preserved as stromatolites and thrombolites, and can also cause water column precipitation of carbonate mud that settles to lake and sea floors.

Introduction

However, cyanobacterial calcification is not obligatory and is directly dependent on environmental conditions. This accounts for apparent discrepancies between the geological ranges of organic-walled and calcified cyanobacterial fossils. Calcified cyanobacteria have not been recognized in marine sediments older than ~1200 Ma ago (Kah and Riding, 2007), whereas there is evidence that cyanobacteria appeared in the late Archaean or Palaeoproterozoic, in the range ~2900–2150 Ma ago (Cavalier-Smith et al., 2006; Hofmann, 1976). This mid-Proterozoic appearance of sheath-calcified cyanobacteria is thought to reflect the development of CO_2 -concentrating

mechanisms (CCMs) as atmospheric CO₂ levels declined (Riding, 2006). Calcified cyanobacterial fossils remained conspicuous components of marine stromatolites and thrombolites through much of the Neoproterozoic, Palaeozoic, and Mesozoic, but became vanishingly scarce in the Cenozoic, including present-day seas (Riding, 1982), probably due to decline in seawater saturation for CaCO₃ minerals (Riding, 1993, p. 514; 2000, p. 200; Kempe and Kazmierczak, 1994). Cyanobacterial calcification is thus a good example of “induced,” as opposed to “controlled” biocalcification. Its close environmental dependence can be used to interpret changes in past conditions such as carbonate saturation state and the availability of inorganic carbon for photosynthesis. In addition to their sedimentological importance, calcified cyanobacteria can therefore assist in the reconstruction of seawater chemistry and atmospheric composition over long geological time scales.

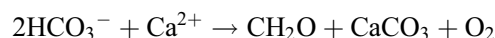
Controls on calcification

The ability of cyanobacteria to grow and reproduce whether they are calcified or uncalcified illustrates the non-obligate nature of their calcification (Pentecost and Riding, 1986). Two factors that directly influence cyanobacterial calcification are the saturation state of ambient waters and the mechanism of photosynthetic uptake of inorganic carbon (Riding, 2006).

Carbonate saturation state: Cyanobacterial calcification requires waters in which CaCO₃ precipitation is thermodynamically favored (Pentecost, 1981; Kempe and Kazmierczak, 1994; Merz-Preiß and Riding, 1999). It has been very common in marine environments at times since the Neoproterozoic, but these episodes are markedly episodic and can be interspersed by long periods when cyanobacterial calcification is scarce (Riding, 1992). Cyanobacterial sheath calcification has not been confidently recognized in present-day marine environments, and is rare to absent throughout the Cenozoic (Riding, 1982; Arp et al., 2001). In contrast, cyanobacterial calcification is locally well-developed in present-day calcareous streams and lakes, and is often significantly involved in the formation of tufa and oncoids (Golubic, 1973; Pedley, 1990; Pentecost, 2005). In streams, calcification reflects increased saturation state that results from warming and degassing of spring waters, especially in turbulent zones, together with the stimulus of photosynthetic carbon removal (Merz-Preiß and Riding, 1999; Bissett et al., 2008). In lakes, precipitation is stimulated by seasonal warming as well as the activity of phytoplankton blooms that include cyanobacteria (Kelts et al., 1978; Thompson and Ferris, 1990). These present-day environments are vulnerable to pollutants such as agricultural fertilizers. Cyanobacterial calcification has declined over the past century in temperate climate hardwater streams and lakes of Europe and North America, largely in response to these anthropogenic changes (Pentecost, 2005, pp. 283–287),

among which phosphate inhibition of CaCO₃ precipitation (Raistrick, 1949) may be important (Hägele et al., 2006).

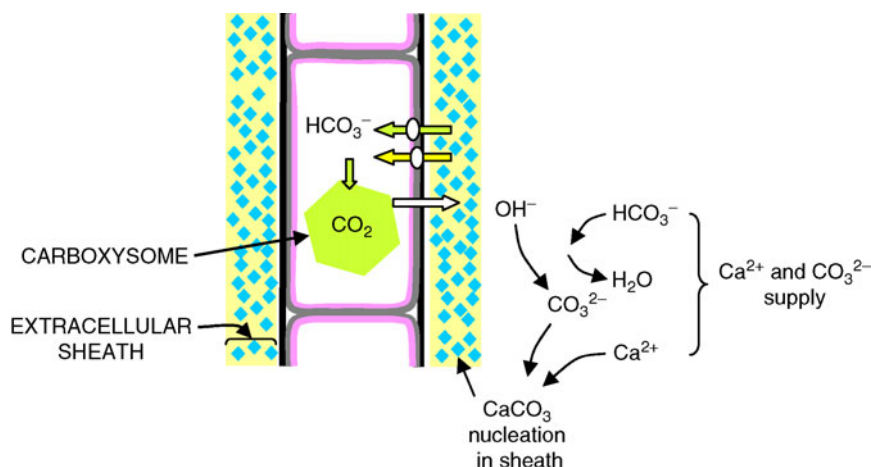
CO₂-concentrating mechanisms: Photosynthetic carbon uptake can directly influence cyanobacterial calcification. Diffusive entry of CO₂ into the cell may not significantly affect ambient pH, but active bicarbonate uptake increases pH near the cell (Miller and Colman, 1980) that promotes calcification (Thompson and Ferris, 1990). Bicarbonate (HCO₃[−]) is actively transported into the cell and intracellularly converted to CO₂ for photosynthesis. These processes lead to increased pH in the immediate vicinity of the cell. Where ambient waters are sufficiently saturated for CaCO₃ minerals then this localized pH increase can trigger the nucleation of CaCO₃ crystallites on or near the cell surface or in the enveloping mucilaginous sheath (Figures 1 and 2):



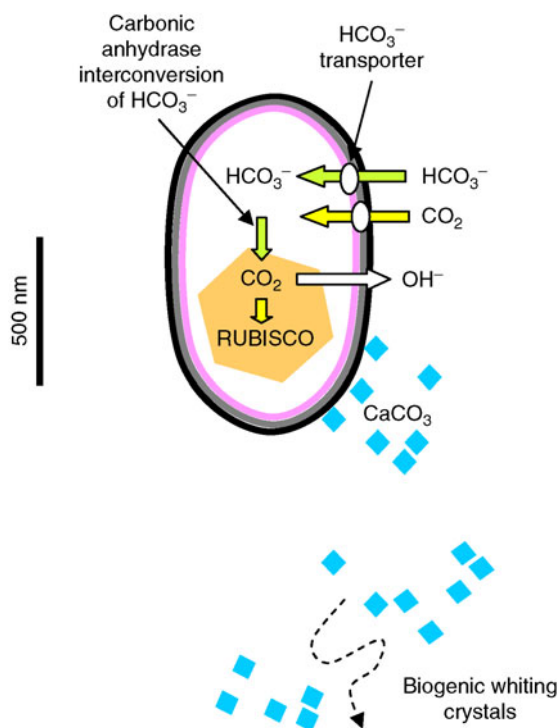
Active bicarbonate uptake and its conversion within the cell to CO₂ by carbonic anhydrase are adaptations to reduced availability of CO₂. They constitute CCMs (Kaplan and Reinhold, 1999). CCM induction can be triggered by localized carbon limitation, e.g., within microbial mats or phytoplankton blooms, and also by fall in global atmospheric CO₂ levels. Modeled estimates suggest that atmospheric CO₂ has fluctuated widely during the Phanerozoic, up to levels that are 25 or more times higher than present atmospheric level (PAL) (Berner and Kothavala, 2001). CCMs are well-developed in cyanobacteria (Kaplan et al., 1980; Giordano et al., 2005) and experiments suggest that they are induced when ambient CO₂ falls below a critical threshold that is roughly equivalent to 10 PAL (Badger et al., 2002). It therefore seems likely that CCM induction plays a significant role in cyanobacterial calcification (Thompson and Ferris, 1990; Merz, 1992), especially at times in the geological past when CO₂ levels have been below 10 PAL (Riding, 2006, p. 302).

Sites of calcification

Calcification in cyanobacteria is close, but external, to the cell. CaCO₃ crystals nucleate either within the protective mucilaginous sheath, or on or close to the cell surface (Thompson and Ferris, 1990; Merz, 1992). Sheath impregnation by crystallites (Figure 1) can produce coherent tubular and shrub-like calcified structures that preserve the sheath morphology and can be preserved as microfossils for hundreds of millions of years (Riding, 1991). In contrast, if isolated crystals form near the cell surface, they do not form a preservable shape but can be released as allochthonous particles (“whittings”) (Figure 2). These can accumulate as masses of micron-size carbonate mud sediment on lake and sea floors and can also survive as ancient geological deposits. However, they are not known to possess features that distinguish them as



Calcified Cyanobacteria, Figure 1 Model of in vivo sheath calcification in a filamentous cyanobacterium related to CO_2 -concentrating mechanism (CCM)-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CCMs involve active carbon transport into the cell and conversions that liberate OH^- ions. Calcification is stimulated by photosynthetic carbon uptake and OH^- release which elevates sheath pH. If ambient carbonate saturation is already elevated, with raised pH, extracellular HCO_3^- converts into CO_3^{2-} , further increasing saturation state that promotes CaCO_3 nucleation in the sheath.

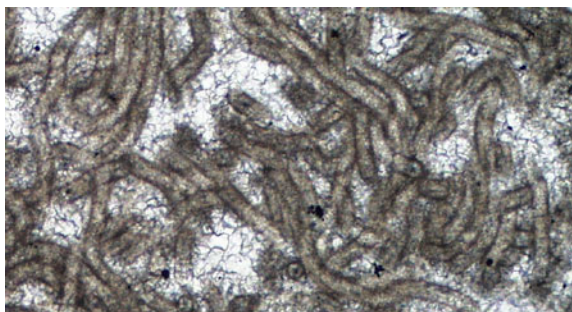


Calcified Cyanobacteria, Figure 2 Model of in vivo sheath calcification in a picoplanktic coccoid cyanobacterium related to CCM-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CaCO_3 nucleation (blue diamonds) occurs on and near the cell surface. The crystals can ultimately be sedimented to the lake or sea floor as carbonate mud.

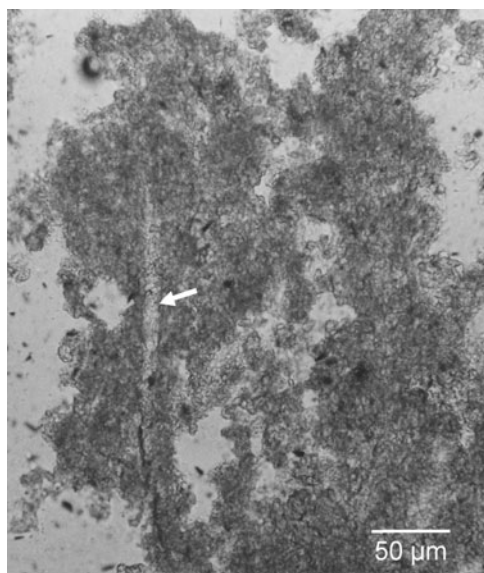
cyanobacterially induced precipitates, and cannot at present be differentiated from carbonate mud of other origins.

Sheath calcification: The protective mucilaginous sheath that envelops benthic calcified cyanobacteria provides a diffusion limited site that enhances the pH rise resulting from carbon uptake (Figures 3 and 4). The sheath is a structured form of EPS (Drews and Weckesser, 1982) providing support, stability, and protection against physical damage, dehydration, and grazers (Dudman, 1977). It can contain the pigment scytonemin that acts as a barrier to ultraviolet radiation (Garcia-Pichel and Castenholz, 1991; Proteau et al., 1993; Dillon and Castenholz, 1999), binds nutrients and metals (Yee et al., 2004), and facilitates gliding motility (Stal, 1995, p. 4; Hoiczky, 1998). Sheath calcification can be partial or complete, and can be limited to the sheath interior (sheath impregnation) or form an external crust (sheath encrustation) (Riding, 1977). For example, it can occur as isolated crystals (Pentecost, 1987, Fig. 1d), form a crystalline network (e.g., Friedmann, 1979, Fig. 9), or create a relatively solid tube of closely juxtaposed crystals (e.g., Couté and Bury, 1988, pl. 2).

Although saturation state with respect to carbonate minerals and CCM induction appear to be key controls on cyanobacterial sheath calcification over geological timescales (Riding, 2006), differences in degree of calcification between different species/strains of cyanobacteria in the same environment also indicate taxonomic specificity for calcification (Golubic, 1973; Merz, 1992; Défarge et al., 1994). Further research is required to elucidate the extent to which such specificity may reflect differences in sheath structure and sheath development, CCM induction, or other factors.



Calcified Cyanobacteria, Figure 3 *Girvanella*, calcified cyanobacterial sheath, early mid-Ordovician, Lunnan, Tarim, China. Width of view 1 mm. Thin-section courtesy Jia-Song Fan.



Calcified Cyanobacteria, Figure 4 Thin-section of present-day oncoid microfabric showing calcified shrub-like sheath surrounding space left by the strand of cells (trichome, arrowed). The cyanobacterium is thought to be the oscillatoriacean *Schizothrix calcicola*, and the calcified sheath closely resembles the microfossil *Angulocellularia* (also *Angusticellularia*), which is locally common in Cambro-Ordovician reefs (Riding and Voronova, 1982). Squaw Island, Canandaigua Lake, New York State, USA.

In addition to in vivo sheath calcification, postmortem sheath degradation by heterotrophic bacteria can result in partial external calcification (Chafetz and Buczynski, 1992), although in culture experiments this may in part be related to the growth medium used (Arp et al., 2002). This incomplete and irregular calcification of decomposing sheaths contrasts with sheath impregnation that produces well-defined tubiform fossils such as *Girvanella* in which wall-thickness remains constant in individual specimens (Riding, 1977, 2006).

Biogenic whiting precipitation: In contrast with relatively large and distinct calcified fossils, such as *Girvanella*, that result from sheath calcification, CaCO_3 precipitates associated with coccoid cyanobacterial blooms are most noticeable as “whittings”. These are ephemeral milk-white patches in freshwater calcareous lakes and shallow tropical seas formed by dense masses of suspended small CaCO_3 crystals (Cloud, 1962). Whiting is a descriptive term, and all whittings are not necessarily biogenic. In addition to the triggering effect of phytoplankton photosynthesis, they could reflect essentially abiogenic CaCO_3 crystal nucleation in the water column, and also – in very shallow water – bottom mud re-suspended by currents or fish (Shinn et al., 1989).

Biogenic cyanobacterial whittings are documented by studies of seasonal blooms of unicellular picoplanktic (cell size in range 0.2–2 μm) cyanobacteria, such as *Synechococcus*. These benefit from efficient CCMs (Badger and Price, 2003) where dissolved inorganic carbon (DIC) availability is limited, as under bloom conditions (Rost et al., 2003). Together with diatoms and other planktic algae, *Synechococcus* is implicated in stimulating biogenic whiting precipitation in present-day freshwater calcareous oligotrophic lakes (Thompson and Ferris, 1990; Dittrich et al., 2004; Lee et al., 2004). Since a sheath is lacking in picoplankton such as *Synechococcus*, calcification is instead localized on a paracrystalline surface layer that provides a binding site (Thompson, 2000, p. 253). This surface layer can be shed, producing biogenic whiting crystals that are deposited from suspension, either individually or as poorly structured aggregates along with organic cells, on lake beds.

In addition to lakes, picoplanktic cyanobacteria are widespread in the open ocean and in nearshore marine environments. They form blooms in Florida Bay, and marine strains of *Synechococcus* calcify under experimental conditions (Lee et al., 2004). There has been much debate concerning whether marine whittings in shallow tropical seas, such as the Bahama Banks, have a similar origin to those in temperate calcareous lakes, and could therefore potentially account for abundant lime mud production on ancient carbonate platforms. If marine whittings on the Bahama Banks are water column precipitates, then they would be important sources of carbonate mud and could be triggered by planktic cyanobacteria (Robbins and Blackwelder, 1992; Robbins et al., 1997). However, CaCO_3 precipitation in freshwater lakes is favored by low pH buffering, whereas in present-day seawater buffering limits pH fluctuation, thereby reducing the response to photosynthetic removal of CO_2 and HCO_3^- . Several studies have suggested that Great Bahama Bank whittings are not due to water column precipitation (Broecker and Takahashi, 1966; Morse et al., 1984; Broecker et al., 2000). For example, whiting CaCO_3 has a $^{14}\text{C}/^{12}\text{C}$ ratio that differs from that of inorganic carbon in the whiting water, but is similar to that of the seafloor sediment. In addition, the saturation state of Bahama Bank waters appears to be too low for pseudohomogeneous precipitation of CaCO_3 .

(Broecker et al., 2001, p. 591; Morse et al., 2003). Broecker et al. (2000) concluded that resuspension of sediment is the dominant process involved in marine whittings on the Bahama Banks. Further work is required to resolve this question, but present-day seawater saturation state in general may be too low to permit CaCO_3 nucleation even within blooms of picoplankton that are operating CCMs.

Nonetheless, biogenic whittings may have occurred under different conditions of seawater chemistry in the geological past, and could therefore have been important sources of fine-grained carbonate. Within plankton blooms, photosynthetic uptake can significantly deplete pCO_2 (Riebesell et al., 1993; Rost et al., 2003), and it is likely that selective pressure for picoplankton to induce CCMs first developed under bloom conditions.

Fossil calcified cyanobacteria

Calcified cyanobacterial fossils have long been noticed in limestones, although they have often been confused with other organisms. Sheath calcified cyanobacteria are morphologically simple fossils, mainly with the appearance of shrub-like dendritic masses (Figure 4) and tangled or erect, sometimes radially arranged, tubes (Figure 3). Densely micritic branched filaments such as *Epiphyton*, and chambered clusters such as *Renalcis* have also often been regarded as possible calcified cyanobacteria. At the same time, because of their general lack of distinguishing features, many of these fossils have often also been compared with calcified green and red algae, and also with foraminifers and sponges. For example, when Nicholson and Etheridge (1878) named *Girvanella* they compared it with foraminifers, and it has subsequently variously been regarded as a calcareous sponge (Seely, 1885), green alga (Rothpletz, 1891), and red alga (Korde, 1973). Bornemann (1886) recognized its cyanobacterial nature, and Pollock (1918) identified it as a calcified sheath. Individual calcified cyanobacteria are normally microscopic, but they commonly form much larger aggregates that are significant components of stromatolites, oncoids, thrombolites, dendrolites, reef crusts, and freshwater tufa deposits that range up to decametric in size.

Three main morphological groups can be distinguished (Riding, 1991): tubes, dendritic shrubs and filaments, and hollow chambers.

Tubes: *Girvanella*, originally described from the Middle Ordovician of Scotland, is very simple: unbranched, often irregularly tangled, tubular filaments of uniform diameter, usually <50 microns, and a thin wall of even thickness (Figure 3). Similar tubes, but often organized into cable-like strands, include *Batinivia*, *Botominella*, *Cladogirvanella*, *Razumovskia*, and *Subtifloria*. *Obruchevella* is coiled. *Ortonella* was described from the Mississippian of England (Garwood, 1914) where it forms oncoids (spherical stromatolite nodules) and stromatolitic crusts. It consists of tubes similar in size to those of *Girvanella*, but distinguished by being erect and branched. Somewhat similar forms include *Bajanophyton*,

Bija, *Botomaella*, *Cayeuxia*, and *Hedstroemia*. Pia (1927, pp. 36–40) recognized that very small tubiform fossils such as *Girvanella* and *Ortonella* were cyanobacteria when he placed them in a new group, the Porostromata.

Dendritic shrubs and filaments: *Angusticellularia* (also named *Angulocellularia*) described from the Cambrian of Siberia by Vologdin (1962) is a submillimetric vertically orientated and irregularly dendritic shrub-like mass of dense micrite (Figure 4). In contrast, narrow branched micritic filaments, usually not tubiform but in some cases with light-colored transverse bands, comprise a group that includes *Epiphyton*, originally described from the Cambrian of Sardinia by Bornemann (1886), and among others, *Epiphytonoides*, *Gordonophyton*, and *Tubomorphophyton*.

Hollow chambers: *Renalcis*, described from the Cambrian of southern Siberia by Vologdin (1932), is a hollow thick-walled chambered microfossil that typically forms irregular submillimetric clusters. Broadly similar fossils, such as *Izhella* and *Shuguria*, are distinguished by wall thickness and number of chambers. Some form millimetric dendritic growths. *Chabakovia* filaments consist of thin-walled chambers. In contrast, *Gemma* and *Tarthina* have few chambers with very thick walls.

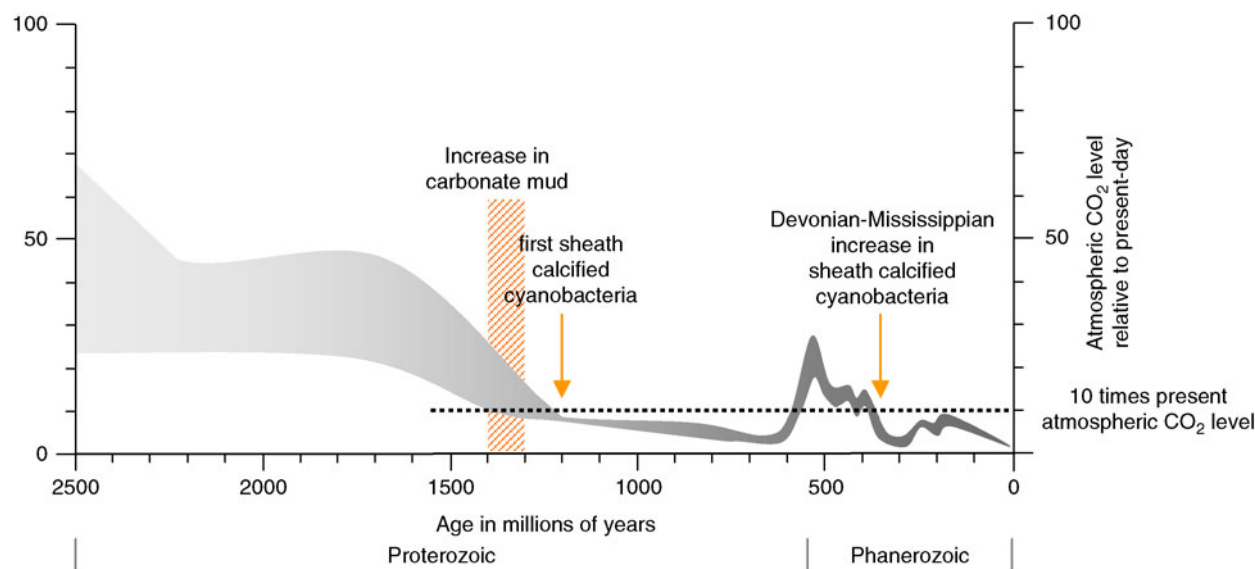
There is considerable mingling of characters among some of these generally simply organized fossils that can make them difficult to distinguish within these broad groups (Pratt, 1984; Riding and Voronova, 1985; Woo et al., 2008). Dense filaments and chambered forms are much more problematic, whereas tubiform and shrub-like forms can often be compared with present-day sheath calcified cyanobacteria. These include sedimentologically important Cambrian and late Devonian fossils such as *Epiphyton* and *Renalcis* that have some resemblances to cyanobacteria but are not identical to modern examples, and are generally referred to as calcimicrobes. Some *Epiphyton* closely resemble red algae (Luchinina and Terleev, 2008).

Proterozoic record and secular controls

The possibilities that marine carbonate saturation state and changes in atmospheric CO_2 level could be reflected in the geological record of cyanobacterial calcified microfossils and carbonate mud (Riding, 2006, p. 309) focuses attention on the mid-Proterozoic interval when calcified sheaths first appeared (Kah and Riding, 2007) and carbonate mud became abundant (Sherman et al., 2000, p. 290) (Figure 5).

CCM induction

Badger et al. (2002) suggested that cyanobacterial CCMs developed in the late Palaeozoic as CO_2 declined and O_2 increased. However, both modeling and palaeosol proxies (Kasting, 1987; Sheldon, 2006) suggest that similar decline in CO_2 occurred in the mid-Proterozoic. It can therefore be suggested that the Mesoproterozoic appearance of calcified sheath microfossils reflects development of CCMs as CO_2 fell below a critical threshold that may



Calcified Cyanobacteria, Figure 5 Increase in marine carbonate mud accumulation ~ 1400 – 1300 Ma ago (Sherman et al., 2000) and first appearance of sheath-calcified cyanobacteria ~ 1200 Ma ago (Kah and Riding, 2007) compared with decline in estimated atmospheric CO_2 values. It is suggested that decline in CO_2 to $\sim 10\times$ present atmospheric level favored development of CCMs that promoted cyanobacterial sheath calcification and whiting production (Riding, 2006). Incorporation of calcified filaments in microbial carbonates gave rise to calcified microbe thrombolites and dendrolites. Inferred Proterozoic CO_2 trend based on Sheldon (2006), Kah and Riding (2007), Hyde et al. (2000), and Ridgwell et al. (2003). Phanerozoic CO_2 trend from Berner and Kothavala (2001, Fig. 13). Threshold for CCMs based on Badger et al. (2002). Decline in CO_2 level below $10\times$ present-level was repeated ~ 350 Ma ago and coincides with marked Devonian-Mississippian increase in calcified cyanobacteria (see Arp et al., 2001, Figs. 3d and 8b).

have been $\sim 10\times$ PAL (Badger et al., 2002; Riding, 2006; Kah and Riding, 2007). Under high early Proterozoic CO_2 levels, cyanobacterial photosynthesis may have relied on CO_2 diffusion that may not have significantly altered pH near the cell surface. Falling CO_2 levels would have encouraged active carbon uptake to maintain photosynthesis and this, in turn, promoted calcification (Figures 1 and 5).

Carbonate mud: Carbonate mud appears to have been scarce in the late Archaean (Sumner and Grotzinger, 2004), but it increased in abundance during the Proterozoic (Kah and Grotzinger, 1992) and was widespread in the Neoproterozoic (Herrington and Fairchild, 1989). Much of it is thought to have been produced by whittings stimulated by phytoplankton photosynthesis (Grotzinger, 1989, 1990). A significant increase in carbonate mud occurred ~ 1400 – 1300 Ma ago (Sherman et al., 2000, p. 290), prior to the appearance of sheath calcification (Kah and Riding, 2007). This “water column factory” transformed carbonate platform sedimentation by creating extensive micrite-rich subtidal deposits (Sherman et al., 2000, p. 290). The timing of this increase in carbonate mud therefore broadly coincided with the first appearance of *Girvanella*-like and other sheath-calcified fossils (see below), and may be linked to CCM development in cyanobacteria, but in this case to planktic forms, possibly similar to present-day *Synechococcus* (Riding, 2006). Muddy carbonate platforms were the deposits in which distinctive molar tooth facies developed (James et al.,

1998; Sherman et al., 2000, p. 290). It can therefore be speculated that mid-late Proterozoic increase in micrites and molar tooth facies, together with *Girvanella* and thrombolites, all reflect decline in atmospheric CO_2 that stimulated CCM induction and calcification in cyanobacteria (Riding, 2006) (Figure 5).

Cyanobacterial sheath calcification: The first appearance of calcified cyanobacteria in the mid-Proterozoic – interpreted to reflect the first development of CCMs in photosynthetic organisms (Riding, 2006) – marked a radical change from stromatolitic to thrombolitic fabrics. Mesoproterozoic examples reported from the ~ 1200 Ma Society Cliffs Fm are currently the oldest known *Girvanella*-like calcified filaments, and are associated with micritic bush-like structures also interpreted as calcified cyanobacteria (Kah and Riding, 2007). The presence of filamentous fabrics had long been recognized in late Proterozoic and early Palaeozoic stromatolites. Microstructure with filament moulds was termed Canaliphorida and Filiformita by Komar (1976, 1989, see Bertrand-Sarfati et al., 1994, p. 182, Fig. 18). Aitken (1989, pp. 15–16) described “dendriform” and “lamelliform elements” as important components of Little Dal reefs in the Mackenzie Mountains. He regarded both as stromatolites with “unusual” or “unique” characteristics: thin-walled tubes and *Renalcis*-like objects in dendriform element, and a reticulate “ladder-rung” arrangement that “may be formed by a meshwork of tubes” in lamelliform element. He remarked that these fabrics “are not typically

stromatolitic” and that “sediment trapping may not have been the dominant process in their formation” (p. 15). Dendriiform and lamelliform elements look quite similar in two of his illustrations (Figs. 10 and 13). Subsequently, Turner et al. (1993, 2000a, Fig. 10b, 2000b) compared the tubules with *Girvanella* and noted that the lamelliform fabric consists of alternating dark layers of “calcimicrobial filaments” and lighter “more cement-rich” areas. Little Dal “hollow tubules with micritic walls” are figured by Batten et al. (2004, Fig. 9b). Knoll and Semikhatov (1998, p. 410, Figs. 3 and 4) described well-preserved “filmy or platy” microstructure in early Neoproterozoic *Baicalia lacera* stromatolites from the Chernaya Rechka Fm, Igarka, Siberia. They found it to be associated with “a distinctly filamentous microstructure” in which “laminae comprising densely interwoven to scattered, vertically, or subhorizontally oriented filaments are interspersed with layers of spongy or dense microspar.” They interpreted the 8–10 μm tubes as “sheaths of LPP-type (*Lyngbya*, *Phormidium*, *Plectonema*) cyanobacteria and preserved as drusy microspar encrustations” (Knoll and Semikhatov, 1998, p. 411). Similar *Baicalia lacera* fabrics in the $\sim 1\text{Ga}$ Burovaya Formation of west-central Siberia locally contain calcified tubes resembling *Siphonophycus* (Petrov and Semikhatov, 2001, p. 270). The lamelliform element shown by Turner et al. (2000a, Fig. 10b) resembles the distinctive “filmy” microstructure of similar age *Baicalia lacera* (Petrov and Semikhatov, 2001, Figs. 5b and 6a) which also has steeply dipping laminae and, as noted above, quite possibly filamentous microstructure too. In these examples, layers of filamentous fabric are generally interleaved with lighter, sparry, layers. If the sparry layers were lacking, the deposit would be indistinguishable from “skeletal stromatolite” (Riding, 1977) and “porostromate stromatolite” (Monty, 1981). In addition to the Little Dal and Chernaya Rechka examples, calcified filaments reminiscent of *Girvanella* are relatively widespread elsewhere

in the Neoproterozoic, e.g., in the ~ 750 – 700 Ma Draken Fm, ~ 725 – 675 Ma Svanbergfjellet Fm, and $\sim 700\text{ Ma}$ Upper Eleonore Bay Supergroup, Greenland (refs in Knoll and Semikhatov, 1998, p. 413). Throughout the Neoproterozoic, these thrombolites with calcified microbial fabrics (Kennard and James, 1986) are intimately associated with stromatolites but typically occur in deeper subtidal environments (Aitken, 1967).

Marine carbonate saturation

Inception of cyanobacterial calcification would have required overall seawater carbonate saturation to be relatively high. Grotzinger (1990) has argued that seawater saturation state progressively reduced during the Proterozoic and that this could account for concomitant decline in stromatolite abundance. Nonetheless, the widespread continued development of microbial carbonates into the Neoproterozoic, together with calcified sheaths themselves, indicates that saturation state was not low. This may have changed during widespread glaciations that occurred in the late Proterozoic ~ 700 – 570 Ma ago (Walter et al., 2000). Sheath-calcified cyanobacteria appear to have been scarce during “Snowball” glaciations. Lower temperature and $p\text{CO}_2$ levels would have decreased seawater saturation state, hindering microbial calcification generally. Shields (2002) attributed decline in Molar Tooth facies $\sim 750\text{ Ma}$ ago to reduction in carbonate saturation. Since cooling would also have favored diffusive entry of CO_2 into cells (Raven et al., 2002) it could have slowed CCM development, further reducing cyanobacterial calcification. As Earth emerged from these snowball glaciations, global warming and O_2 rise could have reactivated CCM development, and rising temperature, calcium, and $p\text{CO}_2$ levels would have increased seawater saturation state, stimulating calcification (Riding, 2006). Calcified cyanobacteria became relatively diverse



Calcified Cyanobacteria, Figure 6 Early Cambrian (Botomian) dendrolite-thrombolite reef, $\sim 70\text{ m}$ thick, near Tafraoute, Anti-Atlas Mountains, Morocco. Figures in foreground for scale.

in the earliest Cambrian (Nemakit-Daldynian) (Riding and Voronova, 1984) and were important components of Cambrian reefs (Riding, 2000; Rowland and Shapiro, 2002).

Calcmicrobial reefs and the Cambrian radiation

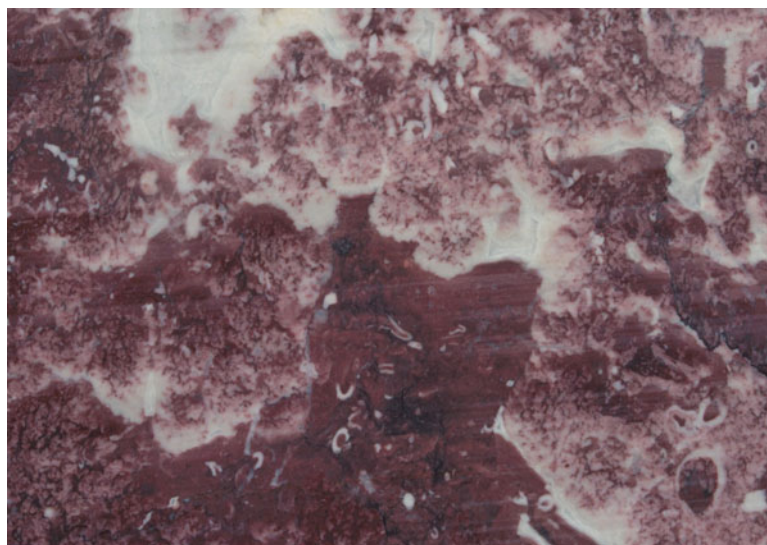
Calcified microfossils such as *Epiphyton*, *Renalcis*, and *Angusticellularia* are major components of Cambrian reefs (Figures 6 and 7) and similar forms reappeared in the late Devonian. The widespread development of microbial reefs in the early Cambrian may have directly contributed to marine invertebrate diversification. Reefs constructed by calcified microbes appeared in the latest Neoproterozoic, ~550 Ma ago (Grotzinger et al., 2000) and were common in Siberia, Altai Sayan, and Mongolia during the Nemakit-Daldynian (543–526 Ma ago) (Riding, 2002). In the Tommotian, 526–522 Ma ago, this reef-building association was augmented by archaeocyath sponges, with calcified microbes generally being volumetrically dominant (Rowland and Shapiro, 2002), and the reefs became large and cavernous. The exceptional biodiversity that characterizes reefs in general is largely due to the process of reef bioconstruction itself (Cocito, 2004). Rapid accretion of framework with synoptic relief and internal growth cavities creates substrates and spaces that differ in incident light, water movement, and sedimentation, as well as accessibility. This increased habitat size and partitioning generates heterogeneity that promotes biodiversity. For example, Cambrian trilobites show peak diversity in reefal environments (Westrop and Adrain, 2001), and comparison of level-bottom and reef communities in general shows that early Cambrian reefs were centers of diversity (Burzin et al., 2001, Fig. 10.2) with number of reef-building species comparable to that of late

Neogene fossil reefs (Kiessling, 2002, Fig. 20). The expansion of these globally distributed reef communities corresponded with rapid diversification of the shallow marine biota, especially during the Tommotian-early Botomian (~526–517 Ma ago) (Zhuravlev 2001, Fig. 8.1), suggesting a direct link between microbial carbonate development and metazoan evolution.

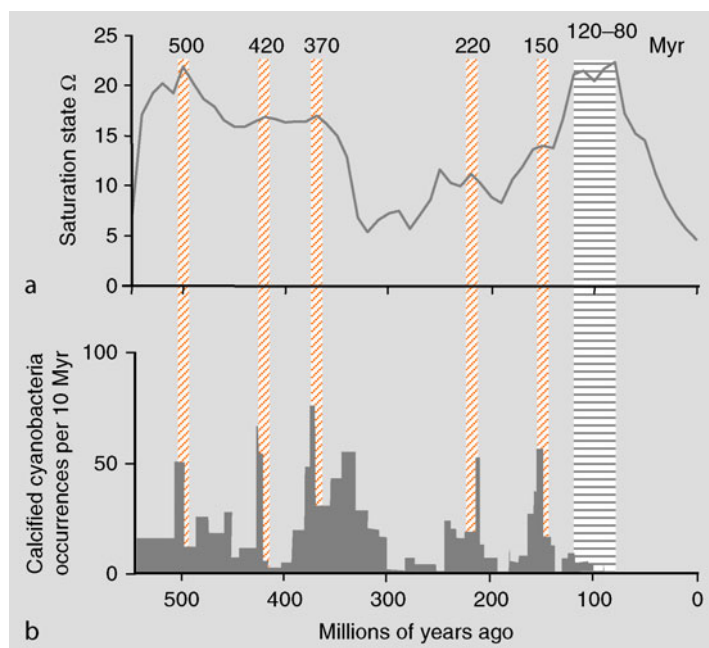
Phanerozoic record and secular controls

Cyanobacterial sheath calcification: Following their Mesoproterozoic inception, sheath-calcified cyanobacteria remained generally common in shallow-water marine carbonates during much of the Palaeozoic and until the late Jurassic (Arp et al., 2001) (Figure 8b). The abundance of sheath-calcified cyanobacteria during the early Palaeozoic (when CO₂ levels are thought to have been high, see Berner and Kothavala, 2001), suggests that CCMs continued to be induced even when pCO₂ substantially exceeded 10 PAL. This may reflect calcification in habitats subject to carbon limitation that induced CCMs, such as microbial mats and reefs (Riding, 2006). Nonetheless, enhancement of CCMs by overall decline in atmospheric CO₂ could be reflected by increased calcified-sheath abundance in the Mississippian, ~335 Ma ago (Riding, 2006) (Figure 5). However, it seems that this increase in calcified-sheath abundance promoted by falling CO₂ was itself terminated by subsequent steep decline in seawater saturation state ~325 Ma ago, before the end of the Mississippian (Figure 8a).

Broad similarities between calcified-sheath abundance and carbonate saturation are suggested by peaks in the late Cambrian (~500 Ma), late Silurian (~420 Ma), late Devonian (~370 Ma), late Triassic (~220 Ma), and late



Calcified Cyanobacteria, Figure 7 Early Cambrian calcimicrobial reef framework, showing clusters of mainly pendant filaments, early cement and geopetal cavity fill. Near Taroudannt, Anti-Atlas Mountains, Morocco. Width of view ~35 cm.



Calcified Cyanobacteria, Figure 8 Comparison of (a) Marine saturation ratio (Ω) for calcite, calculated from estimates of seawater and atmospheric composition, and (b) abundance of marine sheath-calcified cyanobacteria (Arp et al. 2001, Fig. 3d) (from Riding and Liang, 2005, Fig. 5). Obliquely ruled areas indicate five intervals approximately 500, 420, 370, 220, and 150 millions of years (Myr) ago where peaks of calculated saturation ratio broadly coincide with increased abundances of calcified cyanobacteria. This correspondence supports the view that cyanobacterial calcification has been increased at times of generally elevated seawater saturation for CaCO_3 minerals. The horizontally ruled area indicates the interval ~120–80 Myr ago in which high calculated saturation contrasts with low abundance of calcified cyanobacteria. This anomaly could reflect lowered Cretaceous carbonate saturation due to widespread deposition of pelagic carbonate by coccolithophore algae and globigerine foraminifers, since biological removal of CaCO_3 is not incorporated into the calculation of saturation values.

Jurassic (~150 Ma) (Figure 8), supporting the view that saturation state has significantly influenced the degree of cyanobacterial sheath calcification over geological time (Riding and Liang, 2005).

Reduced abundance of sheath-calcified cyanobacteria ~120–80 Ma ago contrasts with calculated saturation state which is elevated during this interval. This anomaly is thought to reflect reduction in actual (as opposed to calculated) saturation state due to burial of CaCO_3 precipitated as calcified skeletons by plankton such as coccolithophore algae and globigerine foraminifers (Riding and Liang, 2005). Such biological removal of calcium is not included in the input used to calculate the saturation values shown. This effect of planktic calcifiers on the seawater system may have continued into the Palaeogene. During the past 50 Ma, from the early Eocene onward calculated saturation has declined steeply in response to low values of Ca^{2+} ions and pCO_2 , and this is consistent with scarcity of marine calcified cyanobacteria. Consequently, although low pCO_2 values during the Cenozoic (see Berner and Kothavala, 2001) are expected to induce cyanobacterial CCMs, the absence of sheath calcification in marine cyanobacteria presumably reflects the overriding effect of low carbonate saturation.

Biogenic whittings: It can be inferred that, following the development of CCMs in the mid-Proterozoic, picoplanktic cyanobacterial activity could have been an important source of marine carbonate mud whenever inorganic carbon could be a limiting resource, as in plankton blooms (Rost et al., 2003), even when overall atmospheric CO_2 levels were high. If this is correct, then the principal factor determining biogenic whiting occurrence would have been carbonate saturation state. Calculations suggest that saturation was relatively elevated during much of the early-mid Palaeozoic and parts of the Mesozoic (Riding and Liang, 2005; and Figure 8a).

The origins of micrite are notoriously difficult to interpret even in present-day seas, and it is known that calcified green algae can also be significant producers (Stockman and Ginsburg, 1967). How can the possible contribution of biogenic whittings therefore be assessed in the geological past? One line of reasoning is to assume that sheath-calcified cyanobacteria reflect generally similar conditions to those that favor picoplanktic whiting precipitation. The geological record of sheath-calcified fossils such as *Girvanella* is relatively well-documented, and they show marked fluctuations in abundance (Arp et al., 2001, Fig. 3d) (Figure 8b). It could therefore be predicted that picoplanktic mud should show a similar pattern of

abundance, with peaks for example in the late Cambrian, Devonian-Mississippian, and late Jurassic. It should be possible to test whether carbonate mud was relatively abundant at these times. It is also possible that biogenic whittings – and sheath calcification – should have been further intensified whenever $p\text{CO}_2$ levels fell to levels near or below ~ 10 PAL, as for example ~ 350 Ma ago (Figure 5).

Cenozoic decline in seawater carbonate saturation, suggested by calculations based on modeled values (Riding and Liang, 2005, Figs. 5a and 8a), would be expected to have reduced biogenic whiting precipitation. Certainly, sheath calcification is scarce to absent during most of the past 100 Ma (Arp et al., 2001, Fig. 3d) (Figure 8b). In this context, it is not surprising that there is uncertainty whether present-day marine whittings on the Bahaman Banks are biogenic or mainly reflect sediment resuspension (Broecker et al., 2000). From a geobiological perspective, however, even if present-day marine conditions do not greatly favor phytoplankton-stimulated precipitation of whittings, these may have been very important at times in the geologic past environment when carbonate saturation state was sufficiently high. If this is correct, biogenic whittings could account for a substantial part of the carbonate mud that accumulated on shallow shelves between the late Mesoproterozoic and Mesozoic.

Summary

Calcified cyanobacteria are easily overlooked fossils, yet their long history coupled with their sensitivity to environmental controls on their calcification, make them potentially significant indices of changes in seawater chemistry and atmospheric CO_2 level throughout the Proterozoic and Phanerozoic. Locally they are also sedimentologically important reef components, and they have played a major role in the formation of calcimicrobial thrombolites that are especially widespread in the Neoproterozoic and early Palaeozoic. At the present day their vanishing scarcity in marine environments is thought to reflect relatively low seawater carbonate saturation that has persisted throughout the Cenozoic. However, their local abundance in oncoids and tufas of freshwater calcareous streams and lakes, and their involvement in lacustrine whittings, are reminders of the ability of cyanobacteria to promote intense CaCO_3 deposition when conditions that include ambient saturation state, are favorable.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Aitken, J. D., 1989. Giant “algal” reefs, middle/upper Proterozoic Little Dal group (>770 , <1200 Ma), Mackenzie Mountains, N. W.T., Canada. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Area*, Canadian Society of Petroleum Geologists, Memoir, Vol. 13, pp. 13–23.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2002. Calcification of cyanobacterial filaments: Girvanella and the origin of lower Paleozoic lime mud: comment. *Geology*, **30**, 579–580.
- Badger, M., and Price, D., 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany*, **54**, 609–622.
- Badger, M. R., Hanson, D., and Price, G. D., 2002. Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Functional Plant Biology*, **29**, 161–173.
- Batten, K. L., Narbonne, G. M., and James, N. P., 2004. Paleoenvironments and growth of early Neoproterozoic calcimicrobial reefs: platform Little Dal Group, northwestern Canada. *Precambrian Research*, **133**, 249–269.
- Berner, R. A., and Kothavala, Z., 2001. GEOCARB III. A revised model of atmospheric CO_2 over Phanerozoic time. *American Journal of Science*, **301**, 182–204.
- Bertrand-Sarfati, J., Freytet, P., and Plaziat, J. C., 1994. Microstructures in tertiary nonmarine stromatolites (France). Comparison with Proterozoic. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 155–191.
- Bissett, A., de Beer, D., Schoon, R., Shiraishi, F., Reimer, A., and Arp, G., 2008. Microbial mediation of stromatolite formation in karst-water creeks. *Limnology and Oceanography*, **53**(3), 1159–1168.
- Bornemann, J., 1886. Die Versteinerungen des cambrischen Schichtensystems der Insel Sardinien nebst vergleichenden Untersuchungen über analoge Vorkommnisse aus anderen Ländern 1. *Nova Acta der Kaiserslichen Leopoldinisch-Carolinischen Deutschen Akademie der Naturforscher*, **51**(1), 1–147.
- Broecker, W. S., and Takahashi, T., 1966. Calcium carbonate precipitation on the Bahama Banks. *Journal of Geophysical Research*, **71**, 1575–1602.
- Broecker, W. S., Sanyal, A., and Takahashi, T., 2000. The origin of Bahamian whittings. *Geophysical Research Letters*, **27**, 3759–3760.
- Broecker, W. S., Langdon, C., Takahashi, T., and Peng, T. H., 2001. Factors controlling the rate of CaCO_3 precipitation on the Great Bahama Bank. *Global Biogeochemical Cycles*, **15**, 589–596.
- Burzin, M. B., Debrenne, F., and Zhuravlev, A. Yu., 2001. Evolution of shallow-water level-bottom communities. In Zhuravlev, A. Yu., and Riding, R. (eds.), *The Ecology of the Cambrian Radiation*. New York: Columbia University Press, pp. 217–237.
- Cavalier-Smith, T., Brasier, M., and Embley, T. M., 2006. Introduction: how and when did microbes change the world? *Philosophical Transactions of the Royal Society, B*, **361**, 845–850.
- Chafetz, H. S., and Buczynski, C., 1992. Bacterially induced lithification of microbial mats. *Palaios*, **7**, 277–293.
- Cloud, P. E., Jr., 1962. Environment of calcium carbonate deposition west of Andros Island, Bahamas. *US Geological Survey Professional Paper*, **350**, 138.
- Cocito, S., 2004. Bioconstruction and biodiversity: their mutual influence. *Scientia Marina*, **68**(Supplement 1), 137–144.
- Couté, A., and Bury, E., 1988. Ultrastructure d'une Cyanophycée aérienne calcifère cavernicole: Scytonema julianum Frank (Richter) (Hormogonophicidae, Nostocales, Scytonemataceae). *Hydrobiologia*, **160**, 219–239.
- Défarge, C., Trichet, J., and Couté, A., 1994. On the appearance of cyanobacterial calcification in modern stromatolites. *Sedimentary Geology*, **94**, 11–19.

- Dillon, J. G., and Castenholz, R. W., 1999. Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *Journal of Phycology*, **35**, 673–681.
- Dittrich, M., Kurz, P., and Wehrli, B., 2004. The role of autotrophic picocyanobacteria in calcite precipitation in an oligotrophic lake. *Geomicrobiology Journal*, **21**, 45–53.
- Drews, G., and Weckesser, J., 1982. Function, structure and composition of cell walls and external layers. In Carr, N. G., and Whitton, B. A. (eds.), *The Biology of Cyanobacteria*. Oxford: Blackwell, pp. 333–357.
- Dudman, W. F., 1977. The role of surface polysaccharides in natural environments. In Sutherland, I. (ed.), *Surface Carbohydrates of the Prokaryotic Cell*. London: Academic Press, pp. 357–414.
- Friedmann, E. I., 1979. The genus *Geitleria* (Cyanophyceae or Cyanobacteria): distribution of *G. calcarea* and *G. floridana* n. sp. *Plant Systematics and Evolution*, **131**, 169–178.
- Garcia-Pichel, F., and Castenholz, R. W., 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *Journal of Phycology*, **27**, 395–409.
- Garwood, E. J., 1914. Some new rock-building organisms from the Lower Carboniferous beds of Westmorland. *Geological Magazine*, 6th decade, **1**, 265–271, pls. 20–21.
- Giordano, M., Beardall, J., and Raven, J. A., 2005. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*, **56**, 99–131.
- Golubic, S., 1973. The relationship between blue-green algae and carbonate deposits. In Carr, N., and Whitton, B. A. (eds.), *The Biology of Blue-Green Algae*. Oxford: Blackwell, pp. 434–472.
- Grotzinger, J. P., 1989. Facies and evolution of Precambrian carbonate depositional systems. The emergence of the modern platform archetype. In Crevello, P. D., Wilson, J. L., Sarg, J. F., and Read, J. F. (eds.), *Controls on Carbonate Platform and Basin Development*. Tulsa, OK: SEPM special publication number, 44, pp. 79–106.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., Watters, W. A., and Knoll, A. H., 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology*, **26**, 334–359.
- Hägele, D., Leinfelder, R., Grau, J., Burmeister, E.-G., and Struck, U., 2006. Oncoids from the river Alz (southern Germany): tiny ecosystems in a phosphorus-limited environment. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 378–395.
- Herrero, A., and Flores, E. (eds.), 2008. *The Cyanobacteria: Molecular Biology, Genomics and Evolution*. UK: Caister Academic Press, 484 pp.
- Herrington, P. M., and Fairchild, I. J., 1989. Carbonate shelf and slope facies evolution prior to Vendian glaciation, central East Greenland. In Gayer, R. A. (ed.), *The Caledonide Geology of Scandinavia*. London: Graham and Trotman, pp. 263–273.
- Hofmann, H. J., 1976. Precambrian microflora, Belcher Islands, Canada: significance and systematics. *Journal of Paleontology*, **50**, 1040–1073.
- Hoiczky, E., 1998. Structural and biochemical analysis of the sheath of *Phormidium uncinatum*. *Journal of Bacteriology*, **180**, 3923–3932.
- Hyde, W. T., Crowley, T. J., Baum, S. K., and Peltier, W. R., 2000. Neoproterozoic ‘snowball Earth’ simulations with a coupled climate/ice-sheet model. *Nature*, **405**, 425–429.
- James, N. P., Narbonne, G. M., and Sherman, A. G., 1998. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic. *Journal of Sedimentary Research*, **68**, 716–722.
- Kah, L. C., and Grotzinger, J. P., 1992. Early Proterozoic (1.9 Ga) thrombolites of the Rocknest Formation, Northwest Territories, Canada. *Palaaios*, **7**, 305–315.
- Kah, L. C., and Riding, R., 2007. Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. *Geology*, **35**, 799–802.
- Kaplan, A., and Reinhold, L., 1999. The CO₂-concentrating mechanism of photosynthetic microorganisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 539–570.
- Kaplan, A., Badger, M. R., and Berry, J. A., 1980. Photosynthesis and the intracellular inorganic carbon-pool in the blue green algae *Anabaena variabilis*. Response to external CO₂ concentration. *Planta*, **149**, 219–226.
- Kasting, J. F., 1987. Theoretical constraints on oxygen and carbon dioxide concentrations in the Precambrian atmosphere. *Precambrian Research*, **34**, 205–228.
- Kelts, K., Hsü, K. J., and Lerman, A., 1978. Freshwater carbonate sedimentation. In Lerman, A. (ed.), *Lakes; Chemistry, Geology, Physics*. New York, NY: Springer-Verlag, pp. 295–323.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. *Bulletin de la Institut Océanographique (Monaco)*, **13**, 61–117.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaaios*, **1**, 492–503.
- Kiessling, W., 2002. Secular variations in the Phanerozoic reef ecosystem. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*, Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 72, pp. 625–690.
- Knoll, A. H., and Semikhatov, M. A., 1998. The genesis and time distribution of two distinctive Proterozoic stromatolite microstructures. *Palaaios*, **13**, 408–422.
- Komar, V. A., 1976. Classification of stromatolites according to microstructure. In ‘*Palaeontology of the Precambrian and Early Cambrian*’, Novosibirsk, USSR: All Union Symposium, pp. 41–43 (in Russian).
- Komar, V. A., 1989. Classification of the microstructures of the Upper Precambrian stromatolites. *Himalayan Geology*, **13**, 229–238.
- Korde, K. B., 1973. *Vodorosli kembriya (Cambrian Algae)*. Nauka, Moscow: Academy of Sciences of the U.S.S.R., Transactions of the Palaeontological Institute, Vol. 139, pp. 1–350 (in Russian).
- Lee, B. D., Apel, W. A., and Walton, M. R., 2004. Screening of cyanobacterial species for calcification. *Biotechnology Progress*, **20**, 1345–1351.
- Luchinina, V. A., and Terleev, A. A., 2008. The morphology of the genus *Epiphyton* Bornemann. *Geologia Croatica*, **61**(2–3), 105–111.
- Merz, M. U. E., 1992. The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**, 81–102.
- Merz-Preiß, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams: ambient environment, chemical thresholds and biological processes. *Sedimentary Geology*, **126**, 103–124.
- Miller, A. G., and Colman, B., 1980. Evidence for HCO₃-transport in the blue-green alga (cyanobacterium) *Coccochloris penicostis*. *Plant Physiology*, **65**, 397–402.
- Monty, C. L. V., 1981. Spongiostromate vs. porostromate stromatolites and oncolites. In Monty, C. (ed.), *Phanerozoic Stromatolites*. Berlin: Springer-Verlag, pp. 1–4.
- Morse, J. W., Millero, F. J., Thurmond, V., Brown, E., and Ostlund, H. G., 1984. The carbonate chemistry of Grand Bahama Bank waters – after 18 years another look. *Journal of Geophysical Research – Oceans*, **89**, 3604–3614.

- Morse J. W., Gledhill D. K., and Millerog, F.J., 2003. CaCO_3 precipitation kinetics in waters from the Great Bahama Bank; implications for the relationship between bank hydrochemistry and whittings. *Geochimica et Cosmochimica Acta*, **67**, 2819–2826.
- Nicholson, H. A., and Etheridge, R. Jr., 1878. *A Monograph of the Silurian Fossils of the Girvan District in Ayrshire with Special Reference to those Contained in the "Gray Collection": Volume I, Fasciculus I (Rhizopoda, Actinozoa, Trilobita)*. Edinburgh, London: W. Blackwood and Sons, 1–135, pls 1–9.
- Pedley, H. M., 1990. Classification and environmental models of cool freshwater tufas. *Sedimentary Geology*, **68**, 143–154.
- Pentecost, A., 1981. The tufa deposits of the Malham district, north Yorkshire. *Field Studies* **5**, 365–387.
- Pentecost, A., 1987. Growth and calcification of the freshwater cyanobacterium *Rivularia haematites*. *Proceedings of the Royal Society of London, Series B*, **232**, 125–136.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer, p. 445.
- Pentecost, A., and Riding, R., 1986. Calcification in cyanobacteria. In Leadbeater, B. S. C., and Riding, R. *Biomining in Lower Plants and Animals*. Oxford: Clarendon Press. Systematics Association Special Volume 30, pp. 73–90.
- Petrov, P. Yu., and Semikhatov, M. A., 2001. Sequence organization and growth patterns of late Mesoproterozoic stromatolite reefs: an example from the Burovaya Formation, Turukhansk Uplift, Siberia. *Precambrian Research*, **111**, 257–281.
- Pia, J., 1927. Thallophtya. In Hirmer, M. (ed.), *Handbuch der Paläobotanik*. Munich: Oldenbourg, Vol. 1, pp. 31–136.
- Pollock, J. B., 1918. Blue-green algae as agents in the deposition of marl in Michigan lakes. *Report of the Michigan Academy of Science*, **20**, 247–260.
- Pratt, B. R., 1984. *Epiphyton and Renalcis* – diagenetic microfossils from calcification of coccoid blue-green algae. *Journal of Sedimentary Research*, **54**, 948–971.
- Proteau, P. J., Gerwick, W. H. et al., 1993. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. *Experientia*, **49**, 825–829.
- Raistrick, B., 1949. The stabilisation of the supersaturation of calcium carbonate solutions by anions possessing O-P-O-P-O chains. *Faraday Discussion Society*, **5**, 234–237.
- Raven, J. A., 2002. Evolution of cyanobacterial symbioses. In Rai, A. N., Bergman, B., and Rasmussen, U. (eds.), *Cyanobacteria in Symbiosis*. Dordrecht: Kluwer, pp. 329–346.
- Raven, J. A., Johnston, A. M., Kübler, J. E., Korb, R., McInroy, S. G., Handley, L. L., Scrimgeour, C. M., Walker, D. I., Beardall, J., Clayton, M. N., Vanderklift, M., Fredriksen, S., and Dunton, K. H., 2002. Seaweeds in cold seas: evolution and carbon acquisition. *Annals of Botany*, **90**, 525–536.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Riding, R., 1977. Calcified *Plectonema* (blue-green algae), a Recent example of *Girvanella* from Aldabra Atoll. *Palaeontology*, **20**, 33–46.
- Riding, R., 1982. Cyanophyte calcification and changes in ocean chemistry. *Nature*, **299**, 814–815.
- Riding, R., 1991. Calcified cyanobacteria. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 55–87.
- Riding, R., 1992. Temporal variation in calcification in marine cyanobacteria. *Journal of the Geological Society (London)*, **149**, 979–989.
- Riding, R., 1993. Phanerozoic patterns of marine CaCO_3 precipitation. *Naturwissenschaften* **80**, 513–516.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**(Supplement 1), 179–214.
- Riding, R., 2002. Structure and composition of organic reefs and carbonate mud mounds: concepts and categories. *Earth-Science Reviews*, **58**, 163–231.
- Riding, R., 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology*, **4**, 299–316.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Riding, R., and Voronova, L., 1982. Recent freshwater oscillatoriacean analogue of the Lower Palaeozoic calcareous alga *Angulocellularia*. *Lethaia*, **15**, 105–114.
- Riding, R., and Voronova, L., 1984. Assemblages of calcareous algae near the Precambrian/Cambrian boundary in Siberia and Mongolia. *Geological Magazine*, **121**, 205–210.
- Riding, R., and Voronova, L., 1985. Morphological groups and series in Cambrian calcareous algae. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology: Contemporary Research and Applications*. Berlin: Springer-Verlag, pp. 56–78.
- Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V., 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature*, **361**, 249–251.
- Robbins, L. L., and Blackwelder, P., 1992. Biochemical and ultrastructural evidence for the origin of whittings. A biologically induced calcium carbonate precipitation mechanism. *Geology*, **20**, 464–468.
- Robbins, L. L., Tao, Y., and Evans, C. A., 1997. Temporal and spatial distribution of whittings on Great Bahama Bank and a new lime mud budget. *Geology*, **25**, 947–950.
- Rost, B., Riebesell, U., Burkhardt, S., and Sültemeyer, D., 2003. Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography*, **48**, 55–67.
- Rothpletz, A., 1891. Fossile Kalkalgen aus den Familien der Codiaceen und der Corallineen. *Zeitschrift der Deutschen geologischen Gesellschaft*, **43**(2), 295–322, Berlin.
- Rowland, S. M., and Shapiro, R. S., 2002. Reef patterns and environmental influences in the Cambrian and earliest Ordovician. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 72, pp. 95–128.
- Seely, H. M., 1885. A new genus of Chazy sponge, *Streptochoetus*. *American Journal of Science*, Series 3, **30**, 355–357, 3 text-figs.
- Sheldon, N. D., 2006. Precambrian paleosols and atmospheric CO_2 levels. *Precambrian Research*, **147**, 148–155.
- Sherman, A. G., James, N. P., and Narbonne, G. M., 2000. Sedimentology of a late Mesoproterozoic muddy carbonate ramp, northern Baffin Island, Arctic Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 67, pp. 275–294.
- Shields, G. A., 2002. 'Molar-tooth microspar': a chemical explanation for its disappearance ~750 Ma. *Terra Nova*, **14**, 108–113.
- Shinn, E. A., Steinen, R. A., Lidz, B. H., and Swart, P. K., 1989. Whittings, a sedimentologic dilemma. *Journal of Sedimentary Research*, **59**, 147–161.
- Stal, L. J., 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist*, **131**, 1–32.
- Stockman, K. W., and Ginsburg, R. N. 1967. The production of lime mud by algae in south Florida. *Journal of Sedimentary Research*, **37**, 633–648.
- Sumner, D. Y., and Grotzinger, J. P., 2004. Implications for Neoproterozoic ocean chemistry from primary carbonate mineralogy of the Campbellrand-Malmani platform, South Africa. *Sedimentology*, **51**, 1–27.

- Thompson, J. B., 2000. Microbial whittings. In Riding, R., and Agramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer-Verlag, pp. 250–260.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N. W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. SEPM special publication number, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaios*, **15**, 87–111.
- Vologdin, A. G., 1932. *Arkheotsiaty Sibiri. Vypusk 2: Fauna kembriyskikh izvestnykh Altaya*. Moscow; Leningrad: State Sci.Tech. Geol. Explor. Publishing House p. 106 (in Russian with English resume).
- Vologdin, A. G., 1962. *Drevneyshie vodorosli SSSR*. Moscow: U.S.S.R. Academy of Sciences Publishing House, 656 pp. (in Russian).
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research* **100**, 371–433.
- Westrop, S. R., and Adrain, J. M. 2001. Sampling at the species level: impact of spatial biases on diversity gradients. *Geology*, **29**, 903–906.
- Whitton, B. A., and Potts, M. (eds.), 2000. *The Ecology of Cyanobacteria, their Diversity in Time and Space*. Dordrecht: Kluwer, 669 pp.
- Woo, J., Chough, S. K., and Han, Z. 2008. Chambers of epiphyton thalli in microbial buildups, zhangxia formation (Middle Cambrian), Shandong Province, China. *Palaios* **23**, 55–64.
- Yee, N., Benning, L. G., Phoenix, V. R., and Ferris, F. G. 2004. Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environmental Science and Technology*, **38**, 775–782.
- Zhuravlev, A. Yu., 2001. Biotic diversity and structure during the Neoproterozoic-Ordovician transition. In Zhuravlev, A. Yu., and Riding, R. (eds.), *The Ecology of the Cambrian Radiation*, New York: Columbia University Press, pp.173–199.

Cross-references

Alkalinity
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Carbonate Environments
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Organomineralization
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCITE PRECIPITATION, MICROBIALY INDUCED

Tanja Bosak

Massachusetts Institute of Technology, Cambridge, MA, USA

Definition

Microbially induced calcite precipitation describes the formation of calcium carbonate minerals from a solution due to the presence of microbial cells, biosynthetic products, or metabolic activity.

Calcium carbonate precipitation

The most basic requirement for the precipitation of calcium carbonate (CaCO_3) minerals, calcite, and aragonite is that the product of the concentrations of calcium $[\text{Ca}^{2+}]$ and carbonate ions $[\text{CO}_3^{2-}]$ exceeds the solubility product of calcite (Equation 1) and aragonite, respectively.

$$[\text{Ca}^{2+}][\text{CO}_3^{2-}] > 10^{-8.35} \quad (1)$$

The solubility of carbonate minerals depends on the temperature and pressure, decreasing with increasing temperatures and increasing with the increasing pressure. When a solution is in equilibrium with carbon dioxide, $[\text{CO}_3^{2-}]$ is determined by pH. In solutions that are undersaturated or not highly saturated, such as modern seawater, the biological activity can strongly control the precipitation of CaCO_3 . Biological processes exert considerably less control on the precipitation of CaCO_3 in environments characterized by high temperature, high pH, and high evaporation and degassing rates such as soda lakes, hot springs, caves, and freshwater tufas.

Microbial processes that promote the precipitation of CaCO_3

Culture-dependent and culture-independent studies have shown that microbes (Bacteria and Archaea) can induce extracellular precipitation of calcium carbonate minerals by:

- Increasing the local pH and the concentration of carbonate ion
- Promoting the nucleation of calcium carbonate minerals and removing the kinetic inhibitors of CaCO_3 precipitation.

Increase in local pH and the concentration of carbonate ion

Because the concentration of carbonate ion increases with the increasing pH, the precipitation of calcium carbonate minerals will also increase with the increasing pH. Many microbial metabolic processes can increase the pH and/or the concentration of carbonate ions: sulfate reduction in marine sediments (Dupraz and Visscher, 2005; Van Lith et al., 2003; Visscher et al., 2000), oxygenic

photosynthesis in freshwater streams and lakes (Merz-Preiß and Riding, 1999; Thompson et al., 1997), anaerobic methane oxidation coupled with sulfate reduction in marine sediments (Reitner et al., 2005), denitrification, degradation of amino-acids and small organic acids (Chekroun et al., 2004), and degradation of calcium oxalate in soils (Braissant et al., 2002). Whether microbes are able to significantly increase local pH and $[\text{CO}_3^{2-}]$ will largely depend on the buffering of the medium by dissolved inorganic carbon. Microbial activity in poorly buffered solutions such as lakes and streams is more likely to influence the saturation state of CaCO_3 (Merz-Preiß and Riding, 1999) than in well-buffered solutions such as soda lakes, hot springs, and solutions analogous to the early soda oceans (Arp et al., 1999a, b; Bosak and Newman, 2005).

Precipitation of calcium carbonate minerals also can be inhibited by the adsorption of various metabolites such as phosphate, sulfate, and organic moieties onto the faces of the growing crystals (Bosak and Newman, 2005; Liu and DeYoreo, 2004; Plant and House, 2002; Teng et al., 1998). Microbial growth and metabolic processes that lower the concentration of these ions in the solution can promote the precipitation of minerals even when they do not significantly influence pH and $[\text{CO}_3^{2-}]$ (Bosak and Newman, 2005; Kawaguchi and Decho, 2002).

Nucleation of calcium carbonate minerals on organic surfaces

Minerals can nucleate either homogeneously on the surface of preexisting carbonate grains, or heterogeneously on organic or other surfaces. When the external calcium carbonate sediment is sparse or absent, nucleation is the most energetically expensive step in the precipitation of calcium carbonate minerals. Organic matter can lower the free energy barrier required to form the initial nuclei, thus promoting mineral formation. Nucleation of CaCO_3 on organic surfaces (i.e., heterogeneous nucleation) occurs when negatively charged surface groups such as carboxylate, phosphate, and sulfate complex bind calcium. Although this process can initially inhibit the precipitation of minerals, once the local concentrations of calcium and carbonate has exceeded the binding capacity of the organic matrix, or when the matrix is partially degraded, mineral nucleation occurs preferentially on the organic template (Arp et al., 2003; Braissant et al., 2003; Decho et al., 2005). Heterogeneous nucleation can increase the number of crystal nuclei even when the bulk medium is supersaturated with calcium carbonate by more than an order of magnitude (Arp et al., 1999b; Bosak and Newman, 2003).

Heterogeneous nucleation of CaCO_3 can take place on the cell surfaces and envelopes, or within the exopolymeric matrix surrounding microbial cells. Examples of the former are the calcification of the sheaths surrounding cyanobacterial cells (Merz-Preiß and Zankl, 1993) and the whitening events caused by the precipitation of calcite on the outer S-layer of some planktonic

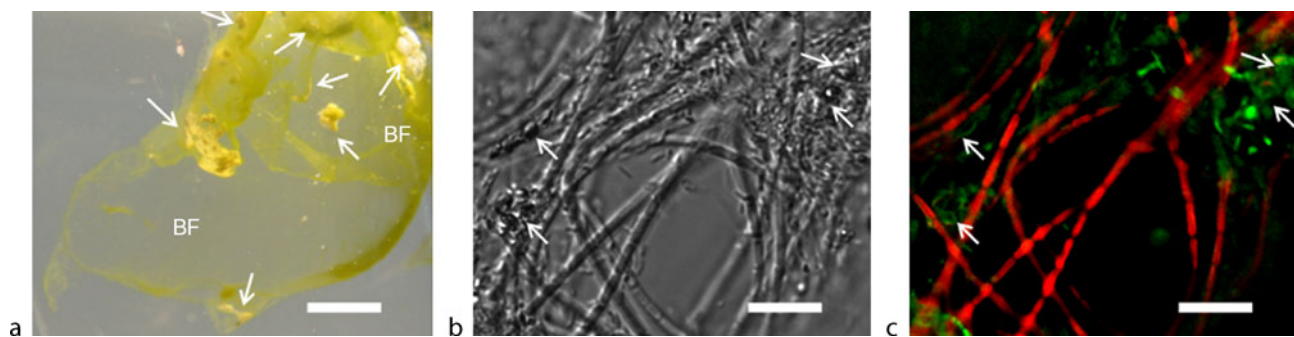
cyanobacteria (Thompson et al., 1997). These processes are most commonly observed in freshwater environments and require actively photosynthesizing cyanobacteria (Thompson et al., 1997). Cyanobacteria encrusted by CaCO_3 are considerably less abundant in modern marine calcium carbonates (Arp et al., 2001). Similarly, the relationship between marine whittings and cyanobacterial presence and activity has not been demonstrated conclusively (Thompson, 2000). Instead, in marine photic zones, carbonate minerals precipitate mainly within the extracellular exopolymeric matrix in areas of heterotrophic degradation (Chafetz and Buczynski, 1992; Dupraz and Visscher, 2005; Gautret et al., 2006; Van Lith et al., 2003; Visscher et al., 2000). In addition to the nucleation on exopolymeric substances or cell surfaces, CaCO_3 also preferentially nucleates around submicron vesicles that are secreted by sulfate-reducing bacteria (Aloisi et al., 2006). The nucleation of CaCO_3 on these vesicles may be a common mechanism in the environment, because outer-membrane vesicles are secreted by many Gram-negative bacteria (Beveridge, 1999; Kuehn and Kesty, 2005).

Recognition of microbially induced CaCO_3 precipitates in the rock record

Even when microbes do not induce measurable changes in the chemical composition of the bulk solution, they can alter the pH and carbonate chemistry, remove kinetic inhibitors, and promote mineral nucleation and precipitation at a microscale (Figure 1). When minerals precipitate, surrounded by microbial cells and microbial aggregates (biofilms) (Figure 1), they can preserve remnants of organic matter, such as acidic amino acids and microbial casts, long after the original cells have been degraded (Bosak et al., 2004a; Sprachta et al., 2001). Furthermore, CaCO_3 precipitated in the presence of organic compounds often has distinct shapes (Bosak and Newman, 2005; Braissant et al., 2003; Chafetz and Buczynski, 1992; Teng et al., 1998), although similar shapes can also be produced abiotically.

Biological influence on the morphology of carbonate crystals and structures is most prominent in skeleton-building organisms. Macroscopic and microscopic eukaryotic organisms such as calcareous algae, protists, corals, and mollusks precipitate hard body parts. Many of these organisms precipitate CaCO_3 by genetically encoded mechanisms that result in shells, skeletons, and carbonate grains with species-specific shapes. In contrast to these products of biologically controlled calcification, the shapes of microbially induced carbonate minerals can be distinguished from abiotically precipitated grains rarely (Golubic et al., 2000). Consequently, the potential identification of biosignatures of microbially induced carbonates in modern and past sedimentary carbonates can be controversial (Grotzinger and Knoll, 1999).

Recognition of microbially induced carbonates is also impeded by the difficulty in distinguishing between CaCO_3 structures that grew *in the presence* of microbes

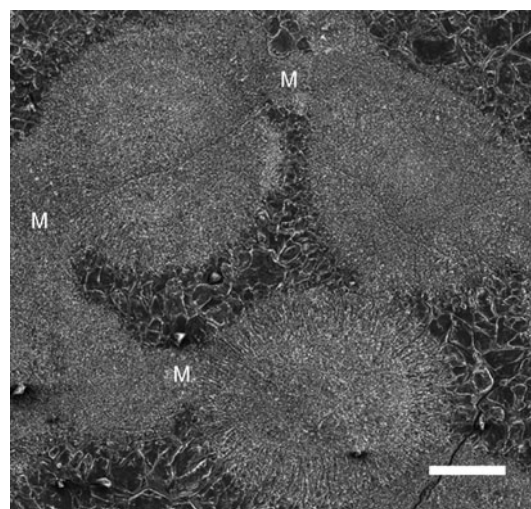


Calcite Precipitation, Microbially Induced, Figure 1 Aragonite precipitated on cyanobacterial biofilms (BF) floating in solution. (a) Photograph of a thin cyanobacterial biofilm with visible aragonite precipitates that preserve the biofilm. *White arrows* mark the densest mineral aggregates. (b) Transmitted-light micrograph of cyanobacterial filaments trapping small mineral grains (*white arrows*) in lightly mineralized areas. (c) Epifluorescence micrograph (deconvolved) of the same field of view as in (b). Cyanobacterial filaments fluoresce *red* and the nuclei of other microbes are stained *green*. Note that the mineral grains are surrounded by microbial aggregates that are not cyanobacteria (and are, presumably, heterotrophic). Scale bar: 5 mm in a, 5 μ m in b and c.

or organic matter from structures that grew *because* of microbial activity. Some examples of carbonate grains and structures with often ambiguous origin include peloids, ooids (Figure 2), whittings, fossil stromatolites, and nanobacteria. Recent developments in analytical techniques such as X-ray microscopy have enabled the detection of small quantities of fossil organic matrices in fossil carbonates at the microscale, thereby establishing that some fossil carbonates grew in the presence of microbes (Benzerara et al., 2006; Lepot et al., 2008). Despite the inclusion of organic matter, the role of biology in forming these structures remains unknown. As an alternative approach, experimental and quantitative models of the morphogenesis of sedimentary structures at the macroscale and microscale have been developed in an attempt to understand which fossil structures and shapes require biological processes (Batchelor et al., 2004; Bosak et al., 2004b; Dupraz et al., 2006; Grotzinger and Rothman, 1996; Reid et al., 2000; Suess and Futterer, 1972).

Summary

The potential of microbes to induce precipitation of calcium carbonate has sparked scientific interest for almost a century. The calcium carbonate (CaCO_3) minerals, calcite and aragonite, often precipitate in association with microbial cells and biofilms in marine and terrestrial sedimentary environments such as reefs, lakes, streams, beaches, soils, hot springs, and caves. Being ubiquitous and relatively insoluble, these minerals have helped preserve bacterial and archaeal cells and biofilms throughout geologic time (Figure 1). Microbes promote the precipitation of calcium carbonate metabolically, by increasing the pH and the alkalinity of their surroundings, or by the secretion of substances that facilitate the mineral nucleation. The recognition of microbially influenced calcium carbonate precipitates in the rock record is often challenging because abiotic carbonate precipitation can produce



Calcite Precipitation, Microbially Induced, Figure 2 An example of carbonate grains of controversial origin. Scanning electron micrograph of a thick polished section of late Jurassic ooids (rounded grains with layered interior structure) precipitated on the Adriatic Platform, Croatia, in low-energy, shallow water settings (Husinec and Reid, 2006). Ooids from low-energy environments were commonly interpreted as largely inorganic precipitates that adsorb organic molecules (Davies et al., 1978). More recently, cyanobacteria and other microbes have been shown to contribute to the formation of low-energy ooids and meniscus cements (M) (Brehm et al., 2006; Hillgartner et al., 2001; Plee et al., 2008). Although microbes may have contributed to the formation of ooids shown here, these grains do not preserve any textural evidence of the former presence of microorganisms. Scale bar: 100 μ m.

crystals with similar shapes and chemical signatures. Current research in the field of microbial carbonates tackles this challenge by conducting culture-dependent and culture-independent studies of microbial mineral precipitation, employing analytical techniques that can detect

small quantities of organic matter in fossil carbonates, and by developing theoretical models that explain the shape of macroscopic carbonate sedimentary structures. Microbially induced precipitation of carbonate minerals is likely to garner even more interest in the future with a primary focus on carbon sequestration, the production of biomimetic materials, and the conservation of ornamental stone.

Bibliography

- Aloisi, G., Gloter, A., Kruger, M., Wallmann, K., Guyot, F., and Zuddas, P., 2006. Nucleation of calcium carbonate on bacterial nanoglobules. *Geology*, **34**, 1017–1020.
- Arp, G., Reimer, A., and Reitner, J., 1999a. Calcification in cyanobacterial biofilms of alkaline salt lakes. *European Journal of Phycology*, **34**.
- Arp, G., Thiel, V., Reimer, A., Michaelis, W., and Reitner, J., 1999b. Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. *Sedimentary Geology*, **126**, 159–176.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Batchelor, M. T., Burne, R. V., Henry, B. I., and Jackson, M. J., 2004. A case for biotic morphogenesis of coniform stromatolites. *Physica A-Statistical Mechanics and Its Applications*, **337**, 319–326.
- Benzerara, K., Menguy, N., Lopez-Garcia, P., Yoon, T.-H., Kazmierczak, J., Tylliszczak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *Proceedings of the National Academy of Sciences*, **103**, 9440–9445.
- Beveridge, T. J., 1999. Structures of gram-negative cell walls and their derived membrane vesicles. *Journal of Bacteriology*, **181**, 4725–4733.
- Bosak, T., and Newman, D. K., 2003. Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, **31**, 577–580.
- Bosak, T., and Newman, D. K., 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. *Journal of Sedimentary Research*, **75**, 190–199.
- Bosak, T., Souza-Egipsy, V., Corsetti, F. A., and Newman, D. K., 2004a. Micrometer-scale porosity as a biosignature in carbonate crusts. *Geology*, **32**, 781–784.
- Bosak, T., Souza-Egipsy, V., and Newman, D. K., 2004b. An abiotic model for peloid formation. *Geobiology*, **2**, 189–198.
- Braissant, O., Cailleau, G., Dupraz, C., and Verrecchia, E. P., 2003. Bacterially induced mineralization of calcium carbonate in terrestrial environments: the role of exopolysaccharides and amino acids. *Journal of Sedimentary Research*, **73**, 485–490.
- Braissant, O., Verrecchia, E., and Aragno, M., 2002. Is the contribution of bacteria to terrestrial carbon budget greatly underestimated? *Naturwissenschaften*, **89**, 366–370.
- Brehm, U., Krumbein, W. E., and Palinska, K. A., 2006. Biomicrospheres generate ooids in the laboratory. *Geomicrobiology Journal*, **23**, 545–550.
- Chafetz, H. S., and Buczynski, C., 1992. Bacterially induced lithification of microbial mats. *Palaaios*, **7**, 277–293.
- Chekroun, K. B., Rodriguez-Navarro, C., Gonzalez-Munoz, M. T., Arias, J. M., Cultrone, G., and Rodriguez-Gallego, M., 2004. Precipitation and growth morphology of calcium carbonate induced by *Myxococcus xanthus*: implications for recognition of bacterial carbonates. *Journal of Sedimentary Research*, **74**, 868–876.
- Davies, P. J., Bubela, B., and Ferguson, J., 1978. The formation of ooids. *Sedimentology*, **25**, 703–730.
- Decho, A. W., Visscher, P. T., and Reid, R. P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography Palaeoclimatology Palaeoecology*, **219**, 71–86.
- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Dupraz, C., Pattisina, R., and Verrecchia, E. P., 2006. Translation of energy into morphology: simulation of stromatolite morphospace using a stochastic model. *Sedimentary Geology*, **185**, 185–203.
- Gautret, P., De Wit, R., Camoin, G., and Golubic, S., 2006. Are environmental conditions recorded by the organic matrices associated with precipitated calcium carbonate in cyanobacterial microbialites? *Geobiology*, **4**, 93–107.
- Golubic, S., Seong-Joo, L., and Browne, K., 2000. Cyanobacteria: architects of sedimentary structures. In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth and Planetary Sciences*, **27**, 313–358.
- Grotzinger, J. P., and Rothman, D. H., 1996. An abiotic model for stromatolite morphogenesis. *Nature*, **383**, 423–425.
- Hillgartner, H., Dupraz, C., and Hug, W., 2001. Microbially induced cementation of carbonate sands: are micritic meniscus cements good indicators of vadose diagenesis? *Sedimentology*, **48**, 117–131.
- Husinec, A., and Reid, J. F., 2006. Transgressive oversized radial ooid facies in the Late Jurassic Adriatic Platform interior: low-energy precipitates from highly supersaturated hypersaline waters. *GSA Bulletin*, **118**, 550–556.
- Kawaguchi, T., and Decho, A. W., 2002. A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO₃ polymorphism. *Journal of Crystal Growth*, **240**, 230–235.
- Kuehn, M., and Kesty, N., 2005. Bacterial outer membrane vesicles and the host–pathogen interaction. *Genes and Development*, **19**, 2645–2655.
- Lepot, K., Benzerara, K., Brown, G. E., and Philippot, P., 2008. Microbially influenced formation of 2,724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Liu, X. Y., and DeYoreo, J. J., 2004. *Nanoscale Structure and Assembly at Solid-Fluid Interfaces*. Boston: Kluwer Academic Publishers.
- Merz-Pleiß, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams: ambient environment, chemical thresholds and biological processes. *Sedimentary Geology*, **126**, 103–124.
- Merz-Pleiß, M., and Zankl, H., 1993. The influence of the sheath on carbonate precipitation by cyanobacteria. *Bollettino della Società Paleontologica Italiana*, (Spec Vol), pp. 325–331.
- Plant, L. J., and House, W. A., 2002. Precipitation of calcite in the presence of inorganic phosphate. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **203**, 143–153.
- Plee, K., Ariztegui, D., Martini, R., and Davaud, E., 2008. Unravelling the microbial role in ooid formation - results of an in situ experiment in modern freshwater Lake Geneva in Switzerland. *Geobiology*, **6**, 341–350.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., Macintyre, L. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early

- lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Sprachta, S., Camoin, G., Golubic, S., and Le Campion, T., 2001. Microbialites in a modern lagoonal environment: nature and distribution, Tikehau atoll (French Polynesia). *Palaeogeography Palaeoclimatology Palaeoecology*, **175**, 103–124.
- Suess, E., and Futterer, D., 1972. Aragonitic ooids 1: experimental precipitation from seawater in the presence of humic acid. *Sedimentology*, **19**, 129–139.
- Teng, H. H., Dove, P. M., Orme, C. A., and De Yoreo, J. J., 1998. Thermodynamics of calcite growth: baseline for understanding biomineral formation. *Science*, **282**, 724–727.
- Thompson, J. B., 2000. Microbial whittings, In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin Heidelberg: Springer, pp. 250–260.
- Thompson, J. B., Schultze-Lam, S., Beveridge, T. J., and DesMarais, D. J., 1997. Whiting events: biogenic origin due to the photosynthetic activity of cyanobacterial phytoplankton. *Limnology and Oceanography*, **42**, 133–141.
- Van Lith, Y., Warthmann, R., Vasconcelos, C., and McKenzie, J. A., 2003. Sulphate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology*, **1**, 71–79.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.

Cross-references

Alkalinity
 Algae (Eukaryotic)
 Animal Skeletons, Advent
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Carbonate Environments
 Carbonates
 Cold Seeps
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Hydrothermal Environments, Terrestrial
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Nan(n)obacteria
 Organomineralization
 Pedogenic Carbonates
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCIUM BIOGEOCHEMISTRY

Anton Eisenhauer
 IFM-GEOMAR, Christian Albrechts Universität zu Kiel,
 Kiel, Germany

Definition

Calcium (Ca) has the atomic number 20 and is an alkaline earth metal with an atomic mass of 40.978 amu. The

important role of Ca in biogeochemical processes is based on its chemical versatility, which is related to its highly adaptable coordination geometry, its divalent charge, modest binding energies, fast reaction kinetics, and its inertness in redox reactions (Williams, 1974). Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element in the Earth's crust and the seventh most abundant element in the ocean. It is essential for living organisms, particularly in cell physiology, shell formation, and calcification and hence, is usually the most common metal in many animals.

Calcium isotopes and their application in Biogeochemistry

There are seven Ca isotopes present in nature of which three isotopes (^{42}Ca (0.646%), ^{43}Ca (0.135%) through ^{44}Ca (2.086%)) are stable, one isotope (^{40}Ca (69.941%)) is radiogenic, one isotope (^{41}Ca) is cosmogenic, and two more isotopes (^{46}Ca (0.004%) and ^{48}Ca (0.187%)) are radioactive having extremely long half-lives (^{46}Ca : $2.8 \cdot 10^{15}$ years; ^{48}Ca : $> 4 \cdot 10^{19}$ years). The cosmogenic isotope ^{41}Ca is radioactive (half live of 103,000 years) and is produced by neutron activation of ^{40}Ca in the upper soil column. ^{41}Ca has received much attention in stellar studies because it decays to ^{41}K , a critical indicator of solar system anomalies. ^{40}Ca is the radiogenic daughter product of ^{40}K decay, along with ^{40}Ar . While K–Ar dating has been used extensively in the geological sciences, the prevalence of ^{40}Ca in nature has impeded its use in dating. Techniques using mass spectrometry and a double spike isotope dilution have been used for K–Ca age dating (Nägler and Villa, 2000).

Natural Ca isotope fractionation has, among other isotope systems such as oxygen (d^{18}O) and carbon (d^{13}C) fractionation, been applied for the study of calcification processes and the so-called vital effect (c.f. Gussone et al., 2003; Lemarchand et al., 2005). Latter effect describes the deviation of the expected inorganic isotope fractionation value or element partitioning coefficient (e.g., Sr/Ca, Mg/Ca, etc.) from the measured one in calcifying organisms such as coccolithophores, foraminifer, corals, and other calcifying organisms.

The more or less species-dependent Ca isotope fractionation of marine calcifying organisms as a function of the adjacent seawater temperature can be applied for the reconstruction of the dynamic of Ca concentrations throughout the Earth's history (c.f. Griffith et al., 2008; Farkas et al., 2007; Heuser et al., 2005; Fantle and DePaolo, 2005; De LaRocha and DePaolo, 2000) and for the reconstruction of past sea surface temperatures (c.f. Nägler et al., 2000; Hippler et al., 2006).

The dynamic of calcium in the ocean

Calcium is the second most abundant divalent cation (~ 10 mmol/l), besides magnesium (Mg, ~ 52 mmol/l). Calcium is considered to be a conservative element in the ocean water column, with a residence time of about

one million year. A number of scientific studies show that continental and oceanic weathering, mid-ocean ridge hydrothermal activity, and carbonate sedimentation are the major factors controlling the marine Ca cycle (Hart, 1973; Thompson, 1983; Zhu & Macdougall, 1998; Berner, 2004). Besides continental weathering and riverine influx, comprising about 70% of the Ca flux to the ocean, submarine magmatism and associated hydrothermal activity at the mid-ocean ridges as well as seafloor weathering are also recognized to be major sources for the marine Ca budget (Berner & Berner, 1996). A number of studies indicated that Ca is released from the oceanic crust during water–rock reactions ranging from high-temperature hydrothermal interactions to seafloor weathering at low temperatures below about 150°C (Berner, 2004; Amini et al., 2008). Ca-bearing mineral phases such as aragonite, calcite, anhydrite, and subordinately hydrous Ca-silicates (e.g., prehnite, zeolites) are formed during different stages of ocean crust alteration.

Marine calcification

The dynamic of Ca in the ocean is of particular interest because of the formation of biogenic and nonbiogenic calcium carbonate (CaCO_3), known as the process of calcification, and its interference and close linkage with the global carbon cycle (c.f. De LaRocha & DePaolo, 2000; Heuser et al., 2005). In particular, the formation of calcareous skeletons by marine planktonic organisms and their subsequent sinking to depth generate a continuous rain of CaCO_3 to the deep ocean and the underlying sediments. This is important in regulating marine carbon cycling and ocean–atmosphere CO_2 exchange. The present rise in atmospheric CO_2 levels causes a significant decrease (acidification) in surface ocean pH and a major change of carbonate chemistry. Laboratory experiments have shown that such changes slow down calcification in corals and coralline macroalgae. Over geological time scales, the Ca balance in the ocean is believed to be directly related to pCO_2 variations in the atmosphere, and it thus may interfere with climate changes on geological time scales (e.g., Demicco et al., 2003; Fantle and DePaolo, 2005).

Summary

Future progress in instrumental analytic will allow high-resolution measurement of Ca elemental and isotope partitioning in inorganic and biological systems, allowing to trace elemental pathways from a bulk solution to the site of calcification.

Bibliography

- Amini, M., Eisenhauer, A., Holmden, C., Boehm, F., Hauff, F., and Jochum, K. P., 2008. $\delta^{44/40}\text{Ca}$ variability in igneous rocks. *Geochimica et Cosmochimica Acta* **72**(12), A19–A19.
- Berner, R. A., 2004. A model for calcium, magnesium and sulfate in seawater over Phanerozoic time. *American Journal of Science*, **304**, 438–453.

- Berner, E. K., and Berner, R. A., 1996. *Global Environment: Water, Air, and Geochemical Cycles*. Upper Saddle River: Prentice Hall.
- De La Rocha, C. L., and DePaolo, D. J., 2000. Isotopic evidence for variations in the marine calcium cycle over the Cenozoic. *Science*, **289**, 1176–1178.
- Demicco, R. V., Lowenstein, T. K., and Hardie, L. A., 2003. Atmospheric pCO_2 since 60 Ma from records of seawater pH, calcium, and primary carbonate mineralogy. *Geology*, **31**(9), 793–796.
- Fantle, M. S., and DePaolo, D. J., 2005. Variations in the marine Ca cycle over the past 20 million years. *Earth and Planetary Science Letters*, **237**, 102–117.
- Farkas, J., Böhm, F., et al. 2007. Calcium isotope record of Phanerozoic oceans: Implications for chemical evolution of seawater and its causative mechanisms. *Geochimica et Cosmochimica Acta* **71**(21), 5117–5134.
- Griffith, E. M., Paytan, A., Caldeira, K., Bullen, T. D., and Thomas E., 2008. A dynamic marine calcium cycle during the past 28 million years. *Science*, **322**, 1671–1674.
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H., Lea, D. W., Bijma, J., and Nägler, T. F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochim Cosmochim Acta*, **67**(7), 1375–1382.
- Hart, S. R., and Zindler, A., 1989. Isotope fractionation laws: a test using calcium. *International Journal of Mass Spectrometry and Ion Physics*, **89**, 287–301.
- Hippler, D., Eisenhauer, A., and Thomas, F. N., 2006. Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140 ka. *Geochim Cosmochim Acta*, **70**, 90–100.
- Heuser, A., Eisenhauer, A., Böhm, F., Wallmann, K., Gussone, N., Pearson, P. N., Nägler, T. F., and Dullo, W.-C., 2005. Calcium isotope ($\delta^{44/40}\text{Ca}$) variations of Neogene planktonic foraminifera. *Paleoceanography*, **20**.
- Lemarchand, D., Wasserburg, G. J., and Papanastassiou, A., 2004. Rate-controlled calcium isotope fractionation in synthetic calcite. *Geochim Cosmochim Acta*, **68**(22), 4665–4678.
- Nägler, T. F., Villa, I. M., 2000. In pursuit of the ^{40}K branching ratios: K–Ca and ^{39}Ar – ^{40}Ar dating of gem silicates. *Chem Geol* **169**, 5–16.
- Nägler, T., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -isotopes: new powerful tool for reconstruction of past sea surface temperatures. *Geochemistry, Geophysics, Geosystems*, **1**, doi: 2000GC000091.
- Thompson, G., 1983. *Hydrothermal Fluxes in the Ocean*. New York: Academic Press.
- Williams, R. J. P., 1974. Calcium ions: their ligands and their function. *Biochemical Society Symposia*, **39**, 133–138.
- Zhu, P., and Macdougall, J. D., 1998. Calcium isotopes in the marine environment and the oceanic calcium cycle. *Geochim Cosmochim Acta*, **62**(10), 1691–1698.

Cross-references

[Animal Biocalcification, Evolution](#)
[Calcified Cyanobacteria](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbonate Environments](#)
[Carbonates](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Dolomite, Microbial](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

CAP CARBONATES

Joachim Reitner
University of Göttingen, Göttingen, Germany

Definition

Cap carbonates are unusual deposits of dolostones and limestones, often enriched in barite, that sharply overlie Neoproterozoic glacial deposits. They are from 2 to 50 m thick and occur on shallow platforms, shelves, and



Cap Carbonates, Figure 1 Marinoan ca. 635 My cap carbonate from Namibia (Fransfontein, Otavi Mountains). Characteristic features of this cap carbonate (mainly dolomite) are syndementarily formed Zebra Limestone-type fabrics. The horizontal fractures were originally cemented with large aragonitic crystals. The remaining pore spaces are filled up with cherts.



Cap Carbonates, Figure 2 Sturtian ca. 750 My old cap carbonate from Namibia (Rasthof cap carbonate, Baesvalkte). These black laminated carbonates are lithified microbial mats. Very common are “roll up structures” which are known from many microbial mat systems, even from modern ones.

slopes worldwide, even in regions otherwise lacking in carbonate strata.

Glaciation events during the late Neoproterozoic (Cryogenian–Ediacaran) are widespread on Earth, reflecting up to four global ice ages (“Snowball Earth Hypothesis”) between 730 and 580 Ma. Numerous publications dealing with these events exist (for example, see Chapter by Hoffman P.F., Snowball Earth, this volume; Shields, 2005; Jiang et al., 2003). Some of them exhibit characteristic, microcrystalline cap dolostones, and also limestones, which is unique and reflects a climate paradox. Particularly the Marinoan glaciation ca. 635 Ma ago is peculiar, because it provides evidence for low altitude glaciation at equatorial latitudes. Three models have been proposed to explain an increase in alkalinity inducing these global carbonate formation events:

(1) Overturn of an anoxic deep ocean; (2) enormous rates of chemical weathering in the hinterland after glaciation (Snowball Earth Hypothesis); (3) destabilization of methane clathrates and increase of alkalinity caused by anaerobic oxidation of methane (AOM). However, none of the hypotheses sufficiently explains the consistent sequence of precipitation events observed within cap carbonate successions. The formation of massive dolostones is very problematic and hardly understood. Massive microbial sulfate reduction may explain this event. Barite precipitation is also very common. The source of sulfate is most probably the weathered hinterland. Caused by seawater oversaturated with aragonite, diagenetically very early large aragonite crystals were formed. It is suggested that cap carbonates are primarily formed by a microbially mediated precipitation. Possibly, the highly oxygenated shallow marine environments of the postglacial Neoproterozoic oceans provided excellent conditions for the evolutionary development of new microbial communities, phototrophic organisms, and of course, the rapid development of Metazoa (Figures 1 and 2).

Bibliography

- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopes evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Shields, G. A., 2005. Neoproterozoic cap carbonates: a critical appraisal of existing models and plume world hypothesis. *Terra Nova*, **17**, 299–310.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Animal Biocalcification, Evolution](#)
[Calcite Precipitation, Microbially Induced](#)
[Calcium Biogeochemistry](#)
[Carbonate Environments](#)
[Cold Seeps](#)
[Dolomite, Microbial](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Permafrost Microbiology](#)
[Snowball Earth](#)

CARBON (ORGANIC, CYCLING)

Amber K. Hardison, Elizabeth A. Canuel
Virginia Institute of Marine Science, Gloucester Point,
VA, USA

Definition

Organic carbon (OC) refers to any carbon that occurs in compounds of ultimately biological origin that can be oxidized.

Introduction

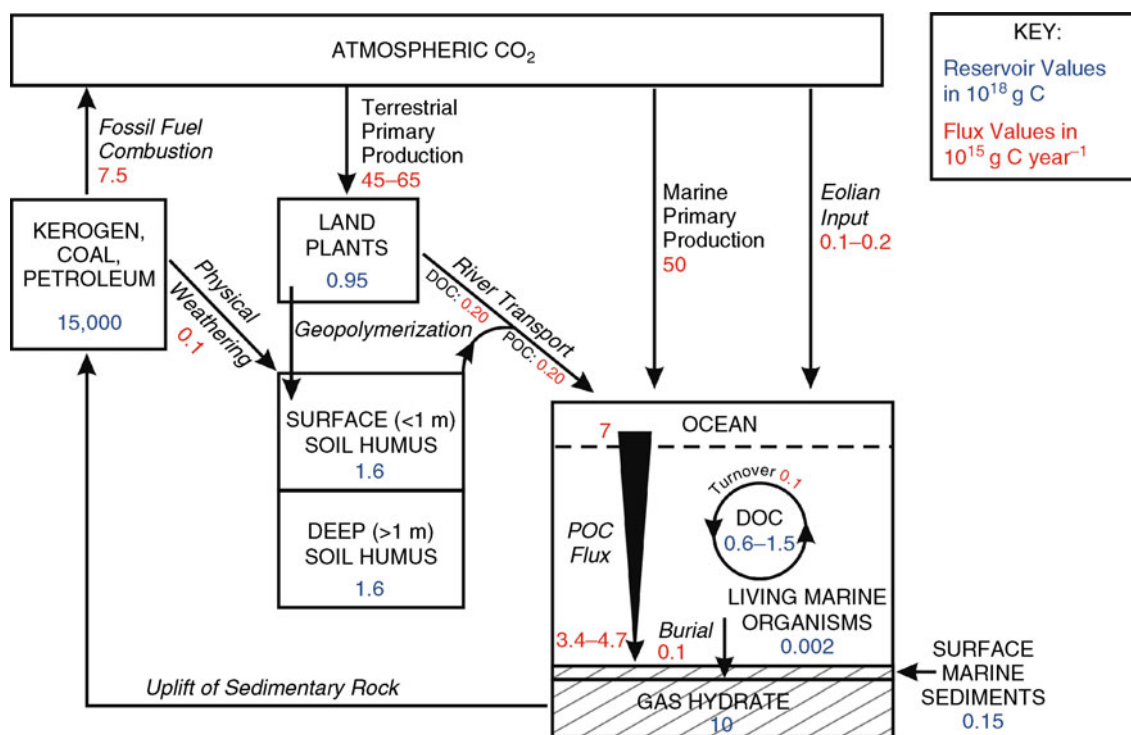
Carbon is made up of organic and inorganic fractions, and the biochemical and geochemical cycling of these forms through the Earth processes is referred to as the global carbon cycle. The global carbon cycle is closely linked to other global cycles such as oxygen, nitrogen, and sulfur because of the important role these elements play in biological processes. Organic carbon (OC) is present in both living and nonliving systems and ultimately fuels all of the Earth's biogeochemical processes (Summons, 1993); therefore, understanding the formation, cycling, and preservation of OC is essential to understanding the cycling of

all bioelements between living and nonliving reservoirs, the formation of economically important petroleum and coal deposits, and predicting how human activity may change the natural balance of these systems.

Much of the OC created during photosynthesis or by chemosynthetic autotrophs is decomposed by heterotrophic organisms. Heterotrophic processes occur in the water column of marine and aquatic environments, as well as in sediments, soils, and rocks. Much of the decomposition of organic matter (OM) occurs under low temperature (<50°C) and pressure during diagenesis. Diagenesis occurs primarily through biological processes (microbes and larger organisms that live in surface sediments) and over depths ranging from microns to hundreds of meters. The OC preserved following diagenesis is subject to further alteration by the processes of catagenesis and metagenesis, which occur at higher temperatures and pressures and lead to the formation of petroleum and natural gas.

Reservoirs

The cycling of OC involves fluxes to and from the major OC reservoirs (Figure 1). Sedimentary rocks are the largest OC reserve, making up approximately 99.5% of the



Carbon (Organic, Cycling), Figure 1 The global organic carbon (OC) cycle, adapted from Hedges (1992). The OC cycle involves fluxes to and from the major surficial and deep reservoirs. Reservoirs are denoted by capital letters, with values in blue. Fluxes due to physical processes (italicized) and primary production (not italicized) are denoted with *arrows*. Flux terms are presented in lower case font, with values given in red. Information presented in this figure is based on published values from Schlesinger (1991), Longhurst (1995), Hedges et al. (1997), Kvenvolden (2002), Richey (2004), IPCC (2007).

total global OC. Despite the relative size of this reservoir, OC only represents one-fifth of the total sedimentary carbon, since inorganic carbon is the dominant fraction (Hedges, 1992). Sedimentary OC occurs primarily as kerogen, coal beds, and petroleum reservoirs, which ultimately come from biogenic processes or sources. Kerogen is an amorphous, highly insoluble particulate OM that is thought to form by two pathways – selective preservation and geopolymerization, both of which occur under low temperature and pressure conditions (Killops and Killops, 2005). Selective preservation involves the formation of kerogen from biomacromolecules that are resistant to microbial degradation. Geopolymerization occurs through a random series of condensation and polymerization reactions involving humic substances. Petroleum is defined as naturally occurring fluid (liquid and gases) that evolves from kerogen. Petroleum is formed primarily from the remains of microbes (algae and bacteria), although some types of petroleum are formed from higher plants. In contrast, coal is a solid and is formed from the remains of higher plants. The most common type of coals, humic coals, is formed through the processes of peatification and coalification, which involve both biological and geochemical processes (Killops and Killops, 2005). The OC in ancient sedimentary rocks provides these economically important fossil fuels as well as a molecular record of life, which has allowed the construction of evolutionary trees and an understanding of the geologic timing of past events and the response of natural systems to tectonic, environmental, and biological change (Brocks and Pearson, 2005). Historically, sedimentary OC affects biologically mediated cycles on geologic time scales, although humans have recently accelerated this cycle through fossil fuel combustion (7.5×10^{15} g C year⁻¹; IPCC, 2007).

The active (surficial) pools of OC include soil humus, land and marine plant tissue, seawater dissolved OC (DOC, <0.5 μ m diameter), and surface marine sediments and gas hydrates (Kvenvolden, 2002; Hedges, 1992). Dead and living particulate OC (>0.5 μ m) are also present in freshwater and seawater pools; however, the DOC pool dominates. With the exception of plant tissue and gas hydrates, most of the active pools of OC are poorly characterized. Terrestrial plant tissue can be further classified as woody biomass (~75%), nonwoody biomass (~15%), and litter (~10%) (Olson et al., 1985). Gas hydrates contain mostly methane and some ethane molecules, and may serve as a large reservoir for OC, although global estimates of the size of this sink are still uncertain (see Kvenvolden, 2002). Soil OM is made up of plant remains (detritus), soil microbes, and humic substances.

Production and fluxes

The major fluxes between organic pools are driven by marine and terrestrial primary production. Photosynthesis, the harvesting of light energy to convert inorganic carbon

to reduced (organic) forms in the tissue of plants, is the primary mode of primary production. However, chemosynthesis by microbial organisms, which uses chemical energy rather than sunlight, is also important. Photosynthesis is carried out by prokaryotes such as cyanobacteria as well as eukaryotic algae and higher plants. The fixation of carbon by plants over the Earth's history accounts for the oxygen in our present atmosphere, thus the cycles of carbon and oxygen are closely linked over geologic time (Hedges, 2002; Hedges and Keil, 1995). In fact, to maintain current atmospheric oxygen levels, rapid cycling of carbon in the surficial pools is required. These pools exchange on relatively short timescales (10^2 – 10^4 years). In contrast, the OC that remains in the sedimentary system cycles slowly (~ 10^8 year; Schlesinger, 1991). Sedimentary reservoirs only become exchangeable through geologic uplift, followed by oxidative weathering of OC by exposure to the atmosphere.

Net global primary production (NPP) on land (45 – 65×10^{15} g C year⁻¹) is dominated by more labile nonwoody plant tissues such as leaves, grasses, and herbaceous annuals (Schlesinger, 1991). The highest gross terrestrial production (82×10^{15} g C year⁻¹) and storage as biomass (56%) occur in the tropics, while polar regions contribute the least (8×10^{15} g C year⁻¹, 0.5%; Taube, 1992). Within these regions, forests are the most productive continental biomes and deserts and tundra are the least (Taube, 1992), reflecting global patterns of temperature and precipitation. Marine primary productivity ($\sim 50 \times 10^{15}$ g C year⁻¹) is dominated by open water (pelagic) phytoplankton productivity because open water constitutes approximately 75% of the total ocean area (Ducklow and McCallister, 2005; Longhurst, 1995). However, when normalized to area, coastal and upwelling areas together account for nearly two thirds of the total oceanic productivity because a greater supply of nutrients is available to support phytoplankton in these areas (Longhurst, 1995). Other primary producers along the coasts (i.e., seagrass, marsh plants, macroalgae, mangroves) are locally important sources of OC.

Delivery of OC (as both dissolved and particulate fractions) from the land to coastal marine environments by rivers accounts for 0.4×10^{15} g C year⁻¹ (Meybeck, 1982; Ittekkot, 1988). However, there remains considerable uncertainty in our ability to adequately quantify carbon exchange from land to the coastal ocean and in our understanding of the processes influencing the fate of terrigenous carbon in coastal sediments (Berner, 1982; Sarmiento and Sundquist, 1992; Hedges and Keil, 1995; Schlünz and Schneider, 2000). Generally, the total annual riverine OC load to the ocean is proportional to the total annual river flow, which is dominated by a small set of rivers that transport the bulk of the fresh water from land to sea (Schlesinger, 1991). However, seasonal and annual/interannual variations in hydrology as well as large events such as storms and hurricanes influence the input, dispersion, and cycling of sediment OM in coastal environments, thus making it difficult to trace terrigenous OM.

In addition, recent molecular and isotopic evidence suggests that OC in seawater and marine sediments is marine, rather than terrestrial, in nature, which suggests that further remineralization of terrestrial material must occur in rivers prior to delivery to the coastal ocean (Schl nz and Schneider, 2000; Richey, 2004; Mayorga et al., 2005) or in the marine environment (Hedges et al., 1997). This is an area requiring further research. Eolian (air) flux from the land to the sea approximates 0.1×10^{15} g C year⁻¹, although this estimate remains uncertain due to methodological difficulties (Romankevich, 1984; Zafiriou et al., 1985).

Decomposition and preservation

Only ~0.1% of global NPP is ultimately preserved within sediments; however, it is this OC that will eventually become part of the sedimentary record and may be transformed into fossil fuels. The remaining 99.9% of global NPP is remineralized to inorganic carbon, nutrients, and water. Remineralization represents a sink for oxygen, linking the global cycles of OC and oxygen. Microfauna, bacteria and fungi (on land) are responsible for the breakdown of OM. Bacterial and fungal decomposition of OM involves a series of steps; macromolecules must first be broken down using extracellular enzymes into dissolved polymers, which are then broken down into low molecular weight monomers (Tissot and Welte, 1984).

The rates and extent of decomposition are influenced by environmental, biological, and physical factors. First, there must be a sufficient supply of labile OM to be degraded. The depth-integrated rate of OM remineralization in marine sediments correlates closely with carbon flux to the sediment surface (Henrichs, 1993). Interestingly, the fraction of OC that is *not* remineralized is also highly correlated with the carbon flux to the sediment surface. Thus, the efficiency of OM decomposition appears to be lower in systems that receive the highest input of OC compared to systems with lower accumulation rates (Henrichs and Reeburgh, 1983). Second, like most metabolic processes, decomposition rates are proportional to temperature, resulting in global patterns of decomposition rates (e.g., higher degradation rates in the tropics than in tundra systems) (Schlesinger, 1991). Oxygen is also a controlling factor for decomposition. Recent evidence suggests that oxygen exposure time, rather than oxygen concentration, may be an important determinant of the extent to which OM is decomposed (Hartnett et al., 1998). OM decomposition occurs under both oxic and anoxic conditions; however, the rates and bacterial communities involved in the processes differ. Oxygen is used preferentially as the terminal electron acceptor during decomposition and results in the most efficient mineralization (i.e., the highest energy yield). Once oxygen is depleted, bacteria utilize other electron acceptors such as sulfate and nitrate. Aerobic decomposition is facilitated

by microbes as well as higher organisms with a range in enzyme capabilities. Anaerobic decomposition is carried out exclusively by microorganisms, and complete remineralization is typically dependent on a consortium of cooperating organisms. Further, anaerobic decomposition processes differ in terrestrial and marine environments because sulfate is abundantly present in marine systems but limited in terrestrial systems. As a result, sulfate reduction is the dominant anaerobic pathway for oxidizing OM in marine systems, while fermentation and methanogenesis dominate in freshwater systems (Henrichs, 1993). The chemical and structural makeup of OM also plays a role in its susceptibility to decomposition (e.g., number of double bonds and functional group composition). For example, nitrogen-rich compounds such as proteins and polynucleotides are usually decomposed preferentially to carbon-rich structural compounds (Wakeham and Canuel, 2006). Additionally, the architecture of the OM may be an important control on its decomposition. Architecture refers to both physical structure (e.g., conformation) as well as OM associations with organic and inorganic matrices (see below).

Decomposition and preservation of OC are closely linked processes, since only OC that is not decomposed is available for preservation, but there are additional factors that may enhance preservation (Burdige, 2007; Wakeham and Canuel, 2006). For instance, the amount of OC that reaches the sediment surface and has the potential to be buried depends in part on productivity in the overlying photic zone and water depth. Sufficiently high productivity is essential for OC to reach the sediment with the potential for preservation (Killops and Killops, 2005). In addition, physical or chemical protection of OM plays a key role in controlling preservation. Protection by binding to inorganic ions, clays, and organic residues decreases the surface area of OM that is exposed to bacteria for degradation. This protection or encapsulation of OM explains why compounds that appear to be labile, or readily available for decomposition, have been found in sedimentary OC deposits. Lastly, the amount of time that OM is exposed to oxic conditions determines the extent to which oxygen-sensitive compounds, compounds that specifically require oxygen for degradation, are preserved (Burdige, 2007).

Preservation of OC occurs predominantly in anoxic marine sediments. More than 80% of preserved OC is located in deltaic-shelf environments associated with fine grain sediments (Bernier, 1982) that support the physical or chemical protection model, since these sediments provide the most potential surface area with which OM can bind. This has likely been true for millions of years, as current petroleum deposits are found in areas that were formerly coastal, fine grained sediments. Major depositional environments include lacustrine environments (open, closed lakes), peat swamps and coal, and continental margins, enclosed or silled basins, and the Black Sea (Killops and Killops, 2005).

Conclusion

The global OC cycle involves exchange of OC between surface and subsurface (buried) reservoirs on time scales of less than one year to millions of years. These fluxes are driven by the production and cycling of OC through biological, physical, and geological processes. The global OC cycle is intimately linked with other global cycles and is sensitive to human-induced perturbations. Humans may have reduced total NPP by up to 40% due to wildfires and pollution, which may be the largest sink for primary production to support a single species in the history of life on the Earth (Vitousek et al., 1986). Fossil fuel combustion and destruction of forests (especially tropical) have decreased the global pool of OC and increased atmospheric carbon dioxide, which has been linked to global climate change. Global atmospheric concentrations of CO₂, methane (CH₄), and nitrous oxide (N₂O) have increased markedly as a result of human activities since preindustrial times (i.e., 1,750). In 2005, concentrations of atmospheric CO₂ (379 ppm) and CH₄ (1,774 ppb) exceeded the natural range over the last 650,000 years (IPCC, 2007). Understanding the changes in the size and composition of the OC reservoir through space and time will help us to better predict the changes in its global cycle as well as the associated cycles of other biologically important elements.

Bibliography

- Berner, R. A., 1982. Burial of organic carbon and pyrite sulfur in the modern ocean: its geochemical and environmental significance. *American Journal of Science*, **282**, 451–473.
- Brocks, J., and Pearson, A., 2005. Building the biomarker tree of life. In Banfield, J. F., Cervinisilva, J., and Neelson, K. M. (eds.), *Molecular Geomicrobiology. Reviews in Mineralogy and Geochemistry*. Chantilly, VA: Mineralogical Society of America and Geochemical Society, Vol. 59, pp. 233–258.
- Burdige, D. J., 2007. Preservation of organic matter in marine sediments. *Chemical Reviews*, **107**, 467–485.
- Ducklow, H. W., and McCallister, S. L., 2005. The biogeochemistry of carbon dioxide in the coastal oceans. In Robinson, A. R., and Brink, K. (eds.), *The Sea, Volume 13, The Global Coastal Ocean – Multiscale Interdisciplinary Processes*. New York: Wiley, pp. 269–315.
- Ducklow, H. W., Purdie, D. A., LeB. Williams, P. J., and Davies, J. M., 1986. Bacterioplankton: a sink for carbon in a coastal marine plankton community. *Science*, **232**, 865–867.
- Hartnett, H. E., Keil, R. G., Hedges, J. I., and Devol, A. H., 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. *Nature*, **391**, 572–574.
- Harvey, H. R., Tuttle, J. H., and Bell, J. T., 1995. Kinetics of phytoplankton decay during simulated sedimentation - changes in biochemical-composition and microbial activity under oxic and anoxic conditions. *Geochimica et Cosmochimica Acta*, **59**, 3367–3377.
- Hedges, J. I., 1992. Global biogeochemical cycles: progress and problems. *Marine Chemistry*, **39**, 67–93.
- Hedges, J. I., 2002. Sedimentary organic matter preservation and atmospheric O₂ regulation. In Gianguzza, A., Pelizzetti, E., and Sammartano, S. (eds.), *Chemistry of Marine Water and Sediments*. Berlin: Springer, 508 pp.
- Hedges, J. I., and Keil, R. G., 1995. Sedimentary organic matter preservation: an assessment and speculative synthesis. *Marine Chemistry*, **49**, 81–115.
- Hedges, J. I., Keil, R. G., and Benner, R., 1997. What happens to terrestrial organic matter in the ocean? *Organic Geochemistry*, **27**, 195–212.
- Henrichs, S. M., 1993. Early diagenesis of organic matter: the dynamics (rates) of cycling of organic compounds. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 101–117.
- Henrichs, S. M., and Reeburgh, W. S., 1983. Anaerobic mineralization of marine sediment organic matter: rates and the role of anaerobic processes in the oceanic carbon economy. *Geomicrobiology*, **5**, 191–237.
- IPCC, 2007. Climate Change 2007: The physical science basis. In Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M., and Miller, H. L. (eds.), *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. New York: Cambridge University Press, 996 pp.
- Ittekkot, V., 1988. Global trends in the nature of organic matter in river suspensions. *Nature*, **332**, 436–438.
- Killops, S. D., and Killops, V. J., 2005. *An Introduction to Organic Geochemistry*. Oxford: Blackwell.
- Kvenvolden, K. A., 2002. Methane hydrate in the global organic carbon cycle. *Terra Nova*, **14**, 302–306.
- Longhurst, A., 1995. Seasonal cycles of pelagic production and consumption. *Progress in Oceanography*, **36**, 77–167.
- Mayorga, E., Aufdenkampe, A. K., Masiello, C. A., Krusche, A. V., Hedges, J. I., Quay, P. D., Richey, J. E., and Brown, T. A., 2005. Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers. *Nature*, **436**, 539–541.
- Meybeck, M., 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science*, **282**, 401–450.
- Olson, J. S., Garrels, R. M., Berner, R. A., Armentano, T. V., Dyer, M. I., and Yaalon, D. H., 1985. The natural carbon cycle. In Trabalka, J. R. (ed.), *Atmospheric Carbon Dioxide and the Global Carbon Cycle*. Washington, DC: U.S. Department of Energy, pp. 175–213.
- Richey, J. E., 2004. Pathways of atmospheric CO₂ through fluvial systems. In Fields, C. (ed.), *Toward CO₂ Stabilization: Issues, Strategies, and Consequences, A SCOPE/GCP Rapid Assessment Project*. Washington, DC: Island Press, pp. 329–340.
- Romankevich, E. A., 1984. *Geochemistry of Organic Matter in the Ocean*. Berlin: Springer.
- Sarmiento, J. L., and Sundquist, E. T., 1992. Revised budget for the oceanic uptake of anthropogenic carbon dioxide. *Nature*, **365**, 589–593.
- Schlesinger, W. H., 1991. *Biogeochemistry: An Analysis of Global Change*. San Diego, CA: Academic.
- Schlünz, B., and Schneider, R. R., 2000. Transport of terrestrial organic carbon to the oceans by rivers: re-estimating flux and burial rates. *International Journal of Earth Sciences*, **88**, 599–606.
- Summons, R. E., 1993. Biogeochemical cycles: a review of fundamental aspects of organic matter formation, preservation, and composition. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 3–21.
- Taube, M., 1992. Evolution of matter and energy. In Craig, P., and Hutzinger, O. (eds.), *The Natural Environment and the Biogeochemical Cycles*. New York: Springer, pp. 200.
- Tissot, B. P., and Welte, D. H., 1984. *Petroleum Formation and Occurrence*. Berlin: Springer, pp. 699.
- Vitousek, P., Ehrlich, P. R., Ehrlich, A. H., and Matson, P. A., 1986. Human appropriation of the products of photosynthesis. *BioScience*, **36**, 368–373.

- Wakeham, S. G., and Canuel, E. A., 2006. Degradation and preservation of organic matter in marine sediments. In Volkman, J. K. (ed.), *Handbook of Environmental Chemistry*. Berlin: Springer, Vol. 2, Part N, pp. 295–321.
- Zafiriou, O. C., Gagosian, R. B., Peltzer, E. T., Alford, J. B., and Loder, T., 1985. Air-to-sea fluxes of lipids at Enewetak Atoll. *Geophysical Research*, **90**(D1), 2409–2423.

Cross-references

[Aerobic Metabolism](#)
[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Methane Oxidation \(Aerobic\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

CARBON (ORGANIC, DEGRADATION)

Steven T. Petsch
 University of Massachusetts Amherst, Amherst, MA,
 USA

Synonyms

Carbon biodegradation; Carbon remineralization

Definition

Organic carbon (OC). In geochemistry, carbon that occurs in materials of ultimately biological origin and that can be oxidized. Organic matter (OM) is the material that contains organic carbon (OC).

Degradation. Transformations of OM within the range of temperatures, pressures and environmental conditions found in or near the Earth surface environments. If biological activity is confirmed to cause the transformations, it is termed biodegradation.

Remineralization. Degradation of OM that results in net conversion of OC to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- .

Introduction

The Earth exists in a near balance between photosynthesis and respiration. Photosynthesis, by which inorganic carbon is chemically reduced to biologically-available OC using light energy, generates an estimated 107 Pg C of net new production each year (1 Pg = 10^{15} g), nearly evenly distributed among terrestrial (57 Pg C year⁻¹) and marine (50 Pg C year⁻¹) environments (Field et al., 2007; Sabine et al., 2004). Of this biomass, over 90 Pg C is remineralized each year to inorganic carbon

through aerobic respiration on land and in the oceans, leaving 17 Pg year⁻¹ to be redistributed among different pools of nonliving OM (Field et al., 2007; Sabine et al., 2004). These pools include soils, aquatic and marine sediments, and nonliving OM in the world's oceans. Degradation of organic carbon in these pools balances the crustal carbon cycle, such that total global respiration plus net OC remineralization are believed to match global rates of photosynthesis (Bernier, 2003). Maintaining or slowly shifting this balance has been a critical component of the coupled atmosphere–ocean climate system throughout the Earth's history, and has served to maintain an equable amount of atmospheric oxygen (O_2) for the past 600 million years (Bernier, 1989, 2003).

Degradation of OC in soils

Soils contain complex mixtures of weathered rocks, new minerals formed within soils, and OM. The world's soils contain an estimated 3,200 Pg C (Sabine et al., 2004 and references therein). Much of this OM derives from terrestrial vegetation. As dead vegetation ages within soils, it is degraded by a combination of chemical reactions and biological processes. Soluble components of decaying vegetation may be leached out of soils and relocated by groundwater movement. Organic acids and chelating agents released by plant roots further facilitate the dissolution and hydrolysis of vegetation. Soil macrofauna, especially detritivores such as earthworms, serve to physically mix soil horizons from different depths, moving OM-rich surface leaf litter down to more water- and mineral-rich horizons and reducing the OC and nutrient content of surface soils (Bohlen et al., 2004). Detritivores also accelerate the formation and disintegration of soil carbon aggregates, resulting in overall larger aggregates of fresh (less-degraded) OM mixed to greater depth within soils (Blair et al., 1995; Cortez, 1998). This activity increases soil porosity and permeability, allowing access to oxygen to greater depth in soils to support aerobic respiration processes.

Throughout in the soils, microbial processes both positively and negatively influence soil OM degradation. Fungal hyphae and other microbial exopolymers bind soil carbon and mineral particles together into aggregates. These exopolymers add to the stock of soil OC contributed by terrestrial vegetation, and support mechanisms of physical protection of OM against further degradation; aggregate-bound OM is much less susceptible to degradation than unassociated OM (Baldock and Skjemstad, 2000). Other microorganisms participate in the degradation of soil OM as they obtain energy and cellular carbon through various metabolic processes. While aerobic respiration is confined to shallow depths of the soil where O_2 can penetrate, anaerobic processes extend throughout the soil profile, from O_2 -free microenvironments within surface soils to deeper O_2 -free soils and weathered regolith.

Soil OC degradation is limited by the availability and reactivity of both OM and electron acceptors that fuel anaerobic respiration. Sulfate is not an abundant

electron acceptor in most soils, while ferric iron and nitrate can be. Soil OM is also susceptible to degradation other than respiration. Fermentative degradation of soil OM breaks down terrestrial inputs and biopolymers into small molecules including low molecular weight organic acids, alcohols, and sugar monomers. Methanogenesis too is an important mechanism of OC degradation in soils, consuming fermentation products of such as methanol, H_2 , and acetate, sustaining the fermentative breakdown of soil OM.

Many components of soil OM are fairly susceptible to degradation and do not persist in soil environments. These include carbohydrate biopolymers (e.g., cellulose, chitin, and other polysaccharides), proteins, and nucleic acids. Other components such as lipids, lignin, and black carbon are much more resistant to degradation, and can persist for millennia as intact compounds or transitional degradation products in soils (Derenne and Largeau, 2001). Lipids commonly found preserved in soils include highly aliphatic waxes, free hydrocarbons such as *n*-alkanes and isoprenoids, and biopolymers such as cutan and suberan. Lignin is a structural biopolymer in plant cell walls that derives from condensation of phenolic precursors. Black carbon comprises a broad suite of aromatic and graphitized organic materials that form during incomplete combustion of terrestrial biomass or fossil fuels; these materials are believed to be extremely resistant to degradation (Schmidt and Noack, 2000). During degradation in soils, soil OM components are remineralized at rates depending on a complex combination of intrinsic reactivity (polymeric material being more resistant than individual monomers), physical protection, (inside mineral aggregates or encapsulated within more resistant biopolymers) and lability (proteins, nucleic acids, and carbohydrates being more reactive than lipids and lignin).

Degradation of OC in the oceans

OC occurs in both dissolved and particulate forms in the oceans. Approximately $50 \text{ Pg C year}^{-1}$ derives from marine primary production driven by phytoplankton (Field et al., 2007; Sabine et al., 2004 and references therein). Rivers deliver an estimated 0.5 Pg of OC to the oceans each year. Primary production is confined to the photic zone of the world ocean, where light and nutrients combine in sufficient abundance to support photosynthetic activity. Within and immediately below the photic zone is a zone of intense heterotrophic activity, in which grazing zooplankton and aerobic heterotrophic bacteria consume particulate OM (Lee et al., 2004). The heterotrophic activity in near-surface waters has several impacts on OC degradation in the oceans. One is that $>90\%$ of primary production is effectively remineralized in the upper 200 m of the marine water column, leaving only a small fraction of surface particulate OM for export to the deep ocean (Suess, 1980; Honjo et al., 2008). Second is that mineral ballasting (association of OM with biogenic minerals produced by plankton or with minerals delivered

from continents to the surface ocean by winds and river) increases the sinking rate of OM through the water column, reducing the time available for degradation in the ocean (Lee et al., 2004; Armstrong et al., 2002). Aggregation of particulates in surface waters results in increased particle size and sinking velocity, further accelerating the export of less-degraded particulate OM to a depth (Sheridan et al., 2002). Lastly, heterotrophic grazing of surface particulates results in net evolution of the composition of sinking particulates, as more labile or easily degraded components are consumed (Lee et al., 2004; Wakeham et al., 1997).

In living biomass of the photic zone, OM that can be identified at the molecular level comprises $>80\%$ of total OC. Between 200 and 1,000 m water depth, molecularly uncharacterized material comes to dominate total OM, such that much of the material delivered to seafloor sediments cannot be assigned to typical compound classes such as lipids, carbohydrates, and amino acids (Lee et al., 2004). Some of this relative increase in uncharacterized material may result from selective preservation of photic zone precursors, while some may be added to sinking particulates as exopolymeric material generated by heterotrophic bacteria that feed on sinking particulate matter. There are also changes observed in the molecularly characterizable fraction of sinking particulate OM. Lipids resistant to degradation, such as long-chain saturated fatty acids and alkenones, are substantially enriched in relative abundance compared with initial photic zone biomass, while pigments, many carbohydrates, and polyunsaturated fatty acids are readily lost from particulates within or below the photic zone (Lee et al., 2004).

This background picture of OC degradation is confounded by the role of mineral surfaces. Minerals, whether delivered to surface waters from the continents by winds and rivers, or produced by plankton in the water column, make up the bulk of sinking particulate mass. OM can be sorbed to mineral particles, and with their greater density, OM-associated particulates can sink rapidly through the water column, helping to export relatively undegraded OC beyond the photic zone. In this way, terrestrially derived OM, which may be protected from degradation by association with detrital minerals or OM macromolecules, can be delivered to deep sea sediments. More generally, marine OM may be protected from degradation by adsorption in mesopores of sinking mineral particles, or OM mineral aggregates taken together may resist degradation, where OM is located in interstices between the mineral grains (Hedges et al., 2001). Clearly, degradation of OM within the marine water column reflects a complex interplay of inherent resistance of various organic materials across a continuum of lability, coupled with mechanisms of both speeding the rate of sinking through the water column and increasing protection from degradation by the association of OM with biogenic and terrigenous minerals.

This discussion of heterotrophic degradation of OC applies to an oxygenated water column. In several locations around the globe, large proportions of the water column are

anoxic. These include the Black Sea, the Cariaco Basin, and seasonally in the Arabian Sea, eastern tropical North Pacific and California Borderlands Basins, among others. Lack of oxygen effectively shuts down aerobic respiration in these regions, allowing the roles of anaerobic microbial processes to dominate OC degradation. In these anoxic basins, the extents of OM degradation as a fraction of primary production are lower than in open ocean sites, leading to higher sediment OC contents. Although the rates of degradation under oxic and anoxic conditions may be similar, the overall proportion of total OM that resists degradation may be greater under anoxic conditions (Van Mooy et al., 2002).

Degradation of OC in marine sediments

Efficient heterotrophic processes in the water column mean that only a small fraction ($<1\%$) of primary production reaches the sea floor. Of this, most is degraded within the upper meters of sediment, leading to OC burial of approximately $160 \text{ Tg C year}^{-1}$ (Berner, 1989; Hedges and Keil, 1995). Clearly, a large fraction of ocean carbon inputs ($\sim 50 \text{ Pg C year}^{-1}$) is degraded in both the water column and shallow sediments.

There is a positive relationship between sediment accumulation rate (either cm year^{-1} or $\text{g}_{\text{carbon}} \text{ cm}^{-2} \text{ year}^{-1}$) and OC burial efficiency, where burial efficiency is the fraction of OC ultimately preserved at depth in sediment relative to OC delivered to the sediment-water interface (Burdige, 2007; Aller, 1998; Canfield, 1994). At very low sedimentation rates, the sediment layers are buried very slowly, and aerobic processes have ample time to remineralize the OM that is delivered to these sediments, thereby explaining the low total OC contents of many low sedimentation rate marine settings. Closer to shore as sedimentation rates increase, rapid burial means that anoxic conditions dominate at shallower sediment depths, and anaerobic degradation processes become more important. Nonetheless, sediments underlying oxygen-deficient waters do not uniformly exhibit high carbon burial efficiency, indicating that oxygen alone is not the primary driver controlling OM degradation rates (Burdige, 2007). Burial efficiencies among a range of marine sediment types range between 10% and 20%. Thus, very little primary production escapes degradation processes to become incorporated into the sedimentary record.

Controls on OC degradation in sediment are similar to those operating in soils and in the marine water column: selective degradation of inherently resistant components, physical protection through association with mineral or organic polymeric surfaces, and availability of electron acceptors and/or appropriate substrates for nonrespiration degradation processes such as fermentation and methanogenesis (Burdige, 2007). Oxygen does not limit OC degradation. Most marine sediments are anoxic within centimeters of the sediment-water interface. Denitrification, sulfate-, iron- and manganese-reduction, fermentation, and methanogenesis are all active components of OM degradation. Oxygen exposure time is nonetheless

a useful proxy for understanding the extent of OC degradation from one environment to the other. Longer cumulative oxygen exposure times result in more OC degradation in sediment and overall lower OM burial efficiency (Aller, 1998; Hartnett et al., 1998).

Most OC is buried in shallow, continental margin sediments. These environments have been targets of numerous investigations over the years, and have provided insights into OC degradation processes in the uppermost few meters of buried sediments (see Martens et al., 1992). Deeply buried marine sediment such as from the Woodlark Basin and Peru Margin in turn demonstrate microbial activity hundreds of meters deep in sediments, indicating OC degradation can persist at slow rates for geologic spans of time (D'Hondt et al., 2004; Wellsbury et al., 2002).

Degradation of OC in ancient sedimentary rocks

The single largest pool of OC on the Earth (15 million Pg C) is represented by OM contained in ancient sedimentary rocks, a mass that dwarfs all other pools of OC combined (Field et al., 2007; Sabine et al., 2004). Traditionally, OC in sedimentary rocks has been viewed as resistant to degradation other than high temperature oil and gas generation or graphitization. Degradation can occur where sedimentary rocks are exposed at the Earth's surface through erosion and soil formation (Petsch et al., 2000, 2001). One unexpected theme emerging from this work is that OC degradation during rock weathering is not completely efficient. Highly aged OC is detected in river particulates from watersheds draining sedimentary lithologies (Longworth et al., 2007; Goni et al., 2005; Blair et al., 2003; Leithold et al., 2006).

However, very little of the total pool of sedimentary OM is exposed in the Earth surface environments; most remains deeply buried in the Earth's crust for millions of years. The persistence of OC in sedimentary rocks for long expanses of geologic time, and its preservation up to the present day, would suggest that degradation processes must largely cease after the burial of OM in sediments. Yet, microbial degradation of deeply buried OM has been described in a number of subsurface environments. These include clear signatures of hydrocarbon biodegradation in many petroleum systems (Head et al., 2003, 2006; Jones et al., 2008) and OM-rich sedimentary rocks (Formolo et al., 2008), coupled with molecular biological analyses that support an interpretation of an active subsurface biosphere involved in OC degradation (Waldron et al., 2007; Jones et al., 2008). These subsurface OC degradation processes are particularly valued for their role in natural gas generation in petroleum systems and other sedimentary basins.

Summary

OC degradation represents the suite of processes by which OM is transformed and remineralized, mainly through biological activity, within soils, aquatic systems,

sediments, and sedimentary rocks. The diverse forms and compositions of natural OM result in a wide range of rates of degradation, as some components are inherently resistant to degradation while others are labile and are thus quickly consumed following biosynthesis. The inherent resistance of some components of OM results in selective preservation in soils and sediments. This selective preservation is attenuated by mechanisms of physical protection, either by association with mineral surface or encapsulation of labile components within more recalcitrant organic matrices. In this way, nominally labile OC can be preserved for greater extents of time or transported across longer distances than would otherwise be the case. Future investigations of OC degradation will likely focus on (1) improved construction of carbon budgets for soils, the oceans, and sediments; (2) the mechanism(s) by which certain compound classes and organic materials remain resistant to biological degradation; (3) improved understanding of the association of organic materials with mineral surfaces, their selective adsorption/desorption behavior, and the physical scale of these relationships; (4) the roles that terrestrial inputs of OM to marine sediments and recycling of ancient OM during rock weathering play in controlling the overall reactivity and preservation of OC in sediments; and (5) improved understanding of couplings between OM composition and degradation processes active within deep subsurface sedimentary environments.

Bibliography

- Aller, R. C., 1998. Mobile deltaic and continental shelf muds as suboxic, fluidized bed reactors. *Marine Chemistry*, **61**, 143–155.
- Armstrong, R. A., Lee, C., Hedges, J. I., Honjo, S., and Wakeham, S. G., 2002. A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. *Deep-Sea Research II*, **49**, 219–236.
- Baldock, J. A., and Skjemstad, J. O., 2000. Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry*, **31**, 697–710.
- Berner, R. A., 1989. Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over Phanerozoic time. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **75**, 97–122.
- Berner, R. A., 2003. The long term carbon cycle, fossil fuels and atmospheric composition. *Nature*, **426**, 323–326.
- Blair, J. M., Parmelee, R. W., and Lavelle, P., 1995. Influences of earthworms on biogeochemistry. In Hendrix, P. F. (ed.), *Earthworm Ecology and Biogeography in North America*. Boca Raton, FL: Lewis, pp. 128–154.
- Blair, N. E., Leithold, E. L., Ford, S. T., Peeler, K. A., Holmes, J. C., and Perkey, D. W., 2003. The persistence of memory: the fate of ancient sedimentary organic carbon in a modern sedimentary system. *Geochimica et Cosmochimica Acta*, **67**, 63–73.
- Bohlen, P. J., Pelletier, D. M., Groffman, P. M., Fahey, T. J., and Fisk, M. C., 2004. Influence of earthworm invasion on redistribution and retention of soil carbon and nitrogen in northern temperate forests. *Ecosystems*, **7**, 13–27.
- Burdige, D. J., 2007. Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? *Chemistry Reviews*, **107**, 467–485.
- Canfield, D. E., 1994. Factors influencing organic carbon preservation in marine sediments. *Chemical Geology*, **114**, 315–329.
- Cortez, J., 1998. Field decomposition of leaf litters: relationships between decomposition rates and soil moisture, soil temperature and earthworm activity. *Soil Biology and Biochemistry*, **30**, 783–793.
- Derenne, S., and Largeau, C., 2001. A review of some important families of refractory macromolecules: composition, origin and fate in soils and sediments. *Soil Science*, **166**, 833–847.
- D'Hondt, S., Jorgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K. U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegel, J., Padilla, C. N., and Acosta, J. L. S., 2004. Distributions of microbial activities in deep seafloor sediments. *Science*, **306**, 2216–2221.
- Field, C. B., Sarmiento, J., and Hales, B., 2007. The carbon cycle of North America in a global context. In King, A. W., Dilling, L., Zimmerman, G. P., Fairman, D. M., Houghton, R. A., Marland, G., Rose, A. Z., and Wilbanks, T. J. (eds.), *The North American Carbon Budget and Implications for the Global Carbon Cycle. U.S. Climate Change Science Program, Synthesis and Assessment Product 2.2*. Asheville, NC: National Oceanic and Atmospheric Administration, National Climatic Data Center, pp. 21–29.
- Formolo, M. F., Martini, A. M., Petsch, S. T., Nüsslein, K., and Salacup, J., 2008. Hydrocarbon biodegradation in sedimentary rocks linked to atmospheric methane variation during deglaciation. *Geology*, **36**, 139–142.
- Goni, M. A., Yunker, M. B., Macdonald, R. W., and Eglinton, T. I., 2005. The supply and preservation of ancient and modern components of organic carbon in the Canadian Beaufort Shelf of the Arctic Ocean. *Marine Chemistry*, **93**, 53–73.
- Hartnett, H. E., Keil, R. G., Hedges, J. I., and Devol, A. H., 1998. Influence of oxygen exposure time on organic carbon preservation in continental margin sediments. *Nature*, **391**, 572–574.
- Head, I., Jones, D. M., and Röling, W., 2006. Marine microorganisms make a meal of oil. *Nature Reviews Microbiology*, **4**, 173–182.
- Head, I. M., Jones, D. M., and Larter, S. R., 2003. Biological activity in the deep subsurface and the origin of heavy oil. *Nature*, **426**, 344–352.
- Hedges, J. I., and Keil, R. G., 1995. Sedimentary organic-matter preservation – an assessment and speculative synthesis. *Marine Chemistry*, **49**, 81–115.
- Hedges, J. I., Baldock, J. A., Gelinas, Y., Lee, C., Peterson, M. L., and Wakeham, S., 2001. Evidence for non-selective preservation of organic matter in sinking marine sediments. *Nature*, **409**, 801–804.
- Honjo, S., Manganini, S. J., Krishfield, R. A., and Francois, R., 2008. Particulate organic carbon fluxes to the ocean interior and factors controlling the biological pump: a synthesis of global sediment trap programs since 1983. *Progress in Oceanography*, **76**, 217–285.
- Jones, D. M., Head, I. M., Gray, N. D., Adams, J. J., Rowan, A. K., Aitken, C. M., Bennett, B., Huang, H., Brown, A., Bowler, B. F. J., Oldenburg, T., Erdmann, M., and Larter, S. R., 2008. Crude-oil biodegradation via methanogenesis in subsurface petroleum reservoirs. *Nature*, **451**, 176–181.
- Lee, C., Wakeham, S., and Arnosti, C., 2004. Particulate organic matter in the sea: the composition conundrum. *Ambio*, **33**, 565–575.
- Leithold, E. L., Blair, N. E., and Perkey, D. W., 2006. Geomorphologic controls on the age of particulate organic

- carbon from small mountainous and upland rivers. *Global Biogeochemical Cycles*, **20**, GB3022, doi:10.1029/2005GB002677.
- Longworth, B. E., Petsch, S. T., Raymond, P. A., and Bauer, J. E., 2007. Linking lithology and land use to sources of dissolved and particulate organic matter in headwaters of a temperate, passive-margin river system. *Geochimica et Cosmochimica Acta*, **71**, 4233–4250.
- Martens, C. S., Haddad, R. I., and Chanton, J. P., 1992. Organic matter accumulation, remineralization, and burial in an anoxic coastal sediment. In Whelan, J. K., and Farrington, J. W. (eds.), *Productivity, Accumulation and Preservation of Organic Matter in Recent and Ancient Sediments*. New York: Columbia University Press, p. 82.
- Petsch, S. T., Berner, R. A., and Eglinton, T. I., 2000. A field study of the chemical weathering of ancient sedimentary organic matter. *Organic Geochemistry*, **31**, 475–487.
- Petsch, S. T., Smernik, R. J., Eglinton, T. I., and Oades, J. M., 2001. A solid state ^{13}C -NMR study of kerogen degradation during black shale weathering. *Geochimica et Cosmochimica Acta*, **65**, 1867–1882.
- Sabine, C. L., Heiman, M., Artaxo, P., Bakker, D. C. E., Chen, C. T. A., Field, C. B., Gruber, N., LeQuéré, C., Prinn, R. G., Richey, J. E., Romero-Lankao, P., Sathaye, J. A., and Valentini, R., 2004. Current status and past trends of the carbon cycle. In Field, C. B., and Raupach, M. R. (eds.), *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World*. Washington, DC: Island Press, pp. 17–44.
- Schmidt, M. W. I., and Noack, A. G., 2000. Black carbon in soils and sediments: analysis, distribution, implications and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.
- Sheridan, C. C., Lee, C., Wakeham, S. G., and Bishop, J. K. B., 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific ocean. *Deep-Sea Research I*, **49**, 1983–2008.
- Suess, E., 1980. Particulate organic carbon fluxes in the oceans – surface productivity and oxygen utilization. *Nature*, **288**, 260–263.
- Van Mooy, B. A. S., Keil, R. G., and Devol, A. H., 2002. Impact of suboxia on sinking particulate organic carbon: enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta*, **66**, 457–465.
- Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. L., 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochimica et Cosmochimica Acta*, **61**, 5363–5369.
- Wakeham, S. G., Lee, C., and Hedges, J. I., 2002. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. *Deep-Sea Research I*, **47**, 1535–1568.
- Waldron, P., Petsch, S. T., Martini, A. M., and Nüsslein, K., 2007. Archaeal community structure and metabolic activity across a salinity gradient within a methane-producing sedimentary basin. *Applied and Environmental Microbiology*, **73**, 4171–4179.
- Wellsbury, P., Mather, I., and Parkes, R. J., 2002. Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. *FEMS Microbiology Ecology*, **42**, 59–70.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Black Shales](#)
[Carbon \(Organic, Cycling\)](#)

[Deep Biosphere of Sediments](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shales](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)

CARBON CYCLE

The carbon cycle is a biogeochemical cycle that describes the movement of carbon between its different compartments (reservoirs) on the Earth, namely biosphere, atmosphere, hydrosphere, pedosphere, and geosphere. Please refer to entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#).”

CARBON ISOTOPES

Please refer to “[Isotopes and Geobiology](#)” and “[Biomarkers \(Organic, Compound-Specific Isotopes\)](#).”

CARBONATE ENVIRONMENTS

Eberhard Gischler

J.W. Goethe-Universität, Frankfurt am Main, Germany

Definition

Carbonate environments occur both in the terrestrial and marine realms as well as in transitional zones between the land and the sea. The variety of environments spans from high-elevation continental lakes to the deep sea, from the equator to latitudes of about 60°, and they include dry and wet climate realms. The majority of carbonates are produced in the sea, currently in more or less similar proportions in neritic and pelagic settings. Carbonate production is to a large part a consequence of biological activity, either directly as in shell and skeleton formation or indirectly as a result of metabolic reactions, which trigger precipitation. The highest rates of carbonate production per unit time and space are probably reached in tropical coral reefs where several kilograms of calcium carbonate form on 1 m² during 1 year's time.

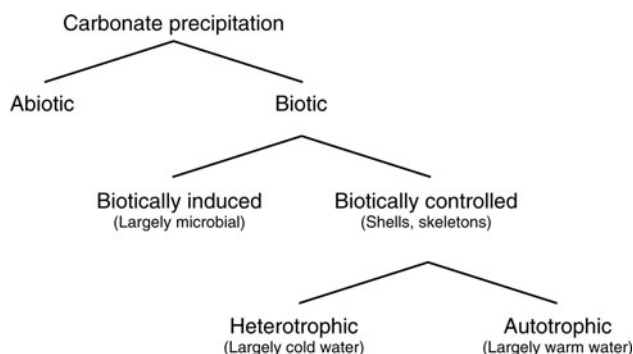
Introduction

Modern carbonate sediments are largely a product of biologic activity, either enzymatically controlled like in calcium carbonate (CaCO₃) shell and skeleton formation of aquatic organisms or indirectly by the metabolic activity

of organisms, which change water chemistry so that carbonate precipitation is enhanced (Milliman, 1974; Scholle et al., 1983; Scoffin, 1987; Tucker and Wright, 1990; Flügel, 2004; Schlager, 2005). The former refers to common carbonate producers such as corals, mollusks, calcareous algae, brachiopods, bryozoa, echinoderms, sponges, foraminifera, or coccolithophorids. The latter includes microbes and algae, which are of great importance in the formation of microbialites (stromatolites and thrombolites), tufa, in the initiation of carbonate precipitation, e.g., by removing carbon dioxide (CO_2) during photosynthesis, or as photosymbionts in reef corals, larger benthic foraminifera, giant clams, and sponges (Figure 1).

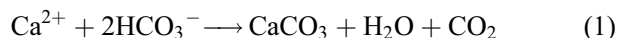
Possible exceptions of biologic production of carbonate include some carbonate muds and non-skeletal carbonate particles such as ooids, peloids, and aggregate grains that are currently forming in the Bahama Banks, the Persian Gulf, restricted platforms, or certain lakes. Peloids include both cemented fecal pellets as well as micritized or recrystallized skeletal grains (Purdy, 1968). Recrystallization is a common pattern in carbonate sediments and rocks, which is a geologically fast process (Bathurst, 1971; Reid and Macintyre, 1998). Carbonate cements are another common phenomenon in which biogenic *versus* abiogenic precipitation is discussed (Schneidermann and Harris, 1985; Schlager, 2005). Classic examples of relatively fast and abundant carbonate cementation include tropical coral reef frameworks (Grammer et al., 1993) and beachrock (Gischler, 2007).

The majority of carbonate sediments by far are produced in the sea. Presently, about half of this carbonate originates in the pelagic realm and largely includes shells of planktonic and benthic foraminifera, pteropod mollusks, and coccolithophorids. The other half is produced in the neritic realm by corals, mollusks, calcareous algae, brachiopods, bryozoa, and echinoderms. It is estimated that this 50:50 ratio between pelagic and neritic carbonate sedimentation changed to 90:10 during Quaternary lowstands of sea level (Milliman and Droxler, 1996).



Carbonate Environments, Figure 1 Carbonate formation in terrestrial, transitional, and marine environments. (Modified from Schlager 2005.)

Today, carbonate minerals commonly formed in the sea are aragonite and high-magnesium calcite (calcite with 4–21% MgCO_3). After sodium (Na^+), magnesium (Mg^{2+}) (1,300 ppm) and calcium (Ca^{2+}) (410 ppm) are the second and third most common cations in the ocean, respectively. Carbonate as HCO_3^- is the third most common anion (140 ppm) after chloride (Cl^-) and sulphate (SO_4^{2-}). Calcium carbonate is precipitated after the formula:



In freshwater environments, for comparison, where ion concentrations in general and the magnesium/calcium ratio in particular are much lower, low-magnesium calcite typically is precipitated. Subaerial exposure surfaces of limestone are characterized by dissolution, and form typical karst features.

Dolomite ($\text{MgCa}(\text{CO}_3)_2$) may form in limited quantity both in the ocean and in lakes. Preconditions for dolomite precipitation are a high Mg/Ca-ratio and elevated salinity. Modern examples of dolomite formation include the deep sea, certain lakes, and sabkhas, which are intertidal carbonate areas in an arid climate. The majority of dolomite presently exposed on the earth's surface, however, was formed secondarily in the burial environment, by dolomitization of carbonate rocks from magnesium-rich solutions.

The analysis of non-skeletal grains such as ooids and cements throughout the marine geologic record has revealed that the mineralogy of precipitated carbonate has changed between aragonite/high-magnesium calcite and low-magnesium calcite (Sandberg, 1983). This observation, known as the Sandberg-Cycles, is a consequence of changing Mg/Ca-ratios in the ocean as controlled by plate tectonic activity. When lower spreading-rates, colder climate, and lower sea levels are experienced during the periods such as today's or the late Paleozoic icehouse worlds, aragonite predominates over low-magnesium calcite (high Mg/Ca). When spreading-rates and sea level were high and the climate was warm, such as during Devonian or Cretaceous greenhouse worlds, low-magnesium calcite precipitation was favored (low Mg/Ca). Meanwhile, additional fossil and aquarium studies have shown that quite a number of carbonate-producing organisms also change their skeleton or shell mineralogy according to Mg/Ca-ratios of seawater (Stanley and Hardie, 1998). Investigations in Precambrian carbonate rocks have supported the contention that carbonate concentrations in the ocean also fluctuated during Earth history, thereby influencing carbonate deposition (Knoll et al., 1993). In the Proterozoic, carbonate concentrations were so high that in places cements were physicochemically precipitated on the seafloor. Carbonate concentrations decreased in the Cambrian when shell and skeleton production exploded. Carbonate concentrations presumably sank again in the Mesozoic when calcareous plankton such as planctonic foraminifera and coccolithophorids

evolved. The Soda Ocean model of Kempe and Degens (1985) speculates that calcium concentrations in the ocean also dramatically decreased with shell and skeleton evolution during the end of the Precambrian through the Cambrian. These authors interpreted shell and skeleton formation as calcium expelling as a consequence of cell poisoning.

In the following, highlights of typical carbonate environments are discussed in a cross section going from the terrestrial, coast, and shallow marine to deep marine settings (Figure 2).

Terrestrial environments

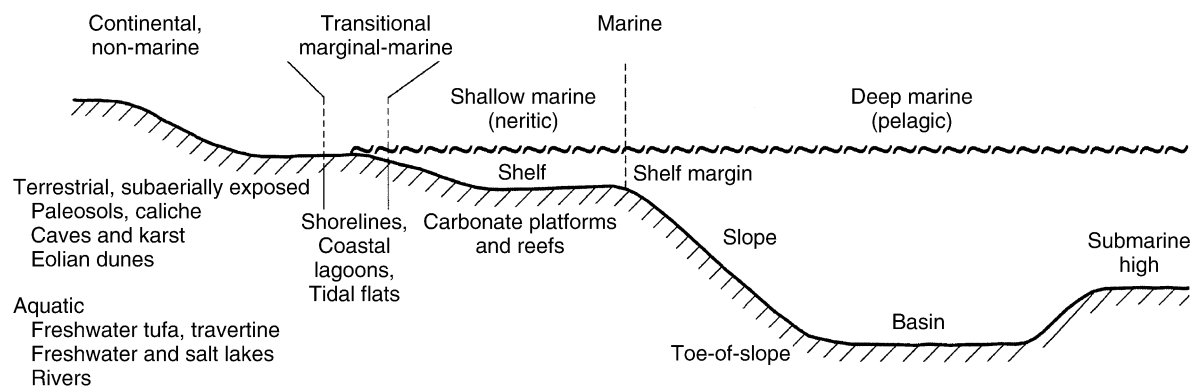
Springs

Carbonate deposits are normally very rare in running freshwater because of the low ion contents of the water. Turbulence that causes CO_2 degassing may lead to

low-magnesium calcite precipitation. Also, pressure release or cooling of hot waters at springs may bring about calcite precipitation. The limestone product is usually termed tufa, which forms under ambient temperatures and may contain calcified remains of bacteria, algae, and plants (Chafetz and Folk, 1984; Ford and Pedley, 1996). Travertine, which forms in hot water (hydrothermally), is dense, usually clearly laminated, and largely lacks algae and plant remains. Typical morphologies of tufa deposits include barrages, cascades, or terraces with pools. Ooids may form in pools that hold water. Tufa deposits are restricted to the late Cenozoic, which presumably is a consequence of limited preservation potential (Figure 3).

Lakes

Lacustrine carbonates occur in both freshwater and saline lake environments (Matter and Tucker, 1978; Hakanson



Carbonate Environments, Figure 2 Carbonate environments in a land-to-sea cross section. (Modified from Flügel, 2004.)



Carbonate Environments, Figure 3 Photo of sliced Quaternary tufa deposit on Casino building of Goethe University, Frankfurt, Germany, with microbial lamination and calcified remains of algal thalli.

and Jansson, 1983; Anadon et al., 1991). Freshwater lakes in humid climates such as Lake Constance, Germany, Lake Zurich, Switzerland, or Green Lake in North America are usually perennial. Their size can be as small as $<10 \text{ km}^2$ as in Green Lake to more than 500 km^2 as in the example of Lake Constance. They are characterized by fine-grained carbonate deposits, usually in an annually laminated fashion (Figure 4). Fine-grained siliciclastics are more common during winter. Low-magnesium calcite is precipitated preferentially during warm summer months, enhanced by photosynthetic activity of phytoplankton that withdraws CO_2 . Other carbonate producers are usually ostracods, gastropods, pelecypods, and the charophytic alga *Chara*. The thalli of this alga may calcify and form characteristic tubes. Reproductive cells

(oogonia) are calcified also. Diatoms occur as well. In the case of high carbonate content of the lake water, microbial activity may occur and produce stromatolites, thrombolites, and/or oncolites (Figure 5).

Saline lakes in arid climates such as the Great Salt Lake in North America or the Dead Sea have considerable sizes of 4,400 and 600 km^2 , respectively. Saline lakes are often ephemeral. They are also characterized by abundant carbonate deposition. Controlled by the Mg/Ca-ratio, either low-magnesium calcite, high-magnesium calcite, aragonite, magnesite (MgCO_3), or dolomite is precipitated. Under high evaporation rates, sulphates and chlorides such as gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and halite (NaCl) may form. In the case of the Great Salt Lake, aragonitic ooids occur in abundance in shallow areas along shore. Also, microbial buildups and sheets are found, which are to large parts due to cyanobacterial activity. Along shore of saline lakes, caliche may be common due to fluctuating lake levels that expose larger areas. When springs are present, tufa or travertine is formed as sheets, mounds, and towers. The latter is characteristic of Mono Lake in California where spectacular towers of tufa are found.



Carbonate Environments, Figure 4 Photo of annually layered Holocene lake sediment from core, Lake Zurich, Switzerland. Light layer is carbonate; dark layer is siliciclastics. Thicker gray bed is a turbidite. Scale in centimeters. (Photo courtesy of Flavio Anselmetti.)

Subaerial exposure

Subaerial exposure of limestone leads to dissolution by meteoric waters. Typical landforms that may have considerable relief are termed karst (James and Choquette, 1988). Landforms include dolines, towers, karren, or poljes. Dissolution may produce cavities and larger caves. Calcium carbonate is re-precipitated in limestone caves in the form of speleothems such as stalactites and stalagmites. Cave pearls or pisoids (“diagenetic ooids”) may form in ponds. Residues of dissolution together with organic material may later become soils on limestone such as laterite or terra rossa. In warm and/or arid regions, carbonate-rich soils form deposits that are called caliche or calcrete (Wright and Tucker, 1991). A caliche is characterized by patterns such as brecciation, clotted textures, root holes or rhizoids, and low-magnesium calcite cements, sometimes in a needle fiber texture (“whiskers”). Laminated soilstone crusts are a special case of calcrete that can resemble stromatolitic formations. Soilstone crusts form by precipitation of calcium carbonate from evaporating pore water. The identification of subaerial exposure surfaces in the fossil record is of great importance, e.g., for the reconstruction of fluctuations in sea level and for sequence stratigraphy (Figures 6 and 7).

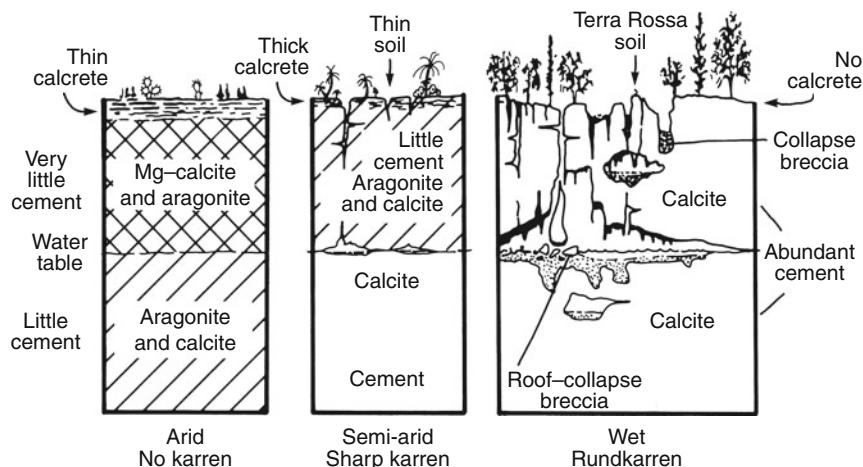
Transitional environments

Beaches and shores

Carbonate beaches are usually formed by sand-sized particles that originate from the adjacent shallow seabed (Inden and Moore, 1983). Shells and skeletons of organisms as well as non-skeletal grains occur. Beach zones include the shoreface, the foreshore, and the backshore. The shoreface lies below the lower tide level and is characterized by cross-bedding. The foreshore is situated between



Carbonate Environments, Figure 5 Photo of lacustrine stromatolites, Bacalar, Yucatan Peninsula, Mexico.



Carbonate Environments, Figure 6 Scheme of karst profiles in various climates. (From James and Choquette, 1988; with permission of Springer, NY.)

low- and high-tide levels. The backshore lies above the high-tide level. Sediments of the foreshore and backshore zones exhibit parallel lamination and beds dip gently toward the sea (Figure 8).

A striking feature in the tropical/subtropical realm, and common in latitudes up to 60° , is beachrock. Beachrock forms in the intertidal zone by rapid cementation of sedimentary particles by marine aragonite and high-magnesium calcite cements. The formation of beachrock is not fully understood, and is assigned to physicochemical and/or biological processes (Gischler, 2007). The strictly intertidal position of beachrock supports the contention that cements precipitate from evaporating pore water during low tide. Also, beachrock appears to be common in windward and rather exposed sites indicating that

flushing of pores by water rich in calcium carbonate is of importance. Typical sediment structures of beachrock are seaward dipping beds. Keystone vugs are small cavities in beaches and beachrock that are produced when air escapes from the beach (Figure 9).

Behind the backshore, in the landward direction, dunes may occur that are a product of eolian deposition (Abegg et al., 2001). Carbonate eolianites are common behind carbonate beaches, and are found in abundance along the eastern coast of the Yucatan Peninsula, on the islands of the Bahamas and Bermuda, along the Trucial Coast (southern Persian Gulf), and on the Balearic Islands in the Mediterranean Sea. High angle cross-bedding as well as rhizoids and caliche, indications of the meteoric realm, are characteristic. *Microcodium* are small spherical bodies



Carbonate Environments, Figure 7 Hand specimen of caliche with laminated crust. Miami Limestone from the Everglades, Florida.

of radiating calcite that forms among rhizoids in eolianites. Carbonate cements include meteoric types composed of low-magnesium calcite.

Tidal flats

As indicated by the name, tidal flats are broad and low-relief areas, which are usually flooded during high tide and subaerially exposed during low tide (Ginsburg, 1975). Classic modern carbonate-dominated examples include the humid tidal flats west of Andros Island on Great Bahama Bank (Shinn et al., 1969) and the arid examples on the southwestern side of the Persian Gulf (Kendall and Skipwith, 1969). Tidal flats are dissected by tidal channels, which are lined by levees. Levees usually enclose low-relief ponds. Beach ridges form the transition to the adjacent subtidal realm. In the case of humid tidal flats, algae such as *Batophora* and microbial mats as well as mangroves thrive along the channels and further inland in the so-called marsh. In the Bahamas, microbial mats are largely formed by the genera *Scytonema* and *Schizothrix*. Gastropods of the genus *Batillaria* occur in great abundance. Benthic foraminifera are present. In arid tidal flats or sabkhas, which transition into the desert on the landward side, microbial mats occur along channels. Also, burrows of crabs may be abundant along the channels. Nebkhas are small mounds that are produced by halophile plants that baffle sediment around their roots (Figure 10).

Sediments on tidal flats are usually poorly sorted muds, silts, and sands. They are horizontally layered and are largely derived during storms from the adjacent seabed, from precipitation, or from eolian transport. Fine-grained sediment is usually found in ponds. Mud cracks and

wrinkled, dried-out, and curled microbial mats are a typical phenomenon. Sands make up levees and beach ridges. A characteristic sedimentary structure is the bird's eyes structure, millimeter-sized fenestral pores that result from gas-bubble formation. In the fossil record, these structures are good indicators of the intertidal and shallow subtidal realm. As a consequence of evaporation on tidal flats, cemented carbonate crusts are formed that may contain dolomite. In the arid realm, gypsum and/or anhydrite is precipitated within the sediment and forms nodules, enterolithid folds, discs, and chicken-wire textures (Figure 11).

Marine environments

Carbonate shelves and platforms (Figure 12)

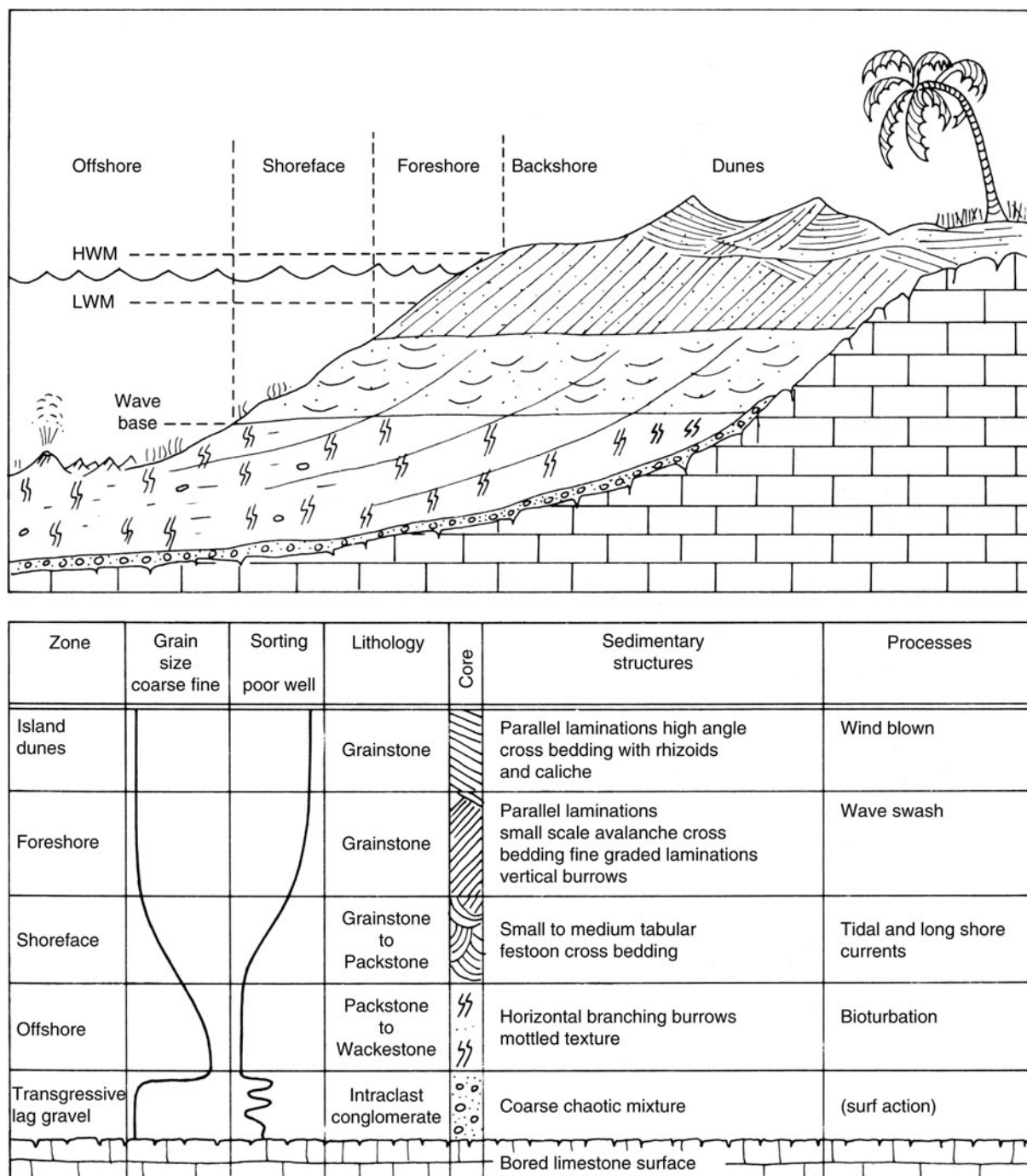
Restricted shelves and platforms

These carbonate environments are shallow (0–20 m) and characterized by partial or complete enclosure by islands, reefs, or shoals. Modern examples include Florida Bay (Ginsburg, 1956; Stockman et al., 1967), the Gulf of Batabano (Hoskins, 1964), Cuba, or Shark Bay, western Australia (Logan et al., 1974). The partial or complete enclosure of these platforms results in restricted circulation and in elevated water temperature and salinity. This in turn leads to low diversity biota of mollusks, benthic foraminifera, crustaceans, and echinoderms. Burrowing crustaceans (*Callianassa*) are responsible for abundant bioturbation (Figure 13). Tolerant corals such as *Solenastrea* and *Siderastrea* are occasionally found in Florida Bay. Sea grasses (*Thalassia*, *Syringodium*, *Posidonia*, and *Cymodocea*) are common, which act as bafflers of sediment. In Florida Bay, the codiacean algae *Penicillus* and *Halimeda* occur in great abundance. Dasycladacean algae such as *Acetabularia* are common. In Shark Bay, stromatolites thrive in intertidal and subtidal areas. Their abundance is classically explained by the exclusion of grazers due to the high salinities in the bay.

Sediments in Florida Bay are largely of skeletal origin. Dominant carbonate producers are mollusks, foraminifera, and the alga *Penicillus*, which is the most important source of aragonite mud (Stockman et al., 1967). Mud banks, elongated hillock-type accumulations of mud, are densely covered by sea grass (Bosence, 1995). Both skeletal (mollusk, foraminifera) and non-skeletal (peloidal) grain types are found in the Gulf of Batabano. Likewise, Shark Bay sediments include skeletal grains of mollusks and foraminifera as well as mud from the breakdown of *Penicillus*. Non-skeletal grains include ooids that are formed in shallow areas, peloids, and reworked lithoclasts from underlying Pleistocene limestone (Figures 14–16).

Open-rimmed shelves and platforms

Intensively studied examples of rimmed carbonate shelves and platforms include the Florida Reef Tract, the Belize shelf and barrier reef, Central America, and the Queensland shelf and Great Barrier Reef, NE Australia. These environments are largely characterized by normal marine



Carbonate Environments, Figure 8 Scheme of beach zonation and sediments. (From Inden and Moore, 1983; with permission of American Association of Petroleum Geologists.) HWM: high water mark; LWM: low water mark.

conditions. Water depths range from 0 to 200 m. The areas covered are several 1,000 km² to several 100,000 km² in the case of the Queensland shelf. In between the coast and platform margins, low and high islands as well as patch reefs and faroes (small circular lagoon reefs) of coral can be found.

Carbonate sediments on these rimmed platforms are almost exclusively of skeletal origin and facies belts more or less parallel to the coast (Ginsburg, 1956; Maxwell, 1968; Purdy and Gischler, 2003; Purdy et al., 2003). On the Belize and Queensland shelves, siliciclastics derived from the hinterland mix with carbonate sediment. As



Carbonate Environments, Figure 9 Tropical beach with beachrock outcrop. Loggerhead Key, Dry Tortugas, South Florida.

a consequence, facies are siliciclastics-dominated near the coast. Marls are found in the middle shelf. Largely carbonate sediments occur at the outer platform and platform margins. Central shelf marls are rich in fragments of mollusks and foraminifera. Mud content is high. In Belize and NE Australia, pelagic biota such as pteropods and planktonic foraminifera, respectively, are found in these platform areas due to the high depths of the shelves. Shelf reefs and outer platforms and margins are predominated by mostly coarse-grained sediments rich in coral, coralline red algae, *Halimeda*, and benthic foraminifera (Figure 17).

Platform margins are several kilometers wide and water depths are between 0 and 50 m. Coral reefs are widespread with the common genera *Acropora*, *Porites*, *Montastraea*, *Diploria*, *Platygyra*, *Pocillopora*, and *Millepora*. The reef crest proper is at sea level. The shallow forereef slope is often characterized by spur-and-groove systems (Shinn et al., 1981) (Figure 18). In the case of Belize, a steep sometimes vertical slope occurs in the deep forereef. This bypass margin was investigated in detail by James and Ginsburg (1979). The Florida and Queensland margins with lower angles are examples of depositional margins (Read, 1985).

Isolated platforms

The largest known isolated carbonate platforms include the Bahamas in the Atlantic and Great Chagos Bank in the Indian Ocean, which each cover 10,000 to 100,000 km², respectively. In the Bahamas archipelago, Great Bahama Bank (Purdy, 1963; Ginsburg, 2001) is probably the best known example (Figure 19). Great Bahama Bank is a shallow, low-relief submarine bank,

which is surrounded by deep water. Water depths on the bank only rarely exceed 6 m. The bank has very high and steep bypass margins, especially on the eastern side. The steepness is presumably the result of both biological construction and erosion. On the eastern side, a well-developed coral reef rim with abundant *Acropora* exists in shallow water. Otherwise, the margins of Great Bahama Bank are largely predominated by ooid sand shoals. These shoals are very shallow, high-energy environments where ooids form and are moving in the form of up to several kilometer-long rippled sand waves, more or less parallel to the bank margins. Joulter Cays ooid shoal in the northeastern corner of the bank is intensively studied (Harris, 1979). The western bank margin also has long and wide ooid shoals. Near the southern end of Tongue of the Ocean, ooid sand waves are oriented perpendicular to the bank margin. The eastern interior part of Great Bahama Bank is covered by the Pleistocene island of Andros, which gets as high as 30 m above present sea level. Most of the platform interior is covered by shallow water as mentioned above. Benthic marine animals such as gastropods (*Strombus*) and echinoids (*Oreaster*) are rather rare. Codiacean algae such as *Penicillus* and sea grass (*Thalassia*) are common. Salinities are above normal marine on the bank and may reach 45‰ close to Andros Island. Sediments are mostly non-skeletal and include peloidal muds as well as aggregate grain ("grapestone") and peloidal sands (Purdy, 1963). Skeletal sands only occur around the eastern reefal margin and in a small band on the western margin.

The origin of carbonate mud on Great Bahama Bank is a matter of debate since several decades [see discussion in

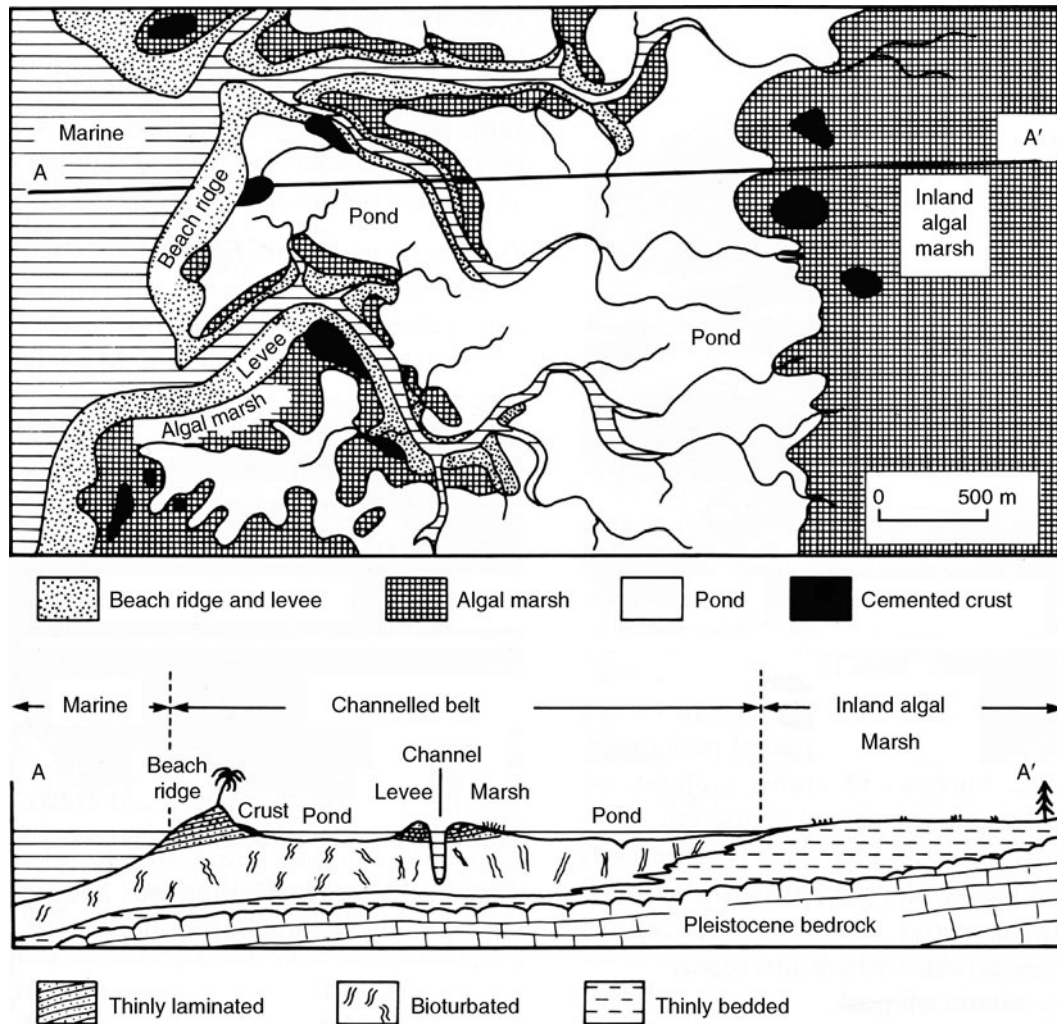


Carbonate Environments, Figure 10 Photos from tidal flats. (a) Aerial view of western part of Andros Island, Bahamas. (Photo courtesy of Robert Ginsburg.) (b) Microbial mat on sabkha in Khiran, southern Kuwait.

Gischler and Zingeler (2002)]. Mud largely consists of micron-sized aragonite needles. It potentially originates from codiacean algae such as *Penicillus*, from physico-chemical precipitation in the warm and saline surface waters of the bank, or from biologically (bacterially) induced precipitation of calcium carbonate. Commonly occurring clouds of suspensions of carbonate mud and water on the bank, known as “whittings,” have been described as expressions of spontaneous precipitation phenomena. However, there is also evidence that whittings are simply a consequence of bottom sediment stirring either by fish or by currents.

More than 2 m high subtidal stromatolites were discovered by Dill et al. (1986) on the Bahamas in normal marine seawater near the Exuma islands. Previously, only small microbial buildups were known from the Bahamas. These stromatolites develop within very strong currents and significant bottom sediment movement. Apparently sediment movement largely excludes grazers and triggers carbonate accumulation via trapping and binding.

Seismic profiling and deep drilling has revealed that the geologic history of Great Bahama Bank was not simply a process of aggradation (Ginsburg, 2001). A complex interplay of slow subsidence and sea-level change has



Carbonate Environments, Figure 11 Tidal flat scheme from Andros Island, Bahamas. (From Scoffin, 1987; with permission of Blackie and Son Ltd.)

modified the bank during the Neogene in that shallow water banks repeatedly aggraded, backstepped, and prograded, thereby filling deep seaways and moving the western margin of Great Bahama Bank by 30 km to the west into the Straits of Florida.

Carbonate ramps

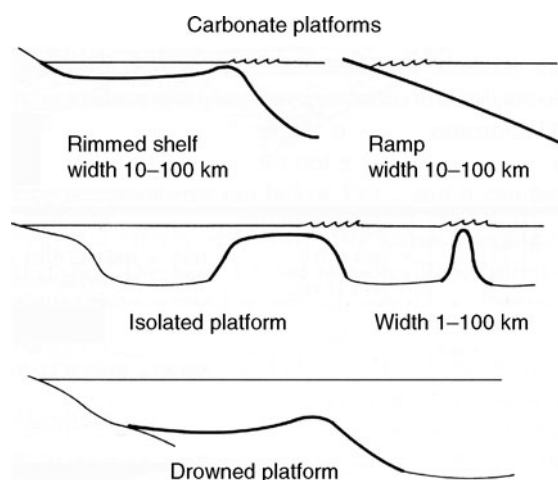
The term ramp was introduced to the carbonate literature for unrimmed carbonate shelves with a low-angle dip ($<1^\circ$) toward the open sea (Burchette and Wright, 1992; Wright and Burchette, 1998). The classical modern example is the southwestern Persian Gulf. Other modern ramp examples include the western Florida margin or Campeche Bank, Mexico. Geometrically, there are homoclinal and distally steepened ramps (Figure 20). Depositional environments are usually divided into inner, middle, and outer ramps. The inner ramp environment is above fair-weather wave base (FWWB); the mid-ramp between FWWB, and

the storm wave base (SWB); the outer ramp below SWB. In the southern Gulf, inner ramp environments consist of shoals and barrier facies with ooid-skeletal sands. Smaller reefs are found just seaward of the barriers. Back barrier sediments are usually fine-grained peloidal and mollusk muds and sands. Mollusk-rich silts and sands are common on the mid-ramp. Outer ramps are characterized by carbonate and argillaceous muds ("marl") with abundant mollusks. In the northern Gulf, barriers are largely lacking. In contrast to the southern Gulf, which is tide-dominated, the northern Persian Gulf ramp is largely wave-dominated. On the inner ramp, quartz grains derived from the adjacent mainland desert may be present in addition to ooid shoals. Small coral patch reefs may also be found in the inner ramp zone. Mid-ramp and outer ramp deposits are also dominated by mollusk-rich silts and sand and argillaceous muds, respectively. Pinnacle-type patch reefs of coral occur in the outer ramp.

Characteristic storm beds or tempestites in mid-ramp settings were identified by Aigner (1982) in the European Triassic Muschelkalk beds. Tempestites exhibit characteristics such as sharp bases, sole marks, graded bedding, hummocky cross-stratification, and ripple marks. Because there are no larger outcrops in modern ramps and shelves showing complete sedimentary bodies or large-scale sedimentary structures, our knowledge of storm beds is largely based on the fossil record.

Temperate- and cool-water shelves and platforms

Apart from the abundant typical warm water and low latitude deposits, about one third of the modern carbonate



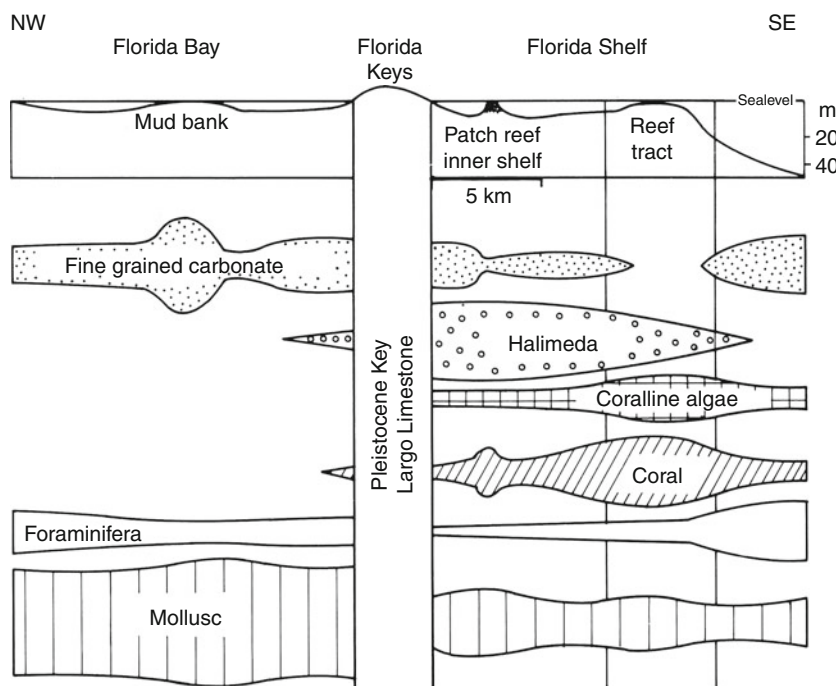
Carbonate Environments, Figure 12 Scheme of carbonate platform types and morphologies. (Modified from Tucker and Wright, 1990.)

shelves and platforms are characterized by temperate and cool-water carbonate sedimentation (Lees and Buller, 1972; James and Clarke, 1997). Prominent and well-studied modern examples are the Great Australian Bight or the Mediterranean realm. Temperatures in the cool-water realm are below 20°C and heterotrophic carbonate producers predominate. They include mollusks, echinoderms, bryozoa, foraminifera, coralline algae, barnacles, and serpulids ("heterozoan association"). Constituent carbonate particles of the same taxa are also present in warm-water carbonates; however, there, they are swamped by carbonate grains from rapidly growing producers that have photosymbionts such as corals and codiacean algae as well as by mud and non-skeletal carbonate formation as in ooids, peloids, and cements ("photozoan association"). There are also cases where heterozoan carbonates occur in warm-water settings, e.g., in western Florida or to some degree on Campeche Bank, Mexico, as well as in several fossil examples. Elevated nutrient contents and primary production are usually responsible, and hamper abundant photozoan carbonate production (Figure 21).

Temperate and cool-water carbonates usually form unrimmed shelves or platforms, and they are strongly influenced by hydrodynamic energy. Submarine cementation is rather weak. In modern examples, kelp (brown algae) forests occur in the constantly wave-dominated inner ramp. Siliciclastic sediment may derive from local sources from the mainland. Sand waves and dunes are found on the mid-ramp, which is prone to storm-reworking. Sediments on inner and mid-ramps are mostly coarse-grained and frequently redeposited. The outer ramp environment may contain fine-grained sediment with bryozoan, sponge, or non-zooxanthellate coral mounds.



Carbonate Environments, Figure 13 Underwater photograph of *Callianassa* mounds, Florida Bay.



Carbonate Environments, Figure 14 Sediment distribution in Florida Bay and the reef tract. (Modified from Ginsburg, 1956.)



Carbonate Environments, Figure 15 SEM photo of carbonate mud from Belize with abundant aragonite needles and nanograins, which originate from codiacean algae.

Reefs

Reefs are classical objects of geobiological research because they are rock formations built by the biological activity of organisms. Reefs normally form topographic highs above the surrounding sea floor. Reef organisms

comprise large numbers of taxa, which either construct a framework or baffle and bind sediment, which is rapidly consolidated. In addition, there are reef dwellers, which do not necessarily contribute to reef growth, and, there are reef-destroying organisms, which bore into, dissolve, or



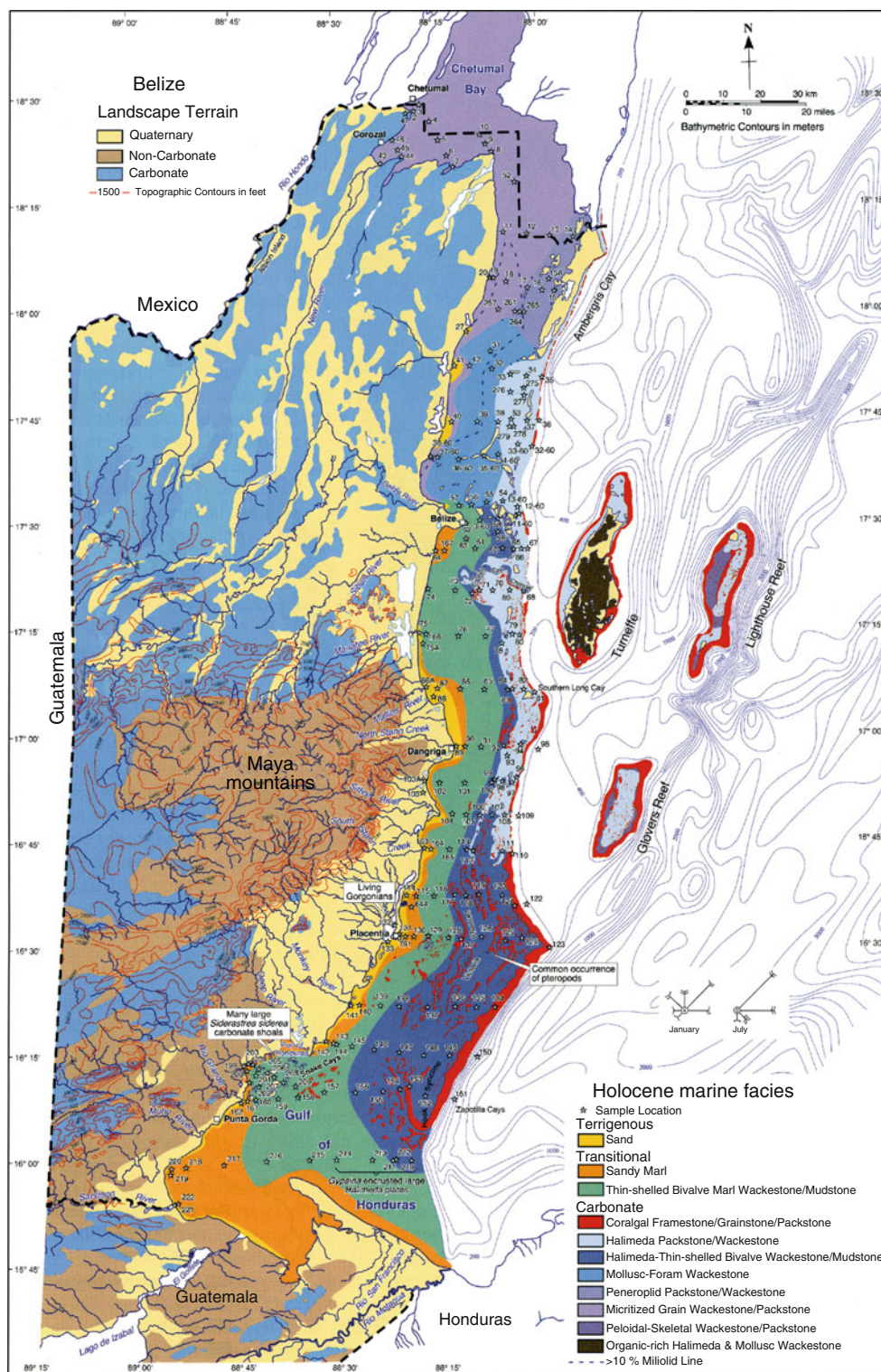
Carbonate Environments, Figure 16 Underwater photographs of calcareous codiacean algae (a) *Penicillus* and (b) *Halimeda*, Belize.

erode reef limestone. This ecological guild concept of constructors, bafflers, binders, dwellers, and destroyers was developed for reef organisms by Fagerstrom (1987), who compiled a geological history of reef building. Like Wilson (1975) before, he stressed that reef building is a geologically old phenomenon that reaches back into the Archaean, however, reef-building organisms have repeatedly changed with time. Recently, Wood (1999), Stanley (2001), and Kiessling et al. (2002) have provided additional monographs and editions on the long history of reef building. The most abundant groups of reef builders during earth history have been microbes, corals, sponges, bryozoa, and rudist bivalves. Reef destroyers include boring sponges, bivalves, worms, and microborers as well as grazing gastropods, echinoderms, and fish. Reef building

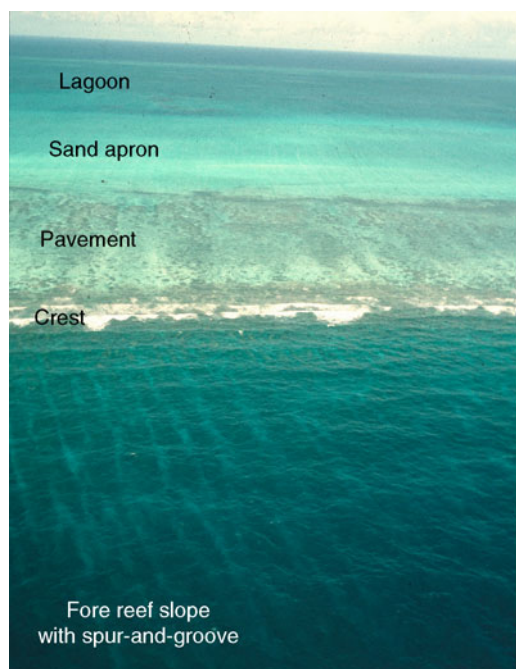
is a complex interplay of reef construction, destruction, sedimentation, and cementation, processes which occur at the same time and eventually produce what geoscientists would term reef limestone (Scoffin, 1992) (Figure 22).

Tropical reefs

Modern tropical reefs cover an area of about 600,000 km² in tropical-subtropical latitudes around the globe. Reefs thrive in warm waters between 18 and 30°C, normal marine salinity, and clear and oligotrophic waters. They represent the most diverse marine ecosystem with several tens of thousand of species including taxa from all known animal phyla. Scleractinian corals and red coralline algae are the dominant builders (Figure 23). Other calcareous

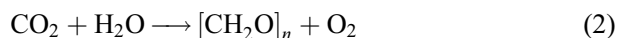


Carbonate Environments, Figure 17 Carbonate sediment distribution on the Belize shelf and platforms. (With permission of Purdy and Gischler, 2003.)



Carbonate Environments, Figure 18 Aerial photograph of zoned margin in Lighthouse Reef, Belize.

algae such as codiaceans, mollusks, benthic foraminifera, and echinoderms are important sediment producers on tropical reefs. Reef-building Scleractinian corals live in symbiosis with dinoflagellates (“zooxanthellae”), which live in their tissues and photosynthesize. The symbionts are both important for the coral metabolism by providing food and also enhance skeletal growth of the aragonite coral skeleton because they consume carbon dioxide.



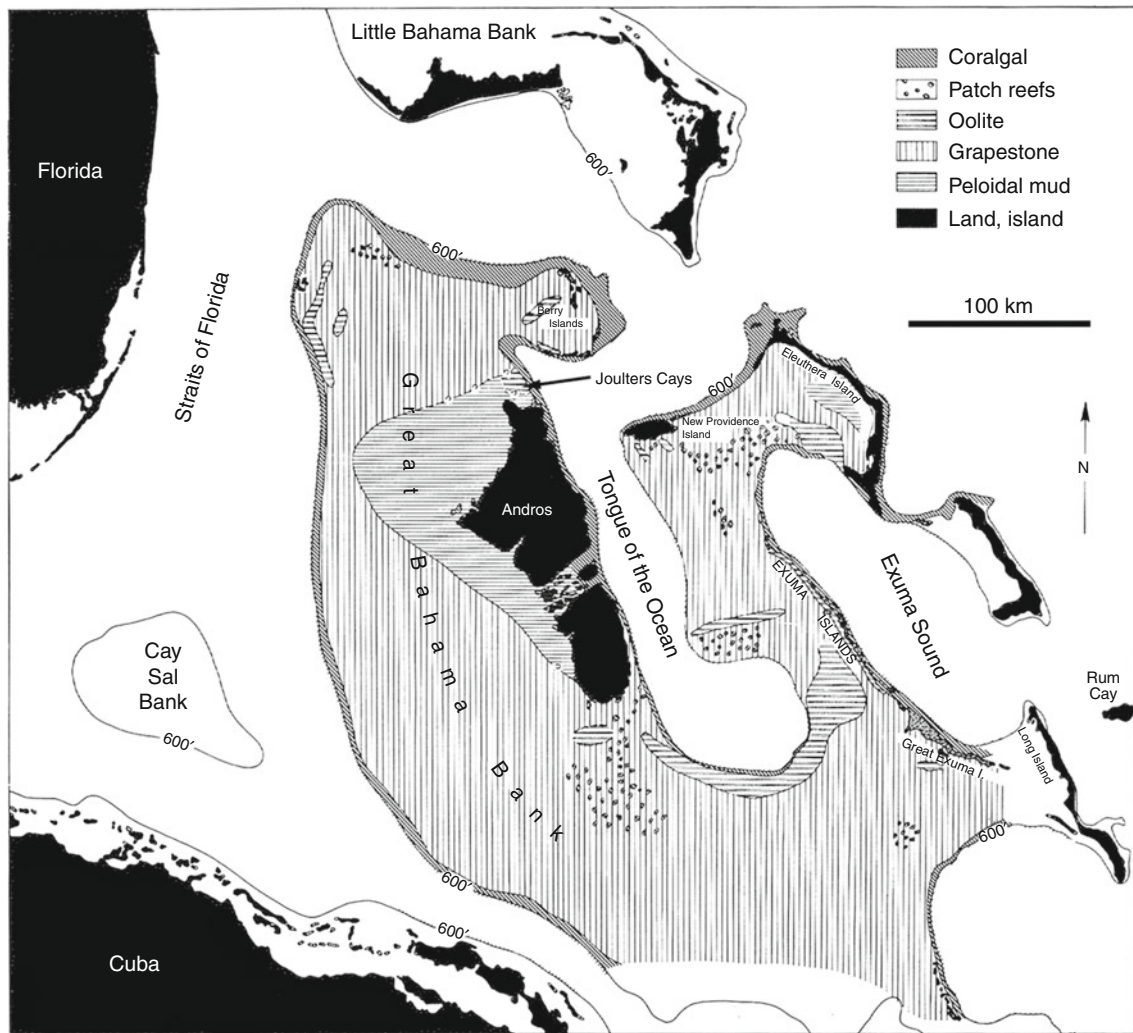
Because of the symbiosis, the tops of tropical reefs are at or close to sea level. Fossil coral reefs are therefore used as gauges for past sea levels. The annual banding of coral skeletons and the longevity of colonial reef corals makes them ideal archives of historical climate change (“sclerochronology”).

Since Darwin (1842), tropical reefs are morphologically subdivided into fringing reefs, barrier reefs, and atolls. Fringing reefs occur close to the coast with a shallow moat or lagoon in between reef and coastline. These reefs are abundant in the Caribbean Sea and the Indonesian archipelago. Barrier reefs are larger structures that are separated from the coast by a deep channel, like in northeastern Australia, New Caledonia, or Belize. The Great Barrier Reef is the longest barrier reef with more than 2,000 km in length. Barrier reefs in the sense of Darwin surround volcanic islands like, e.g., Bora Bora in the Tuamotu archipelago. Atolls are circular reefs, which enclose a deep lagoon. There are about 400 atolls worldwide. They are most common in the Pacific and Indian

Oceans, and have a volcanic basement. According to Darwin’s (1842) elegant subsidence theory, fringing reef, barrier reef, and atoll are genetically connected by the simple process of subsidence of a volcanic island. Tayama (1952) carried the theory even further and explained the formation of small circular reefs with shallow or filled lagoons, so-called table reefs, as the final stage in reef development before drowning and seamount (guyot) formation. Even so, the subsidence theory does not explain large barrier reefs that are attached to large continental land masses, and, it neglects the significance of eustatic sea-level change, which was first considered in Daly’s (1910) glacial control theory. Indeed, drilling on Enewetak Atoll in the 1950s has demonstrated about 1.3 km of shallow-water reef limestone on top of an Eocene volcano, but numerous hiatuses were found, which are the result of repeated subaerial exposure. The antecedent karst model of Purdy (1974) takes into account the importance of limestone dissolution by meteoric waters during eustatic lowstands of sea level, which are even able to produce fringing, barrier, and atoll reef morphologies as well as spur-and-groove patterns in subaerially exposed limestone and limestone-volcanic islands. Recent studies have shown that modern barrier reefs are comparably young features that only came into existence about 400–600 ky ago when high amplitude, eccentricity-driven eustatic sea-level changes became more and more dominant (International Consortium of Great Barrier Reef Drilling, 2001; Multer et al., 2002).

Tropical reefs are characterized by ecological zonation, with zones running more or less parallel to bathymetry (see Figure 18). Modern reefs also exhibit provincialism between Atlantic and Indo-Pacific realms. For high sea-level greenhouse climates in earth history, like in the Devonian or Jurassic and Cretaceous, however, no evidence of provincialism was found. Common reef zones include from outboard to inboard the fore reef, reef crest, back reef, sand apron, and lagoon. Surface sediments are coarse sands of coral and calcareous algae in reefal environments and more fine-grained, mollusk- and foraminifer-rich sediments in lagoonal areas. Several shallow lagoons of Caribbean reefs are rich in non-skeletal grains. Milliman (1974) explained this difference to Indo-Pacific reefs, which he termed the “ooid problem,” by the shallower and smaller lagoons, in which water exchange is much faster. Carbonate-rich water circulates more rapidly and thereby preferentially produces non-skeletal grains such as ooids, peloids, and aggregates by submarine cementation (Figure 24). However, the discovery of abundant modern non-skeletal grains such as ooids and cemented fecal pellets in the shallow lagoons of Aitutaki Atoll (Cook Islands) and Bora Bora (Tuamotus) in the south Pacific opposes this widely held concept (Rankey and Reeder 2009; Gischler 2010).

Drilling in the barrier reef around the island of Tahiti (Montaggioni and Camoin, 1993) and investigations in cavities of the Great Barrier Reef (Reitner, 1993) have shown the importance of abundant microbial activity in



Carbonate Environments, Figure 19 Sediment map of the Great Bahama Bank. (With permission of Traverse and Ginsburg, 1966.) Bank margin is defined by 600 ft (180 m) depth contour.

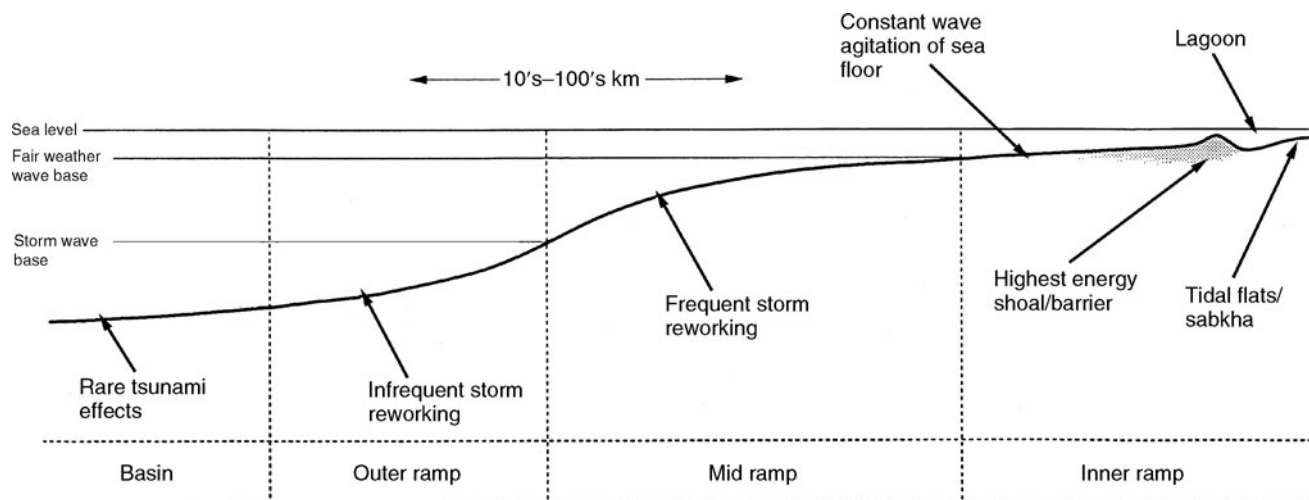
tropical coral reef cavities. Especially in Tahiti, stromatolite-type formations are quite abundant, even though microbial activity was apparently most widespread during the early Holocene. In the Great Barrier Reef example, sponges and sponge tissue decay are additional agents besides microbes that trigger carbonate formation.

Deep- and cold-water reefs and mounds

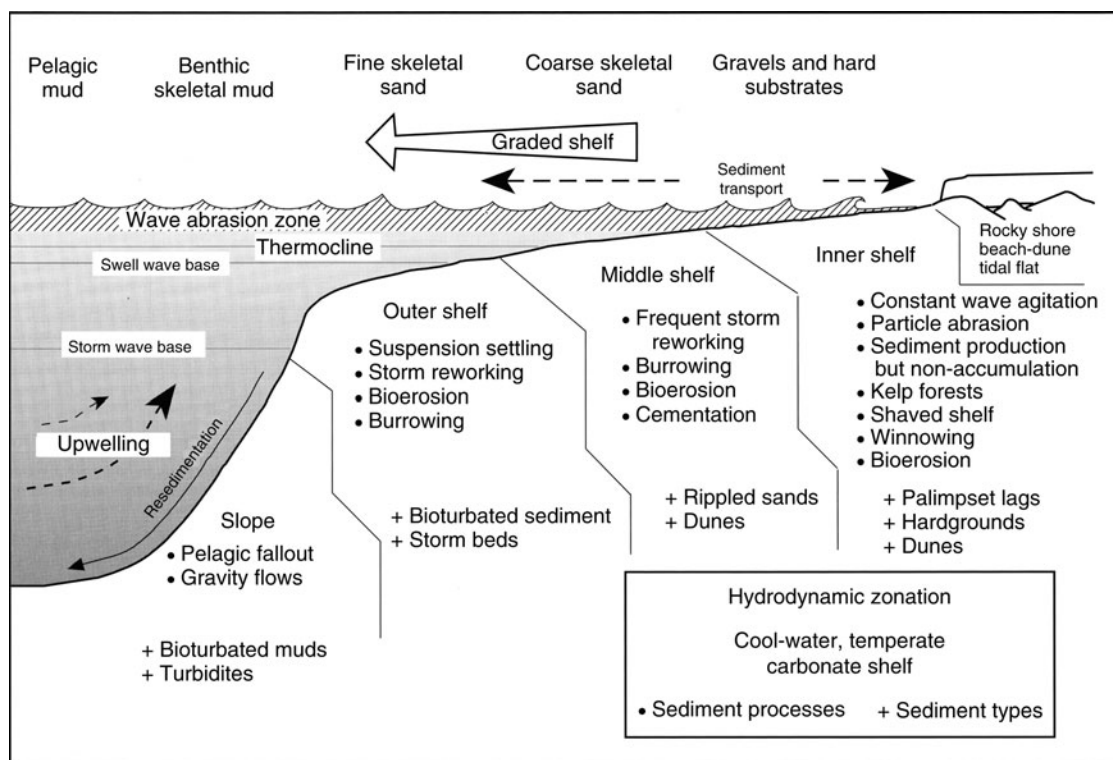
After the first discovery of abundant coral reefs in deep and cold water off the Scandinavian coast (Teichert, 1958), it took several decades before these reef structures were investigated in great detail (Freiwald and Roberts, 2005; Roberts et al., 2006). These reefs are largely built by non-zooxanthellate branched corals such as *Lophelia*, *Madrepora*, or *Cladocora* in water depths below 200 m and temperatures between 4 and 2°C. Reef thickness may reach more than 30 m and individual reef structures have lengths of more than 10 km. Species richness in the

north Atlantic examples is around 1,300 taxa. Deep-water reef foundations are usually submarine topographic highs such as, e.g., iceberg plough marks. Meanwhile, it has become clear that deep- and cold-water reefs in the northeastern Atlantic are not restricted to the Scandinavian area but are also abundant off the Atlantic shelf margin as far south as the Mediterranean and in the Caribbean.

Somewhat similar deep- and cold-water reefs, termed lithoherms, were discovered in strong currents at the bottom of the Straits of Florida in 600–700 m of water (Neumann et al., 1977). Lithoherms are several 100 m long and up to 50 m high. Their shape is ellipsoidal with the long axis parallel to the current. The faunal elements mainly include non-zooxanthellate corals, crinoids, sponges, and bryozoa. Interstitial muddy to sandy detrital carbonate sediment is abundant and largely a product of baffling. The mounds are cemented at the surface in that concentric, hard carbonate crusts occur. Stromatactis-type fenestral



Carbonate Environments, Figure 20 Scheme of a ramp. (Modified from Burchette and Wright, 1992.)

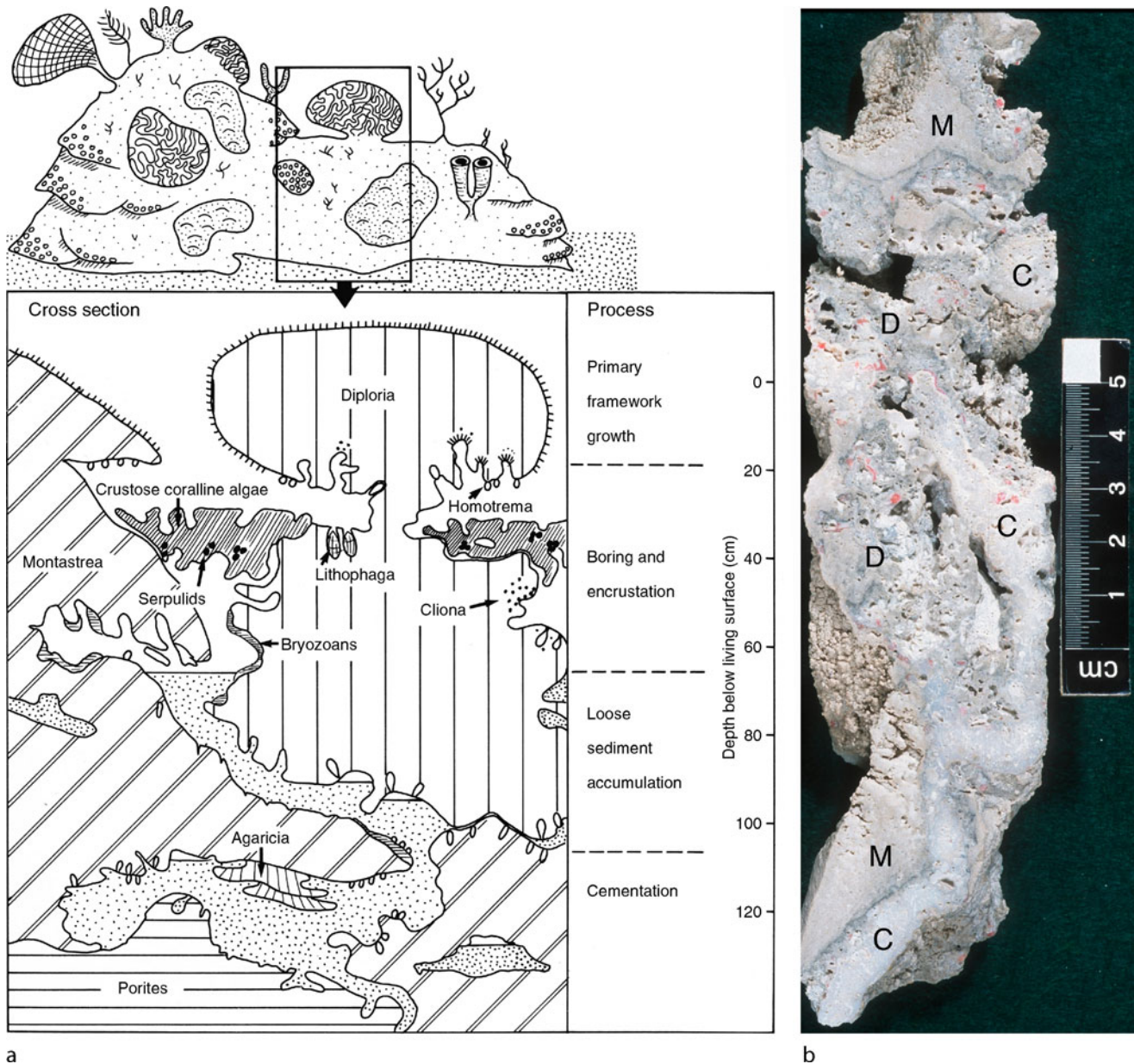


Carbonate Environments, Figure 21 Scheme of cool-water shelves. (Modified from James and Clarke, 1997.)

cavities were discovered and attributed largely to burrowing and subsequent sediment infill.

It is tempting to interpret the Florida lithoherms as modern analogues of fossil deep-water mud mounds. Mud mounds are common in the fossil record, especially in the Devonian and Carboniferous (Monty et al., 1995). The most prominent examples are probably the

Carboniferous ("Waulsortian") mud mounds. Waulsortian mounds or banks also formed in deep water and contain biota with crinoids, sponges, bryozoa, brachiopods, and mollusks. Corals are rare. Stromatactis, large cement and sediment-filled fenestral cavities with flat bases and irregular tops, is very common in Paleozoic mud mounds (Krause et al., 2004). Even so, major differences between

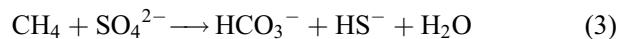


Carbonate Environments, Figure 22 (a) Scheme of reef limestone formation with synchronous processes. (From Scoffin, 1992; with permission of Springer: Berlin, Heidelberg.) (b) Quaternary reef limestone from Belize Barrier Reef. C coral; M microbialite, D detritus.

the lithohermes and the Waulsortian mud mounds appear to be the abundance of carbonate mud in the fossil examples. Lithohermes have higher quantities of coarse sediment. Also, lithohermes are largely biodepositional mounds whereas the Waulsortian ones are microbial mounds in which in situ mud production prevailed (Monty et al., 1995).

Also, mud mounds and deep-water carbonate mounds have been found to be associated with cold seeps or hot vents (Beauchamp and von Bitter, 1992; Mounji et al., 1998). Around locations of seepage of methane (CH_4)

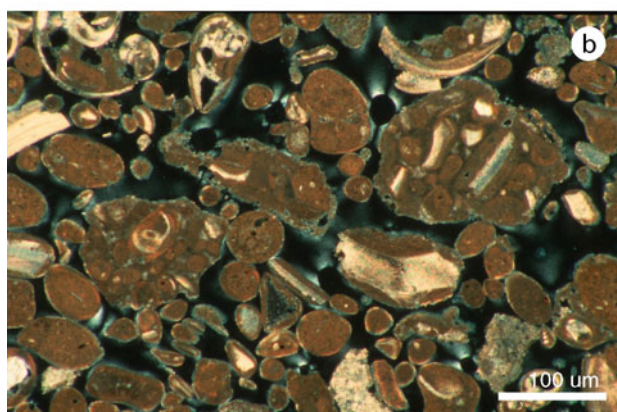
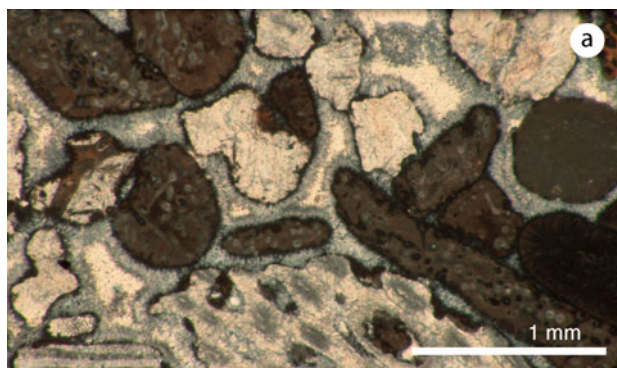
and hydrogen sulfide (H_2S) on the seafloor, authigenic carbonate precipitation may be triggered by bacteria via chemosynthetic processes that produce bicarbonate:



Usually, metazoans such as certain bivalve mollusks (*Caplyptogena*, *Bathymodiolus*, lucinids), vestimentiferan worms (*Riftia*), and thysanid shrimps, which have



Carbonate Environments, Figure 23 Underwater photograph of a reef with abundant acroporids and milleporids. Glovers Reef Atoll, Belize.

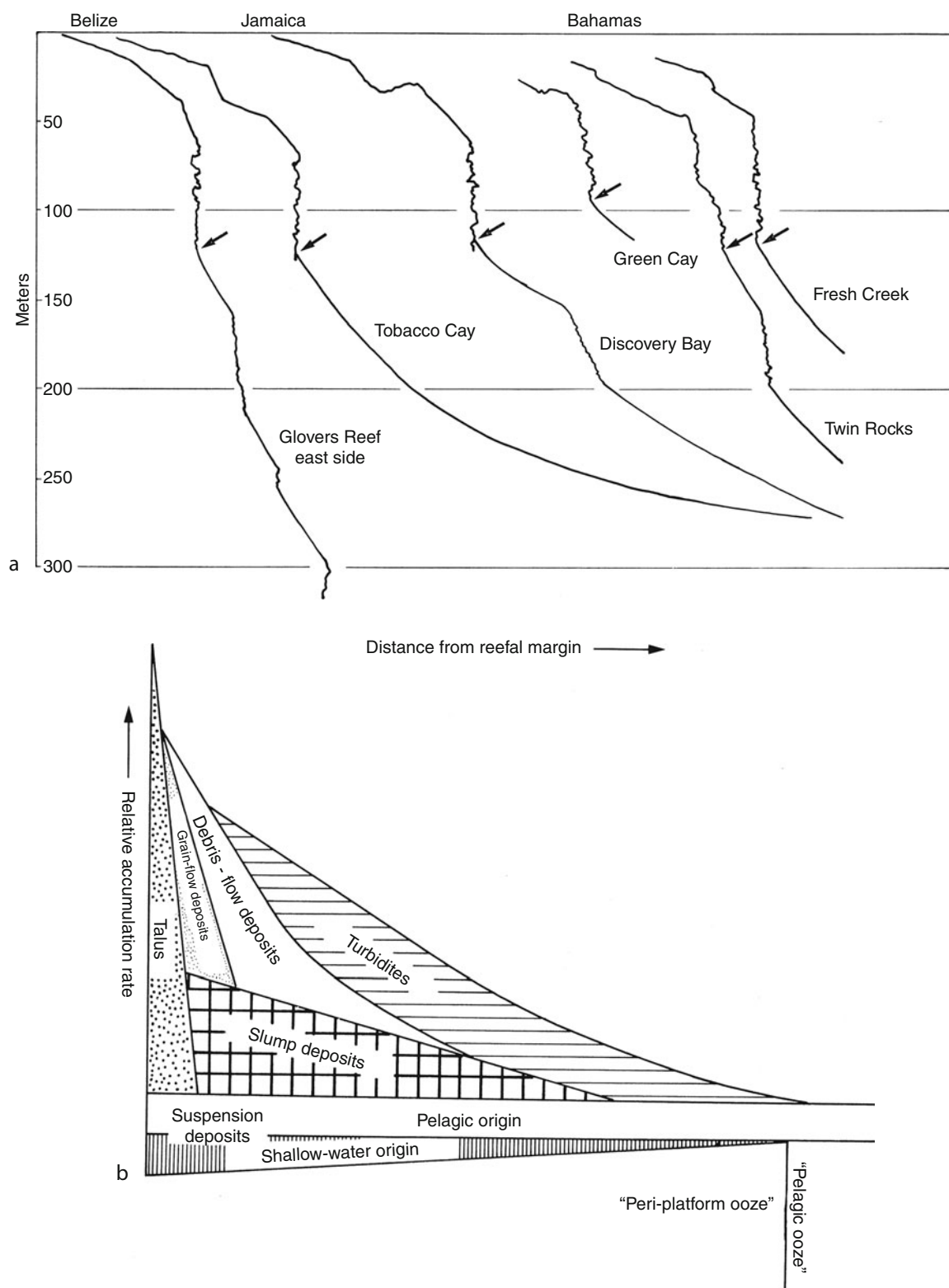


Carbonate Environments, Figure 24 Thin-section photographs of typical (a) skeletal carbonate sediment in beachrock with corals (light) and *Halimeda* (dark) and aragonite needle cement; (b) non-skeletal carbonate sediment with aggregate grains, ooids, and peloids. Kuwait, northern Persian Gulf.

chemosynthetic bacteria in their gills and tissue thrive at these seeps. Interestingly, fossil seeps often have abundant brachiopods (dimerelloids, e.g., *Peregrinella*), which do not have modern representatives that harbor chemosynthetic bacteria (Campbell and Bottjer, 1995). Meanwhile, fossil seep carbonates have been reported from rocks as old as Silurian (Little et al., 1997). Apart from their characteristic fauna and texture, and their patchy distribution, methane seep carbonates may be identified rather easily in the fossil record by geochemical means. Carbon isotopes of these carbonates are typically very strongly depleted (Beauchamp and von Bitter, 1992).

Slope

Carbonate slopes may have lower angles as in depositional margins or higher angles in bypass margins. In general, carbonate and siliciclastic slopes have similar heights, however, carbonate slopes are usually steeper (Schlager, 2005). James and Ginsburg (1979) have compiled slope morphologies of several western Atlantic carbonate margins, which usually exhibit a threefold division (Figure 25). There is a low-angle (20–30°) fore reef slope, a steep slope to vertical wall between ca. 50–150 m and a 50° slope below the wall, which is dominated by carbonate debris and blocks transported down from shallower depths. Foliateous corals, *Halimeda*, crinoids, and sponges may be found colonizing the walls. In general, the slope is characterized by sediment redeposition as seen, e.g., in the occurrence of talus blocks, debris-flows, and slump deposits, as well as breccias (James and Ginsburg, 1979; Schlager, 2005). Limestone turbidites (Meischner, 1964) originating from platform tops occur on lower slopes and in the adjacent

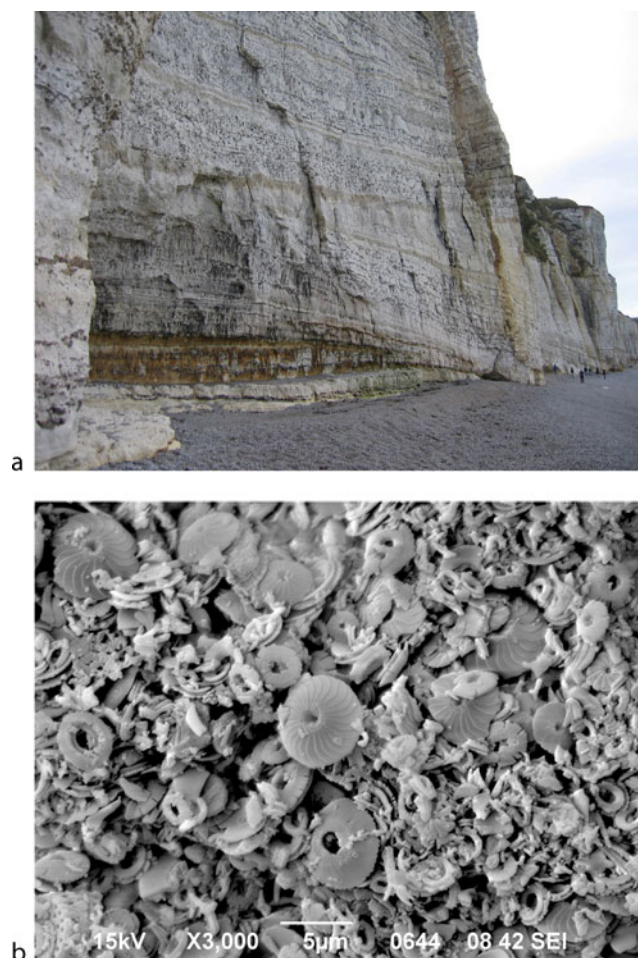


Carbonate Environments, Figure 25 (a) Schemes of fore reef slopes. (Modified from James and Ginsburg, 1979.) (b) Sedimentary processes on slopes. (From Enos and Moore, 1983; with permission of American Association of Petroleum Geologists.)

toe-of-slope among pelitic deposits. Limestone turbidites have similar characteristics as siliciclastic turbidites, with sharp bases, graded beds of debris, and lenticular geometries of individual turbidite bodies. Highstand shedding describes the fact that limestone turbidites in the Quaternary are more common during sea-level highstands when platforms are flooded and abundant carbonate produced as opposed to lowstands of sea level when platforms are subaerially exposed (Droxler and Schlager, 1985). Recent investigations in fossil slope deposits have revealed the formation of so-called auto-micrites as a result of microbial activity on carbonate slopes (Keim and Schlager, 2001). Automicrite facies is apparently a significant agent for platform margin and slope stabilization in these Triassic examples.

Basin

Basinal carbonate environments are in depths between about 200 and 5,000 m (Hsü and Jenkyns, 1974; Crevello and Harris, 1985). The basins of the Atlantic, Indian Ocean, and large parts of southern Pacific Ocean are covered by very fine-grained carbonate sediments, which are termed ooze. Consolidated ooze is called chalk. Carbonate deposition and accumulation is controlled by carbonate supply from the pelagic realm and largely includes tests of planktonic foraminifera, pteropod gastropods, and coccoliths (Figure 26). These particles are transported to the sea floor as “marine snow,” which is largely composed of fecal pellet aggregates. Fecal pellets derive from arthropods such as krill and copepods, and sinking velocities of pellets are much faster as compared to those of individual tests. Other minor contributors of calcium carbonate to the deep sea include shells of ostracods, shells of pseudoplanktonic organisms such as arthropods and bivalve mollusks, and cephalopod shells from *Nautilus*, *Sepia*, and *Spirula*. Carbonate accumulation is also dependent upon the position of the aragonite and calcite compensation depths (ACD, CCD), respectively. The ACD lies at about 2,000–2,500 m and the CCD at about 5,000–5,500 m depth. Below the CCD all calcium carbonate is dissolved. Biota in basinal environments include benthic foraminifera, echinoderms, sponges, and crustaceans. Ichnospecies are *Planolites*, *Chondrites*, and *Zoophycos*. *Thalassinoides*-type bioturbation, often filled by chert, is typical in Cretaceous deep-water limestone. Except for fine lamination, sedimentary structures are usually rare. In Quaternary deposits, carbonate cycles were discovered, which are caused by changing carbonate saturation states during glacial and interglacials. During glacial, lower sea-level positions result in higher carbonate saturation in the pelagic realm, because the neritic carbonate factory is largely shut off. During high sea levels, carbonate production is more or less equally distributed between the neritic and pelagic realm, leading to lower carbonate content in basinal deposits. The genesis of deep-water carbonates during the Paleozoic is somewhat enigmatic, because calcareous plankton only evolved in



Carbonate Environments, Figure 26 (a) Outcrop of Cretaceous chalk in Etretat, northern France. Dark spots are silicified *Thalassinoides* burrows. Erosional surfaces probably caused by currents and/or slumping/sliding. (b) SEM photograph of Pleistocene chalk with abundant coccoliths, Angola Basin, Atlantic.

the Mesozoic. Potential Paleozoic contributors include cephalopods, ostracods, tentaculites, calcispheres, and possibly very fine-grained detritus from the neritic realm. Cephalopod limestone is a typical Paleozoic deposit of shallow basins or deeper submarine rises with no modern counterpart (Wendt and Aigner, 1985).

Summary

Carbonate depositional environments were described in an idealized cross section from terrestrial to transitional, shallow marine, and to deep marine realms. Environments discussed include springs, lakes, subaerial exposure horizons, beaches and shores, tidal flats, restricted, open-rimmed, isolated shelves and platforms, ramps, tropical and cold deep-water reefs and mounds, slopes, and deep sea basins. The great majority of carbonates forms in the

marine realm, and, carbonate formation is largely a product of biological activity. Biological control is either direct, e.g., during growth of shells and skeletons of invertebrates such as corals, mollusks, brachiopods, echinoderms, and sponges, tests of foraminifera or carbonate particles produced by calcareous algae or coccolithophorids. Indirect biological control relates to metabolic processes, e.g., photosynthesis of microbes, which triggers carbonate precipitation. Abiotic formation of carbonates is the exception and includes the formation of some carbonate muds, cements, certain dolomites, and non-skeletal grains such as grapestones, lumps, or ooids. Elevated temperature and salinity, high concentrations of calcium, magnesium, and carbonate ions, low pressure, and CO₂-removal are major environmental parameters that potentially trigger abiotic carbonate formation.

Bibliography

- Abegg, F. E., Harris, P. M., and Loope, D. B. (eds.), 2001. Modern and ancient carbonate eolianites. Society for Sedimentary Geology, SEPM special publications, **71**, 207.
- Aigner, T., 1982. Calcareous tempestites: storm-dominated stratification, upper muschelkalk limestones (Middle Triassic, SW Germany). In Einsele, G., and Seilacher, A. (eds.), *Cyclic and Event Stratification*. Berlin: Springer, pp. 180–198.
- Anadon, P., Cabrera, L., and Kelts, K. (eds.), 1991. Lacustrine facies analysis. International Association Sedimentologists, special publication, **13**, 318 p.
- Bathurst, R. G. C., 1971. Carbonate sediments and their diagenesis. *Developments in Sedimentology*, **12**, 658.
- Beauchamp, B., and von Bitter, P. (eds.), 1992. Chemosynthesis: geological processes and products. *Palaos*, **7**(4), 337–484.
- Bosence, D. W. J., 1995. Anatomy of a recent biotrital mud-mound, Florida Bay, USA. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-mounds: Their Origin and Evolution*. International Association Sedimentologists. Special publication, **23**, pp. 475–493.
- Burchette, T. P., and Wright, V. P., 1992. Carbonate ramp depositional systems. *Sedimentary Geology*, **79**, 3–57.
- Campbell, K. A., and Bottjer, D. J., 1995. Brachiopods and chemosymbiotic bivalves in Phanerozoic hydrothermal vent and cold seep environments. *Geology*, **23**, 321–324.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Petrology*, **54**, 289–316.
- Crevello, P. D., and Harris, P. M., 1985. Deep water carbonates: build-ups, turbidites, debris flows and chalks. Society of Sedimentary Geology (SEPM) Core Workshop, **6**, 527 p.
- Daly, R. A., 1910. The glacial-control theory of coral reefs. *American Journal of Science*, **30**, 297–308.
- Darwin, C. R., 1842. *The Structure and Distribution of Coral Reefs*. London: Smith Elder, 214 p.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P., 1986. Giant subtidal stromatolites forming in normal marine seawater. *Nature*, **324**, 55–58.
- Droxler, A. W., and Schlager, W., 1985. Glacial versus interglacial sedimentation rates and turbidite frequency in the Bahamas. *Geology*, **13**, 799–802.
- Enos, P., and Moore, C. H., 1983. Fore-reef slope. In Scholle, P. A., Bebout, D. G., and Moore, C. H. (eds.), *Carbonate Depositional Environments*. American Association Petroleum Geologists Memoir, Vol. **33**, pp. 507–538.
- Fagerstrom, J. A., 1987. *The Evolution of Reef Communities*. New York: Wiley, 600 p.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks*. Berlin: Springer, 976 p.
- Ford, T. D., and Pedley, H. M., 1996. A review of tufa and travertine deposits of the world. *Earth Science Reviews*, **41**, 117–175.
- Freiwald, A., and Roberts, J. M. (eds.), 2005. *Cold-water Corals and Ecosystems*. Berlin: Springer, 1243 p.
- Ginsburg, R. N., 1956. Environmental relationships of grain size and constituent particles in some South Florida carbonate sediments. *Bulletin American Association Petroleum Geologists Bulletin*, **40**, 2384–2427.
- Ginsburg, R. N. (eds.), 1975. *Tidal Deposits: A Casebook of Recent Examples and Fossil Counterparts*. New York: Springer, 428 p.
- Ginsburg, R. N. (ed.), 2001. Subsurface geology of a prograding carbonate platform margin, great Bahama bank: results of the Bahamas drilling project. Society for Sedimentary Geology, SEPM special publications, **70**, 271 p.
- Gischler, E., 2007. Beachrock and intertidal precipitates. In Nash, D. J., and McLaren, S. J. (eds.), *Geochemical Sediments and Landscapes*. Oxford: Blackwell, pp. 365–390.
- Gischler, E., 2010. Sedimentary facies of Bora Bora, Darwin's type barrier reef (Society Islands, south Pacific): the "oolite problem" reconsidered. *Journal of Sedimentary Research*, under review.
- Gischler, E., and Zingeler, D., 2002. The origin of carbonate mud in isolated carbonate platforms of Belize, Central America. *International Journal of Earth Sciences*, **91**, 1054–1070.
- Grammer, G. M., Ginsburg, R. N., Swart, P. K., McNeill, D. F., Jull, A. J. T., and Prezbindowski, D. R., 1993. Rapid growth of syndepositional marine aragonite cements in steep marginal slope deposits, Bahamas and Belize. *Journal of Sedimentary Petrology*, **63**, 983–989.
- Hakanson, L., and Jansson, M., 1983. *Principles of Lake Sedimentation*. Berlin: Springer, 316 p.
- Harris, P. M., 1979. *Facies anatomy and diagenesis of a Bahamian ooid shoal: Sedimenta*. Miami: University of Miami, Vol. **7**, 163 p.
- Hoskins, C. W., 1964. Molluscan biofacies in calcareous sediments, Gulf of Batabano, Cuba. *American Association Petroleum Geologists Bulletin*, **48**, 1680–1704.
- Hsü, K. J., and Jenkyns, H. C. (eds.), 1974. Pelagic sediments: on land and under the sea. International Association Sedimentologists, special publication, **1**, 447 p.
- Inden, R. F., and Moore, C. H., 1983. Beach environment. In Scholle, P. A., Bebout, D. G., and Moore, C. H. (eds.), *Carbonate Depositional Environments*. American Association Petroleum Geologists Memoir, Vol. **33**, pp. 211–265.
- International Consortium of Great Barrier Reef Drilling, 2001. New constraints on the origin of the Australian Great Barrier Reef: results from an international project of deep coring. *Geology*, **29**, 483–486.
- James, N. P., and Choquette, P. W. (eds.), 1988. *Paleokarst*. New York: Springer, 416 p.
- James, N. P., and Clarke, J. A. D. (eds.), 1997. Cool-water carbonates. Society for Sedimentary Geology, SEPM special publications, **56**, 440 p.
- James, N. P., and Ginsburg, R. N., 1979. The seaward margin of Belize barrier and atoll reefs. International Association Sedimentologists, special publication, **3**, 191 p.
- Keim, L., and Schlager, W., 2001. Quantitative compositional analysis of a Triassic carbonate platform (Southern Alps, Italy). *Sedimentary Geology*, **139**, 261–283.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kendall, C. G. S. G., and Skipwith, P. A. E., 1969. Geomorphology of a recent shallow-water carbonate province: Khor al Bazam,

- Trucial Coast, southwest Persian Gulf. *Geological Society of America Bulletin*, **80**, 865–891.
- Kiessling, W., Flügel, E., and Golonka, J. (eds.), 2002. Phanerozoic reef patterns. Society for Sedimentary Geology, SEPM special publications, **72**, 775 p.
- Knoll, A. H., Fairchild, I. J., and Swett, K., 1993. Calcified microbes in Neoproterozoic carbonates: implications for our understanding of the Proterozoic/Cambrian transition. *Palaaios*, **8**, 512–525.
- Krause, F. F., Scotese, C. R., Nieto, C., Sayhegh, S. G., Hopkins, J. C., and Meyer, R. O., 2004. Paleozoic stromatolites and zebra carbonate mud-mounds: global abundance and paleogeographic distribution. *Geology*, **32**, 181–184.
- Lees, A., and Buller, A. T., 1972. Modern temperate water and warm water shelf carbonate sediments contrasted. *Marine Geology*, **13**, 1767–1773.
- Little, C. T. S., Herrington, R. J., Maslennikov, V. V., Morris, N. J., and Zaykov, V. V., 1997. Silurian hydrothermal-vent community from the southern Urals, Russia. *Nature*, **385**, 146–148.
- Logan, B. W., Read, J. F., Hagan, G. M., Hoffman, P., Brown, R. G., Woods, P. J., and Gebelein, C. D., 1974. Evolution and diagenesis of quaternary carbonate sequences, Shark Bay, Western Australia. *American Association Petroleum Geologists Memoir*, **22**, 358.
- Matter, A., and Tucker, M. E. (eds.), 1978. Modern and ancient lake sediments. International Association Sedimentologists, special publication, **2**, 290 p.
- Maxwell, W. G. H., 1968. *Atlas of the Great Barrier Reef*. Amsterdam: Elsevier, 258 p.
- Meischner, D., 1964. Alldapische Kalke, Turbidite in Riff-nahen Sedimentations becken. In Bouma, A., and Brower, A. (eds.), *Turbidites, Developments in Sedimentology*, Vol. **3**, pp. 156–191.
- Milliman, J. D., 1974. *Marine Carbonates*. Berlin: Springer, 375 p.
- Milliman, J. D., and Droxler, A. W., 1996. Neritic and pelagic carbonate sedimentation in the marine environment: ignorance is not bliss. *Geologische Rundschau*, **85**, 496–504.
- Montaggioni, L. F., and Camoin, G. F., 1993. Stromatolites associated with coralgal communities in Holocene high-energy reefs. *Geology*, **21**, 149–152.
- Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), 1995. Carbonate mud-mounds: their origin and evolution. International Association Sedimentologists, special publication, **23**, 537 p.
- Mounji, D., Bourque, P. A., and Savard, M. M., 1998. Hydrothermal origin of Devonian conical mounds (kess-kess) of Hamar Lakhdad Ridge, Anti-Atlas, Morocco. *Geology*, **26**, 1123–1126.
- Multer, H. G., Gischler, E., Lundberg, J., Simmons, K., and Shinn, E. A., 2002. The key largo limestone revisited: pleistocene shelf edge facies, Florida keys, USA. *Facies*, **46**, 229–272.
- Neumann, A. C., Kofoed, J. W., and Keller, G., 1977. Lithohermes in the straits of Florida. *Geology*, **5**, 4–10.
- Purdy, E. G., 1963. Recent calcium carbonate facies of the Great Bahama Bank. 1. Petrography and reaction groups; 2. sedimentary facies. *Journal of Geology*, **71**, 334–355, 472–497.
- Purdy, E. G., 1968. Carbonate diagenesis: an environmental survey. *Geologica Romana*, **7**, 183–228.
- Purdy, E. G., 1974. Reef configurations: cause and effect. In Laporte, L. F. (ed.), *Reefs in Time and Space*, Society of Sedimentary Geology, SEPM special publication, **18**, pp. 9–76.
- Purdy, E. G., and Gischler, E., 2003. The Belize margin revisited: 1. Holocene marine facies. *International Journal of Earth Sciences*, **92**, 532–551.
- Purdy, E. G., Gischler, E., and Lomando, A. J., 2003. The Belize margin revisited: 2. Origin of Holocene antecedent topography. *International Journal of Earth Sciences*, **92**, 552–572.
- Rankey, E. C., and Reeder, S. L., 2009. Holocene ooids of Aitutaki Atoll, Cook Islands, South Pacific. *Geology*, **37**, 971–974.
- Read, J. F. 1985. Carbonate platform models. *American Association Petroleum Geologists Bulletin*, **69**, 1–21.
- Reid, R. P., and Macintyre, I. G., 1998. Carbonate recrystallization in shallow marine environments: a widespread diagenetic process forming micritized grains. *Journal of Sedimentary Research*, **68**, 928–946.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia): formation and concepts. *Facies*, **29**, 3–29.
- Roberts, J. M., Wheeler, A. J., and Freiwald, A., 2006. Reefs of the deep: the biology and geology of cold-water coral ecosystems. *Science*, **312**, 543–547.
- Sandberg, P. A., 1983. An oscillating trend in Phanerozoic non-skeletal carbonate mineralogy. *Nature*, **305**, 19–22.
- Schlager, W., 2005. Carbonate sedimentology and sequence stratigraphy. Concepts in Sedimentology and Paleontology. Society for Sedimentary Geology (SEPM), **8**, 200.
- Schneidermann, N., and Harris, P. M. (eds.), 1985. Carbonate cements. Society for Sedimentary Geology, SEPM special publications, **36**, 379.
- Scholle, P. A., Bebout, D. G., and Moore, C. H. (eds.), 1983. Carbonate depositional environments. *American Association Petroleum Geologists Memoir*, **33**, 708.
- Scoffin, T. P., 1987. *An Introduction to Carbonate Sediments and Rocks*. Blackie: Glasgow, p. 274.
- Scoffin, T. P., 1992. *Taphonomy of coral reefs: a review*. *Coral Reefs*. Berlin, Heidelberg: Springer, Vol. **11**, pp. 57–77.
- Shinn, E. A., Lloyd, R. M., and Ginsburg, R. N., 1969. Anatomy of a modern carbonate tidal flat, Andros Island, Bahamas. *Journal of Sedimentary Petrology*, **39**, 1202–1228.
- Shinn, E. A., Hudson, J. H., Robbin, D. M., and Lidz, B., 1981. Spurs and grooves revisited: construction versus erosion, Looe Key Reef, Florida. In *Proceedings 4th International Coral Reef Symposium*, Vol. **1**, pp. 475–483.
- Stanley, G. D. Jr. (ed.), 2001. The history and sedimentology of ancient reef systems. *Topics in Geobiology*, **17**, 458.
- Stanley, S. M., and Hardie, L. A., 1998. Secular oscillations in the carbonate mineralogy of reef-building and sediment-producing organisms driven by tectonically forced shifts in seawater chemistry. *Palaeogeography Palaeoclimatology Palaeoecology*, **144**, 3–19.
- Stockman, K. W., Ginsburg, R. N., and Shinn, E. A., 1967. The production of lime mud by algae in South Florida. *Journal of Sedimentary Petrology*, **37**, 633–648.
- Tayama, R., 1952. *Coral Reefs in the South Seas*. Tokyo: Bulletin Hydrographical Office Maritime Safety Agency, Vol. **11**, 292 p. (3 volumes).
- Teichert, C., 1958. Cold- and deep-water coral banks. *American Association Petroleum Geologists Bulletin*, **42**, 1064–1082.
- Traverse, A., and Ginsburg, R. N., 1966. Palynology of the surface sediments of Great Bahama Bank, as related to water movement and sedimentation. *Marine Geology*, **4**, 417–459.
- Tucker, M. E., and Wright, V. P., 1990. *Carbonate Sedimentology*. Oxford: Blackwell, 482 p.
- Wendt, J., and Aigner, T., 1985. Facies patterns and depositional environments of Paleozoic cephalopod limestones. *Sedimentary Geology*, **44**, 263–400.
- Wilson, J. L., 1975. *Carbonate Facies in Geologic History*. New York: Springer, 471 p.
- Wood, R., 1999. *Reef Evolution*. Oxford: Oxford University Press, 414 p.
- Wright, V. P., and Burchette, T. P. (eds.), 1998. Carbonate ramps. Geological Society, special publication, **149**, 465 p.
- Wright, V. P., and Tucker, M. E. (eds.), 1991. Calcretes. International Association Sedimentologists Reprint Series, **2**, 352 p.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Bioerosion
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Carbonates
 Cold Seeps
 Cyanobacteria
 Dolomite, Microbial
 Foraminifera
 Hydrothermal Environments, Marine
 Karst Ecosystems
 Microbial Biomineralization
 Microbial Degradation
 Microbial Ecology of Submarine Caves
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Reefs
 Saline Lakes
 Sediment Diagenesis – Biologically Controlled
 Sinter
 Soda Ocean Hypothesis
 Sponges (Porifera) and Sponge Microbes
 Stromatactis
 Stromatolites
 Symbiosis
 Thrombolites
 Tidal Flats
 Tufa, Freshwater
 Waulsortian Mud Mounds

CARBONATES

Martin Dietzel
 Graz University of Technology, Graz, Austria

Synonyms

Carbonate minerals

Definition

Carbonates. The term carbonates comprises solid phases such as minerals and amorphous substances, which basically consist of cations and triangular carbonate groups.

Introduction

The most common carbonate minerals in natural surroundings are calcite (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$). These minerals comprise about 90 wt.% of carbonates within the Earth's crust and 10–15 wt.% of sedimentary rocks. The proportion of dolomite to calcite increases with decreasing geological age, representing the potential for transformation of calcium carbonate to dolomite at elevated temperature and high molar Mg^{2+} to Ca^{2+} ratio during geological history. Additional carbonate

minerals are numerous, but occur in rather special natural surroundings.

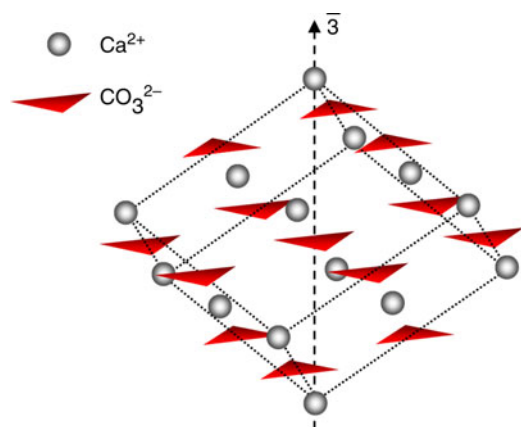
The occurrence and formation of carbonate minerals is mostly related to the precipitation from aqueous solutions and to a lesser extent to the crystallization from carbonate melts. In aquatic systems carbonate formation may be inorganically or biogenically induced (e.g., Morse and Mackenzie, 1990; Dove et al., 2003; Morse et al., 2007). Inorganic precipitation occurs from supersaturated solutions to form evaporites in marine and terrestrial environments, ooids in shallow water, saline lake deposits, speleothems in caves, sinter from spring water, hydrothermal ores, etc. Biogenic precipitation is mostly related to carbonates as skeletal parts and in tissues of marine and non-marine organisms such as foraminifera, coccoliths, corals, bivalves, and prokaryotes. The main mass proportion of carbonates is built from biogenic activity, whereas less arises from inorganic origin.

Once carbonate particles are formed, sedimentation may yield shelf deposits of carbonate sand or mud, deep sea ooze, or mud in lakes. Compaction, cementation, and diagenetic reactions result in lithification to limestone or calcareous marl and sandstone. Under elevated temperatures and pressures, the primary limestone may be transformed to, for example, dolomite rocks or marble.

Crystal structure and chemical composition

The basic units of carbonate minerals are cations and the triangular carbonate groups (CO_3^{2-}). The crystal structure of carbonate minerals is strongly related to the ionic radius of the cation versus the oxygen ion (e.g., Reeder, 1990). As the Ca:O ionic radius ratio in CaCO_3 is close to the upper limit for 6-coordination, CaCO_3 can occur in 6- and 9-coordination of Ca to O yielding calcite and aragonite, respectively. Accordingly, anhydrous carbonate minerals of smaller and larger cations compared to Ca^{2+} crystallize isostructurally to calcite and aragonite, respectively: (1) In the *calcite type* (trigonal carbonates), the carbonate group lies in planes at right angles to the threefold *c*-axis and the metal ions in alternate planes with a sixfold coordination of metal ions to oxygen ions (Figure 1). Metal ions of anhydrous carbonates of the *calcite type* are, e.g., Fe^{2+} , Mg^{2+} , Mn^{2+} , Cd^{2+} , and Zn^{2+} (small cations; see minerals in Table 1). (2) In the *aragonite type* (orthorhombic carbonates), a ninefold coordination of the cation to oxygen ions exists. Respective metal ions of anhydrous carbonates are, e.g., Ba^{2+} , Sr^{2+} , and Pb^{2+} as well as rare earth elements such as Sm, Eu, and Yb (large cations; Table 1).

Beside calcite and aragonite, the third CaCO_3 polymorph vaterite (hexagonal; see Figure 2) and amorphous calcium carbonate phases (ACC) can occur. Hydrus CaCO_3 minerals comprise amorphous $\text{CaCO}_3 \cdot \text{H}_2\text{O}$, monohydrocalciumcarbonate ($\text{CaCO}_3 \cdot \text{H}_2\text{O}$), and ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$). In analogy to calcium, a set of hydrus magnesium carbonates appears: Nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$), landsfordite ($\text{MgCO}_3 \cdot 5\text{H}_2\text{O}$), and hydromagnesite ($\text{Mg}_5(\text{OH})_2(\text{CO}_3)_4 \cdot 4\text{H}_2\text{O}$).



Carbonates, Figure 1 Schematic crystal lattice of calcite.

Carbonates, Table 1 Densities and cation radii, and solubility products ($pK_S = -\log(K_S)$ at 25°C and 1 atm) of common carbonate minerals

Mineral	Formula	Density (g cm ⁻³)	Cation radius (pm)	pK_S
<i>Trigonal</i>				
Calcite	CaCO ₃	2.71	100	8.48
Siderite	FeCO ₃	3.80	78	11.03
Magnesite	MgCO ₃	2.96	72	8.10
Rhodocrosite	MnCO ₃	3.13	83	10.50
Otavite	CdCO ₃	4.26	95	12.10
Smithonite	ZnCO ₃	4.40	74	10.00
Dolomite	CaMg(CO ₃) ₂	2.87	-	17.09
Monohydrocalcite	CaCO ₃ ·H ₂ O	2.43	100	7.15
<i>Orthorhombic</i>				
Aragonite	CaCO ₃	2.93	118	8.34
Witherite	BaCO ₃	4.43	147	8.56
Strontianite	SrCO ₃	3.70	131	9.27
Cerussite	PbCO ₃	6.60	135	13.13
<i>Hexagonal</i>				
Vaterite	CaCO ₃	2.54	100	7.91
<i>Monoclinic</i>				
Ikaite	CaCO ₃ ·6H ₂ O	1.77	100	6.62
<i>Amorphous</i>				
Amorphous calcium carbonate (ACC)	CaCO ₃	-	100	6.40

Ordered double carbonate minerals are dolomite, ankerite (CaFe(CO₃)₂), kutnahorite (CaMn(CO₃)₂), huntite (Mg₃Ca(CO₃)₄), and shortite (Na₂Ca₂(CO₃)₃). In the structure of dolomite, distinct Mg²⁺ and Ca²⁺ layers alternate with CO₃²⁻ layers, whereas in the disordered protodolomite (MgCa(CO₃)₂) Mg²⁺ and Ca²⁺ are randomly distributed in the cation layer, as in Mg calcite. Carbonate minerals may contain hydroxide ions, like the copper carbonates malachite (Cu₂(OH)₂CO₃) and azurite (Cu₃(OH)₂(CO₃)₂), where the respective formation is sensitive to the CO₂ partial pressure. Typical alkali carbonate minerals are natron (Na₂CO₃·10H₂O), nahcolite

(NaHCO₃), trona (Na₃(CO₃)(HCO₃)·2H₂O), and kalicinite (KHCO₃).

Solubility and stability

The solubility of carbonate minerals is expressed by the solubility product, $K_S = (Me^{2+}) \cdot (CO_3^{2-})$ for divalent cations. Parentheses denote the activities of aqueous species at thermodynamic equilibrium. In aqueous environments the stability of minerals to each other is given by the individual values of the solubility product. At standard condition, aragonite and vaterite are more soluble than calcite (lowest K_S -value for calcite in Table 1). Accordingly, calcite is the thermodynamically stable CaCO₃ polymorph. At higher pressure, the more dense phase aragonite is the stable polymorph, although aragonite has a larger cation site due to 9-coordination of Ca to O.

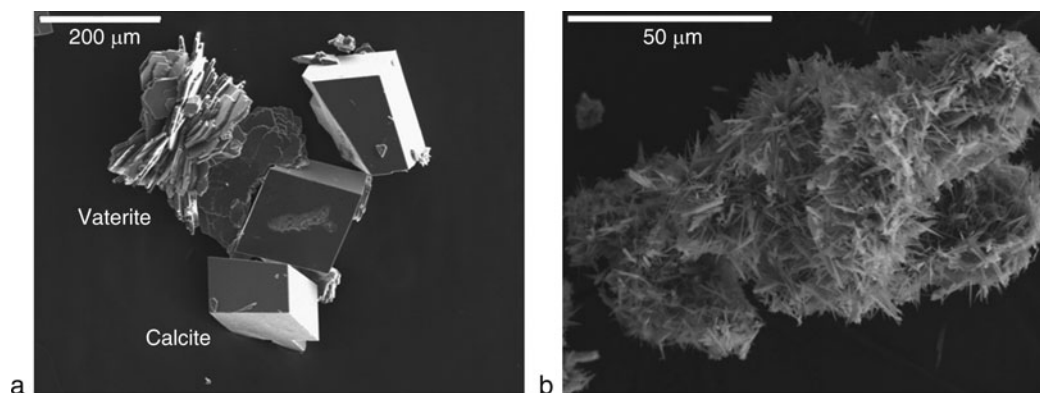
Elevated temperatures cause a decrease of carbonate mineral solubility. Most reliable values of solubility products as a function of temperature are obtained by the empirical equation as compiled by Nordstrom et al. (1990). High pressure favors the dissolution of carbonate minerals as observed in seawater below ≈ 3 –5 km depths, the so-called carbonate compensation depth (CCD, e.g., Morse and Mackenzie, 1990). Deep-sea sediments below the CCD are free of calcite and aragonite.

At elevated pH in alkaline solutions, precipitation of carbonate minerals may occur due to high [CO₃²⁻] to [DIC] ratios ([]: molar concentration, DIC: dissolved inorganic carbon). Components of DIC comprise CO_{2(aq)}, H₂CO₃, HCO₃⁻, and CO₃²⁻, where CO_{2(aq)} is dominant over H₂CO₃ by a factor of 1,000 (see Usdowski, 1982). In acidic solutions the dissolution of carbonates is favored by a high H₂CO₃* content ([H₂CO₃*] = [CO_{2(aq)}] + [H₂CO₃]) and low [CO₃²⁻] to [DIC] ratios.

High ionic solute content generally results in high-dissolution capacities due to a decrease of ion activities versus the concentrations of total dissolved ions. Accordingly, calcite is more soluble in seawater than in freshwater. Seawater is slightly supersaturated with respect to calcite. Supersaturation means that the present ion activity product of the calcium and carbonate ion, $IAP = (Ca^{2+}) \cdot (CO_3^{2-})$, exceeds the K_S value for calcite (saturation degree: $\Omega = IAP/K_S > 1$), and precipitation of calcite may occur. In terrestrial solutions, like drip water in caves and spring water in karst areas, supersaturation with respect to carbonate minerals and sinter formation are mostly related to the degassing of CO₂, which results in an increase of pH.

Precipitation and dissolution

Several carbonate minerals such as calcite, witherite, and strontianite can be easily precipitated from aqueous solutions, whereas others, such as dolomite, are not easily precipitated. In the classical experimental study by Plummer et al. (1978), expressions for the dissolution and precipitation kinetics of calcite are given. Since the Plummer et al. study, various approaches have been used to model the



Carbonates, Figure 2 SEM images of inorganically induced CaCO_3 precipitates with typical shapes. (a) Rhombohedral calcite crystals associated with hexagonal plates of vaterite (CaCl_2 solution without magnesium; 30°C ; pH 9.0). (b) Small aragonite needles (CaCl_2 solution at a molar Mg^{2+} to Ca^{2+} ratio of 2; 20°C ; pH 9.0).

general relationships. Calcite dissolution kinetics is described by empirical rate expressions depending on the reaction rate constant, the reactive surface area, the volume of the solution, and the supersaturation state. More recently, Arakaki and Mucci (1995) interpreted the dissolution of calcite based on the surface complexation theory.

In natural environments, the observed precipitation and dissolution rates (R in $\text{mol m}^{-2} \text{s}^{-1}$) often disagree with the rates estimated from experimental approaches. This may be caused by an incorrect estimation of the reactive mineral surface. Lower rates in natural surroundings may also be due to inhibition by foreign ions or organic matter (e.g., Zaihua et al., 1995).

Gutjahr et al. (1996) showed that in addition to anions like phosphate, the occurrence of divalent ions such as Mg^{2+} , Fe^{2+} , Sr^{2+} , and Ba^{2+} significantly decreases the precipitation rates of calcite. In seawater, the transformation into the more stable phases is hindered by the high Mg^{2+} content. Thus, aragonite and high-Mg calcite are often found in marine sediments, although they are metastable against calcite and low-Mg calcite, respectively (Berner, 1966).

A main aspect of inhibition of carbonate precipitation in natural environments (e.g., biomineralization) and applied systems (e.g., scale formation) is the presence of dissolved organic matter, such as organic acids (e.g., Mann, 2001). For instance, polyaspartic acid can systematically and significantly modify the morphology and growth kinetics of calcite.

Isomorphic substitution

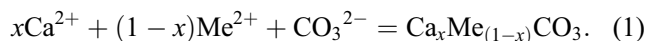
In natural systems, carbonate minerals are generally not pure substances. Impurities can be caused by isomorphic substitution of a host ion by foreign ions in a given crystal lattice. Isomorphic substitution of divalent cations in carbonate minerals depends on the cation radius, which accounts for the crystal structure and the solubility of carbonate minerals. Isomorphic substitution may result

in solid solution formation, as in the irregular but unsymmetrical SrCO_3 – CaCO_3 (aragonite type) solid solution.

Mechanisms of co-precipitation of foreign elements into carbonate minerals are complex and equilibrium thermodynamics may not be appropriate for dealing with many low-temperature reactions. On the other hand, trace element substitution in carbonate minerals can provide information about the environment conditions during formation.

The literature dealing with incorporation of trace elements into carbonate minerals is voluminous, especially with respect to the most abundant calcium and magnesium carbonates. As both the anhydrous magnesium and calcium carbonate crystallize in the *calcite type*, natural skeletal calcite can include up to about 25 mol% MgCO_3 to form so-called Mg calcite, which is isostructural with calcite. Low- and high-Mg calcites are defined by a limit of 4 mol% MgCO_3 with high-Mg calcite being less stable than low-Mg calcite. Only traces of magnesium are incorporated into aragonite as the cation sites are larger due to 9-coordination of Ca to O. Consequently, the larger Sr^{2+} ion is readily incorporated into aragonite, and less Sr^{2+} substitution occurs in calcite.

In the case of a solid CaCO_3 phase, a foreign element is incorporated into the crystal according to the overall expression



The related distribution coefficient is defined by the mole fractions X_{MeCO_3} and X_{CaCO_3} in the solid CaCO_3 and the molar Me^{2+} to Ca^{2+} ratio of the aqueous solution according to $D_{\text{Me}} = (X_{\text{MeCO}_3}/X_{\text{CaCO}_3})/([\text{Me}^{2+}]/[\text{Ca}^{2+}])$. The values of the experimental distribution coefficients for trace elements in carbonate minerals show a systematic behavior, but one that is different from that proposed by a thermodynamic equilibrium approach (e.g., Rimstidt et al., 1998; Lea, 2004; Boettcher and Dietzel, 2010).

The distribution coefficient of Mg^{2+} between calcite and aqueous solution is less than unity ($D_{\text{Mg}} \approx 0.02$ at 25°C). The higher the temperature, the more Mg^{2+} is incorporated into the calcite lattice (e.g., Oomori et al., 1987). In contrast, the distribution coefficients of Sr^{2+} in aragonite is larger than unity, and an increase in temperature results in less incorporation of Sr^{2+} into aragonite (Dietzel et al., 2004). Tesoriero and Pankow (1996) clearly showed that the incorporation of divalent cations into inorganically precipitated calcite is a function of precipitation rate. At low rates, a constant value of the distribution coefficient is reached representing thermodynamic equilibrium. Besides temperature and precipitation rates, the apparent D_{Me} values depend also on the concentrations of additional foreign ions via competitive substitution, the ionic strength of the solution, and the differential partitioning of the foreign cation on nonequivalent crystallographic faces of the carbonate mineral.

Trace element incorporation into carbonate minerals is widely used to estimate conditions of formation. For instance, Mg^{2+} to Ca^{2+} and Sr^{2+} to Ca^{2+} ratios in calcium carbonate minerals of foraminifera, coccoliths, bivalves, or in speleothems are used to estimate paleotemperature and environmental records as well as diagenetic alterations (e.g., Purton et al., 1999; Huang and Fairchild, 2001). The interpretation of datasets for trace element distribution in carbonate minerals is related to saturation states, precipitation rates, temperatures, and metabolisms of the organisms. Accordingly, recent studies of Elderfield et al. (2006) show a carbonate ion saturation state effect on Mg^{2+} incorporation in benthic foraminifera, and Freitas et al. (2006) show that Sr^{2+} to Ca^{2+} ratios in carbonate shell might be controlled by kinetic effects, the latter driven by seasonal variation in shell growth rate that is in turn influenced in part by seawater temperature.

Isotope geochemistry

Stable isotopes of carbon and oxygen of CO_3^{2-} molecules as well as cation isotopes in carbonate minerals are frequently considered in environmental studies to decipher the origin of components, conditions during formation, alteration effects, etc. (e.g., Ripperdan, 2001). For instance, stable oxygen isotopic fractionation between calcite and water has been widely used as a proxy for temperature and environmental changes in paleoceanography, speleology, and applied mineralogy (e.g., Dietzel, 2000; Spötl and Matthey, 2006). The basic concept is referred to the temperature sensitive oxygen isotope fractionation factor between calcite and water, $\alpha_{\text{calcite-water}} = (\delta^{18}\text{O}_{\text{calcite}} + 1,000) / (\delta^{18}\text{O}_{\text{water}} + 1,000)$. For example, Kim and O'Neil (1997) and more recently Coplen (2007) adopted from inorganic precipitation experiments and field studies the $\alpha_{\text{calcite-water}}$ values for equilibrium fractionation. From laboratory experiments and field studies it is widely accepted that the use of $\delta^{18}\text{O}$ as a temperature proxy is complicated by nonequilibrium fractionation (e.g., Kim and O'Neil,

1997; Mickler et al., 2004; Dietzel et al., 2009). In natural biogenic carbonates, the effect of growth rate on $\delta^{18}\text{O}$ values of carbonates is noticeable, with a rapid growth rate resulting in a lower $\delta^{18}\text{O}$ value (e.g., Adkins et al., 2003). Numerous temperature functions are used to estimate $\alpha_{\text{MeCO}_3\text{-water}}$ values for the respective cation content, crystal structure, and organism (e.g., Lea, 2004).

In the case of nontraditional stable isotopes, Mg and Ca isotopic distributions between aqueous cations and carbonate minerals are promising proxies to reconstruct environmental conditions during carbonate formation and to provide insight into geochemical cycles (e.g., Young and Galy, 2004; Tang et al., 2008, respectively). For example, ^{44}Ca is discriminated versus ^{40}Ca during calcium carbonate crystallization. Various models exist for the calcium isotope fractionation during precipitation of calcium carbonate from aqueous solution (e.g., Fantle and DePaolo, 2007). Although, effects of temperature, precipitation rate, crystal structure, and metabolism are identified as main factors for calcium isotope fractionation, future research is required to decipher individual fraction mechanisms.

Biom mineralization

Biom mineralization refers to the process by which living organisms form minerals. The most abundant carbonate biom minerals with respect to quantities and widespread distribution are calcium carbonates such as calcite and Mg calcite, and to a lesser extent aragonite, monohydrocalcite, and ACC (e.g., Weiner and Dove, 2003). Biom mineral phases often have shape, size, crystallinity, isotopic and trace signatures quite unlike their isotopic inorganically formed counterparts as a result of the interaction between organic macromolecules and an amorphous-crystalline phase transformation during early stages of biom mineralization. Sorting out the physiological effects from environmental signals provides a challenge for further scientific research.

Although, ACC is thermodynamically metastable it can be introduced as an important precursor of biom mineralization. Biom mineralization mechanisms and strategies for the biomimetic preparation of functional materials can be followed by transformation of ACC into crystallized calcium carbonates regulated by additives, e.g., functional polymer, proteins, and inorganic ions (Xu et al., 2008).

Han and Aizenberg (2008) showed that the ACC phase can be doped with foreign ions (e.g., Mg^{2+}) and organic molecules (e.g., dyes) and that these compounds later function as growth modifiers of calcite crystals. Such foreign components are incorporated into the crystals during the transformation process of ACC to calcite, which may indicate a vital effect on biogenically induced calcite. Lam et al. (2007) synthesized ACC in the presence of Mg^{2+} and polyaspartic acid. While the first Mg rich ACC precipitates showed short-range structures most similar to aragonite, the initial polyaspartic ACC precipitates possessed short-range structures resembling vaterite. The

influence of these additives on the crystallization of calcium carbonate is apparent even in the precipitation of the first amorphous precursor phase.

Summary

Carbonate minerals have a high diversity and are widely distributed throughout the Earth's crust. The most abundant carbonates contain calcium and magnesium such as calcite, dolomite, Mg calcite, and aragonite. For biogenically formed carbonates, precursor phases such as ACC are of special interest. In aqueous systems the stability of carbonates is related to the solubility product and the kinetics of dissolution and precipitation. Incorporation of foreign cations and isotopes into carbonates is mostly controlled by the composition of the solution, temperature, and precipitation rate. Thus, the chemical and isotopic composition of carbonates can be used as promising proxies to decipher environmental conditions during mineral formation and alteration. Lab experiments will be essential to provide insight about environmental changes throughout the geologic history from field data.

Bibliography

- Adkins, J. F., Boyle, E. A., Curry, W. B., and Lutinger, A., 2003. Stable isotopes in deep-sea corals and a new mechanism for "vital effects". *Geochimica et Cosmochimica Acta*, **67**(6), 1129–1143.
- Arakaki, T., and Mucci, A., 1995. A continuous and mechanistic representation of calcite reaction-controlled kinetics in dilute solutions at 25°C and 1atm total pressure. *Aquatic Geochemistry*, **1**, 105–130.
- Berner, R. A., 1966. Chemical diagenesis of some modern carbonate sediments. *American Journal of Science*, **264**, 1–36.
- Boettcher, M. E., and Dietzel, M., 2010. Metal-ion partitioning during low-temperature precipitation and dissolution of anhydrous carbonates and sulfates. In Prieto, M. (ed.), *Ion partitioning in low temperature aqueous systems: From fundamentals to applications in climate proxies and environmental geochemistry*. EMU Notes in Mineralogy Vol. 10 (in press).
- Coplen, T. B., 2007. Calibration of the calcite-water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochimica et Cosmochimica Acta*, **71**(16), 3948–3957.
- Dietzel, M., 2000. $^{13}\text{C}/^{12}\text{C}$ -signatures of calcite-sinter onto concrete. In Rammlmair, D., et al. (eds.), *Applied Mineralogy*. Rotterdam, The Netherlands: Balkema, pp. 747–748.
- Dietzel, M., Gussone, N., and Eisenhauer, A., 2004. Coprecipitation of Sr^{2+} and Ba^{2+} with aragonite by membrane diffusion of CO_2 between 10 and 50°C. *Chemical Geology*, **203**(1–2), 139–151.
- Dietzel, M., Tang, J., Leis, A., and Köhler, S., 2009. Stable isotopic fractionation during inorganic calcite precipitation – Effects of temperature, precipitation rate and pH. *Chemical Geology*, **268**, 107–115.
- Dove, P. M., De Yoreo, J. J., and Weiner, S., 2003. Biomineralization. *Reviews in Mineralogy and Geochemistry*, **54**, 381 p.
- Elderfield, H., Yu, J., Anand, P., Kiefer, T., and Nyland, B., 2006. Calibrations for benthic foraminiferal Mg/Ca paleothermometry and the carbonate ion hypothesis. *Earth and Planetary Science Letters*, **250**(3–4), 633–649.
- Fantle, M. S., and DePaolo, D. J., 2007. Ca isotopes in carbonate sediment and pore fluid from ODP site 807A: The $\text{Ca}^{2+}_{(\text{aq})}$ -calcite equilibrium fractionation factor and calcite recrystallization rates in Pleistocene sediments. *Geochimica et Cosmochimica Acta*, **71**(10), 2524–2546.
- Freitas, P. S., Clarke, L. J., Kennedy, H., Richardson, C. A., and Abrantes, F., 2006. Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. *Geochimica et Cosmochimica Acta*, **70**(20), 5119–5133.
- Gutjahr, A., Dabringhaus, H., and Lacmann, R., 1996. Studies of the growth and dissolution kinetics of the CaCO_3 polymorphs calcite and aragonite II. The influence of divalent cation additives on the growth and dissolution rates. *Journal of Crystal Growth*, **158**(3), 310–315.
- Han, T. Y. J., and Aizenberg, J., 2008. Calcium carbonate storage in amorphous form and its template-induced crystallization. *Chemistry of Materials*, **20**(3), 1064–1068.
- Huang, Y., and Fairchild, I. J., 2001. Partitioning of Sr^{2+} and Mg^{2+} into calcite under karst-analogue experimental conditions. *Geochimica et Cosmochimica Acta*, **65**(1), 47–62.
- Kim, S. T., and O'Neil, J. R., 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochimica et Cosmochimica Acta*, **61**(16), 3461–3474.
- Lam, R. S. K., Charnock, J. M., Lennie, A., and Meldrum, F. C., 2007. Synthesis-dependant structural variations in amorphous calcium carbonate. *Crystengcomm*, **9**(12), 1226–1236.
- Lea, D. W., 2004. Elemental and isotopic proxies of the past ocean temperature. In Elderfield, H. (ed.), *The Oceans and Marine Geochemistry*, Vol. 6. In Holland H. D., and Turekian K. K. (eds.), *Treatise on Geochemistry*, Oxford: Elsevier, pp. 365–390.
- Mann, S., 2001. *Biomineralization*. Oxford: Oxford University Press, p. 198.
- Mickler, P. J., Banner, J. L., Stern, L., Asmerom, Y., Edwards, R. L., and Ito, E., 2004. Stable isotope variations in modern tropical speleothems: evaluating equilibrium vs. kinetic isotope effects. *Geochimica et Cosmochimica Acta*, **68**(21), 4381–4393.
- Morse, J. W., Arvidson, R. S., and Lüttge, A., 2007. Calcium carbonate formation and dissolution. *Chemical Reviews*, **107**, 342–381.
- Morse, J. W., and Mackenzie, F. T., 1990. Geochemistry of sedimentary carbonates. *Developments in Sedimentology*, **48**, 707.
- Nordstrom, D. K., Plummer, N., Langmuir, D. L., Busenberg, E., and May, 1990. Revised chemical-equilibrium data for major water-mineral reactions and their limitations. In Melchior, D. C., and Bassett, R. L. (eds.), *Chemical modeling of aqueous systems II: revised chemical equilibrium*. *American Chemical Society Symposium Series*, **416**, 398–413.
- Oomori, T., Kaneshima, H., Maezato, Y., and Kitano, Y., 1987. Distribution coefficient of Mg^{2+} ions between calcite and solution at 10–50°C. *Marine Chemistry*, **20**(4), 327–336.
- Plummer, L. N., Wigley, T. M. L., and Parkhurst, D. L., 1978. Kinetics of calcite dissolution in CO_2 -water systems at 5°C to 60°C and 0.0 to 1.0atm CO_2 . *American Journal of Science*, **278**(2), 179–216.
- Purton, L. M. A., Shields, G. A., Brasier, M. D., and Grime, G. W., 1999. Metabolism controls Sr/Ca ratios in fossil aragonitic mollusks. *Geology*, **27**(12), 1083–1086.
- Reeder, R. J., 1990. Carbonates: mineralogy and chemistry. *Reviews in Mineralogy*, **11**, 399 p.
- Rimstidt, J. D., Balog, A., and Webb, J., 1998. Distribution of trace elements between carbonate minerals and aqueous solutions. *Geochimica et Cosmochimica Acta*, **62**(11), 1851–1863.
- Ripperdan, R. L., 2001. Stratigraphic variation in marine carbonate carbon isotope ratios. In Valley, J. W., and Cole, D. R. (eds.),

- Stable Isotope Geochemistry*, Chap. 13. Reviews in Mineralogy and Geochemistry, 43, pp. 637–662.
- Spötl, C., and Matthey, D., 2006. Stable isotope microsampling of speleothems for palaeoenvironmental studies: a comparison of microdrill, micromill and laser ablation techniques. *Chemical Geology*, **235**(1–2), 48–58.
- Tang, J., Dietzel, M., Köhler, S. J., Böhm, F., and Eisenhauer, A., (2008) $\text{Sr}^{2+}/\text{Ca}^{2+}$ and $^{44}\text{Ca}/^{40}\text{Ca}$ fractionation during inorganic calcite formation: II. Ca isotopes. *Geochimica et Cosmochimica Acta*, **72**, 3733–3745.
- Tesoriero, A. J., and Pankow, J. F., 1996. Solid solution partitioning of Sr^{2+} , Ba^{2+} , and Cd^{2+} to calcite. *Geochimica et Cosmochimica Acta*, **60**(6), 1053–1063.
- Uzdowski, E., 1982. Reactions and equilibria in the systems $\text{CO}_2\text{-H}_2\text{O}$ and $\text{CaCO}_3\text{-CO}_2\text{-H}_2\text{O}$ ($0^\circ\text{--}50^\circ\text{C}$). *Neues Jahrbuch für Mineralogie, Abhandlungen*, **144**(2), 148–171.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy and Geochemistry*, 54, pp. 1–29.
- Xu, X. R., Cai, A. H., Liu, R., Pan, H. H., and Tang, R. K., 2008. Amorphous calcium carbonate in biomineralization. *Record of Chemical Progress*, **20**(1), 54–59.
- Young, E. D., and Galy, A., 2004. The Isotope geochemistry and cosmochemistry of magnesium. In Johnson, C. M., Beard, B. L., Albarede, F. (eds.), *Geochemistry of Non-Traditional Stable Isotopes*, Chap. 6. Reviews in Mineralogy and Geochemistry, 55, pp. 197–230.
- Zaihua, L., Svensson, U., Dreybrodt, W., Daoxian, Y., and Buhmann, D., 1995. Hydrodynamic control of inorganic calcite precipitation in Huanglong Ravine, China: field measurements and theoretical prediction of deposition rates. *Geochimica et Cosmochimica Acta*, **59**(15), 3087–3097.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Animal Biocalcification, Evolution
 Animal Skeletons, Advent
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Cap Carbonates
 Carbon Cycle
 Carbon (Organic, Cycling)
 Carbonate Environments
 Cold Seeps
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Isotopes and Geobiology
 Karst Ecosystems
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Molar-tooth Structure
 Mud Mounds
 Nanocrystals, Microbially Induced
 Organomineralization
 Pedogenic Carbonates
 Raman Microscopy (Confocal)
 Reefs
 Saline Lakes
 Siderite
 Soda Ocean Hypothesis
 Speleothems

CATHODOLUMINESCENCE MICROSCOPY

Walter Vortisch

Montanuniversität Leoben, Leoben, Austria

Definition

Cathodoluminescence (CL) is the emission of visible light (also ultraviolet [UV] and infrared) of a solid material that is irradiated by an electron beam (in vacuum). During irradiation CL often changes. Besides CL, the emission of element-specific X-rays and backscattered, secondary and Auger electrons is caused (e.g., Walker and Burley, 1991). Moreover, the sample is heated by the electron beam, which represents an important cause for the alteration of CL characteristics during electron bombardment. Cathodoluminescence microscopy (CLM) combines CL with optical microscopy (in geosciences, generally polarizing microscopy). CL microscopic studies are mostly carried out on thin sections (uncovered, preferably polished), sometimes on up to one cm-thick samples, the latter preferably with polished surface as well. Depending on the instrument used (see below), samples can be studied uncoated or with a coating to avoid charging by the electron beam.

Two types of CL are known: intrinsic and extrinsic (for details see discussion by Remond et al., 2000). Intrinsic CL is purely due to lattice features, generally very weak and only visible if there are no activators present in adequate amounts. Extrinsic CL, the generally observable case, is caused by activators, which occur as trace elements. The activators may be supported by sensitizers that transmit energy to the activators. Quenchers are elements that reduce or even inhibit CL. The significance of sensitizers and quenchers is still in discussion. An important example is iron, which is generally considered as a quencher in carbonate minerals, and lead, which seems to be a sensitizer (e.g., Machel, 2000). In contrast to the view of iron as a quencher, Cazenave et al. (2003) showed in a recent study of doped calcite that Mn-activated CL in calcite can be uninfluenced by iron in a relatively wide range of iron concentrations. The question whether Pb and Zn can be sensitizers in calcite was discussed by Budd et al. (2000), who could not find any evidence for sensitizing by these trace elements.

In geosciences, CLM is a powerful tool for detailed analyses of rocks and minerals, including biogenic mineral substances (for a summary of CL data and earlier literature, see for instance Marshall, 1988, Remond et al., 1992 and Pagel et al., 2000). Intensity and wavelengths (colors) of emitted light may vary strongly for the same mineral phase or even within the same mineral grain, depending on genetically determined features. This is especially significant when a mineral substance is growing or recrystallizing under changing ambient conditions, often causing changes of CL intensity or color, sometimes revealing patterns of complex zoning. In most cases, such effects are not visible by optical microscopy. Therefore, for studies of biogenic mineral substances, CLM can

provide important information. In geosciences, CLM has been used for more than 40 years, resulting in a huge number of publications (for an outline of history, see for instance Pagel et al., 2000). In biology, probably the first CL study was performed by means of an electron microprobe by Cavallier (1975).

Instrumentation

CL studies can be carried out by (1) CLM with cold cathode equipment, (2) CLM with hot cathode equipment, and (3) scanning electron microscopy (SEM) and electron probe micro-analysis (EPMA), if the latter are equipped with CL detector or with an instrumentation for light optical observation/recording. For CLM, CL images can be recorded by conventional camera technique using film, video technique, or digital camera. However, today, digital camera and video techniques have generally replaced film techniques. For detailed and reproducible recording of CL colors, which is important for the determination of activators, optical spectrometers are applied (e.g., Gillhaus et al., 2001). Moreover, CLM can be combined with energy-dispersive X-ray spectroscopy (EDS), providing rapid elemental analysis (see below).

An overview for the application of SEM and EPMA for CL studies is given by Remond et al. (2000). These instruments allow the combination of CL with electron images and elemental analysis by means of EDS or wavelength-dispersive X-ray spectroscopy. Due to the influence of the chemistry of analyzed materials on backscattered electrons (BSE), corresponding electron images are especially interesting in combination with CL. Moreover, SEM offers the advantage of high spatial resolution, including spectrometric recording of CL colors. Monochromatic CL mapping by SEM, with the same resolution as for element mapping by means of EDS, was recently described by Edwards et al. (2007).

For cold cathode CLM, a vacuum chamber with fixed electron gun and x - y stage for sample movement is attached to the stage of a microscope. As the electron beam is directed from above, i.e., from the same side as the objective of the microscope, besides thin sections, up to one cm-thick samples can be studied as well. Care has to be taken with cold cathode CLM systems, to avoid too high energy flux, which can damage thin sections and increase the changes of CL intensity and colors during electron bombardment. Due to low vacuum, residual gas is ionized, preventing electron charging of the sample surface. Therefore, coating is not necessary. A disadvantage of cold cathode CLM systems, often mentioned in the literature (e.g., Walker and Burley, 1991), is that objects with very low CL intensity are not observable, or at least require too long exposure times for conventional camera film techniques. Witkowski et al. (2000), for instance, published excellent CL images of objects with low CL intensity, showing that former problems can be overcome by using a high-sensitivity digital video camera linked to a computer.

Besides optical spectroscopy, EDS is also possible with cold cathode CLM systems (e.g., Vortisch et al., 2003). Cold cathode CLM systems equipped with EDS can also be used for the analysis of opaque and nonluminescent materials as well as finely ground pressed powders (Vortisch, 2004). Cold cathode CLM systems are less expensive and easy to operate. Instrumental working conditions, vacuum, high tension and beam current are now automatically stabilized. The CL images used in this chapter were obtained by means of a cold cathode CLM system.

Hot cathode CLM systems operate at high vacuum, comparable to SEM. To prevent charging during electron bombardment, coating is recommended. Microscope, vacuum chamber with hot cathode, and x - y stage for sample movement are integrated. In contrast to cold cathode CLM systems, the sample may be less heated. Hot cathode CLM systems are generally regarded as being more suitable for objects with low CL intensity. Therefore, many publications including optical spectroscopy for the analysis of CL-activating trace elements are based on hot cathode CLM (e.g., Gillhaus et al., 2001; for interesting examples concerning geobiology, see e.g., Barbin, 2000; see below).

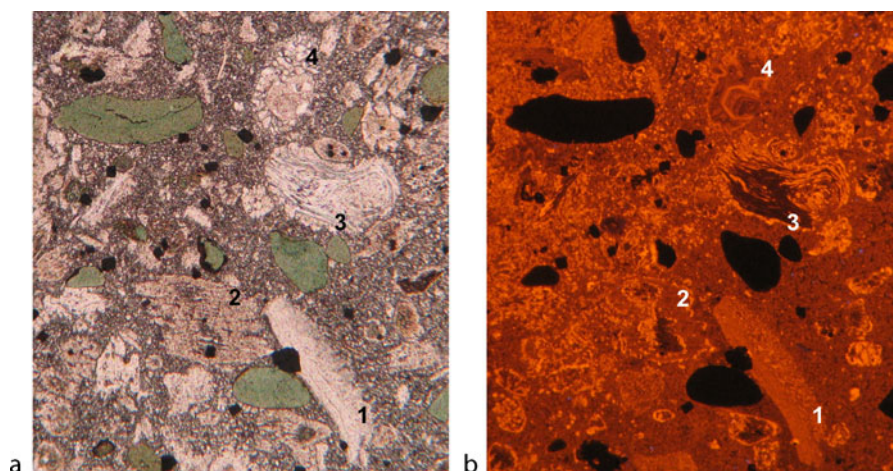
The irradiation of the sample is normally from below, opposite to the objective of the microscope, therefore only transparent objects (i.e., thin sections) can be studied.

CL in geobiology

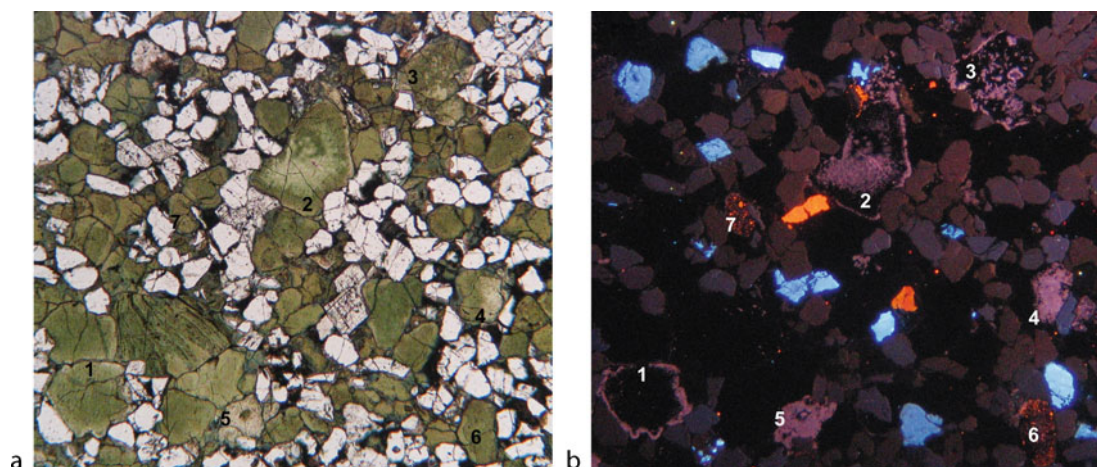
CLM proved to be of special interest in the following areas of geobiology:

- Biogenic minerals in shells, bones, and other mineralized parts of organisms; structures and conditions of growth
- Processes of fossilization and transformation of biogenic mineral matter during diagenesis; mineralization of organic matter
- Microbial mineralization processes

Structures and growth conditions of biogenic minerals often reveal more details when CLM is applied. Barbin et al. (1991a), for instance, were able to distinguish winter growth rings from other parts of the shell of *Pecten maximus* by means of CL, observing an inverse relation between growth velocity and CL intensity. In a recent study of experimental biology, Barbin et al. (2008) were testing the uptake of manganese of the oyster species *Crassostrea gigas* (Mn^{2+} was added as $MnCl_2$ to a tank with seawater where the oysters were living). By means of CLM and microchemical analysis, the authors could prove the daily incorporation of Mn to the growing shell. Mn was incorporated simultaneously to calcitic and aragonitic parts of the shell. CL colors were yellowish for calcite and greenish for aragonite. A comparable differentiation in CL color between calcite and aragonite was observed in the shell of *Halotis* by Hawkes et al. (1996) during



Cathodoluminescence Microscopy, Figure 1 Thin section of Lower Ordovician limestone from Möckleby (southern Öland, Sweden), revealing various stages of diagenetic alteration of carbonate fossils (interpretation Maurits Lindström); optical ((a), plane polarized light) and cathodoluminescence (CL) images (b); height of images: 1.6 mm. 1. Trilobite fragment, completely recrystallized but still clearly separable from surrounding sediment by CL. 2. Recrystallized echinoderm fragment with uniform extinction under crossed polarizers, showing changing chemical conditions during recrystallization. The innermost part with dull CL indicates an (almost) unchanged chemical composition. 3. Brachiopod fragment, consisting of fibrous calcite with sediment matrix entering between the fibers and (later) diagenetically recrystallizing. A larger inner part of this fragment shows very low CL intensity, indicating recrystallization without considerable alteration of the original chemical composition. 4. Series of cement generations filling a small cavity (probably after diagenetically dissolved, siliceous sponge needle), demonstrating the chemically complex early diagenetic history of this limestone layer that was already lithified near the interface sediment/seawater.



Cathodoluminescence Microscopy, Figure 2 Thin section of Upper Cretaceous glauconitic sandstone, showing intergrowth of glauconite (no CL) and calcium phosphate (CaP, pink CL); optical ((a), plane polarized light) and CL images (b); height of images: 2 mm. The intergrowth with CaP (determined by scanning electron microscopy–energy-dispersive X-ray spectroscopy (SEM–EDS)) indicates glauconitization of organic substances, especially fecal pellets (Schinagl and Vortisch, in prep.). 1. Glauconite with an outer rim of CaP. 2, 3. Compared with grain 1, less intensive glauconitization of organic material, indicated by considerable proportions of CaP inside the grains. 4, 5. Grains consisting predominantly of CaP, with minor proportions of glauconite. 6, 7. Glauconite grains with small inclusions of carbonate minerals. *Bright yellow CL*: carbonate (probably dolomite); *bright blue CL*: potassium feldspar; *brownish to violet CL*: quartz.

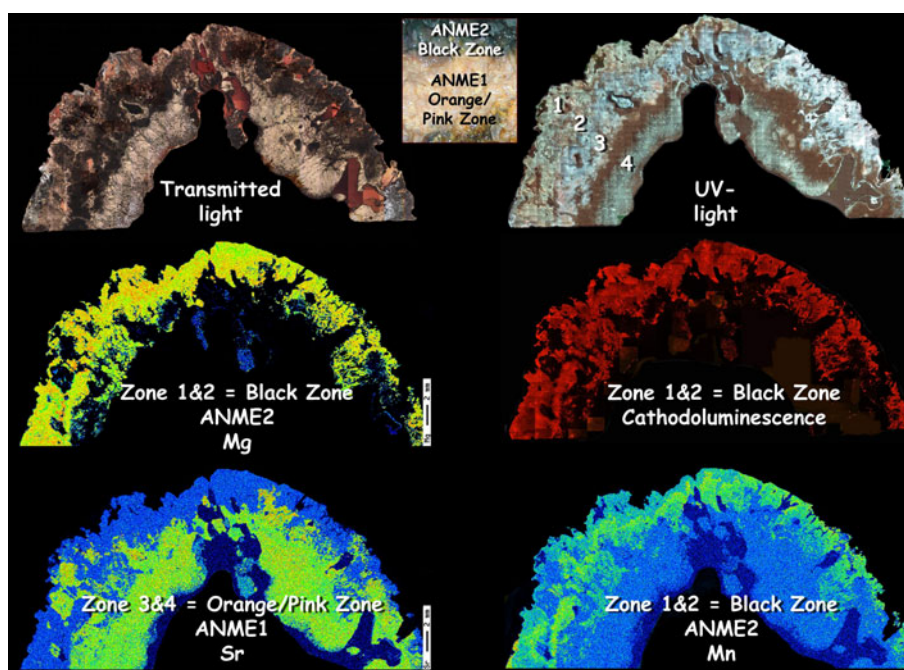
a similar experimental study. CL colors observed by these authors were orange-red for calcite and yellow-green for aragonite.

Biogenic minerals are generally transformed during diagenesis by processes such as recrystallization or replacement. By means of CLM, it is often possible to observe primary structural details even after diagenetic alteration of the biogenic material. Baumgartner-Mora and Baumgartner (1994) have shown good examples for Miocene foraminifera from Costa Rica. When biogenic minerals are investigated with regard to primary stable isotope geochemistry, it is important to know whether the geochemistry of the analyzed material has been changed or not. By means of CLM, Wierzbowski and Joachimski (2007) selected carbonate shells (calcitic and aragonitic), which were nonluminescent or showed only intrinsic bluish CL, for oxygen and carbon isotope analysis, with the argument that diagenetic alteration of carbonate minerals generally results in the enhancement of CL intensity. Barbin et al. (1991b) already recommended the usage of only nonluminescent biogenic carbonate for the investigation of past sea water composition. These authors emphasize that even recent carbonate shells may show

CL of considerable intensity. Figure 1 shows examples for different steps of diagenetic recrystallization of fossils in a Lower Ordovician limestone from Sweden.

The postdepositional mineralization of organic materials may also represent interesting objects for CLM. Götze and Rößler (2000) studied silicified Permian wood. On the base of CL, they concluded that a polyphase process of silicification included an initial hydrothermal phase. Among other analytical procedures, CLM was also applied by Scott and Collinson (2003) for the analysis of Tertiary and Jurassic permineralized woods that contain calcite and Ca phosphate as important mineral phases. Coprolites of Upper Triassic carnivore vertebrates were investigated by Hollocher et al. (2005), who suggest an early onset of mineralization, starting with the formation of apatite. Further minerals are calcite and glauconite. An example for the association of glauconite and apatite, probably due to the mineralization of fecal pellets, is shown in Figure 2.

Reitner et al. (2005, 2006) applied CLM, among other methods, for the study of microbial mineralization processes in methane-derived carbonate buildups in the Black Sea sediments. An example of this work is given in



Cathodoluminescence Microscopy, Figure 3 Thin section of methane-derived carbonates from the Black Sea (Reitner et al., 2005, 2006). Comparison of light, ultraviolet (UV), and CL microscopy with element distribution of Mg, Sr, and Mn. Length of individual image 40 mm. (Light optical image and CL image compiled of about 200 images, UV image of about 600 single images.) The carbonates were precipitated within thick microbial mats, mainly constructed of anaerobic methane-oxidizing microbial communities. The microbial mats are differentiated in an outer black zone, characterized by the so-called ANME 2 (ANAerobic METHanotrophs) microbial community, and an inner orange/pink zone characterized by the so-called ANME 1 microbial community. ANME 2 community precipitates high Mg–calcite (HMGC), which is rich in Mn. The incorporation of Mn within the calcite crystal lattice causes a strong CL of the HMGC. ANME 1 community precipitates a Sr-rich nonluminescent aragonite.

Figure 3. Microbial mats with their methane–sulfate redox systems are the place where strongly luminescent high Mg–calcite is formed as a first carbonate phase. This first carbonate phase interfingers with nonluminescent aragonite, with the latter finally creating the thrombolitic fabric of the towers (Reitner et al., 2005). Another example for the significance of microbial activity at the methane–sulfate interface for early mineralization of marine sediments, also studied by means of CLM, was described by Meister et al. (2007). This work reports on early, near-surface dolomite cementation in Quaternary sediments from the Peru continental margin.

Conclusions

CLM is successfully applied in all areas of geobiology where mineral substances are involved. Important examples are biogenic minerals in shells, bones, and other mineralized parts of organisms; their structures and conditions of growth and processes of fossilization; mineralization of organic matter; microbially influenced processes of mineralization and formation of sedimentary structures. Minerals often studied by CLM are carbonates, phosphates, and silica minerals.

Acknowledgments

I am grateful to late Prof. Dr. Maurits Lindström for the interpretation of the Lower Ordovician sample (Figure 1), to Prof. Dr. Joachim Reitner for providing Figure 3 (including figure caption), to Jay Morgan for important discussions and language corrections in the manuscript, and to Dr. Reinhard Gratzner for his help with graphical work.

Bibliography

- Barbin, V., 2000. Cathodoluminescence of carbonate shells: bio-chemical vs diagenetic processes. In Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D. (eds.), *Cathodoluminescence in Geosciences*. Berlin: Springer, pp. 303–329.
- Barbin, V., Schein, E., Roux, M., Decrouez, D., and Ramseyer, K., 1991a. Stries de croissance révélées par cathodoluminescence dans la coquille de *Pecten maximus* (L.) récent de la rade de Brest (Pectinidae, Bivalvia). *Geobios*, **24**, 65–70.
- Barbin, V., Ramseyer, K., Debenay, J. P., Schein, E., Roux, M., and Decrouez, D., 1991b. Cathodoluminescence of recent biogenic carbonates: an environmental and ontogenetic fingerprint. *Geological Magazine*, **128**, 19–26.
- Barbin, V., Ramseyer, K., and Elfman, M., 2008. Biological record of added manganese to seawater: a new efficient tool to mark in vivo growth lines in the oyster species *Crassostrea gigas*. *International Journal of Earth Sciences (Geologische Rundschau)*, **97**, 193–199.
- Baumgartner-Mora, C., and Baumgartner, P. O., 1994. Shell structure of fossil foraminifera studied by cathodoluminescence. *European Microscopy and Analysis*, **28**, 29–32.
- Budd, D. A., Hammes, U., and Ward, W. B., 2000. Cathodoluminescence in calcite cements: new insights on Pb and Zn sensitizing, Mn activation, and Fe quenching at low trace-element concentrations. *Journal of Sedimentary Research*, **70**, 217–226.
- Cavallier, J. F., 1975. Sur l'utilisation de la cathodoluminescence en biologie. *Journal de Microscopie de Biologie Cellulère*, **22**, 371–380.
- Cazenave, S., Chapoulie, R., and Villeneuve, G., 2003. Cathodoluminescence of synthetic and natural calcite: the effects of manganese and iron on orange emission. *Mineralogy and Petrology*, **78**, 243–253.
- Edwards, P. R., Martin, R. W., and Lee, M. R., 2007. Combined cathodoluminescence hyperspectral imaging and wavelength dispersive X-ray analysis of minerals. *American Mineralogist*, **92**, 235–242.
- Gillhaus, A., Richter, D. K., Meijer, J., Neuser, R. D., and Stephan, A., 2001. Quantitative high resolution cathodoluminescence spectroscopy of diagenetic and hydrothermal dolomites. *Sedimentary Geology*, **140**, 191–199.
- Götze, J., and Rößler, R., 2000. Kathodolumineszenz-Untersuchungen an Kieselhölzern – I. Silifizierungen aus dem Versteinerten Wald von Chemnitz (Perm, Deutschland). *Veröffentlichungen des Museums für Naturkunde in Chemnitz*, **23**, 35–50.
- Hawkes, G. P., Day, R. W., Wallace, M. W., Nugent, K. W., Bettiol, A. A., Jamieson, D. N., and Williams, M. C., 1996. Analyzing the growth and form of mollusc shell layers, in situ, by cathodoluminescence microscopy and Raman spectroscopy. *Journal of Shellfish Research*, **15**, 659–666.
- Hollocher, K. T., Alcobér, O. A., Colombi, C. E., and Hollocher, T. C., 2005. Carnivore coprolites from the Upper Triassic Ischigualasto Formation, Argentina: chemistry, mineralogy, and evidence for rapid initial mineralization. *Palaio*, **20**, 51–63.
- Machel, H. G., 2000. Application of cathodoluminescence to carbonate diagenesis. In Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D. (eds.), *Cathodoluminescence in Geosciences*. Berlin: Springer, pp. 271–301.
- Machel, H. G., Mason, R. A., Mariano, A. N., and Mucci, A., 1991. Causes and measurements of luminescence in calcite and dolomite. In Barker, C. E., and Kopp, O. C. (eds.), *Luminescence Microscopy and Spectroscopy: Qualitative and Quantitative Applications*. Tulsa: Short course 25, SEPM (Society for Sedimentary Research), pp. 9–25.
- Marshall, D. J., 1988. *Cathodoluminescence of Geological Materials*. London: Unwin Hyman.
- Meister, P., McKenzie, J. A., Vasconcelos, C., Bernasconi, S., Frank, M., Gutjahr, M., and Schrag, D. P., 2007. Dolomite formation in the dynamic deep biosphere: results from the Peru Margin. *Sedimentology*, **54**, 1007–1031.
- Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D., 2000. Cathodoluminescence in geosciences: an introduction. In Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D. (eds.), *Cathodoluminescence in Geosciences*. Berlin: Springer, pp. 1–22.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 66–79.
- Reitner, J., Schäfer, N., and Thiel, V., 2006. Biosignatures and architecture of methane-derived carbonates at cold seeps from the Black Sea. *Schriftenreihe der Deutschen Gesellschaft für Geowissenschaften (Lecture in Abstract Book Sediment 2006 Göttingen)*, **45**, p.136.
- Remond, G., Cesbron, F., Chapoulie, R., Ohnenstetter, D., Roques-Carnes, C., and Schvoerer, M., 1992. Cathodoluminescence applied to the microcharacterization of mineral materials: a present status in experimentation and interpretation. *Scanning Microscopy*, **6**, 23–68.
- Remond, G., Phillips, M. R., and Roques-Carnes, C., 2000. Importance of instrumental and experimental factors on the interpretation of cathodoluminescence data from wide band gap materials.

- In Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D. (eds.), *Cathodoluminescence in Geosciences*. Berlin: Springer, pp. 59–126.
- Schinagl, W. and Vortisch, W., (in preparation). Sedimentation and diagenesis of an Upper Cretaceous sandstone from eastern Austria.
- Scott, A. C., and Collinson, M. E., 2003. Non-destructive multiple approaches to interpret the preservation of plant fossils: implications for calcium-rich permineralizations. *Journal of the Geological Society*, **160**, 857–862.
- Vortisch, W., 2004. Application of combined cathodoluminescence and energy-dispersive X ray analysis of mineral raw materials. In Pecchio, M., Andrade, F. R. D., D'Agostino, L. Z., Kahn, H., Sant'Agostino, L. M. Tassinari, M. L. (eds.), *Applied Mineralogy – Developments in Science and Technology*. São Paulo, Brazil: International Council for Applied Mineralogy do Brasil, ICAM-BR, Vol. 1, pp. 169–172.
- Vortisch, W., Harding, D., and Morgan, J., 2003. Petrographic analysis using cathodoluminescence microscopy simultaneous with energy-dispersive X-ray spectroscopy. *Mineralogy and Petrology*, **79**, 193–202.
- Walker, G., and Burley, S. D., 1991. Luminescence petrography and spectroscopic studies of diagenetic minerals. In Barker, C. E., and Kopp, O. C. (eds.), *Luminescence Microscopy and Spectroscopy: Qualitative and Quantitative Applications*. Tulsa: Short course 25, SEPM (Society for Sedimentary Research), pp. 83–96.
- Wierzbowski, H., and Joachimski, M., 2007. Reconstruction of late Bajocian-Bathonian marine palaeoenvironments using carbon and oxygen isotope ratios of calcareous fossils from the Polish Jura Chain (central Poland). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 523–540.
- Witkowski, F. W., Blundell, D. J., Gutteridge, P., Horbury, A. D., Oxtoby, N. H., and Quing, H., 2000. Video cathodoluminescence microscopy of diagenetic cements and its applications. *Marine and Petroleum Geology*, **17**, 1085–1093.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Biofilms and Fossilization](#)
[Biogeochemical Cycles](#)
[Biosignatures in Rocks](#)
[Calcareous Algae](#)
[Calcite Precipitation, Microbially Induced](#)
[Calcium Biogeochemistry](#)
[Cap Carbonates](#)
[Carbonate Environments](#)
[Cold Seeps](#)
[Clay Authigenesis, Bacterial](#)
[Deep Biosphere of Sediments](#)

[Dolomite, Microbial](#)
[Foraminifera](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Silicification – Bacteria \(or Passive\)](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Molar-tooth Structure](#)
[Nanocrystals, Microbially Induced](#)
[Pedogenic Carbonates](#)
[Phosphorus, Phosphorites](#)
[Radiolaria](#)
[Reefs](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Silica Biomineralization, Sponges](#)
[Tufa, Freshwater](#)

CHEMOLITHOTROPHY

Volker Thiel
 University of Göttingen, Göttingen, Germany

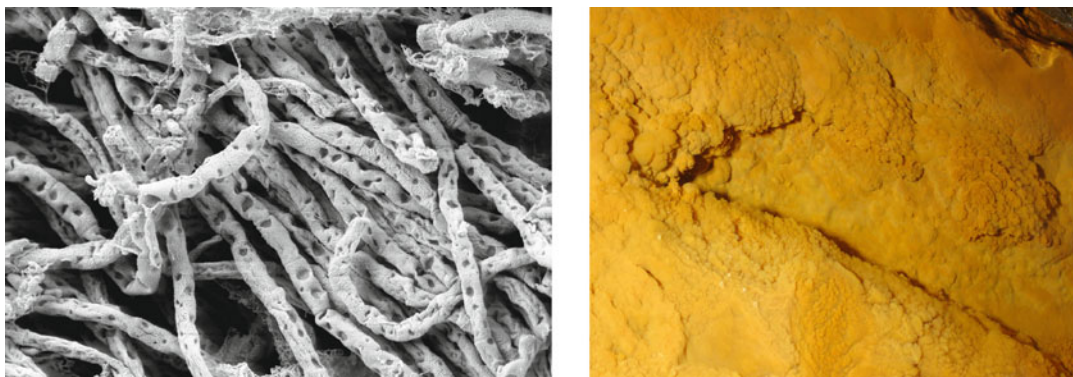
The term “Chemolithotrophy” refers to the gain of energy for cell biosynthesis and maintenance from the oxidation of inorganic compounds (= electron donors), in the absence of light (Kelly and Wood, 2006). The process was first described by the Russian microbiologist Sergej Winogradsky (1887, 1888).

Chemolithotrophy is a strategy unique to some prokaryotes (i.e., Bacteria and Archaea), the so-called chemolithotrophs. Whereas there is no known macrofauna possessing the capability of chemolithotrophy, some animals such as particular tubeworms and bivalves can form symbioses with chemolithotrophs, (e.g., at cold seeps or in hydrothermal environments). There exist many inorganic electron donors that can fuel chemolithotrophs involving geologic, biologic, and anthropogenic sources. Reduced sulfur, nitrogen and iron species and hydrogen are the most common substrates (Table 1). If chemolithotrophs are able to use

Chemolithotrophy, Table 1 Some common reactions performed by chemolithotrophs

Electron donor	Reaction	Organism	$\Delta G_0'$ (kJ/reaction)
Hydrogen	$\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$	Hydrogen bacteria	–237.2
Sulfide	$\text{HS}^- + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{S}^0 + \text{H}_2\text{O}$	Sulfur bacteria	–209.4
Sulfur	$\text{S}^0 + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	Sulfur bacteria	–587.1
Ammonium	$\text{NH}_4^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$	Nitrifying bacteria	–274.7
Nitrite	$\text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^-$	Nitrifying bacteria	–74.1
Iron	$\text{Fe}^{2+} + \text{H}^+ + \frac{1}{4} \text{O}_2 \rightarrow \text{Fe}^{3+} + \frac{1}{2} \text{H}_2\text{O}$	Iron oxidizing bacteria	–32.9

Source: Adapted from Madigan and Martinko (2006)



Chemolithotrophy, Figure 1 *Left:* Filamentous sulfur bacteria forming a bacterial mat in the Tunnel of Äspö, Sweden. These chemoautotrophs oxidize sulfide to elemental sulfur. Holes in the filaments are preparation artifacts that reflect the former location of intracellular sulfur granules. The filaments are approximately 1 μm in diameter (Scanning electron microscope image courtesy of J. Reitner). *Right:* Massive iron-oxidizing microbial mats developing at an underground spring discharging water rich in reduced iron (Fe^{2+}). The chemolithotrophic bacterium *Gallionella ferruginea* is a main component of the microbial community at this site (Tunnel of Äspö, Sweden) and is responsible for the production of large amounts of orange coloured iron oxides and hydroxides. The area displayed is about 1 m wide.

CO_2 as their carbon source (most are) they are referred to as chemolithoautotrophs. Well-known examples of chemolithotrophs relevant in geobiology are sulfur-oxidizing bacteria (e.g., *Beggiatoa*; *Thiomargarita*) and iron-oxidizing bacteria (see entries “*Fe(II)*-Oxidizing Prokaryotes,” “*Gallionella*”) (Figure 1).

Bibliography

- Kelly, D., and Wood, A. P., 2006. The chemolithotrophic prokaryotes. *Prokaryotes*, 2, 441–456.
- Madigan, M., and Martinko, J. (eds.), 2006. *Brock Biology of Microorganisms*, 11th edn. Upper Saddle River, NJ: Prentice Hall.
- Winogradsky, S., 1887. Über Schwefelbakterien. *Botanische Zeitung*, 45, 489–507, 513–523.
- Winogradsky, S., 1888. Über Eisenbakterien. *Botanische Zeitung*, 46, 261–270.

Cross-references

Acetogens
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Bacteria
 Beggiatoa
 Cold Seeps
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Hydrothermal Environments, Marine
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Thiomargarita

CHERTS

Volker Thiel
 University of Göttingen, Göttingen, Germany

Chert is a sedimentary rock mainly consisting of micro- or cryptocrystalline (=submicroscopic) quartz (SiO_2) varieties.

Cherts often have an “indirect” biogenic origin as they result from re-precipitation of dissolved opal-A shells derived from microorganisms such as diatoms (diatomite, diatomaceous cherts, see also entry “*Diatoms*”) and radiolarians (radiolarite, see also entry “*Protozoa (Heterotroph, Eukaryotic)*”). Postsedimentary dissolution of these shells leads to the accumulation of silica-rich fluids within pore waters, thus, inducing precipitation of metastable cristobalite, also known as opal-CT (Konhauser, 2007). Opal-CT may, under increasing temperatures and pressures, be converted into stable quartz. Chert may also originate from microbial silification, and it may form, with or without microbial mediation, in hydrothermal environments (Konhauser et al., 2004; Jones et al., 2004, see also entries “*Sinter*” and “*Hot Springs and Geysers*”). Depending on the content of clay and other minerals, chert may grade into siliceous shales or -mudstones. The color of cherts may vary greatly with the content of accessory elements. Iron (hematite), for instance, gives rise to red varieties called “jasper.” Banded cherts, with often colorful, rhythmic layers, are called “agate.”

Chert may occur as extensive layers (bedded cherts) within sedimentary formations as well as in concretionary

nodules. For example, vast deposits of bedded cherts, alternating with iron minerals, are found within the Precambrian “Banded Iron Formations” (see entry). “Flintstones” on the other hand, are dark-colored chert nodules that precipitated in chalk such as in the Upper Cretaceous Maastrichtian Formation in Middle Europe. Here, the SiO₂ mainly derived from diagenetically dissolved, and re-precipitated, sponge spicules. Deposits with a high content of sponge-derived SiO₂ dispersed in sedimentary rocks are called “spiculites.”

Cherts may provide an excellent matrix for the long-term preservation of macro- and microfossils as well as chemical and isotopic signatures, due to their density and resistance. Examples are the Rhynie Chert that uniquely preserved an early Devonian community of plants, animals, bacteria, and fungi (Taylor et al., 2009), and numerous Precambrian cherts that provide an exciting view on the early dawn of life on Earth (Derenne et al., 2008; Westall et al., 2006, see entry “*Biosignatures in Rocks*”).

Bibliography

- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J.-N., 2008. Molecular evidence for life in the 3.5 billion-year old Warrawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu Geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Taylor, T. N., Taylor, E. L., and Krings, M., 2009. *Paleobotany – The Biology and Evolution of Fossil Plants*. Amsterdam: Elsevier (Academic Press).
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006. The 3.466 Ga Kitty’s Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications, 405, pp. 105–131.

Cross-references

Algae (Eukaryotic)
 Banded Iron Formations
 Biosignatures in Rocks
 Diatoms
 Hot Springs and Geysers
 Hydrothermal Environments, Terrestrial
 Microbial Silicification – Bacteria (or Passive)
 Pore Waters
 Protozoa (Heterotroph, Eukaryotic)
 Sinter
 Silica Biomineralization, Sponges
 Sponges (Porifera) and Sponge Microbes

CHONDRITES

Chondrites are the most common class of stony meteorites. Chondrites largely consist of iron- and magnesium silicate minerals and remain unaffected from melting and differentiation. Therefore, chondrites are considered to reflect the elemental composition of the original solar nebula. *Carbonaceous chondrites* are a rare variety of chondrites containing numerous organic compounds, including amino acids. See “*Meteoritics*” for further reading.

CHROOCOCCIDIOPSIS

Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

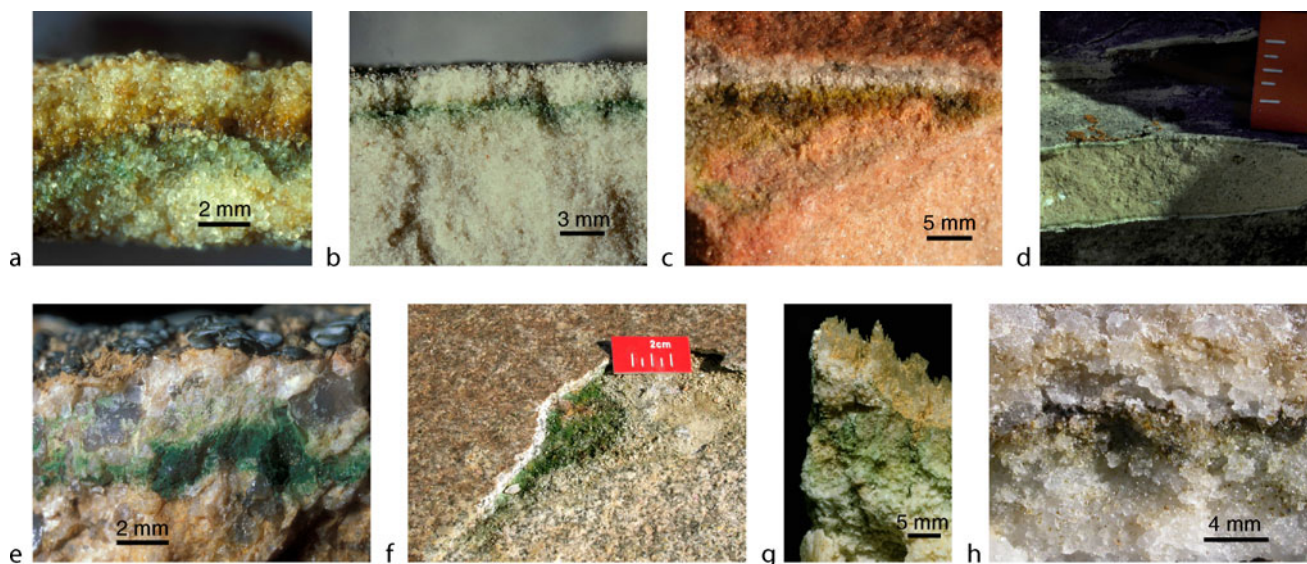
The unicellular cyanobacterial genus *Chroococcidiopsis* was first described by Geitler (1933) from Sumatra, where it was found in warm springs.

Definition

The cyanobacterial genus *Chroococcidiopsis* is defined as having more or less spherical cells surrounded by a thin, firm, colorless, sometimes layered extra-cellular polysaccharide sheath (EPS). The cell has an S-layer of a special ribbon-like type, not found in other cyanobacteria so far (Büdel and Rhiel, 1985). It often occurs in large agglomerations of spherical or irregular shape. Fully grown cells (usually 2–6 µm in diameter) divide in two modes: (1) after one or two binary divisions with planes rectangular to each other, resulting daughter cells continue to divide in different planes without intermediate growth (successive multiple divisions); (2) by successive or almost spontaneous irregular cell division (multiple fission) without intermediate growth. In both types, the final daughter cells are very small (1–3 µm in diameter) and are released by rupture of the sheath envelope.

Presently, the genus *Chroococcidiopsis* comprises 14 different species (Komárek and Anagnostidis, 1998). *Chroococcidiopsis* is closely related to the heterocyst-containing, filamentous cyanobacteria (Nostocales, Stigonematales, see entry “*Cyanobacteria*”) and forms their natural sister group (Fewer et al., 2002). The current species concept needs revision.

In terms of ecology, *Chroococcidiopsis* is most interesting because of the wide variety of habitats it colonizes. Among these are different extreme biotopes such as thermal or mineral springs (see entry “*Hydrothermal Environments, Terrestrial*”), and extremely hot and cold deserts (Antarctica), where it is epilithic or endolithic on rocks (Figure 1a–h; see entry “*Endoliths*”). The genus is a common photobiont of the cyanolichens of the



Chroococcidiopsis, Figure 1 *Chroococcidiopsis* endolithic in rocks. (a) Sandstone of the Clarens formation, Golden Gate Highlands National Park, South Africa. (b) Tshipise Sandstone Plateau, Limpopo Province, South Africa. (c) Beacon Sandstone, Dry Valleys, Antarctica. (d) Cocconino Sandstone, Colorado Plateau, Arizona, USA. (e) Granite inselberg, Limpopo Province, South Africa. (f) Granite rock boulder, Dry Valleys, Antarctica. (g) Gypsum from a sebkah, Tunisia. (h) Calcite rock outcrop, Dry Valleys, Antarctica.

Lichinomycetes group (see entry “*Fungi and Lichens*”), mostly growing on rock surfaces of hot deserts. As an endolith, it contributes to biogenic weathering (see entry “*Bioerosion*”) of silicate rocks by photosynthetic alkalization (Büdel et al., 2004).

Bibliography

- Büdel, B., and Rhiel, E., 1985. A new cell wall structure in a symbiotic and a free-living strain of the blue-green alga genus *Chroococcidiopsis* (Pleurocapsales). *Archiv für Mikrobiologie*, **143**, 117–121
- Büdel, B., Weber, B., Kühl, M., Pfan, H., Sültemeyer, D., and Wessels, D. C. J., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268
- Fewer, D., Friedl, T., and Büdel, B., 2002. *Chroococcidiopsis* and heterocyst-differentiating cyanobacteria are each others’ closest living relatives. *Molecular Phylogenetics and Evolution*, **41**, 498–506
- Geitler, L., 1933. Diagnosen neuer Blaualgen von den Sunda-Inseln. *Archiv für Hydrobiologie*, **4**(Supplement 12 Tropische Binnengewässer), 622–634
- Komárek, J., and Anagnostidis, K., 1998. *Cyanoprokaryota 1. Teil Chroococcales*. Jena: Gustav Fischer.

Cross-references

[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biological Volcanic Rock Weathering](#)
[Cyanobacteria](#)
[Desert Varnish](#)
[Endoliths](#)
[Extreme Environments](#)
[Fungi and Lichens](#)
[Geomycology](#)

CLAY AUTHIGENESIS, BACTERIAL

Kurt O. Konhauser
 University of Alberta, Edmonton, Alberta, Canada

Definition

The formation of clay minerals in situ through the direct or indirect action of bacteria.

Overview

Clays are one of the largest and most diverse assemblage of minerals known. The vast majority of clay minerals are aluminum silicate in composition, with various other cations substituted into the crystal lattice. The replacement of either Si or Al can lead to an excess of negative charge, which is either neutralized by the adsorption of additional cations onto the outer mineral surfaces or within the interlayer spaces.

Within the past 2 decades, field studies have led to the general recognition that bacteria can mediate the formation of clay minerals. Some clays form as replacement products from the alteration of primary minerals. In this regard, the bacterial community growing on the rock surface potentially function initially as the weathering agents (through the production of organic acids or ligands that facilitate mineral dissolution) and later as reactive surfaces for the nucleation of secondary clay phases. For instance, Konhauser et al. (2002) documented that highly altered rocks found within active steam vents at Kilauea Volcano, Hawaii, contained bacteria with small (<500 nm in diameter) grains of smectite $[(\text{Al}_{1.6}\text{Fe}_{0.4})(\text{Si}_{3.8}\text{Al}_{0.2})\text{O}_{10}(\text{OH})_2\cdot\text{K}_{0.2}]$ attached directly to the outer cell walls.

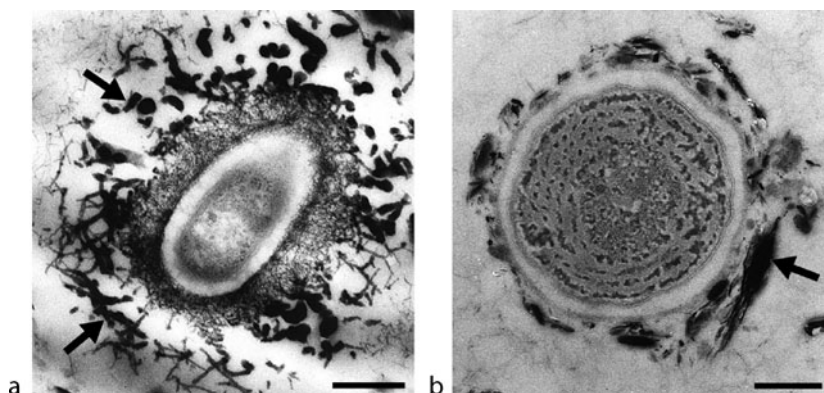
Many of the elements now incorporated into the clays originated when the volcanic glass dissolved and released them into the pore waters. Leveille et al. (2002) similarly documented the formation of kerolite $[\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O}]$ in association with microbial mats colonizing the surfaces of walls and ceilings within basaltic sea caves on the north coast of Kauai, Hawaii. That microorganisms were in some way involved in mineral formation was suggested by the lack of kerolite within basalt vesicles near the rock surface or in the carbonate-rich evaporitic coatings found on other exposed surfaces without the presence of microbial mats.

Other clays form in environments far removed from the actual sites of weathering. The best examples come from biofilms retrieved from the sediment–water interface of lakes and rivers. Ferris et al. (1987) initially described complex (Fe,Al)-silicates on bacterial cells growing in metal-contaminated lake sediment in northern Ontario. These precipitates ranged from poorly ordered and uncharacterized phases to crystalline forms of the Fe-rich chlorite, chamosite $[(\text{Fe})_3(\text{Si}_3\text{Al})\text{O}_{10}(\text{OH})_2]$. Since then, similar clayey precipitates have been reported from various rivers around the world (e.g., Konhauser et al., 1998). What is particularly remarkable about bacterially mediated clays is that they share a number of properties, irrespective of the chemical composition of the waters from which they were sampled: (1) They are generally amorphous to poorly ordered structures. (2) All have grains sized $<1\ \mu\text{m}$, although the majority are $<100\ \text{nm}$. (3) The grains have a composition dominated by iron, silicon, and aluminium in varying amounts.

Based on the observations from natural studies, a general sequence of events leading to clay biomineralization can be adduced (Konhauser and Urrutia, 1999). In the initial stages, a bacterium adsorbs any number of different free aqueous iron species, i.e., Fe^{2+} , Fe^{3+} , $\text{Fe}(\text{OH})^{2+}$, and $\text{Fe}(\text{OH})_2^+$, depending on solution chemistry and redox potential. Adsorption can occur on either the cell wall,

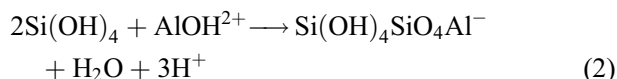
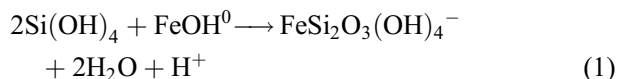
which is frequently lined with anionic carboxyl and phosphate functional groups, or to the more neutrally charged carbohydrate-rich extracellular layers that surround the cell, including sheaths and capsules – the latter are the organic components that make up the bulk of biofilms. If the soluble iron concentration around the cell surface exceeds the solubility product of ferric hydroxide, then the reaction between the bound iron with dissolved iron will lead to the development of small ($\sim 100\ \text{nm}$ in diameter), dense, mineral aggregates on the outer cell surface (Figure 1a). Indeed, it has been shown experimentally that a continuum exists between cationic iron sorption and precipitation reactions at bacterial surfaces; with the three stages of (1) sorption, (2) surface site saturation and (3) precipitation, all evident in Langmuir-type isotherms relating the solid-phase concentration of Fe(III) to the equilibrium proton and soluble Fe(III) concentrations (Warren and Ferris, 1998). Alternatively, bacteria can attract preformed nanometer-sized ferric hydroxide particles from suspension (Glasauer et al., 2001), thereby negating the need for the nucleation step. In most solutions, iron is only found in trace amounts compared to other ions, particularly silica. Under those conditions, the adsorbed/particulate iron may instead serve as kinetically favorable site for the development of more complex precipitates of variable clay composition, morphology, and structure (Figure 1b).

Why these clays form is likely as follows. In the pH range of most natural water bodies, negatively charged counter-ions, or those molecules that are neutrally charged but exhibit residual surface electronegativity (e.g., oligomeric and colloidal silica species), accumulate near the solution-adsorbed iron/solid interface to neutralize the net positive charge of iron. Two surface species of iron oxide exist in this pH range; FeOH_2^+ and $\text{Fe}(\text{OH})^0$, but the majority of the surface charge is positive at circumneutral pH. The initial (Fe,Al)-silicate phases then form via hydrogen bonding between the hydroxyl groups



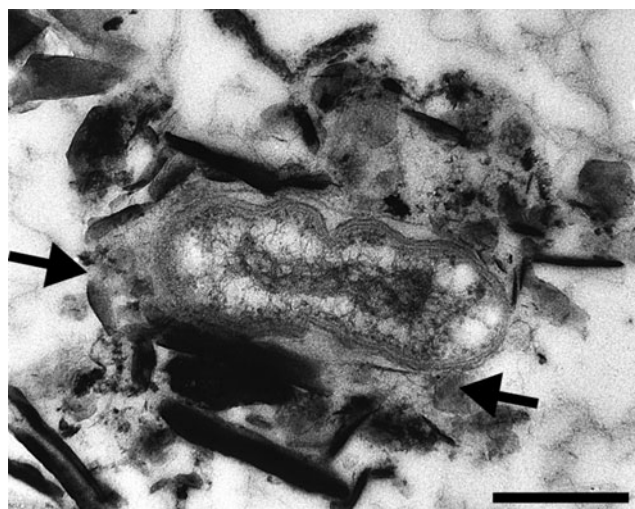
Clay Authigenesis, Bacterial, Figure 1 Transmission electron micrographs (TEM) of bacteria (stained) from the Rio Solimões in Brazil. (a) Fe-mineralized capsule with formation of dense Fe-rich aggregates on the outer edges (arrow). (b) Partially encrusted cyanobacterium with various amorphous clay precipitates (arrow) tangential to the cell wall and surrounding sheath. Scale bars = 500 nm.

associated with the cell-bound iron and the hydroxyl groups in the dissolved silica, aluminum, or aluminosilicate complexes (e.g., Taylor et al., 1997; Davis et al., 2002). Exactly how these reactions occur in nature has not been ascertained, but we do know that dimeric silica (the species that accounts for more than 99% of the oligomeric silica in natural waters) is highly reactive toward iron hydroxide surfaces (Reaction 1), and that oligomeric silica exhibits a strong affinity for dissolved aluminum, forming soluble hydroxy-aluminosilicate ions (e.g., Reaction 2) that subsequently react with cell-bound iron.



This arrangement of ions forms an electric double layer with iron attaching to the bacterial surface as an inner sphere complex, while the silica-aluminosilicate species attach as more diffuse outer layers. The surface charge of these composites is inevitably dependent upon the solution pH, the ionic strength of the solution, and the time of reaction, such that as the particles age more silica (and aluminium) sorb. Interestingly, this mechanism of binding Fe to the bacterial cell surface and subsequent reaction with Si and Al from solution has been shown in experimental systems with *Bacillus subtilis* (e.g., Urrutia and Beveridge, 1994). Accordingly, if the microbial mats are subject to sufficiently concentrated solutions, then continued reaction of the dissolved components to the Fe-bearing solid interface eventually results in the formation of amorphous to poorly ordered clay phases. Often, these reactions lead to the partial and complete encrustation of some bacterial cells as abiological surface reactions accelerate the rate of mineral precipitation: in some biofilms, the density of clayey material surrounding individual cells can extend hundreds of nanometers away from the cell surface (Figure 2). Over time, these hydrous compounds dehydrate, some converting to more stable crystalline forms. This latter material presumably uses some fraction of the precursor surface as a template for its own growth, in effect circumventing the need for direct nucleation (Steeff and van Cappellen, 1990). Once it begins to grow, the more stable clay phase increases its own surface area until it can control the composition of the proximal solution. When this happens, the saturation state of the solution moves below the solubility of the precursor, inhibiting further growth, or even dissolving the precursor.

The reactions above are naturally an oversimplification because a number of inorganic processes are also at play. It is well known that soluble Al–Si complexes precipitate as poorly ordered Al–silicates when a state of supersaturation is achieved (Wada and Wada, 1980). Moreover, Fe ions can readily be incorporated into those structures



Clay Authigenesis, Bacterial, Figure 2 TEM image of highly encrusted bacterium from the Speed River in Ontario, with clay minerals extending over 500 nm away from cell surface. Arrow indicates surrounding capsule in which the clay minerals have formed. Scale bar = 500 nm.

leading to clay-like products (Farmer et al., 1991). All these reactions can conceivably take place in the proximity of bacterial surfaces or within the extensive EPS, particularly since diffusion through the polysaccharide matrix is inherently slow, and a microenvironment can be established that is conducive to mineralization. Additionally, colloidal species of (Fe, Al)-silicate composition that either form initially in the water column or are products of weathering and soil formation, may react directly with the outermost cellular layers. It follows that anything which will neutralize or diminish the charge of the colloids (e.g., bacterial surface if colloids are positively charged or adsorbed iron if the colloids are negatively charged) will cause the particles to flocculate out of solution.

One of the intriguing questions that arises from the observation of bacterial cells completely encrusted in clays is why do they allow for mineralization to occur? It has been established that bacteria have the ability to partially control their surface charge (Doyle, 1989). During metabolism, a membrane-induced proton motive force continuously pumps protons into the wall fabric that effectively compete with metal ions for anionic wall sites (Urrutia et al., 1992). Subsequently, living bacteria may be able to reduce authigenic mineral formation (and detrital mineral adhesion) on their cellular surfaces, unless environmental conditions exceed their capacity to compensate. In most aqueous environments with low dissolved solutes, this condition should not arise. Therefore, the presence of epicellular minerals on bacteria suggests that (1) the cells observed under the electron microscope had lysed prior to mineralization, or (2) that the mineralized matrix is in some way advantageous to the microorganism. Several possibilities for the latter

exist. First, clays provide a good source of exchangeable inorganic nutrients readily available to microbes (Tazaki et al., 1994). Second, clays such as montmorillonite stimulate the respiration of bacteria by maintaining the pH and water content of the environment at a level suitable for growth (Stotzky and Rem, 1966). Third, the formation of an external mineralized matrix may protect the living cells from the detrimental effects of toxin-producing microorganisms (Habte and Barrion, 1984) and predation by grazing protozoans (e.g., Heynen et al., 1988).

Summary

Microorganisms are commonly associated with fine-grained (Fe, Al)-silicates of variable composition. The inorganic phases develop in a predictable manner, beginning with the adsorption of cationic iron to anionic cellular surfaces, supersaturation of the proximal fluid with Fe (III), nucleation and precipitation of a precursor ferric hydroxide phase on the cell surface, followed by reaction with dissolved silica and aluminium, and eventually the growth of an amorphous clay-like phase.

Bibliography

- Davis, C. C., Chen, H. W., and Edwards, M., 2002. Modelling silica sorption to iron hydroxide. *Environmental Science and Technology*, **36**, 582–587.
- Doyle, R. J., 1989. How cell walls of gram-positive bacteria interact with metal ions. In: Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley, pp. 275–293.
- Farmer, V. C., Krishnamurti, G. S. R., and Huang, P. M., 1991. Synthetic allophane and layer-silicate formation in $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-FeO-Fe}_2\text{O}_3\text{-MgO-H}_2\text{O}$ systems at 23°C and 89°C in a calcareous environment. *Clays and Clay Minerals*, **39**, 561–570.
- Ferris, F. G., Fyfe, W. S., and Beveridge, T. J., 1987. Bacteria as nucleation sites for authigenic minerals in a metal-contaminated lake sediment. *Chemical Geology*, **63**, 225–232.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2001. Sorption of Fe Hydr(oxides) to the surface of *Shewanella putrefaciens*: cell-bound fine-grained minerals are not always formed de novo. *Applied and Environmental Microbiology*, **67**, 5544–5550.
- Habte, M., and Barrion, M., 1984. Interaction of *Rhizobium* sp. with toxin producing fungus in culture medium and in a tropical soil. *Applied and Environmental Microbiology*, **47**, 1080–1083.
- Heynen, C. E., van Elsas, J. D., Kuikman, P. J., and van Veen, J. A., 1988. Dynamics of *Rhizobium leguminosarum* biovar *trifolii* introduced in soil; the effect of bentonite clay on predation by protozoa. *Soil Biology and Biochemistry*, **20**, 483–488.
- Konhauser, K. O., and Urrutia, M. M., 1999. Bacterial clay authigenesis: a common biogeochemical process. *Chemical Geology*, **161**, 399–413.
- Konhauser, K. O., Fisher, Q. J., Fyfe, W. S., Longstaffe, F. J., and Powell, M. A., 1998. Authigenic mineralization and detrital clay binding by freshwater biofilms: the Brahmani River, India. *Geomicrobiology Journal*, **15**, 209–222.
- Konhauser, K. O., Schiffman, P., and Fisher, Q. J., 2002. Microbial mediation of authigenic clays during hydrothermal alteration of Basaltic Tephra, Kilauea Volcano. *Geochemistry, Geophysics, and Geosystems*, **3**(12), 1–13.
- Leveille, R. J., Longstaffe, F. J., and Fyfe, W. S., 2002. Kerolite in carbonate-rich speleothems and microbial deposits from Basaltic Sea Caves, Kauai, Hawaii. *Clays and Clay Minerals*, **50**, 514–524.
- Steefel, C. I., and van Cappellen, P., 1990. A new kinetic approach to modeling water–rock interaction: the role of nucleation, precursors, and ostwald ripening. *Geochimica et Cosmochimica Acta*, **54**, 2657–2677.
- Stotzky, G., and Rem, L. T., 1966. Influence of clay minerals on microorganisms. 1. Montmorillonite and kaolinite on bacteria. *Canadian Journal of Microbiology*, **12**, 547–563.
- Taylor, P. D., Jugdaohsingh, R., and Powell, J. J., 1997. Soluble silica and high affinity for aluminium under physiological and natural conditions. *Journal of the American Chemical Society*, **119**, 8852–8856.
- Tazaki, K., Fyfe, W. S., Iizumi, S., Sampei, Y., Watanabe, H., Goto, M., Miyake, Y., and Noda, S., 1994. Clay aerosols and arctic ice algae. *Clays and Clay Minerals*, **42**, 402–408.
- Urrutia, M. M., and Beveridge, T. J., 1994. Formation of fine-grained metal and silicate precipitates on a bacterial surface (*Bacillus subtilis*). *Chemical Geology*, **116**, 261–280.
- Urrutia, M. M., Kemper, M., Doyle, R., and Beveridge, T. J., 1992. The membrane-induced proton motive force influences the metal binding ability of *Bacillus subtilis* cell walls. *Applied and Environmental Microbiology*, **58**, 3837–3844.
- Wada, S. I., and Wada, K., 1980. Formation, composition and structure of hydroxy-aluminosilicate ions. *Journal of Soil Science*, **31**, 457–467.
- Warren, L. A., and Ferris, F. G., 1998. continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Cross-references

[Black Shales](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomineralization](#)
[Microbial Silicification – Bacteria \(or Passive\)](#)
[Microbial Surface Reactivity](#)
[Soils](#)

COCCOLITHOPHORES

Volker Thiel

University of Göttingen, Göttingen, Germany

Coccolithophores (coccolithophorids) are a group of unicellular, marine, planktonic algae belonging to the haptophytes (Prymnesiophyta). The coccolithophores are capable of controlling the intracellular precipitation of calcite onto organic plates and the assembly of the mature carbonate scales at the cell surface. These scales, the coccoliths, are spherical or oval, often intricately patterned and generally less than 20 µm in diameter. Given the abundance of coccoliths in sediments, coccolithophorids have been playing a major role in the global carbon budget. They were very abundant in the Mesozoic era, particularly in the Cretaceous period, in which they became a major component of the “Chalk” lithology (e.g., White Cliffs of Dover, England). Today, coccolithophorids are accounting for about a third of the total marine CaCO_3 production, and coccoliths are a major component of the

modern deep-sea calcareous oozes. The most widespread modern species is *Emiliania huxleyi*. Recent studies showed that calcification and net primary production in *E. huxleyi* have increased by ~40% over the last 220 years due to ocean acidification in response to rising atmospheric CO₂ partial pressures (Iglesias-Rodriguez et al., 2008). Contributions of sedimentary organic matter from coccolithophorids, and other haptophyte algae, can be by tracked by particular lipid biomarkers, the long-chain alkenones. These compounds are often used as a “paleothermometer” for the estimation of past sea surface temperatures (see entry “*Biomarkers (Organic, Compound-Specific Isotopes)*”). For detailed reading and further references please see entry “*Algae (Eukaryotic)*.”

Bibliography

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, V., and Boessenkool, K. P., 2008. Phytoplankton calcification in a high-CO₂ world. *Science*, **320**, 336–340.

COLD SEEPS

Robert G. Jenkins
Yokohama National University, Yokohama City, Japan

Synonyms

Diffusive seep; Ground water seep; Hydrocarbon seep; Methane seep; Oil seep. The terms “seep” and “seepage” can be used as alternates

Definition

Fluid flow containing reduced compounds, e.g., methane or hydrogen sulfide (see entry *Sulfur Cycle*), with almost no temperature anomalies compared with ambient seawater gushing out from or reaching very close proximity to the seafloor.

Introduction

Active water circulation systems can be found at seafloor level and beneath the seafloor along the plate boundaries such as mid-ocean ridges and trenches. It is well known that at ocean ridges, hydrothermal fluid containing reduced compounds and minerals vent out from the seafloor. In contrast to the hydrothermal vents, focused flow containing reduced compounds, e.g., methane and hydrogen sulfide, gushing out from the seafloor can also be found along active and passive continental margins and around diapirs of serpentine and salt rock (halite) with no or less temperature anomalies compared to the ambient seawater. This fluid flow is called a cold seep.

The first cold seep was found at the Florida Escarpment off Oregon in 1984 by the submersible Alvin (Paull et al., 1984). The Alvin found a large number of alleged tube

worms and white clams which were thought to be highly endemic to hydrothermal vents. It turned out that there are fluid flows which contain reduced compounds and which support chemosynthesis-based life, similar to hydrothermal vents (Kulm et al., 1986).

Examples of the reduced compounds contained in cold seeps are methane and hydrogen sulfide. These compounds lead to extensive biogeochemical reaction when the fluid encounters oxic interstitial water which has penetrated into sediments from seawater. The reaction is mainly mediated by microbes as a way of obtaining chemosynthetic energy to synthesize their organic compounds. Primary production based on the chemosynthesis found in cold seeps results in the formation of characteristic life forms similar to those which can also be found in hydrothermal vents.

The important role played by methane in global warming is due to the fact that the greenhouse effect that results from it is more than 20 times that of carbon dioxide (Kvenvolden, 1988). Hence if we think of cold seeps as a pipe connecting the deep subsurface and the oceans, we can understand their importance in terms of global climate change and biogeochemical cycles at the sediment–water interface.

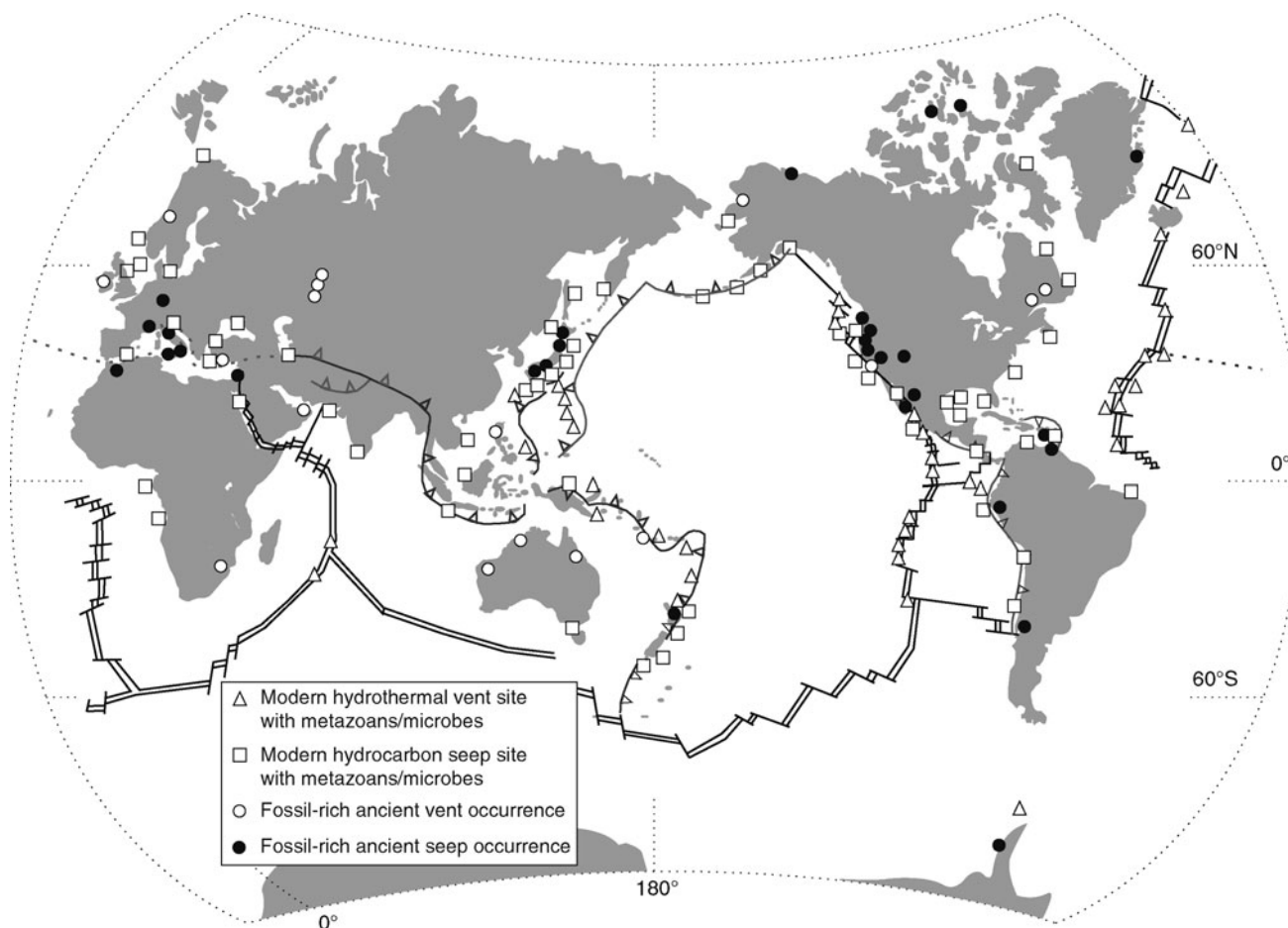
Distribution

Cold seeps are widely distributed along the active and passive continental margins of the world’s oceans (Figure 1; Sibuet and Olu, 1998; Levin, 2005). The depth range of a cold seep varies from shallow water less than 15 m (Montagna and Spies, 1985) to deep water more than 7,400 m in the Japan Trench (Fujikura et al., 1999).

The total number of cold seeps reported to date represent a minimum, because finding a cold seep is significantly more difficult than finding a hydrothermal vent. The difficulties of identification result from the natural characteristics of cold seeps which are difficult to observe by the use of a submersible or remote operated vehicle (ROV).

Natural characteristics of cold seeps

Cold seeps vary in accordance with their natural characteristics. Most cold seeps are very calm, i.e., the low seepage intensity and low fluid flow rate has no effect on the sea floor. The almost total absence of any temperature anomaly in relation to the ambient seawater also makes it difficult to distinguish the flow by visual observation. The occurrence of chemical anomalies is found over too narrow an area and the anomalies usually occur only in the sediment. Thus, this kind of calm cold seep, called a diffusive seep, is rather difficult to find on the basis of geological and geochemical signatures which exist only beneath the sea floor. One good indicator pointing to the existence of a cold seep is the occurrence of chemosynthesis-based life obtaining energy from the reduced compounds contained in the seep fluids (Sibuet and Olu, 1998). Such biota often comprises large bivalves and/or



Cold Seeps, Figure 1 Distribution of modern and ancient cold seeps and hydrothermal vents. (After Campbell, 2006.)

worm tubes which are clearly visible (Figure 2). However, seep-associated infaunal animals (i.e., animals living only in the sediments) and microorganisms are potentially difficult to detect.

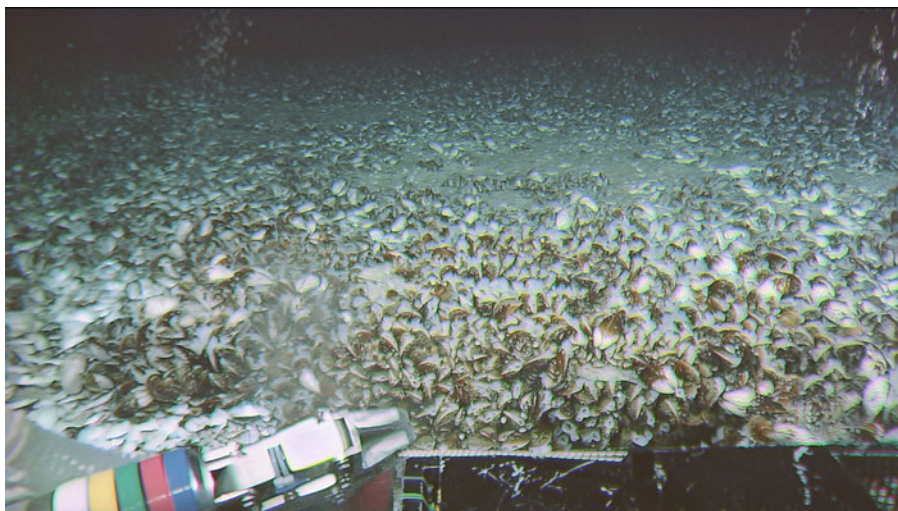
Direct measurements of cold seep fluid chemistry started around the end of the last century (e.g., Tryon and Brown, 2001, 2004). The results show that the fluid pattern and rate of flow are more complex than was previously thought to be the case. Although most cold seeps are calm, there are some effusive and explosive cold seeps. When the saturation level of dissolved methane in sea water is exceeded, the excess methane forms gas bubbles (Figure 3) or gas hydrate (Figure 4) depending on the physical conditions. The bubbly gaseous methane can be found by visual observation (Figure 2) and also by hydroacoustic sounders, like a fisheries sonar (Figure 5; Paull et al., 2007; Aoyama and Matsumoto, 2009). The acoustic sounder makes it possible to see the plume due to the relatively low density of the methane bubbles compared to the ambient sea water. In waters off Niigata, Sea of Japan, methane bubble streams venting out from the sea floor at about 900 m water depth produce a plume

reaching to about 300 m below the sea surface (Figure 5; Aoyama and Matsumoto, 2009).

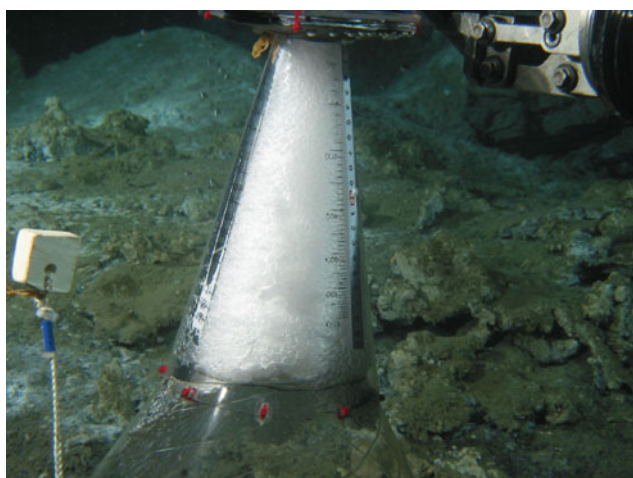
Cold seeps associated with a mud volcano or serpentine diapir show that the flow gushes out together with muddy sediment or brecciated serpentine rocks. It may also happen that methane erupts from the seafloor due to a pressure increase in the sediments caused by an accumulation of methane gas. Such an eruption forms chaotic structures of strata, like landslides, and crater-like topographically depressed areas, so-called pock marks. The size of a pock mark may vary from a few meters to a few hundred meters in diameter and from 1 m to 80 m in depth (Gay et al., 2006).

Pathway and migration of fluid flow

Generally, reduced compounds increase with depth in marine sediments. These reduced compounds can be oxidized by oxidants such as oxygen, nitrate, and sulfate and usually occur at a shallow depth under the sea floor. Although these reactions do also occur elsewhere below the sea floor, substantial reaction occurs in cold seeps



Cold Seeps, Figure 2 Cold seep in the Kuroshima Knoll, Okinawa, southwestern Japan. A dense colony of bathymodiolian bivalves, housing chemosynthetic microbes, covered the sea floor. Gushing out of gaseous bubbly methane can be seen. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).



Cold Seeps, Figure 3 Collecting methane gas bubbles at a cold seep. ROV Hyper-dolphin collected the bubbly methane gas venting out from the cold seep in the waters off Joetsu, Sea of Japan. Putative shell-like gas hydrate covers each gas bubble. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).



Cold Seeps, Figure 4 Outcrop observation of gas hydrate in the waters off Joetsu, Sea of Japan. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).

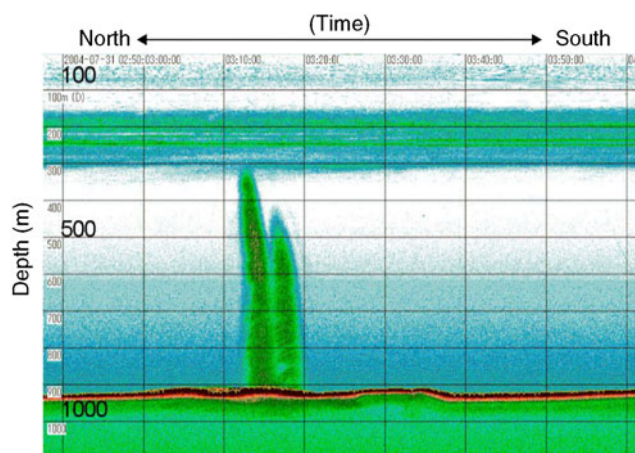
because of the high amounts of reduced compounds brought up to the sea floor from the deep subsurface. Accumulation and migration of reduced compounds are required to form a cold seep.

The pore water containing reduced compounds favors migration through physically weak paths, e.g., faults, and permeable sediments. Tectonic stresses, e.g., plate subduction or diapirs, induce the formation of such pathways. Thus, occurrences of cold seeps are largely dependent on the tectonic background of the area concerned.

Cold seep pathways take the form of a fault or faulted anticline, salt or serpentine diapirs, or a cut bank of strata. These structures can be revealed by seismic profiles, direct observations of the outcrops in the ocean, and drill core samples with chemical profiles of the pore water (Figure 6; Moore et al., 1990, 2001).

Iodine radioisotopes (see entry *Isotopes, Radiogenic*) have recently been identified as a useful tool to determine the age of the strata from which the methane originated, as the iodine will be released at the same time as methane is generated from organic matter, and the diffusion rates of methane and iodine are very similar (Fehn et al., 2003).

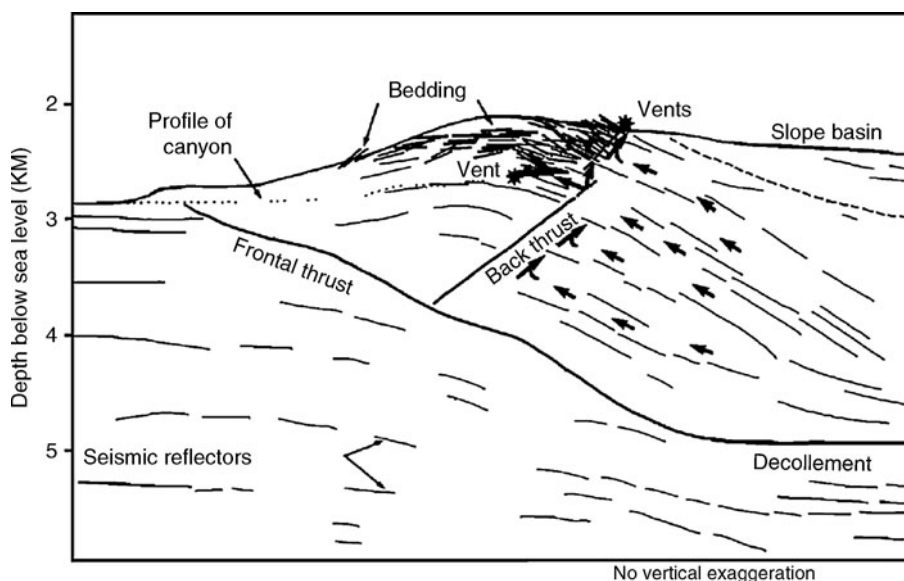
For example, pore water from a cold seep in the Japan Sea passed through and probably originated from strata which sedimented during 30–20 Ma (Tomaru et al., 2007). Faults associated with cold seeps occur particularly on the landward wedge of subduction zones, e.g., the Barbados accretionary wedge, the Cascadia margin and the Japan Trench. Faults created by tectonic stress due to subduction of



Cold Seeps, Figure 5 Methane plumes observed by acoustic sounder at the Umitaka spur, off Joetsu, Sea of Japan. The plumes rose up from the sea floor at depths ranging from ca. 900 m to ca. 300 m. The horizontal axis indicates time. The vehicle obtaining the echogram ran from north to south. (Modified from Aoyama and Matusmoto (2009) (Patent no. US 7539081 B2, Seabed resource exploration system and seabed exploration method by Chiharu Aoyama).

plates play the role of physically weak pathways for the fluid flow. It may also happen that faults cut through a methane reservoir formed under impermeable strata, and the methane then migrates upward through the fault. Indeed, the direction formed by *Calymene* (bivalve) colonies, which mostly live in cold seeps and/or hydrothermal vents, is coincident with a theoretical stress fracture pattern caused by subduction tectonics (Ogawa et al., 1996).

The main driving forces of the migration of pore water containing reduced compounds are density differences and the compression of pore water by increasing pressure within the sediments. The buoyancy of gaseous and dissolved methane and light hydrocarbons in pore water is the main driving force for upward migration. Gaseous methane, the main hydrocarbon gas in the fluid flow, has a strong buoyancy, for example, the density of gaseous methane beneath 3–4 km of water is $200\text{--}300\text{ kg m}^{-3}$ compared with $1,024\text{ kg m}^{-3}$ for seawater (Clennell et al., 2000). Water in which methane is dissolved is also lighter than methane-free water. Thus, the pore water containing gaseous and dissolved methane potentially tends to migrate upward within permeable sediments. Entrapment of the fluid usually occurs where impermeable sediments are found. When strata composed of impermeable sediment are tilted, the fluid migrates within the permeable sediment in a lateral-upward direction directly beneath the impermeable strata. Thus, the hinge zone of an anticline is suitable for the accumulation of methane contained in pore water. Methane-rich fluids may further migrate upwards through faults that cut through the impermeable strata. The fluid also gushes out from outcrops where there is a break in the strata caused by erosion.



Cold Seeps, Figure 6 Interpreted seismic reflection obtained from accretionary prism, off Oregon. Rigid arrows indicate inferred flow paths for fluids. Direct measurements of bedding from the submersible Alvin correlate well with the seismic reflection lines. (After Moore et al., 1990.)

Increasing pressure arising from compaction due to continuous loading of sediments or tectonic stress squeezes out pore water containing reduced compounds into overlying strata. The increasing pressure causes not only pore water migration but also mud diapirs. This is why mud diapirs (or mud volcanoes) are often associated with methane or other reduced compounds.

Heating of the pore water is the other factor causing upward fluid migration. Heating occurs as a result of geothermal activities in the deep subsurface. After heating by geothermal activity in the deep subsurface, the temperature of the fluid drops during upward migration. Thus, the fluid temperature at sea floor level shows no or fewer anomalies in contrast to hydrothermal vents.

Migration of reduced compounds is also caused by diffusion. Although diffusion can be considered as a factor much less important than buoyancy and pressure increase, it is important for the penetration of oxidants from the sea water into sediments.

Chemistry of fluid flow

Major reduced compounds in the fluid flow in cold seeps are methane and other light hydrocarbons, hydrogen sulfide, ammonia, reduced iron, barium, and hydrogen (e.g., Tunnicliffe et al., 2003; Tryon and Brown, 2004). Methane is the most important by virtue of the fact that it is the most common hydrocarbon gas in marine sediments. Methane has a pronounced greenhouse effect and plays an important role both as an energy source and as a carbon source for microbes; furthermore, the anaerobic oxidation of methane (AOM) coupled with sulfate reduction produces hydrogen sulfide which acts as a major energy source for chemosynthesis-based ecosystems.

Elements contained in the fluid flow change with the depth of sediments, because the generation and consumption occur at different depths. Concentrations of reduced compounds generally increase in line with the depth of the sediments. Drastic changes in the chemical profile occur at shallower depths where the reduced compounds encounter oxidants. These reactions take place mainly within sediments, but it sometimes happens that the reduced compounds discharge directly into the sea water column. In such a case, the oxidation reactions take place within the water column.

The drastic chemical changes in cold seeps are generally responsible for substantial biological activities. Methane, the major reduced compound in a cold seep, is generated at a relatively deep subsurface level under anaerobic conditions. When a fluid flow containing methane migrates toward the sea floor and encounters oxidants such as oxygen and sulfate (see entry *Sulfur Cycle*) which penetrated into the sediment from oxic sea water, intensive biogeochemical reactions occur. The most important and extensive of these is AOM which occurs where the methane and sulfate coexist in anaerobic conditions. Following the AOM, oxidation of hydrogen sulfide, formation of pyrite (see entry *Iron Sulfide Formation*), and

precipitation of carbonate minerals occur. When the fluid flow gushes out into well-oxygenated conditions, aerobic oxidation of methane (*Methane Oxidation (Aerobic)*) occurs.

The origin of methane

There are several pathways for the generation of methane of which biogenic and thermogenic processes are the most important. In the case of the former, the methane is produced directly by microbes, and in the case of the latter, the methane is produced through the thermal degradation of organic compounds in the deep subsurface. Methane that is produced neither from organic material nor through organic processes can be found mainly along the edge of hydrothermal vent fields and above serpentine diapirs.

Biogenic methane

Biogenic methane is produced by microbes. The methanogenic microbes (methanogens) belong to the phylum Euryarchaeota within the domain Archaea. Methanogens are obligatory anaerobes, meaning that they live in an anoxic environment which usually occurred below the zone of sulfate reduction. The main substrates linked to the generation of methane are simple carbon-containing molecules such as carbon dioxide, formate, acetate, methanol, and methylamine (Whiticar, 1999). The two simple pathways of methanogenesis, i.e., carbonate reduction and acetate fermentation, are expressed as $\text{CO}_2 + 8\text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ and $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$, respectively. Archaeal methanogenesis promotes large fractionations of carbon isotopes. The stable carbon isotopic composition of methane produced by carbonate reduction generally ranges from -60 to -80‰ (vs. PDB), while the methane produced by the fermentation process ranges from -50 to -60‰ (vs. PDB; Whiticar, 1999). The methane is mostly a unitary hydrocarbon product of the biological process, whereas the thermogenic process explained below produces methane as well as other hydrocarbons such as ethane and propane. However, the recent suggestion that ethane and propane are also produced biologically (Hinrichs et al., 2006) upset the long-held concept of the origin of light hydrocarbons larger than methane, which were thought to be generated by the thermal degradation of organic matter only. The biogenic process is carried out at a relatively shallow depth compared to where thermogenic methane is produced. This is due to the presence of appropriate thermodynamic conditions, i.e., not too high a temperature and pressure, to permit microbial activities.

Thermogenic methane

The methane generated through the degradation of sedimentary organic matter as a result of thermodynamic conditions is called thermogenic methane (Kotelnikova, 2002). The rising temperature and pressure of sediments that accompany the increasing depth accelerates the

degradation of organic compounds. Thus, the production of thermogenic methane generally increases in proportion to the depth of the sediments, usually more than a few hundred meters below the seafloor. Contrarily, a high temperature inhibits microbial activities at a deep subsurface level, thus it follows that the dominant methane in the deep subsurface is thermogenically generated. This process produces not only methane but also ethane, propane, and other light hydrocarbons. There are fewer isotopic effects on carbon and hydrogen from methane produced by thermal maturation compared to biogenic methane. Thus, the carbon isotopic composition of thermogenic methane ranges from -20 to -50‰ (vs. PDB; Whiticar, 1999).

Abiogenic methane

Methane produced from inorganic matter through an inorganic process is called abiogenic methane. Such methane is thought to be generated through the processes of cooling and degassing of mafic igneous rocks and magma and the process of serpentinization of ultramafic rocks. Although the exact mechanism of abiogenic methane formation is still not fully understood, some abiogenic methane formation processes have been revealed (e.g., Horita and Berndt, 1999; Proskurowski et al., 2008). We can now say that abiogenic methane is probably the dominant methane source in hydrothermal vent systems especially along mid-ocean ridges and above serpentine diapirs.

Identification of the origin of methane

Looking specifically at the methane in cold seeps, it has been thought that this originated from the processes of biogenesis and/or thermogenesis. Most abiogenic methane will be found at hydrothermal vents and not at cold seeps except in the case of cold seeps associated with serpentine diapirs. Means of allowing us to estimate the origin of methane are a combination of analyses of the carbon isotopic compositions of the methane and the relative abundance of ethane and propane compared to the amount of methane included in fluid seepage (Schoell, 1988). Specifically, extremely negative carbon isotope values of methane, generally less than -50‰ (vs. PDB), with a high methane/(ethane + propane) ratio indicate that the methane was generated by a biogenic process. In contrast, less negative carbon isotopic composition, generally -20‰ to -50‰ (vs. PDB), with a relatively low methane/(ethane + propane) ratio indicates that the methane was generated by a thermogenic process.

Anaerobic oxidation of methane (AOM)

AOM is a key reaction at cold seeps. Although several probable reactions have been proposed (Valentine, 2002), the details of the AOM reaction are not yet clearly understood. What we can say is that the net reaction of the AOM is expressed as $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$. Thus, the relative amounts of both sulfate and methane regulate the activity of AOM. This reaction occurs

widely in oceans because large amounts of sulfate are contained in sea water whereas the reaction will not occur in freshwater environments such as ponds, lakes, rivers, and rice fields because freshwater lacks sulfate (Whiticar, 1999). It has recently been recognized that AOM by sulfate reduction is carried out by consortia of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria (Elvert et al., 1999; Boetius et al., 2000; Orphan et al., 2001, 2004). The anaerobic methane-oxidizing archaea (ANME) are found in three different phylogenetic clusters belonging to the Euryarchaeota, i.e., ANME-1, -2, and -3. It has also been revealed by genome analysis that methane is oxidized under anoxic conditions by means of a “reverse methanogenesis” process (Hallum et al., 2004). The ecology of each of these archaeal lineages (ANME-1, -2, and -3) differs from that of the others. Thus, the differences reflect their distribution pattern in cold seeps (Elvert et al., 2005; Knittel et al., 2005).

An important function of the AOM reaction is that it prevents the escape of methane, which has a pronounced greenhouse effect, from sediments into the ocean and the atmosphere. The carbon derived from methane is transformed into bicarbonate or carbonate ions. An increase in the ions leads to an increase in alkalinity and thus to the subsequent precipitation of carbonate minerals given the existence of calcium or other appropriate cations (explained below).

Oxidation of sulfides

The hydrogen sulfide (see entry *Sulfide Mineral Oxidation*), a byproduct of the AOM, is used for subsequent reactions. One is the oxidation of sulfides mediated by sulfur-oxidizing bacteria under (sub)oxic conditions, and the other is the formation of pyrite (see entry *Iron Sulfide Formation*) in anoxic conditions. Sulfur-oxidizing (thiotrophic) bacteria, such as *Beggiatoa* (qv), *Thiothrix*, and *Thioploca*, use the hydrogen sulfide as an electron donor and oxidize it by oxygen. The final product of the reaction is sulfate. Most sulfur-oxidizing bacteria are chemolithoautotrophs, but some can use organic compounds for their cell synthesis. The thiotrophic bacteria sometimes form white mats that cover the sea floor and some may live as symbiotic bacteria in animal tissues (e.g., in gills).

Aerobic oxidation of methane (qv)

The aerobic oxidation of methane (see entry *Methane Oxidation (Aerobic)*) is performed by methane-oxidizing (methanotrophic) bacteria, whereas archaea are responsible for AOM. When the methane is released directly into an oxic water column, it will be aerobically oxidized by these methanotrophs. However, in normal marine environments, methane is mostly oxidized anaerobically by AOM within the sediments. Aerobic oxidation of methane becomes important when methane vents out vigorously into oxic sea water.

Authigenic mineral precipitation in cold seeps (qv “microbial mineralization” and “microbially induced sedimentary structures”)

One result of the substantial biological reactions referred to above is the precipitation of several minerals. The minerals most commonly found in cold seeps are carbonate such as aragonite, high magnesian calcite, and low magnesian calcite. The precipitation of these carbonates occurred because of increasing alkalinity resulting from AOM. When AOM occurs at sea-floor level or above the sea floor in euxinic waters, reef-like carbonate build-ups may be formed on the sea floor, as in the Black Sea (Michaelis et al., 2002). Under an oxic water column, the reaction generally occurred within the sediment, thus the carbonate formed beneath the sea floor. At a shallower depth below the sea floor, aragonite and high magnesian calcite can be formed, whereas low magnesian calcite and dolomite are frequently found relatively deeper within the sediments (e.g., Takeuchi et al., 2007). Methane-derived carbonate bodies are sometimes exposed on the sea floor because of surface erosion. Then the carbonate may function as a hard substrate for sessile organisms or carbonate borers.

Pyrite is another common authigenic mineral found in cold seeps. Hydrogen sulfide produced as a result of AOM may combine with reduced iron to form monosulfide, i.e., a precursor of pyrite. Subsequent reaction of the precursor with elemental sulfur or sulfides promotes the formation of pyrite. It is commonly the case that the pyrite crystal aggregation forms a framboidal shape, a so-called framboidal pyrite (for details see Chapter *Iron Sulfide Formation*). The sulfur isotopic composition of pyrite inherits the composition of the hydrogen sulfide source and indicates whether or not the hydrogen sulfide derived from bacterial sulfate reduction (Peckmann and Thiel, 2004).

Relationship to methane gas hydrate

Cold seeps are frequently associated with gas hydrate (Figure 4) where the environment has appropriate physical conditions for gas hydrate formation. The main component of the gas hydrate is methane but carbon dioxide, ethane, and other gaseous molecules are also present. Gas hydrates form in low temperature – high pressure environments where the appropriate concentrations of methane exist (Zhang, 2003). Such environments are usually distributed in the deep sea around continental margins and in permafrost areas on land. The hydrate is one of the largest methane reservoirs in the world. It has been estimated that more than 2,100 Gt of methane are stored in gas hydrates.

Formation of a mass of methane gas hydrate usually occurs within sediments whereas methane rapidly escapes and does not readily accumulate in water columns. If a substantial amount of methane, e.g., bubbly methane gas, is released into a water column, gas hydrate may form as a thin shell on the surface of a gas bubble (Figure 3).

The depth limit of gas hydrate formation and occurrence in the sediments is usually determined by the local geothermal gradient. The gas hydrate itself may act as an impermeable barrier within the sediment. In this case, the methane gas, which has migrated from the deep subsurface, is trapped and accumulates beneath the gas hydrate layer.

The importance of the gas hydrate for cold seeps is that (1) pore water surrounding the methane gas hydrate has an oversaturated level of methane, and (2) the rapid dissociation of methane gas hydrate may result in an episodic increase of cold seeps and sometimes an eruption of methane. When methane-undersaturated water penetrates the sediment and comes into proximity with the gas hydrate, the gas hydrate dissociates so as to create a physical equilibrium between the pore water and the gas hydrate. When the pore water, after taking up a substantial amount of methane, migrates further upward, a continuous methane supply is established between the methane gas hydrate reservoir and the sea floor.

Episodic emission of methane because of the dissociation of methane gas hydrate occurs, for example, in cases where the sea level decreases at the beginning of a glacial period or increasing sea water temperature because of global warming (e.g., Judd et al., 2002). The dissociation of gas hydrates may cause instability of the sea floor inducing landslides (Buffett, 2000). Such an underwater landslide results in additional lowering of pressure and may produce further dissociation of gas hydrate. It should be noted that the meaning of the existence of methane gas hydrate is that the area has large potential for the generation and/or accumulation of methane.

Because the density of gas hydrate is lower than that of sea water, the gas hydrate itself tends to rise upwards. Thus, an insufficient mixture of sediment in the gas hydrate and/or insufficient loading by overlain sediments causes the release of a mass of gas hydrate into a water column.

Ecosystem established at cold seeps – chemosynthesis-based ecosystems

Cold seeps can be considered as chemosynthesis-based ecosystems that support a large amount of life in deep seas (Figures 2 and 7; Sibuet and Olu, 1998). The main primary producers of the ecosystem found in cold seeps are chemoautotrophic microbes, such as sulfur-oxidizing bacteria. For the sulfur-oxidizing bacteria, hydrogen sulfide has the role of an electron acceptor, oxygen that of an electron donor, and carbon dioxide that of a carbon source. Such microbes that metabolize via the chemical reaction of inorganic compounds with an inorganic carbon source are called chemolithoautotrophs. An ecosystem based on chemoautotrophy is called a chemosynthesis-based ecosystem. This kind of ecosystem is a counterpart to the photosynthetic ecosystem found on the surface of the Earth. Chemosynthetic ecosystems establish themselves especially in areas where light does not penetrate such as the



Cold Seeps, Figure 7 Chemosynthesis-based community composed of a dense accumulation of *Bathymodiolus* (mytilid bivalves) from Kuroshima Knoll, off Okinawa, southwest of Japan. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).

deep sea and the subsurface below the sea floor. Within these settings, chemosynthesis-based ecosystems particularly flourish at cold seeps and hydrothermal vents because these are environments where large amounts of reduced compounds encounter oxic waters.

With chemosynthetic microbes as a basis, heterotrophic microbes and animals flourish in cold seeps as members of the chemosynthetic ecosystem. In order to flourish in a oxygen-poor cold seep environment which is instead rich in toxic substances such as hydrogen sulfide, the animals found there often have a detoxification system (Felbeck et al., 1985). Most animals living in cold seeps house chemosynthetic bacteria, such as sulfur-oxidizing bacteria and methane-oxidizing bacteria, and obtain organic matter from those symbionts. These symbiotic chemosynthetic bacteria are called as chemosymbiotic bacteria. The animals living in cold seeps are generally endemic at a higher taxon level and diverge from the animals belonging to photosynthetic ecosystems. Major animals living in a cold seep environment are vesicomyid, mytilid, thyasirid, lucinid, and solemyid bivalves, provannid, acmaeid and neomphalid gastropods, and vestimentiferan tube worms. Crabs, sea urchins, sponges, and barnacles are occasionally found as minor faunal elements. Nectobenthic animals, i.e., animals that can live near the sea floor, such as hydrothermal vent shrimps, are relatively fewer in number at cold seeps than in hydrothermal vent fields. This is because the reduced compounds, their energy source, are often consumed already within the sediments, and only occasionally appear above the sea floor. In contrast, infaunal animals, i.e., animals that live in the sediment, are more abundant in cold seeps than in hydrothermal vents, because the cold seeps are usually covered by rich soft sediments whereas fresh

(recently formed) hard igneous rocks are exposed at hydrothermal vent fields.

The biotic composition of cold seeps is controlled by physicochemical factors including the quantity of reduced compounds such as methane and hydrogen sulfides. One good example of the biotic elements controlled by methane accumulations is seen at Hydrate Ridge (Cascadia Margin, off Oregon). *Beggiatoa* (sulfur-oxidizing bacteria) mats, *Calyptogena* (vesicomyid bivalve), and *Acharax* (solemyid bivalve) all flourish in this cold seep area in a zoned distribution. A large concentration of hydrogen sulfide was detected in the *Beggiatoa* zone, decreasing toward the *Acharax* zone through the *Calyptogena* zone (Sahling et al., 2002). A similar regulation of distribution caused by the concentration of hydrogen sulfide occurred at the species level. Two species of *Calyptogena* (bivalve), *C. kilmeri* and *C. pacifica*, coexisted in a cold seep but they were roughly segregated. The *C. kilmeri* lived where there was a much higher concentration of hydrogen sulfide and the other lived where the concentration of hydrogen sulfide was much lower (Barry et al., 1997).

The biotic component that lives in cold seep environments looks similar to those found in other reduced environments such as hydrothermal vents, sunken vertebrate carcasses, and driftwood (see Chapter *Whale and Wood Falls*). Actually, the components are mostly the same at a higher taxonomic level (i.e., genus or family level), although they are restricted to living in a single type of habitat at the species level (Peck et al., 1997), except for vertebrate carcasses (Smith and Baco, 2003). Twenty species found in a cold seep environment also live on/around vertebrate carcasses. Thus, vertebrate carcasses may play a role as a dispersal stepping stone prior to living in a cold seep at least for some species. It is generally thought that the macro organisms in hydrothermal vent environments have evolved from cold seep species (Hecker, 1985; McLean 1985; Craddock et al., 1995). In addition, molecular data indicate that mytilid bivalves living in a cold seep environment adapt to such a reduced environment via whale carcasses as an evolutionary stepping stone (Distel et al., 2000; Smith and Baco, 2003).

Ancient cold seeps

Recognition of ancient cold seeps

Numerous ancient cold seeps dating from various ages have been found in a variety of tectonic settings (Figures 1 and 8; see review in Campbell, 2006). The identification of ancient cold seeps depends on revealing features which indicate the presence of methane and other hydrocarbons which escaped from the sea floor. In order to make these features evident, a comprehensive approach based on geological, geochemical, and paleontological techniques is a primary requirement.

It is well known that carbonate minerals are forming in modern cold seep environments as a result of increased alkalinity due to AOM (Ritger et al., 1987; Aharon,



Cold Seeps, Figure 8 Late Cretaceous cold seep deposit (*above*) and dense accumulation of lucinid bivalves associated with the carbonate (*below*) from South Dakota, USA. The carbonate rock was induced by anaerobic oxidation of methane at a late Cretaceous active cold seep in the Western Interior Seaway, located in the eastern part of what is today the Rocky Mountains in northwestern America.

2000). Methane-derived carbonates are potentially imprinted with environmental information concerning the location where they were formed. Shapes, textures, and imprinted chemical signatures of modern cold seep

deposits can be seen as a good analog facilitating the recognition of ancient cold seeps. A conduit pipe such as a carbonate chimney (Takeuchi et al., 2007), although they are generally formed under the sea floor in contrast to chimneys at hydrothermal vents, is a good example indicating the route of fluid. Cold seep carbonates often have chaotic fractured structures. The fracture is usually ductile and/or accompanied by brittle sediment deformations. The deformations are probably due to active fluid venting. Microbially induced carbonate cements, e.g., clotted fabrics and isopachous fibrous cements, are frequently found in the carbonate. The influence of methane and microbial activity can be detected by further biogeochemical analysis (Peckmann and Thiel, 2004).

The carbon isotopic compositions of the carbonate reflect the values of the carbon source. Methane, the main gaseous component of cold seeps, generally shows negative carbon isotopic compositions in the case of thermogenic and biogenic methane (see above). The carbon isotopic compositions of thermogenic and biogenic methane commonly range from -30 to -50‰ and -50 to -80‰ (vs. PDB), respectively, and are thus distinguished from other carbon sources in the marine environment. These values will be imprinted at roughly the same level in the carbon isotopic compositions of the methane-derived carbonate. Considering that the $\delta^{13}\text{C}$ -values of carbonate are often somewhat higher than the values of the source methane because of the admixture of other carbon sources (Peckmann and Thiel, 2004), values ca. lower than -30‰ (vs. PDB) suggest that a major portion of the carbonate carbon is derived from methane.

Lipid biomarkers provide information on ancient microbial activities. It has been revealed that AOM is mediated by microbial consortia composed of archaea and sulfate-reducing bacteria (Orphan et al., 2002). Cell membrane lipids produced by those microbes have a strong propensity to be preserved in sediments (Peckmann and Thiel, 2004). Analyzing the carbon isotopic composition of each of these biomarkers (see entry *Biomarkers (Organic, Compound-Specific Isotopes)*) tells us whether or not the microbes used methane as the carbon source for their biosynthesis. The carbon isotopic compositions of lipids derived from methanotrophic microbes sometimes reach as low as -120‰ or even lower. On the basis of this information, the presence of isotopically depleted lipid biomarkers derived from archaea will be a good indicator for the occurrence of AOM (Elvert et al., 2005).

Oldest cold seep

Ancient cold seeps with chemosymbiotic macrofossils have been traced back to at least Silurian (Barbieri et al., 2004). As it is difficult to find and recognize ancient cold seeps without macrofossils, there is practically no record of older cold seeps. However, a possible cold seep occurring after the severe glacial period around 600 Ma, i.e., the period known as “Snowball Earth,” has recently been

proposed. The evidence for a cold seep related to the dissociation of methane gas hydrate is the strongly negative carbon isotopic composition of the “cap carbonate” from south China (Jiang et al., 2003). However, cap carbonates on a glacial deposit formed around 600 Ma are widely distributed throughout the world. If all the cap carbonates were formed under the influence of dissociating methane gas hydrate, numerous cold seeps would have appeared at that time. It is easy to imagine that widespread cold seeps existed in the Precambrian because biogenic methane already existed at 3.5 Ga (Ueno et al., 2006). The apparent lack of cold seeps in the Precambrian, apart from the cap carbonate, is probably due to their having been overlooked because of a lack of associated macro fossils.

Changes in cold seeps through time

The number of cold seeps varies through the Phanerozoic. In the Paleozoic, there are only five ancient cold seep localities. In spite of the fact that a stratigraphic change in carbon isotope ratios shows a global negative shift at the Permian/Triassic boundary, probably caused by the release of a large amount of methane into the ocean and the atmosphere, probably from methane gas hydrate, there are no records of ancient cold seeps from the Permian and Triassic. However, the number of records of ancient cold seeps in post-Triassic marine sediments has increased. More than 15 localities have been found in the Mesozoic and in an abundance of localities, more than a dozen, from the Cenozoic (Campbell, 2006). The cause of the drastic increase in cold seeps since the late Mesozoic might be due to an increase of organic flux into sediments resulting from increasing planktonic productivity and diversity during the Mesozoic Era (Tappan and Loeblich, 1973; COSOD II, 1987) leading to increasing generation of methane in marine sediments.

The biotic components of cold seeps changed through the Phanerozoic (Campbell and Bottjer, 1995; Little and Vrijenhoek, 2003). Mollusks are the most dominant organism in modern cold seeps, despite the dominance of brachiopods in the Paleozoic and mid-Mesozoic. The brachiopods were replaced by mollusks, mainly by bivalves, in the late Mesozoic (Campbell and Bottjer, 1995; Kiel and Peckmann, 2008). The last abundant occurrence of brachiopods in cold seeps is found from the Campanian, late Cretaceous (ca. 80 Ma), from the Omagari, Hokkaido, Japan (Hikida et al., 2003; Kaim et al., 2010). Vesicomyid and bathymodiolian bivalves, which flourished in modern cold seeps, entered the cold seep environment during the Eocene (Ca. 40 Ma; Kiel, 2006; Amano and Kiel, 2007; Amano et al., 2008). It should be noted that the fossil record of vesicomyid bivalves has recently been reexamined and its origin changed from Cretaceous to Eocene (Amano and Kiel, 2007; Amano et al., 2008; Kiel et al., 2008). Most living molluscan genera which lived in ancient cold seeps originated in the late Mesozoic to Paleogene (see basic data in Kiel and Little, 2006 and updated data in Amano et al.,

2007; Jenkins et al., 2007; Campbell et al., 2008; Kaim et al., 2008; Kiel et al., 2008; Kaim et al., 2009).

Impact on climate change

Judd et al. (2002), indicate that the input of greenhouse gases from the sea floor into the world's oceanic and atmospheric systems may be an important component of the atmospheric carbon budget, because the greenhouse potential of methane is more than 20 times that of carbon dioxide. A catastrophic release of methane may therefore produce global climate changes. Such an event due to the rapid escape of methane into the atmosphere happened at the Paleocene–Eocene boundary (ca. 55.5 Ma). At that time, the rapid dissociation of methane hydrate and subsequent release of methane into the atmosphere resulted in global warming and an increase of 6°C in the sea surface temperature (Dickens et al., 1995). A similar methane release outburst is thought to have occurred at the end-Permian forming one of the biggest known biodiversity crises (see entry *Critical Intervals in Earth History*) (Ryskin, 2003; Retallack and Krull, 2006). Kennet et al. (2002) proposed that the periodic glacial-interglacial cycle during Quaternary was controlled by the decomposition of methane gas hydrate and the subsequent release of methane gas into the atmosphere. Evidence of these methane releases comes mostly from carbon isotopic anomalies which were recorded in carbonate and organic material. The release of isotopically depleted biogenic methane induces negative carbon isotope excursions because of the transfer of ¹³C-depleted carbon from methane to carbon dioxide.

Summary

Cold seeps have been the object of considerable attention because of their role as a connecting pipe between the lithosphere, hydrosphere, and biosphere on the Earth. Reduced compounds contained in cold seep fluid result in substantial biogeochemical reactions. The establishment of a cold seep requires specific geological and biological background conditions, such as tectonic stress to make a route for the fluid, sedimentary organic matter supply to provide a source of methane and other hydrocarbons, microbial and/or geothermal activities to form the hydrocarbons, and microbial activities accounting for the oxidation of reduced compounds. Revealing the processes and mechanisms has largely progressed during the last 2 decades. For example, it was revealed at the time of the transition from the twentieth to the twenty-first century that anaerobic methane-oxidizing archaea and sulfate-reducing bacteria are responsible for the anaerobic oxidation of methane. However, there are still many questions about cold seeps that remain unresolved, e.g., the long-term behavior of cold seeps, response to climate change and details of various biological processes and mechanisms. Gaining an understanding of cold seeps is a primary requirement because a cold seep provides a condensed version of

geobiochemical processes related to methane. Thus, the knowledge that we can obtain from the research on cold seep may help us to understand a much wider range of geobiological phenomena taking place on Earth.

Furthermore, methane, the main component contained in cold seep fluids, has a potentially large effect on global warming. It is evident from several geological records through the Phanerozoic that dissociation of methane gas hydrate has drastic effects in terms of global climate change. Methane is also attractive to human beings because of its large potential as a future energy source. In fact, the methane amount stored in gas hydrate beneath the sea floor and permafrost is more than 2,100 Gt. However, as noted above, methane has a tremendous potential to bring about global climate change on the Earth. Thus, any form of intervention in the natural gases in marine sediments should be carefully carried out on the basis of sufficient knowledge of what will happen when the methane is released from the sea floor. Adopting this stance implies a very strong requirement to reveal the entire range of processes and mechanisms within a cold seep.

Bibliography

- Aharon, P., 2000. Microbial processes and products fueled by hydrocarbons at submarine seeps. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 270–281.
- Amano, K., Jenkins, R. G., and Hikida, Y., 2007. A new gigantic *Nucinella* (Bivalvia: Solemyoidea) from the Cretaceous cold-seep deposit in Hokkaido, northern Japan. *Veliger*, **49**, 84–90.
- Amano, K., Jenkins, R. G., Kurihara, Y., and Kiel, S., 2008. A new genus for *Vesicomya inflata* Kanie & Nishida, a lucinid shell convergent with that of Vesicomys, from Cretaceous strata of Hokkaido, Japan. *Veliger*, **50**, 255–262.
- Amano, K., and Kiel, S., 2007. Fossil vesicomyid bivalves from the North Pacific region. *Veliger*, **49**, 270–293.
- Aoyama, C., and Matsumoto, R., 2009. Acoustic surveys of methane plume by quantitative echo sounder in Japan Sea and the estimate of the seeping amount of the methane hydrate bubbles. *Journal of Geography*, **118**, 156–174 (Japanese with English abstract).
- Barbieri, R., Ori, G. G., and Cavalazzi, B., 2004. A Silurian cold-seep ecosystem from the Middle Atlas, Morocco. *Palaio*, **19**, 527–542.
- Barry, J. P., Kochevar, R. E., and Baxter, C. H., 1997. The influence of pore-water chemistry and physiology on the distribution of vesicomyid clams at cold seeps in Monterey Bay: implications for patterns of chemosynthetic community organization. *Limnology and Oceanography*, **42**, 318–328.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Buffett, B. A., 2000. Clathrate hydrates. *Annual Reviews in Earth and Planetary Sciences*, **28**, 477–507.
- Campbell, K. A., 2006. Hydrocarbon seep and hydrothermal vent paleoenvironments and paleontology: past developments and future research directions. *Palaeogeography Palaeoclimatology Palaeoecology*, **232**, 362–407.
- Campbell, K. A., and Bottjer, D. J., 1995. Brachiopods and chemosymbiotic bivalves in Phanerozoic hydrothermal vent and cold seep environments. *Geology*, **23**, 321–324.
- Campbell, K. A., Peterson, D. E., and Alfaro, A. C., 2008. Two new species of *Retiskenea* (Gastropoda: Neomphalidae) from Lower Cretaceous hydrocarbon seepcarbonates of northern California. *Journal of Paleontology*, **82**, 140–153.
- Clennell, M. B., Judd, A., and Hovland, M., 2000. Movement and accumulation of methane in marine sediments: relation to gas hydrate systems. In Max, M. D. (ed.), *Natural Gas Hydrate in Ocean and Permafrost Environments*. The Netherlands: Kluwer, pp. 105–122.
- COSOD II, 1987. *Report of the Second Conference on Scientific Ocean Drilling*. European Science Foundation, Strasbourg, France, 142 pp.
- Craddock, C., Hoeh, W. R., Gustafson, R. G., Lutz, R. A., Hashimoto, J., and Vrijenhoek, R. J., 1995. Evolutionary relationships among deep-sea mytilids (Bivalvia: Mytilidae) from hydrothermal vents and cold-water methane/sulfide seeps. *Marine Biology*, **121**, 477–485.
- Dickens, G. R., O'Neil, J. R., Rea, D. K., and Owen, R. M., 1995. Dissociation of oceanic methane hydrate as a cause of the carbon isotope excursion at the end of the Paleocene. *Paleoceanography*, **10**, 965–971.
- Distel, D. L., Baco, A. R., Chuang, E., Morrill, W., Cavanaugh, C., and Smith, C. R., 2000. Do mussels take wooden steps to deep-sea vents? *Nature*, **403**, 725–726.
- Elvert, M., Hopmans, E. C., Treude, T., Boetius, A., and Suess, E., 2005. Spatial variations of methanotrophic consortia at cold methane seeps: implications from a high-resolution molecular and isotopic approach. *Geobiology*, **3**, 195–206.
- Elvert, M., Suess, E., and Whiticar, M. J., 1999. Anaerobic methane oxidizing associated with marine gas hydrates: superlight C-isotopes from saturated and unsaturated C₂₀ and C₂₅ irregular isoprenoids. *Naturwissenschaften*, **86**, 295–300.
- Fehn, U., Snyder, G. T., Matsumoto, R., Muramatsu, Y., and Tomaru, H., 2003. Iodine dating of pore waters associated with gas hydrates in the Nankai area, Japan. *Geology*, **31**, 521–524.
- Felbeck, H., Powell, M. A., Hand, S. C., and Somero, G. N., 1985. Metabolic adaptations of hydrothermal vents animals. *Bulletin of the Biological Society of Washington*, **6**, 261–272.
- Fujikura, K., Kojima, S., Tamaki, K., Maki, Y., Hunt, J., and Okutani, T., 1999. The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan Trench. *Marine Ecology Progress Series*, **190**, 17–26.
- Gay, A., Lopez, M., Ondreas, H., Charlou, J. L., Sermondadaz, G., and Cochonat, P., 2006. Seafloor facies related to upward methane flux within a Giant Pockmark of the Lower Congo Basin. *Marine Geology*, **226**, 81–95.
- Hallum, S. J., Putnam, N., Prestion, C. M., Detter, J. C., Rockhsar, D., Richardson, P. M., and DeLong, E. F., 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science*, **305**, 1457–1462.
- Hecker, B., 1985. Fauna from a cold sulfur-seep in the Gulf of Mexico: comparison with hydrothermal vent communities and evolutionary implications. *Bulletin of the Biological Society of Washington*, **6**, 465–473.
- Hikida, Y., Suzuki, S., Togo, Y., and Ijiri, A., 2003. An exceptionally well-preserved fossil seep community from the Cretaceous Yezo Group in the Nakagawa area, Hokkaido. *Paleontological Research*, **7**, 329–342.
- Hinrichs, K. U., Hayes, J. M., Bach, W., Spivack, A. J., Hmelo, L. R., Holm, N. G., Johnson, C. G., and Sylva, S. P., 2006. Biological formation of ethane and propane in the deep marine subsurface. *Proceedings of the National Academy of Sciences*, **103**, 14684–14689.
- Horita, J., and Berndt, M. E., 1999. Abiogenic methane formation and isotopic fractionation under hydrothermal conditions. *Science*, **285**, 1055–1057.

- Jenkins, R. G., Kaim, A., Hikida, Y., and Tanabe, K., 2007. Methane-flux-dependent lateral faunal changes in a Late Cretaceous chemosymbiotic assemblage from the Nakagawa area of Hokkaido, Japan. *Geobiology*, **5**, 127–139.
- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopic evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Judd, A. G., Hovland, M., Dimitrov, L. I., Gil, S. G., and Jukes, V., 2002. The geological methane budget at Continental Margins and its influence on climate change. *Geofluids*, **2**, 109–126.
- Kaim, A., Jenkins, R. G., and Waren, A., 2008. Provannid and provannid-like gastropods from the Late Cretaceous cold seeps of Hokkaido (Japan) and the fossil record of the Provannidae (Gastropoda: Abysochrysoidea). *Zoological Journal of the Linnean Society*, **154**, 421–436.
- Kaim, A., Jenkins, R. G., and Hikida, Y., 2009. Gastropods from Late Cretaceous Omagari and Yasukawa hydrocarbon seep deposits in the Nakagawa area, Hokkaido, Japan. *Acta Palaeontologica Polonica*, **54**, 463–490.
- Kaim, A., Bitner, M. A., Jenkins, R. G., and Hikida, Y., 2010. A monospecific assemblage of terebratulide brachiopods in the Upper Cretaceous seep deposits of Omagari, Hokkaido, Japan. *Acta Palaeontologica Polonica*, **55**, 73–84.
- Kennet, J. P., Cannariato, K. G., Hendy, I. L., and Behl, R. J., 2002. *Methane Hydrates in Quaternary Climate Change*. Washington: American Geophysical Union.
- Kiel, S., 2006. New records and species of molluscs from Tertiary cold-seep carbonates in Washington State, USA. *Journal of Paleontology*, **80**, 121–137.
- Kiel, S., Amano, K., and Jenkins, R. G., 2008. Bivalves from Cretaceous cold seep deposits on Hokkaido, Japan. *Acta Palaeontologica Polonica*, **53**, 525–537.
- Kiel, S., and Little, C. T. S., 2006. Cold-seep mollusks are older than the general marine mollusk fauna. *Science*, **313**, 1429–1431.
- Kiel, S., and Peckmann, J., 2008. Paleoecology and evolutionary significance of an Early Cretaceous *Peregrinella*-dominated hydrocarbon-seep deposit on the Crimean Peninsula. *Palaios*, **23**, 751.
- Knittel, K., Losekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Kotelnikova, S., 2002. Microbial production and oxidation of methane in deep subsurface. *Earth-Science Reviews*, **58**, 367–395.
- Kulm, L. D., Suess, E., Moore, J. C., Carson, B., Lewis, B. T., Ritger, S. D., Kadko, D. C., Thornburg, T. M., Embley, R. W., Rugh, W. D., Massoth, G. J., Langseth, M. G., Cochrane, G. R., and Scamman, R. L., 1986. Oregon subduction zone - venting, fauna, and carbonates. *Science*, **231**, 561–566.
- Kvenvolden, K. A., 1988. Methane hydrate – a major reservoir of carbon in the shallow geosphere? *Chemical Geology*, **71**, 41–51.
- Levin, L. A., 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanography and Marine Biology – An Annual Review*, **43**, 1–46.
- Little, C. T. S., and Vrijenhoek, R. C., 2003. Are hydrothermal vent animals living fossils? *Trends in Ecology and Evolution*, **18**, 582–588.
- McLean, J. H., 1985. Preliminary report on the limpets at hydrothermal vents. *Bulletin of the Biological Society of Washington*, **6**, 159–166.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jorgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., and Gulin, M. B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Montagna, P. A., and Spies, R. B., 1985. Meiofauna and chlorophyll associated with *Beggiatoa* mats of a natural submarine petroleum seep. *Marine Environmental Research London*, **16**, 231–242.
- Moore, G. F., Taira, A., Klaus, A., Becker, L., Boeckel, B., Cragg, B. A., Dean, A., Fergusson, C. L., Henry, P., and Hirano, S., 2001. New insights into deformation and fluid flow processes in the Nankai Trough accretionary prism: results of Ocean Drilling Program Leg 190. *Geochemistry, Geophysics, Geosystems*, **2**, 1058.
- Moore, J. C., Orange, D., and Kulm, L. D., 1990. Interrelationship of fluid venting and structural evolution: Alvin observations from the frontal accretionary prism, Oregon. *Journal of Geophysical Research*, **95**, 8795–8808.
- Ogawa, Y., Fujioka, K., Fujikura, K., and Iwabuchi, Y., 1996. En echelon patterns of *Calypptogena* colonies in the Japan Trench. *Geology*, **24**, 807–810.
- Orphan, V., House, C., Hinrichs, K., McKeegan, K., and DeLong, E., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7663–7668.
- Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., and DeLong, E. F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., Ussler, W., Naehr, T. H., House, C. H., Hinrichs, K. U., and Paull, C. K., 2004. Geological, geochemical, and microbiological heterogeneity of the seafloor around methane vents in the Eel River Basin, offshore California. *Chemical Geology*, **205**, 265–289.
- Paull, C. K., Hecker, B., Commeau, R., Freeman-Lynde, R. P., Neumann, C., Corso, W. P., Golubic, S., Hook, J. E., Sikes, E., and Curran, J., 1984. Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. *Science*, **226**, 965–967.
- Paull, C. K., Ussler III, W., Peltzer, E. T., Brewer, P. G., Keaten, R., Mitts, P. J., Nealon, J. W., Greinert, J., Herguera, J.-C., and Elena Perez, M., 2007. Authigenic carbon entombed in methane-soaked sediments from the northeastern transform margin of the Guaymas Basin, Gulf of California. *Deep-Sea Research Part II*, **54**, 1240–1267.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Peck, A. S., Gustafson, R. G., Lutz, R. A., and Vrijenhoek, R. C., 1997. Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalve: Vesicomyidae): results from the mitochondrial cytochrome oxidase subunit 1. *Marine Biology*, **130**, 151–161.
- Proskurowski, G., Lilley, M. D., Seewald, J. S., Fruh-Green, G. L., Olson, E. J., Lupton, J. E., Sylva, S. P., and Kelley, D. S., 2008. Abiogenic hydrocarbon production at Lost City Hydrothermal Field. *Science*, **319**, 604–607.
- Retallack, G. J., and Krull, E. S., 2006. Carbon isotopic evidence for terminal-Permian methane outbursts and their role in extinctions of animals, plants, coral reefs, and peat swamps. In Greb, S. F., and DiMichele, W. A. (eds.), *Wetlands Through Time*. Geological Society of America Special Paper, pp. 249–268.
- Ritger, S., Carson, B., and Suess, E., 1987. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon/Washington margin. *Geological Society of America Bulletin*, **98**, 147–156.
- Ryskin, G., 2003. Methane-driven oceanic eruptions and mass extinctions. *Geology*, **31**, 741–744.

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of microorganisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

and its release to the environment has resulted in Cu pollution in soils and surface waters.

Properties: Copper has two stable isotopes ^{63}Cu and ^{65}Cu with a natural abundance of 69.17 and 30.83, respectively, and a standard atomic weight of 63.546 (Coplen et al., 2002; Wieser, 2007). The copper concentration in the bulk continental crust, in the oceanic crust, and in soils is estimated to be 27 ppm (Rudnick and Gao, 2003), 78 ppm (Wedepohl and Hartmann, 1994), and 39 ppm (Han et al., 2002), respectively. The concentrations of copper in unpolluted seawater is typically around 0.3 ppb and in river water, 1.5 ppb (Gaillardet et al., 2003). The world average concentration of copper in rivers has been estimated as 23.6 nM Cu dissolved load and 100 ppm Cu suspended particulate load (Martin et al., 1993; Martin and Meybeck, 1979; Martin and Thomas, 1994). In natural systems, it occurs in the Cu(I) (cuprous) or Cu(II) (cupric) redox states and rarely as elemental copper. The standard redox potential of the aqueous Cu(I)/Cu(II) couple is at 0.34 V, but the actual redox potential depends strongly on its coordinative environment. This is exploited by organisms using Cu as reactive center of reductases with high redox potentials between +0.2 and +0.8 V.

Copper toxicity: Due to its toxicity to humans, the US-EPA maximum contaminant level goals and EU standards for copper in drinking water has been set at 1.3 (USEPA, 1991) and 2 mg/l (EU, 1998), respectively. Copper is also toxic to marine and terrestrial prokaryotic and eukaryotic organisms (Flemming and Trevors, 1989; Gledhill et al., 1997). Its toxicity derives from its redox reactions producing radical ions similar to Fenton chemistry causing oxidative stress. Phytochelatins (plants) and metallothioneins (animals, fungi) are intracellular metal binding proteins that play an important role in detoxification and homeostasis of Cu and other metal ions.

Copper as a nutrient: Copper-containing oxidoreductase enzymes are widespread among prokaryotic and eukaryotic organisms and mediate important biogeochemical processes, including nitrification, denitrification, and methane oxidation. Examples are particulate methane monooxygenase, ammonia monooxygenase, nitrite reductase, cytochrome *c* oxidase, and superoxide dismutase. It has been suggested that Cu-bearing reductases evolved only after the oxygenation of the Earth atmosphere, considering the low solubility of Cu in sulfidic environments and the high redox potential of organically bound Cu(I)/Cu(II) up to the limit of the stability field of water (Frausto da Silva and Williams, 2001). Copper is part of a metalloenzyme involved in the uptake of iron by diatoms so that a Cu/Fe co-limitation of primary productivity in the ocean is conceivable (Maldonado et al., 2006; Peers et al., 2005). Recently, it was discovered that some methanotrophic bacteria possess a high-affinity Cu uptake system involving methanobactin, a structurally distinct chromopeptide metallophore analogous to iron-specific siderophores (DiSpirito et al., 1998). This highlights the potential role of copper availability and copper uptake in regulating biogeochemical cycles.

Bibliography

- Coplen, T. B., Böhlke, J. K., De Bièvre, P., Ding, T., Holden, N. E., Hopple, J. A., Krouse, H. R., Lamberty, A., Peiser, H. S., Revesz, K., Rieder, S. E., Rosman, K. J. R., Rothe, E., Taylor, P. D. P., Vocke, R. D., and Xiao, Y. K., 2002. Isotope-abundance variations of selected elements (IUPAC Technical Report). *Pure and Applied Chemistry*, **74**, 1987–2017.
- DiSpirito, A. A., Zahn, J. A., Graham, D. W., Kim, H. J., Larive, C. K., Derrick, T. S., Cox, C. D., and Taylor, A., 1998. Copper-binding compounds from *Methylosinus trichosporium* OB3b. *Journal of Bacteriology*, **180**, 3606–3613.
- EU, 1998. The quality of water intended for human consumption. *Council Directive 98/83/EC*.
- Flemming, C. A., and Trevors, J. T., 1989. Copper toxicity and chemistry in the environment – a review. *Water, Air, and Soil Pollution*, **44**, 143–158.
- Frausto da Silva, J. J. R., and Williams, R. J. P., 2001. *The Biological Chemistry of the Elements – The Inorganic Chemistry of life*. Oxford: Oxford University Press.
- Gaillardet, J., Viers, J., and Dupre, B., 2003. Trace elements in river waters. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry*. Oxford: Pergamon.
- Gledhill, M., Nimmo, M., Hill, S. J., and Brown, M. T., 1997. The toxicity of copper(II) species to marine Algae, with particular reference to macroalgae. *Journal of Phycology*, **33**, 2–11.
- Han, F. X., Banin, A., Su, Y., Monts, D. L., Plodinec, M. J., Kingery, W. L., and Triplett, G. E., 2002. Industrial age anthropogenic inputs of heavy metals into the pedosphere. *Naturwissenschaften*, **89**, 497–504.
- Maldonado, M. T., Allen, A. E., Chong, J. S., Lin, K., Leus, D., Karpenko, N., and Harris, S., 2006. Copper-dependent iron transport in coastal and oceanic diatoms. *Limnology and Oceanography*, **51**, 1729–1743.
- Martin, J. M., and Meybeck, M., 1979. Elemental mass-balance of material carried by major world rivers. *Marine chemistry*, **7**, 173–206.
- Martin, J. M., and Thomas, A. J., 1994. The global insignificance of telluric input of dissolved trace metals (Cd, Cu, Ni and Zn) to ocean margins. *Marine chemistry*, **46**, 165–178.
- Martin, J. M., Guan, D. M., Elbazpoulchiet, F., Thomas, A. J., and Gordeev, V. V., 1993. Preliminary assessment of the distributions of some trace elements (As, Cd, Cu, Fe, Ni, Pb, and Zn) in a pristine aquatic environment-The Lena River estuary (Russia). *Marine Chemistry*, **43**, 185–199.
- Peers, G., Quesnel, S. A., and Price, N. M., 2005. Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. *Limnology and Oceanography*, **50**, 1149–1158.
- Rudnick, R. L., and Gao, S., 2003. The composition of the continental crust. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry*. New York: Elsevier.
- USEPA, 1991. Maximum contaminant level goals and national primary drinking water regulation for lead and copper; final rule (40 CFR Parts 141 and 143). *Federal Register*, **56**, 110.
- Wedepohl, K. H., and Hartmann, G., 1994. The composition of the primitive upper earth's mantle. In Meyer, H. O. A., and Leonardos, O. H. (eds.), *Kimberlites, Related Rocks and Mantle Xenoliths*. Rio de Janeiro, Brazil: Companhia de Pesquisa de Recursos Minerais.
- Wieser, M. E., 2007. Atomic weights of the elements 2005. *Journal of Physical and Chemical Reference Data*, **36**, 485–496.

Cross-references

Metals, Acquisition by Marine Bacteria
Siderophores

COSMIC MOLECULAR CLOUDS

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Complex molecular clouds; Interstellar clouds; Interstellar dust and molecules; Molecular clouds

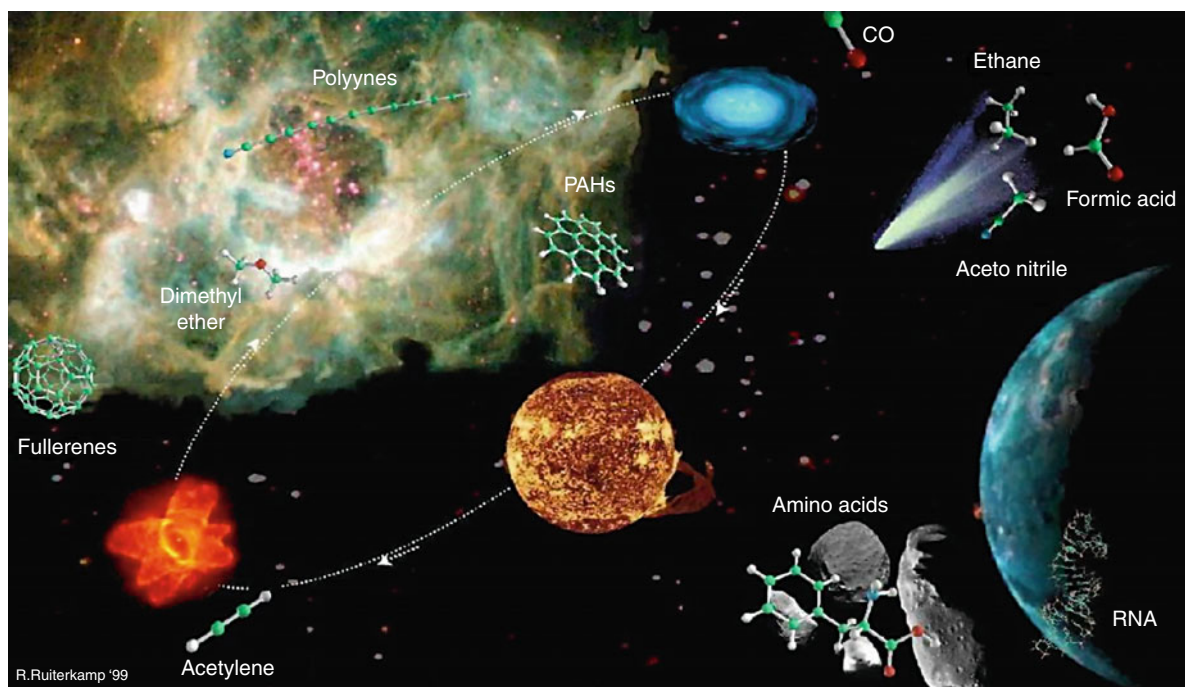
Definition

A cosmic molecular cloud is an accumulation of cosmic matter that is composed of organic and inorganic molecules. The dust grains in a cosmic molecular cloud are mainly composed of crystalline silicates (Watson et al., 2009) covered with ice of H_2O , NH_3 , and CO_2 . The main molecule constituent is H_2 -He, but other molecules also occur, e.g., NH_3 , CO , and complex organic molecules.

Cosmic molecular clouds are denser than the interstellar medium, with gas densities ranging from about 10 molecules per cm^3 on large scales to over 10^6 molecules per cm^3 in cloud cores. These cores are very cold, with temperatures ranging from 8 to 20 K. New stars and protoplanetary discs are formed, when it comes to a gravitational collapse of cosmic molecular clouds. Via nucleosynthesis, these stars produce elements, which may constitute organic molecules (e.g., H, C, N, O, S, P). When

stars burn out, they collapse either via catastrophic explosions or by forming expanding shells, thereby recycling modified material back into the interstellar space and, thus, forming new cosmic molecular clouds (Boss, 2004). Some of the interstellar clouds are enriched in prebiotic organic molecules. These molecules are found in cosmic matter, chondritic asteroids/meteorites, and cosmic dust particles.

Based on spectroscopic wavelength analyses, more than 120 different complex organic molecules could be detected within cosmic molecular clouds, including common PAHs (polycyclic aromatic hydrocarbons), amino acids, sugars ("polyols"), hydrogen cyanide (HCN), and formic acid (Figure 1) (Ehrenfreund and Menten, 2001). The largest organic molecules so far reported from cosmic molecular clouds are cyanodecapentayne (HC_{11}N) and diethyl ether ($\text{C}_2\text{H}_5)_2\text{O}$. Not all of these molecules are part of the gaseous phase, but the more complex ones are related with cosmic dust particles. A well studied cosmic molecular cloud is the Sagittarius B cloud, where the most complex organic molecules like ethyl cyanide, acetone, glycine, acetic acid, and glycolaldehyde were detected (Hollis et al., 2000; Snyder, 1997). Glycolaldehyde may react with ribose to form a molecule, which is a major constituent of RNA. It was hypothesized that organic molecules within a cosmic molecular cloud are formed on 1–2 μm -sized dust grains, e.g., via photochemical reactions. These dust grains have a distinctive shell-like structure. An inner core of silicates is covered by an inner rim



Cosmic Molecular Clouds, Figure 1 Interstellar space with the occurrence of different organic molecules. (From Ehrenfreund and Charnley, 2000.)

containing complex organic compounds like amino acids, “polyols,” and carbonaceous matter such as hydrogenated amorphous carbon or kerogen-like material. The inner rim is covered by an outer rim consisting of “dirty” ice. In that icy outer rim, due to the heavy ultraviolet radiation, radicals are formed and H_2 is dissociated in monoatomic H that may react with other radicals to form organic molecules (Sorrell, 2001; Pearson et al., 2002). An important finding obtained with the Hubble Space Telescope was the observation of protoplanetary discs in the Orion cloud (Megeath et al., 2005). The size of these discs is more or less similar to our solar system. During solar system formation, an interstellar cloud becomes the building block, from which planets, asteroids, and comets form. Comets and carbonaceous chondrites are chemically very similar to star-forming interstellar clouds and are relics of them (Busemann et al., 2006; Wickramasinghe et al., 2009). They carry pristine primitive organic matter and represent a plausible source of prebiotic organic matter on the late Hadean early Earth. The stable isotope compositions of organic matter, e.g., from the Murchison carbonaceous chondrite (CM2), provide clear evidence that the organic matter was formed under conditions in stellar clouds and not on Earth (Sephton et al., 2003, see entry “*Meteoritics*” for further reading). External delivery of water and organic compounds is widely accepted as the origin of pristine earth-related organic matter (Wickramasinghe, 2010) and may have pushed the start of early life with the first microbial organism – *LUCA* – *Last Universal Common Ancestor* (Hoenigsberg, 2003).

Bibliography

- Boss, A. P., 2004. From molecular clouds to circumstellar disks. In Festou, M. C., Keller, H. U., and Weaver, H. A. (eds.), *Comets II*. Tucson: University of Arizona Press, pp. 67–80, 745 pp.
- Busemann, H., Young, A. F., Alexander, C. M. O. D., Hoppe, P., Mukhopadhyay, S., and Nittler, L. R., 2006. Interstellar chemistry recorded in organic matter from primitive meteorites. *Science*, **312**, 727–730.
- Ehrenfreund, P., and Charnley, S. B., 2000. Organic molecules in the interstellar medium, comets and meteorites: a voyage from dark clouds to the early earth. *Annual Review of Astronomy & Astrophysics*, **38**, 427–483.
- Ehrenfreund, P., and Menten, K., 2001. From molecular clouds to the origin of life. In Horneck, G., and Baumstark-Khan, C. (eds.), *Lecture Notes in Physics: Astrobiology: The Quest for the Conditions of Life*. Berlin: Springer, pp. 7–23.
- Hoenigsberg, H., 2003. Evolution without speciation but with selection: *LUCA*, the last universal common ancestor in Gilbert’s RNA world. *Genetics and Molecular Research*, **2**, 366–375.
- Hollis, J. M., Lovas, F. J., and Jewell, P. R., 2000. Interstellar glycolaldehyde: the first sugar. *The Astrophysical Journal*, **540**, L107–L110.
- Megeath, S. T., Flaherty, K. M., Hora, J., Allen, L. E., Fazio, G. G., Hartmann, L., Myers, P. C., Muzerolle, J., Pipher, J. L., Siegler, N., Stauffer, J. R., and Young, E., 2005. A spitzer/IRAC survey of the Orion molecular clouds. In Cesaroni, R., Felli, M., Churchwell, E., and Walmsley, C. M. (eds.), *Massive Star Birth: A Crossroads of Astrophysics, Proceedings IAU Symposium No. 227*, pp. 383–388.
- Pearson, V. K., Sephton, M. A., Kearsley, A. T., Bland, P. A., Franchi, I. A., and Gilmour, I., 2002. Clay mineral-organic matter relationships in the early Solar System. *Meteoritics and Planetary Science*, **37**, 1829–1833.
- Sephton, M. A., Verchovsky, A. B., Bland, P. A., Gilmour, I., Grady, M. M., and Wright, I. P., 2003. Investigating the variations in carbon and nitrogen isotopes in carbonaceous chondrites. *Geochimica et Cosmochimica Acta*, **67**, 2093–2108.
- Snyder, L. E., 1997. The search for interstellar glycine. *Origins of Life and Evolution of Biospheres*, **27**, 115–133.
- Sorrell, W. H., 2001. Origin of amino acids and organic sugars in interstellar clouds. *The Astrophysical Journal*, **555**, L129–L132.
- Watson, D. M., Leisenring, J. M., Furlan, E., Bohac, C. J., Sargent, B., Forrest, W. J., Calvet, N., Hartmann, L., Nordhaus, J. T., Green, J. D., Kim, K. H., Sloan, G. C., Chen, C. H., Keller, L. D., d’Alessio, P., Najita, J., Uchida, K. I., and Houck, J. R., 2009. Crystalline silicates and dust processing in the protoplanetary disks of the Taurus young cluster. *The Astrophysical Journal Supplement Series*, **180**, 84–101.
- Wickramasinghe, J. T., Wickramasinghe, N. C., and Wallis, M. K., 2009. Liquid water and organics in comets: implications for exobiology. *International Journal of Astrobiology*, **8**, 281–290.
- Wickramasinghe, N. C., 2010. The astrobiological case for our cosmic ancestry. *International Journal of Astrobiology*, **9**, 119–129.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Chondrites](#)
[Meteoritics](#)
[Origin of Life](#)

CRITICAL INTERVALS IN EARTH HISTORY

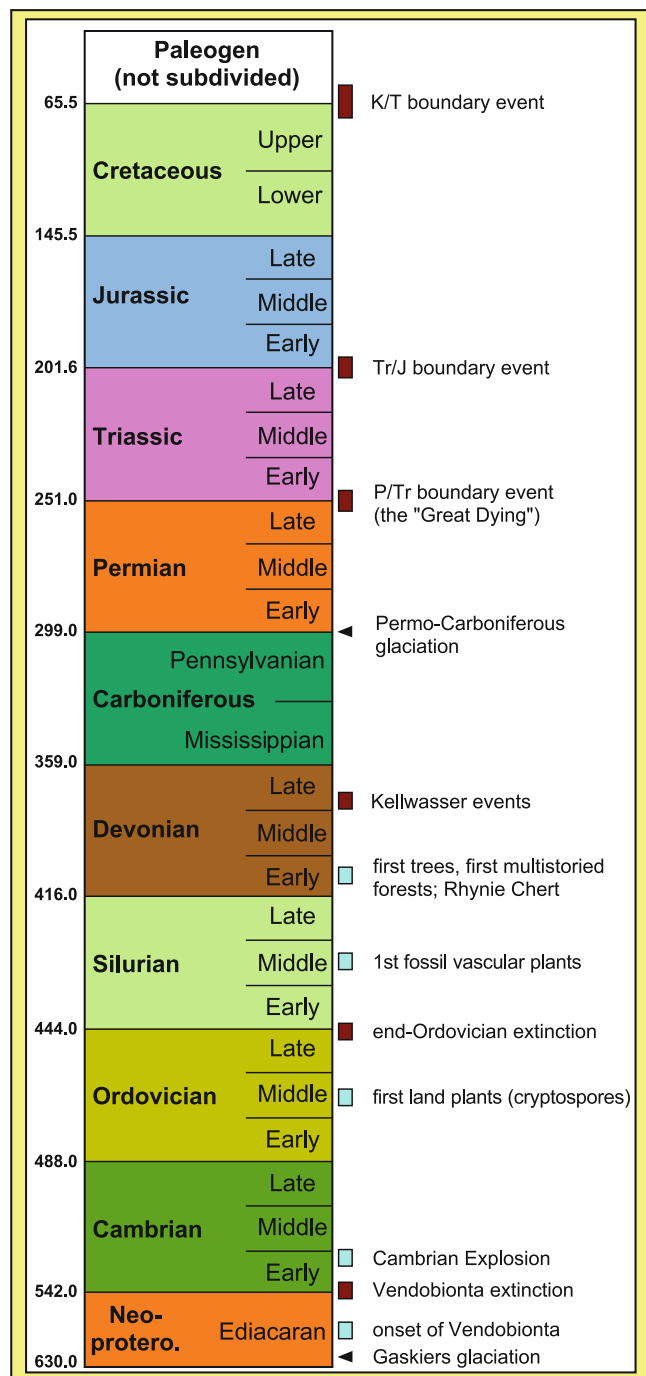
Frank Wiese, Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition

In our human understandings of time, Darwinian evolution is something, which happens very slowly. Consulting the (biogeochemical) fossil record, we notice periods in life’s history, which are characterized by slow and continuous evolution, successive background extinction, and equally successive new speciations. On the other hand, we see also periods, during which a sudden occurrence of new groups and its rapid radiation and dispersal can be seen in the fossil record. *Vice versa*, we also recognize catastrophic extinction events (mass extinctions; see below), reducing the number of known taxa dramatically within a very short time of only thousands of years or even much less.

Among these intervals were phases, in which comparatively short-termed (in a geological sense) pulses of evolution resulted in significant and irreversible evolutionary innovations, which opened new paleobiological pathways, affecting the abiotic and biotic evolutionary landscape likewise. These intervals are what we understand as main critical intervals in earth’s history here, and we believe that three periods merit this designation. These are the

Great Oxygenation Event or the Great Oxidation Event, respectively (GOE, ca. 2.4 Ga), the Cambrian Explosion around 524 Ma (Lower Cambrian), and the evolution of land plants from the Middle Ordovician on (ca. 463 Ma; Figure 1).



Critical Intervals in Earth History, Figure 1 Overview over the most significant critical (late Proterozoic to terminal Cretaceous) intervals considered in the text, and their stratigraphical position. Blue: diversification; dark: extinction.

Critical intervals of subordinate significance are mass extinction events. It is clear that any of these phases of mass extinction reflect severe perturbation in the global ecosystems, and there is no doubt that the biotic recovery from mass extinctions is also a critical period in the regeneration of the biosphere. However, when carefully considering the most significant phases of mass extinction in the Phanerozoic (the "Big Five"; Figure 1), we see that its aftermaths never resulted in the evolution of any new clades. Although critically for the fauna extinct, new taxa always developed in a saturated evolutionary landscape, where no space was for innovations at a clade's scale. These events, thus, represent "merely" a convulsion of the taxonomic inventory and are regarded here as subordinate critical intervals.

The study of critical intervals in the history of life aims at the mapping of the paleobiological and geological changes during these intervals, the interaction between the both, and the attempt to extract possible complex triggering mechanisms for the phenological expression in the (biogeochemical) fossil record within its geological framework. The study of critical intervals is, thus, an ideal example of how geobiology is embedded in interdisciplinary research of classic paleontology, genetics, bio- and isotope geochemistry as well as sedimentary geology, to name some of the disciplines inevitably applied.

Great Oxygenation Event, ca. 2.4 Ga (Great Oxidation Event)

Definition

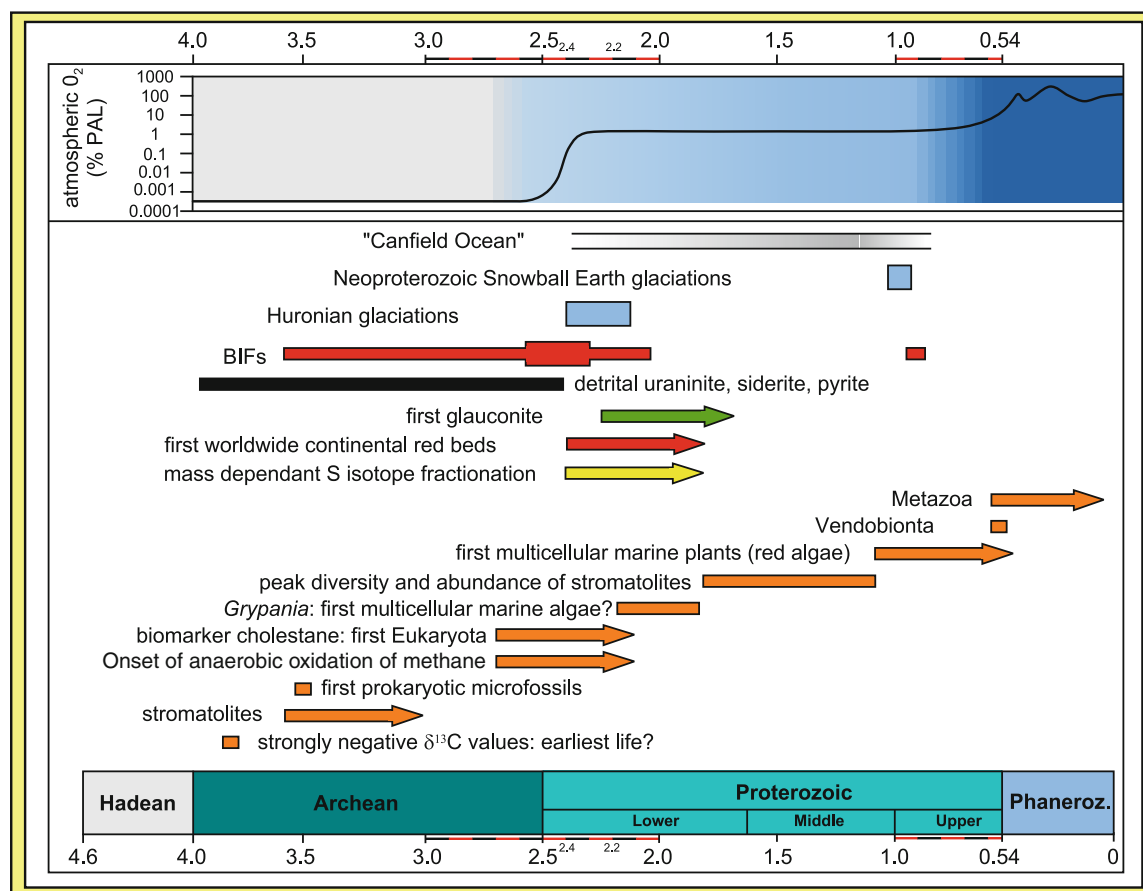
Sedimentological and geochemical data indicate that the atmosphere became suddenly oxygenated up to a pO_2 of ca. 1% of present atmospheric level (PAL) around ~2.4 Ga (Kump, 2008; Figure 2). This period is called the "Great Oxygenation Event" or the "Great Oxidation Event" (GOE). Thus, it marks the boundary between an oxygen-free and an oxygenized world. The GOE approximates stratigraphically the boundary between the Archean and the Proterozoic (2.5 Ga).

Evidences

The evidence for atmospheric oxygenation results from various independent sources, which also provide controversial data (see compilations in Holland, 2006; Falkowski and Godfrey, 2009; Figure 2).

Sedimentological aspects: Prior to the GOE, detrital pyrite, uraninite, and siderite are known from siliciclastic fluvial sediments – minerals that would have been oxidized under the presence of O_2 either in the atmosphere or water – and this clearly indicates the lack of any free oxygen. There are no records of these minerals younger than 2.5–2.3 Ga. Around 2.4 Ga, the first red beds developed in the continental areas, resulting from dispersedly distributed ferric oxide in the sediments, which only could have developed by oxygenation of ferrous iron under the presence of free atmospheric O_2 . A while later, the mineral glauconite (containing ferric iron) occurred for the first time.

The occurrence of banded iron formations (BIFs) from the early Archean on (ca. 3.8 Ga) with its peak occurrence



Critical Intervals in Earth History, Figure 2 Compilation of sedimentological, mineralogical, biogeochemical, paleobiological and climatical trends with an estimate of the O₂ evolution throughout the Archean through Phanerozoic (based on the references cited in the text).

at ca. 2.6 Ga, bearing large amounts of ferric iron, were previously thought to correlate positively with the occurrence of oxygen (see overview in Klein, 2005). However, as shown by Kappler et al. (2005), the precipitation of ferric oxides by anoxygenic phototrophic Fe(II)-oxidizing bacteria could also explain the development of BIFs, which then is not indicative of any O₂ availability.

Geochemical evidence: Geochemically, the period around 2.4 Ga is characterized by the establishment of mass dependent sulfur isotope fractionation (Bekker et al., 2004; Farquhar and Wing, 2003) and a first enrichment of molybdenum in black shales (Scott et al., 2008), both indicative of a progressively oxidized atmosphere. In addition, chromium isotope fractionation points at slightly elevated O₂ levels even earlier than the GOE already at ca. 2.8–2.6 Ga (Frei et al., 2009).

Possible causes for the GOE

There are a variety of models that try to explain the GOE.

Onset of oxygenic photosynthesis/anaerobic oxidation of methane (AOM) (2.7–2.6 Ga). Anaerobic methanogenesis

by archaea (*via* methyl-CoM reductase) was responsible for an estimated atmospheric methane concentration of 1,000 ppm and extreme greenhouse conditions in the early atmosphere. The rise of oxygenic photosynthesis by cyanophyta (at ~2.45 Ga) as suggested by biomarker evidence (Buick, 2008), and the onset of AOM by sulfate reduction (at ~2.7 Ga; Hinrichs and Boetius, 2002) resulted in a significant withdrawal of CO₂ and CH₄ from, and an enrichment of O₂ in the atmosphere. Anbar et al. (2007) proposed “a whiff of oxygen” some ca. 90 Ma before the GOE, and Kaufman et al. (2007), based on sulfur isotopes, suggested slightly oxic conditions in the ocean prior to oxygenation of the atmosphere, but the sudden oxygenation event at 2.4 Ga has also been considered as a direct result of increased microbial activity reaching a ‘critical point’ by some scientists.

Time lag: An alternative approach derives from the assumption that oxygenic photosynthesis had already been established around 3.2 Ga (deduced from kerogen-rich black shales) or even 3.8 Ga (deduced from U–Th–Pb isotopy; see Buick, 2008 for discussion). The time lag

between the onset of oxygenic photosynthesis and the GOE has been explained by two models. Konhauser et al. (2009) observed a decrease in nickel concentration of ocean waters around 2.4 Ga. As nickel is a crucial element in several enzymes of methanogens, a decline would have severely dropped its activity (nickel famine). The stagnation of biogenic methane supply would then have favored a rapid increase of environmental oxygenation. Goldblatt et al. (2006) proposed, based on modeling, an alternative explanation. Assuming the onset of oxygenic photosynthesis at 2.7 Ga, the 300 Ma time-lag is explained by the bistability of the atmospheric oxygen system. A low oxygen steady state system existed at oxygen levels around and below 10^{-5} PAL prior to the GOE. With oxygen levels higher than 10^{-5} PAL, UV shielding becomes more efficient, which resulted in a nonlinear increase of atmospheric oxygen's lifetime and thus, oxygen content. The high-oxygen steady-state system stabilized at values larger than 5×10^{-3} PAL.

Concluding remarks

Even though the timing of the onset of oxygenic photosynthesis is controversially debated, sedimentological and geochemical data clearly point at a time around 2.4 Ga, where the atmosphere became slightly oxygenized with values of ca. $>10^{-5}$ PAL. The presumably microbially induced severe drawdown of the greenhouse gases CH_4 and CO_2 and the increase of O_2 resulted in a temperature drop of estimated 4–9°C (Goldblatt et al., 2006), leading directly to the climate disaster of the Paleoproterozoic Huronian snowball earth glaciations at ca. 2.3–2.1 Ga (Kopp et al., 2005).

The GOE is also regarded as an oxygen catastrophe or oxygen crisis. In fact, from the moment on, when oxygenized environments in the oceans and in/on the ocean floors developed, these oxic environments (which progressively increased) were no longer habitable for anoxic microorganisms. Thus, the GOE also marks a first fundamental and irreversible turnover of shelf microbial community structure and, thus, a first truly critical interval in Earth's history.

The Cambrian Explosion

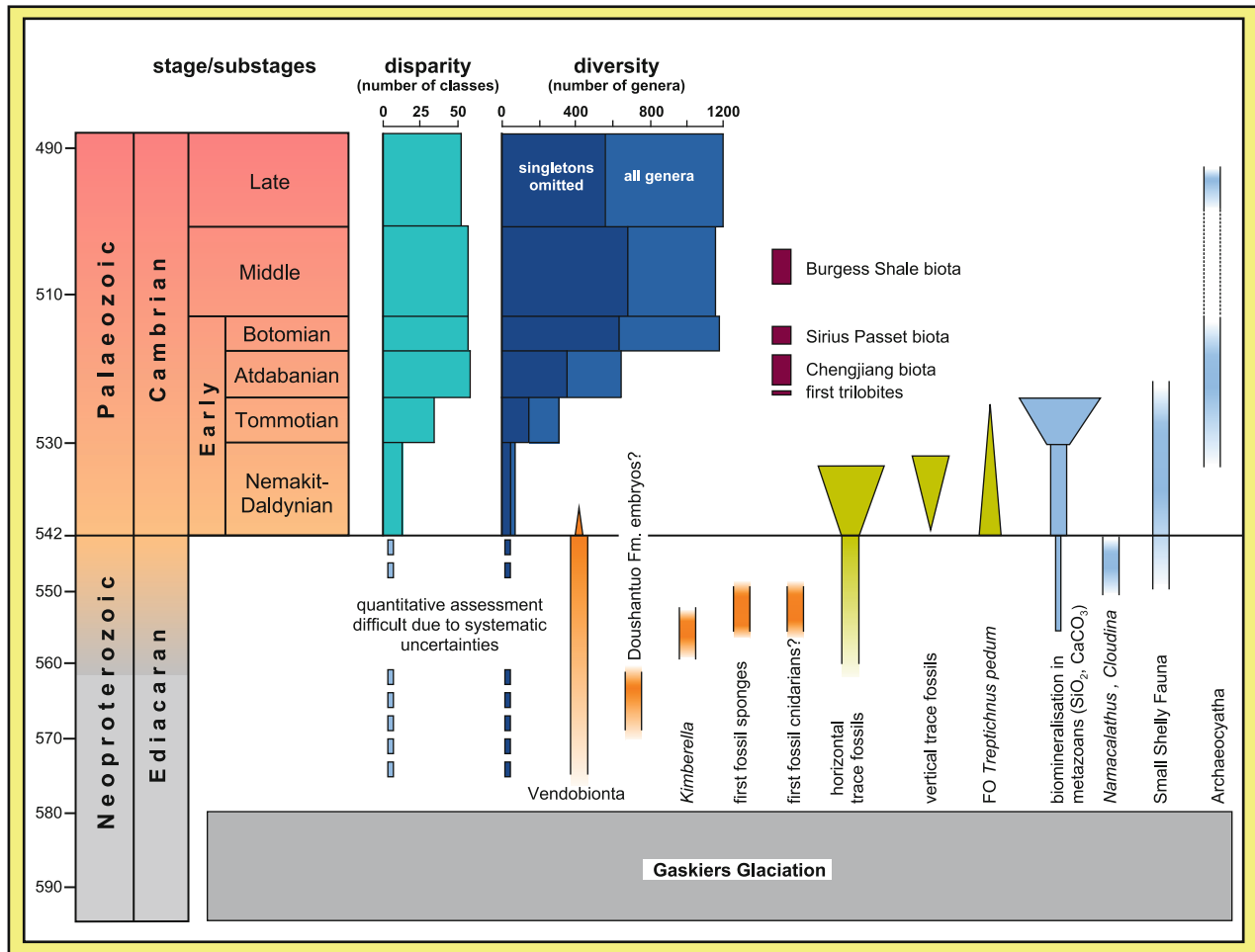
Definition

The Cambrian “Explosion” marks a sudden outburst of morphological disparity (distinctness) and diversity among metazoans during the early Cambrian. Starting around ca. 524 Ma, the number of known genera increased within roughly 10 Ma from something about 200 genera in the Tommotian (Lower Cambrian) to ca. 1,200 genera in the Botomian (highest Lower Cambrian, Figure 3). During this interval, all modern phyla occurred in the sedimentary record, including the vertebrates, thus, being the birth of the “tree of animals” (Shu, 2006). An overview on the ecology of the Cambrian Explosion is given by Zhuravlev and Riding (2001).

The way to the Cambrian Explosion

The explosion of life is impressively documented by the rich and excellently preserved faunas in a number of important Cambrian “fossilagerstätten,” which opened a preservation window to this early period of metazoan evolution and diversification. Maybe, the most famous ones are the Chengjiang and Sirius Passet biota (Lower Cambrian; Figures 4 and 5) and the Burgess Shale biota (Middle Cambrian; Figure 3). There have been repeated discussions in the past, whether we see a true pulse of evolution or whether we face merely exceptional preservational hot-spots, and based on calibration of the molecular clock some authors suggest increasing disparity already around 1 Ga (e.g., Blair and Hedges, 2004). Other molecular clock dating suggests cryptic evolution of the Metazoa in the Cryogenian, but a first radiation pulse occurred at ca. 635–542 My (Peterson et al., 2008). This approximates roughly the first occurrence of animal macrofossils at 575 Ma. However, at the moment, the literature data suggest a common understanding that we see a true expression of metazoan radiation in the fossil record. To appreciate this event in its significance fully, it is desirable to highlight it in its evolutionary context from the first occurrence of larger animal body fossils and trace fossils from ca. 575 Ma on (Figure 3).

The post-GOE times from 2.4 Ga on were a period of evolutionary stasis – the “boring billion” of Kerr (2005) – in which stromatolites had their abundance and diversity peak from ca. 2.0 to 1.0 Ga. Either complex Cyanophyta colonies or possibly the first eukaryotic algae (*Grypania*) occurred by 2.0 Ga, and the first red algae are known since 1.2 Ga (Butterfield et al., 1990). The rise of complex animal life (Metazoa: Parazoa) is dated by some authors around 1 Ga, based on carbonate rock texture (Neuweiler et al., 2009) or by molecular clock dating (e.g., Hedges et al., 2004). After the last Snowball Earth glaciation around 580 Ma (see Hoffman and Schrag, 2002 on the *Snowball Earth* theory), enigmatic body fossil of organisms with unknown taxonomic affinities, the Vendobionta (reaching already sizes up to 2 m!), occurred worldwide at ca. 575 Ma. The Vendobionta show a typical “quilt” bauplan (Figure 6), and they are intimately associated with microbial mats (“elephant skin”), specifically large sulfur bacteria (Steiner and Reitner, 2001). At ca. 560 Ma, unequivocal trace fossils prove the existence of bilaterians (see discussion of problematical trace fossil records from 1 Ga in Jensen, 2003). Dated as 580–570 Ma, the Wengan Phosphorites (Doushantuo Group, China) yielded structures that resemble metazoan embryos, but its metazoan nature has recently been doubted and reinterpreted as phosphatized, large sulfur bacteria with affinities to the *Thiomargarita* group (Bailey et al., 2007). However, possible body fossil of sponge larvae occur also, and by 550 Ma, fossil sponges (Hexactinellida) are known from the Dengying Formation (China). Of the same age is *Kimberella* (White Sea region), which is interpreted as a nonmineralized mollusk, which then marks also the first body fossils of a bilaterian metazoan. The same period



Critical Intervals in Earth History, Figure 3 Summary of evolutionary and paleobiological landmarks in the Upper Proterozoic to Middle Cambrian; mainly based on the compilations of Valentine (2002) and Marshall (2006); with further references therein. Archaeocyatha: dashed lines indicate rare or no records and link to the Upper Cambrian occurrences of Antarctica (Debrenne et al., 1984). Dating of first Cnidaria around 555 Ma after Xiao et al. (2000).

also marks the onset of enzymatic biocalcification. The first mineralized skeletons occur in the form of *Namacalathus* and *Namapoikia* in Namibia around 550 Ma, which could be coralline sponges (Grotzinger et al., 2000; Wood et al., 2002). Calcified tubes of *Cloudina* (Figures 7 and 8) show large resemblance with the deep sea vent and cold seep related *Escarpiia*, a pogonophorid worm. All three taxa occur together within thrombolithic and finely laminated microbial assemblages in the first reef-like structures in the form of small mud mounds (Figure 9).

In the Lower Cambrian (Nemakit-Daldynian), calcareous Archaeocyatha (possible Demospongia) formed the first Cyanophyta/sponge mud mounds. Concomitantly, phosphatic and calcitic microfossils or mineralized sklerites of parts of unknown organisms, the Small Shelly Fauna, SSF, appeared – partly in large abundances – worldwide. Only little later, in the Lower Tommotian, first

shells of brachiopods, mollusks, and arthropods occurred (for an overview of the biotic development prior to the Cambrian Explosion see Valentine, 2002; Xiao and Kaufman, 2007).

Ecologically, most of the Ediacaran period was considered as a peaceful “Garden of Ediacara” by McMenamin (1986), as no evidence of predation is known from this time. Instead, it appears that the biomat-related Vendobionta represented an ecologically and evolutionary very stable steady-state system without any significant innovations for over 30 Ma. Thus, the slowly increasing disparity, which we recognize close to the base of the Cambrian, resulted exclusively from speciation among the Metazoa. Even more, paleobiogeographical and facies affinities (e.g., different species of *Cloudina* occupied different habitats) were now recognizable. And, trouble in paradise became visible by small holes in *Cloudina* – the first evidence of predator–prey interaction (Hua et al., 2003).



Critical Intervals in Earth History, Figure 4 Chengjiang Biota (Lower Cambrian): Anterior parts of *Yunnanzoon lividum* with structures resembling gills or branchial arches. Although previously interpreted as a Chordata, its systematic position is still enigmatic; see also Hou et al. (2004). Scale bar: ca. 10 mm (coll. J. Reitner, GZG Göttingen).

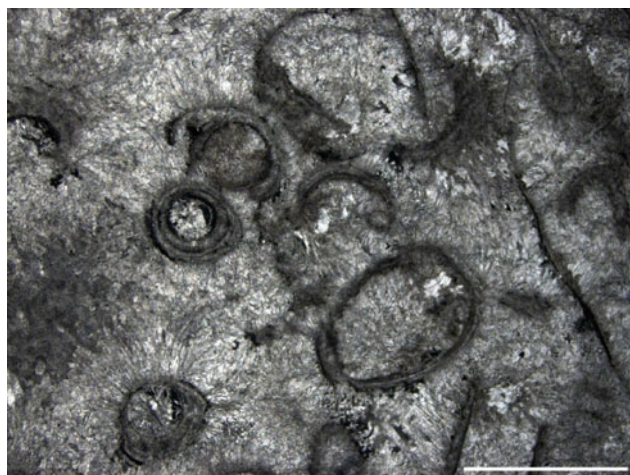


Critical Intervals in Earth History, Figure 5 Chengjiang Biota (Lower Cambrian): The worm *Paleosolex sinensis*, a representative of the Nematophora and a very common element of the Chengjiang fauna; see also Hou et al. (2004). Scale bar: 5 mm (coll. J. Reitner, GZG Göttingen).

The increasing complexity among the Metazoa finds its expression also in progressively more complex burrow systems. While in the Middle Ediacaran sub-biomat miners created simple horizontal furrows on the sediment's surface, terminal Ediacaran burrows became three-dimensional, such as, for example, *Trichophycus pedum*, the biostratigraphical index marker for the base of the Cambrian system (ca. 542 Ma). It is specifically this trend, which was responsible for the first severe extinction among multicellular animals: approximately at the base of the



Critical Intervals in Earth History, Figure 6 Vendobionta: *Ernietta* mass occurrence in small-scale channel deposits, Namibia.



Critical Intervals in Earth History, Figure 7 First calcareous biomineralization: Thin sections of *Cloudina* from the Dridoorvlatke area, Namibia. Cross section; Scale bar: ca. 5 mm.

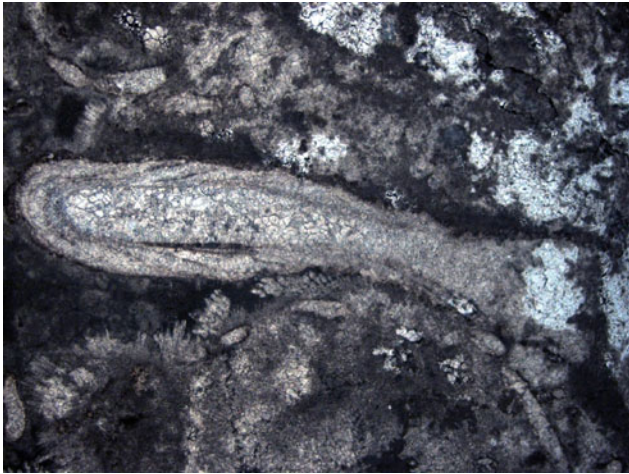
Cambrian, the Vendobionta decreased significantly in abundance to become completely extinct in the Lower Cambrian. Seilacher and Pflüger (1994) suggested that the progressive complexity among infaunal burrowers and burrow intensities resulted in an irreversible destruction of the microbial mats – the essential for the Vendobionta (agronomic revolution).

Possible reasons for the Cambrian Explosion

For the slow but continuous increase of disparity and diversity over ca. 60 Ma in the terminal Ediacaran and its explosive increase around 524 Ma, a number of possible triggering mechanisms are suggested, proposing changes in the abiotic, biotic, and genetic evolutionary landscapes (see, a.o., Marshall, 2006 and Levinton, 2008 for a compilation).

Changes in the abiotic evolutionary landscape (environmental changes)

- (a) *Oxygen level*: An explanation for the increasing disparity in the Ediacaran might be a second oxygenation event to a pO_2 near modern values as deduced from chromium isotopes ($\delta^{33}\text{Cr}$) around 800–542 Ma (Frei et al., 2009) or by 580 Ma (Canfield et al., 2009). Before this time, geographically widespread occurrences of black shales indicate anaerobic deep oceans: due to the aerobic continental weathering of detrital pyrite and, resulting, riverine sulfate input into the



Critical Intervals in Earth History, Figure 8 First calcareous biomineralization: Thin sections of *Cloudina* from the Dridoorrvlakte area, Namibia. Latitudinal section; Same magnification like Fig. 7.

oceans, its bacterial reduction caused deep ocean saturation of H_2S (“Canfield Ocean,” see Canfield, 1999; Figure 2). This caused a reduction of iron, copper, and molybdenum, all less soluble under the presence of hydrogen sulfide. Because these play important roles in the enzymatic metabolism of the Cyanophyta, the depletion of these elements caused malnourishment, which might have blocked evolutionary advance for more than a billion years. Only after the last episode of the Snowball Earth period (Gaskiers Glaciation), molybdenum isotopes indicate a second oxygenation event and progressively oxygenized deeper part of the oceans. This could have increased the capacity for metabolism of larger Metazoa and the evolution of the Vendobionta and first metazoans can be seen in the light of this atmospheric/oceanic change. However, the long delay between the terminal Proterozoic oxygenation event and the Cambrian Explosion of estimated 50–60 Ma makes a direct link unlikely. On the other hand, the increased O_2 amount doubtlessly acted as an indispensable prerequisite for size increase and evolution of the Metazoa.

- (b) *Global carbon cycle*: In the Vendian/Cambrian boundary interval, numerous published $\delta^{13}\text{C}$ curves show vivid fluctuations and a negative $\delta^{13}\text{C}$ peak with up to -12 per mil in the Upper Ediacaran. The observed $\delta^{13}\text{C}$ fluctuations are surely expression of heavy perturbations in the global carbon cycle and, thus, indicate environmental disturbances. There is a wealth on literature on this problematic, and variety of complex and differing explanation models exists, as illustrated by two examples: For the terminal Ediacaran negative $\delta^{13}\text{C}$ peak, Kirschvink and Raub



Critical Intervals in Earth History, Figure 9 Latest Proterozoic mud mounds, exhumed by erosion, with *Namacalathus* (sponge?), *Cloudina*, and thrombolithic microbial limestones. Swartkloof area, Namibia.

(2003) postulated methane release, resulting in a rise of global temperatures as a triggering pulse for the Cambrian Explosion, while Bristow and Kennedy (2008) suggested that the negative excursion resulted from a non-steady-state oxidation of oceanic dissolved organic carbon (DOC), resulting from a rise of atmospheric oxygen (see above).

- (c) *Ocean water chemistry*: The ocean water geochemistry changed progressively from a Proterozoic soda ocean towards a late Proterozoic halite ocean during the Ediacaran (e.g., Hardie, 2003). As seawater carbonate alkalinity triggers the possibility of Ca^{2+} extraction, the decreasing pH values progressively facilitated enzymatic CaCO_3 mineralization. Geochemical data from terminal Proterozoic (~544 Ma) and early Cambrian (~515 Ma) marine halite indicate that seawater Ca^{2+} concentrations increased approximately threefold during the early Cambrian (Brennan et al., 2004). A high Ca^{2+} and low $[\text{SO}_4]^{2-}$ composition of the early Cambrian sea water is recorded by Petrychenko et al. (2005), which then could be responsible for the sudden occurrence of organisms with strongly mineralized shells and sklerites consisting of CaCO_3 .
- (d) *Global sea-level and paleoceanography*: The terminal Ediacaran is a period of global sea-level rise, and the break-up of Rodinia from ca. 850 Ma on created a huge amount of environmentally differentiated shelf areas, which became flooded during this interval. These provided an immense potential for specialization and diversification of shelf organisms. However, from the Ediacaran through the Cambrian, there is a continuous flooding of shelf areas (Miller et al., 2005; Haq and Schutter, 2008), and there seems to be no or only little relation to the Cambrian Explosion.

Changes in genetic and developmental capacities

With the first occurrence of the Bilateria, the bilaterian development system had developed. When we accept the Ediacaran *Kimberella* as a molluscan Bilateria, then the bilaterian development system must predate the Cambrian Explosion significantly. As a small increase of genomic complexity can result in high morphological diversity, the Cambrian Explosion cannot exclusively be explained by developmental innovations (see compilation and discussion in Marshall (2006).

Changes in the biotic environment

Interactions among organisms is difficult to recognize in the fossil record. However, predator–prey relations could be reconstructed in some cases as we see traces of predation, manifested by damages in mineralized skeletons. The first record of predation is known from small holes in *Cloudina*, bioerosive evidence of predation. With the arthropod *Anomalocaris* from the Lower Cambrian (Chengjiang Biota), a first top predator occurred. This shows that predation pressure and the need to predate had well established, which opened a wide window for

any imaginable evolutionary interactions and its morphological manifestation in the form of a biological arms race.

Biomineralization

After all, the Cambrian Explosion is phenologically also an expression of increased biomineralization, why it is also a biomineralization event. We have no knowledge about diversity of any nektonic and benthic nonmineralized bilaterians before the Cambrian Explosion, because they are simply not preserved. To argue that the increase of trace fossil diversity matches that of the body fossil diversity is in a strict sense not feasible because trace fossils are expression of behavior, and it is impossible to deduce body fossil diversity from trace fossil diversity (the same type of trace fossil can be generated by several organisms of different groups).

Concluding remarks

The rise of the Metazoa and increasing bioturbation of the sea floor resulted in the extinction of the Vendobionta – the first period of multicellular animals extinction in Earth's history (if one accepts that the Vendobionta are multicellular eukaryota). The succeeding Cambrian Explosion is still difficult to interpret. Although there are a number of possible explanations for environmental and biological changes during the Lower Cambrian, none of the mechanisms alone provides sufficient argument to exclusively explain the explosive radiation of invertebrates within this short period of only ca. 10 Ma so far.

The rise of land plants

Generalities

The first multicellular marine plants are known since ca. 1.2 Ga (Butterfield et al., 1990; Figure 2), and although molecular data suggest some kind of very primitive land plants around 700 Ma (Heckman et al., 2001), it took further 230 Ma until first multicellular complex land plants (Embryophyta) occurred in the Lower(?) Middle Ordovician (Darriwillian, ca. 465 Ma). These records are phytodebris and cryptospores with uncertain affinities (Steemans and Wellman, 2004; Figure 1). In the Upper Ordovician to Lower Silurian, cryptospore diversity and abundance decreased significantly, while trilete/hilate spores became frequent, radiated, and finally, the first vascular plant remains occurred in the Silurian (Wenlock) ca. 40 Ma later (e.g., Wellman et al., 2003; Gensel, 2008). A first spectacular fossilagerstätte of advanced Embryophyta is the Lower Devonian Rhynie Chert with excellently preserved cellular structures (Figure 10), and it is possibly the best investigated case study of an early terrestrial ecosystem (see Wellman, 2004; Taylor et al., 2005; with additional references therein). Still in the Devonian, first seed plants, trees and multi-storied forests occurred. Overviews over the (early) land plant evolution can be found, a.o., in Graham et al. (2000), Gensel and Edwards (2001) or Gensel (2008).



Critical Intervals in Earth History, Figure 10 Thin section of a piece of Rhynie Chert. Well visible are the excellently preserved cellular structure of the silicified plants remains (*Aglaophyton major*). (Stem cross section ca. 3 mm). (coll. J. Reitner, GZG Göttingen).

Associated environmental changes

There can be no doubt that the occupation of land with its succeeding evolutionary evolutions over ca. 60–80 Ma represents possibly the most significant step in plant evolution since the development of the first eukaryotic plant cell. However, the progressively increasing occupation of the continents by land plants resulted also in significant changes in Paleozoic ecosystem structures and had severe impact on a number of geological processes such as weathering, the development of the atmosphere, and global carbon and hydrological cycles.

Development of land animals

The spread of land plants represented a first significant occurrence and increase of terrestrial primary productivity and, thus, the prerequisite for animal life on the continents. However, DiMichele and Hook (1992) suggested that there existed no direct positive correlation between land plant and early land animal evolution in the sense of herbivory. Instead, the biomass affected the animal food web *via* a detritus chain. The increasing complexity of terrestrial animal ecosystems can be deduced by the progressive diversification of arthropods as well as trace fossil abundance in paleosoils and continental deposits from the Ordovician on (see Retallack and Feakes, 1987; Buatois et al., 1998), and the occurrence of simple food webs are suggested by the finds of predatory arthropods already for the Silurian (Jeram et al., 1990).

Effect on continental weathering, erosion and nutrients

The evolution of plants and the occurrence of deep roots triggered the development of paleosoils, which are known since the Ordovician (see overview in Retallack, 2005). The roots favored physical weathering, and the evolution

of root symbionts such as mycorrhizae acted as a chemical weathering agents. A further pedogenic factor is the increasing amount of bioturbation, which, by its large surface and permeability, favored chemical weathering. The development of soils advanced likewise erosion and an increased continental run-off of nutrients in to the oceans (see Algeo and Scheckler, 1998).

Effect on the atmospheric composition

With the occurrence of land plants, a feedback system between atmospheric CO₂ evolution and land plants established (see Beerling and Berner, 2005). The withdrawal of CO₂ by photosynthesis and by increased weathering of Ca–Mg silicates resulted in progressive drop of atmospheric *p*CO₂ from the Silurian on (Beerling and Berner, 2005).

Concluding remarks

Although the development of continental ecosystems is much less appreciated in the literature than marine environments, the significance of the land plant evolution is enormous. Apart from the above-mentioned geological aspects, the development of continental primary productivity was the base for animal life on land and the progressive establishment of complex ecosystems as we see them today. Plants did the first step, animals followed. The shore leave of the vertebrates in the Devonian would not have been possible without the occurrence of land plants.

Extinction events

Generalities

An extinction event is the – in a geological sense – abrupt decrease of speciation, diversity, and abundance within a geologically very short time. It affects all groups of organisms at various degrees. As mentioned above, we do not consider extinction events as classical critical intervals, as we never see the extinction, or in the aftermath of extinction, the entry, of new phyla. Instead, we see something like a phylogenetic steady-state, where even after heavy extinction no evolutionary playground for complete innovations at high levels was created.

The “Big Five”

Classically, five main extinction events are distinguished in the Phanerozoic, in the literature referred to as “the Big Five.” These are (Figure 1)

1. The Upper Ordovician event with an estimated extinction of ca. 85% of the marine species and ca. 50% of marine genera (see overview in Krug and Patzkowsky, 2004 and Sheehan, 2001).
2. The Upper Devonian Kellwasser and Hangenberg events (Frasnian/Famennian boundary) with an extinction rate of ca. 20% of all animal families and 70–80% of all animal species (see overview in McGhee, 1996; Over et al., 2005; Becker and Kirchgasser, 2007).
3. The Permian–Triassic boundary (P/Tr boundary event) with an extinction of ca. 95% of the shelf biota and

65–70% of the vertebrates. Nicknamed “The Big Dying,” it was the most dramatic mass extinction in the Phanerozoic (see overview in White, 2002; Over et al., 2005; Şengör and Atayman, 2009).

4. At the Triassic–Jurassic boundary (Tr/J boundary event) with a loss of ca. 77% of all species and ca. 41% of all mesobenthic and macrobenthic genera (see overview in Tanner et al., 2004; Hesselbo et al., 2007).
5. At the Cretaceous/Tertiary boundary (K/T boundary event). This event caused extinction of ca. 65% among the species. Among the victims were so popular groups such as ammonoids and dinosaurs (see overview in Ryder et al., 1996; MacLeod et al., 1997).

Causes

There exists a flood of ideas and literature about the possible causes of mass extinctions with complex interactions and feedback mechanisms. Here, some of the popular concepts will be briefly introduced, which either focus at the trigger or the kill mechanisms.

Impact events. The trend-setting discovery was the recognition of an iridium anomaly at the K/T boundary Event by Alvarez et al. (1980), who suggested that a bolides’ impact was responsible for the end-Cretaceous extinction of dinosaurs and a great number of typical Cretaceous marine invertebrates such as ammonites, belemnites, inoceramids, and rudist bivalves. Later, tektites (impact glasses) were discovered in the boundary interval in numerous sections worldwide. Today, the Chicxulub structure on the Yucatan peninsula of Mexico (ca. 170 km diameter) is seen as the associated impact structure, although there is still some controversy about the stratigraphical fit of the extinction and the dating of the impact (Keller et al., 2007). The inferred relation between mass extinction and large impacts resulted in a search of impact evidences such as craters, spherule layers, iridium anomalies, or certain noble gases in fullerenes that correlate stratigraphically with other large extinction events. The Siljan impact structure in Sweden (diameter: ca. 55 km, age: ca. 368 Ma) was shown to correlate with the Upper Devonian Kellwasser-Events, from which also microtektites are known (Claeys et al., 1996). In the case of the Permian/Triassic boundary interval (ca. 250 Ma), the dating of the Araguinha structure of Brazil (diameter ca. 40 km, age: ca. 445.5 $\pm$ 3.5 Ma: Hammerschmidt and Engelhardt, 1995) shows good temporal accordance. For the Triassic/Jurassic boundary extinction (ca. 199.6 Ma, Gradstein et al., 2004), the Manicouagan impact structure, Quebec, Canada (diameter: ca. 100 km) was made responsible (Olsen et al., 1987). However, refined dating shows that its age is 215.5 (Ramezani et al., 2005), postdating the extinction level significantly (see Kelley, 2007 for a discussion of the problematical stratigraphical accuracy of impact – mass extinction correlation). Possible killer are believed to be direct physical effects such as shock waves, heat and fire, impact winter, succeeding warming, collapse of the food chain, or environmental acidification. However, it

appears that of the Big Five only for the K/T boundary extinction, an impact as a possible cause is still seriously discussed.

Flood basalt volcanism. Catastrophic outflow of gigantic flood basalts (continental flood basalt provinces, large igneous provinces) co-occur with the Permian/Triassic boundary event (Siberian flood basalts), the K/T boundary event (Deccan Trapps, India) and the Triassic/Jurassic boundary event (Central Atlantic Magmatic Province, CAMP). They are believed to have triggered mass extinction by the production of large amounts of CO₂ (e.g., Fraiser and Bottjer, 2007) and SO₂ either *via* climate changes resulting in the either warming or cooling or *via* feedback loops causing oceanic anoxia or gas hydrate release (see Wignall, 2001 for a summary and Kelley, 2007).

Anoxic events. Oceanic anoxic events, indicated by the occurrence of laminated marine black shales, are common phenomena in Earth’s history and were made responsible for some extinction events such as the Kellwasser events (late Devonian), the P/Tr and Tr/J boundary events and some subordinate extinctions such as the Cenomanian/Turonian boundary event. For the P/Tr boundary event, even photic zone anoxia was suggested, caused by release of deep-sea originated hydrogen sulfide to the surface (Kump et al., 2005; Meyer et al., 2008; the superanoxic event of Grice et al., 2004). However, oceanic anoxia alone fails to explain the extinction of land plants and animals.

H₂S release into the atmosphere. In the cause of massive oceanic anoxia, hydrogen sulfide is believed to have been released into the atmosphere. H₂S toxicity and hypercapnia are suggested as possible killing mechanisms (Meyer et al., 2008).

Explosive methane release. The release of methane is believed to be an associated process of catastrophic global warming in the context of catastrophic volcanisms and greenhouse gas emission (s.o). However, Ryskin (2003) suggested in the case of the P/Tr boundary event that the explosive release of methane by the collapse and instability of gas hydrates brought anoxic deep waters with hydrogen sulfide to the surface, causing mass extinction of the shelf biota.

Glaciations, warming, cooling. The succession of a severe cooling, followed by warming was made responsible for the end-Ordovician extinction. The model proposes an equator-shift of boreal taxa and extinction of warm-water fauna. *Vice versa*, in the next step, warming caused a poleward-shift of warm waters and extinction among cold-water faunas. In addition, the associated glacioeustatic sea-level low diminished shelf areas and caused stress on the shelf biota (see discussion in Hallam and Wignall, 1997 and an ecological approach in Twitchett, 2006). The reasons for Phanerozoic glaciations are not always clear to elaborate; in the case of the late Ordovician glaciation, withdraw of atmospheric CO₂ as a result of increased continental weathering due to volcanism-induced atmospheric SO₂ emission is suggested (Young et al., 2009).

Sea-level changes. Sea-level changes are observed to co-occur with mass extinctions and are suggested to be a possible triggering mechanism. However, while for the Ordovician a glacio-eustatic sea-level low is suggested to be responsible by some authors (see above), strong transgression with the establishment of anoxia is suggested by others (e.g., Kellwasser Event: Bond and Wignall, 2008). There is no universal model for how sea-level changes effects extinction. A more general discussion on that topic is given by Peters (2008).

Gamma ray burst. For the end-Ordovician extinction, a gamma ray burst was suggested by Melott et al. (2004). It resulted in a depletion of the ozone layer and increased UV radiation, which is believed to be lethal to numerous groups of surface-dwelling organisms and the phytoplankton. Furthermore, a gamma ray burst can produce atmospheric opaque nitrogen dioxide, resulting in an increased back-scatter of sunlight, which provides a feasible mechanism for a global cooling.

Urease dead zones. Wooldbridge (2008) suggested that the pH dependency of the enzyme urease might be the explanation for the end-Devonian, the P/Tr, the Tr/J and the K/T boundary events. Urease catalyzes the hydrolysis of urea into ammonia and CO₂. It enables organisms to access urea as a source for metabolism, and it is a very important enzyme for biomineralization of carbonate and nitrogen accessibility during early stages in ontogenetic development. It is equally important in numerous plants, bacteria, fungi, and invertebrates, but not the vertebrates. At pH 7.9, the enzyme becomes inactivate, forming a dead zone between some pH optima. Thus, any sudden or long-term changes in the CO₂ concentration (e.g., flood basalt volcanisms), and, resulting, fluctuations of pH values crossing the magic 7.9 boundary, might potentially be phases of extinction.

Halogenated gas emission. Based on recent investigations in evaporitic environments, Weissflog et al. (2008) suggested that during evaporation in the central European Zechstein basin (South Permian Basin; Upper Permian) bacterially-derived, toxic volatile halogenated hydrocarbons (HCC; e.g., chloroform, trichloroethylene, tetrachlorethylene) emitted into the atmosphere. They damaged the ozone layer and – by increased UV radiation – the Permian biosphere. Likewise, the phytotoxicologic effect of the HCC provided a direct kill mechanism for Permian biota.

Concluding remarks

When reviewing the literature it becomes clear that for the “Big Five” no single cause mechanism is accepted (Elewa, 2008). A number of possible “killer” are discussed for each period of mass extinction, and in the case of the Permian/Triassic event, 6 potential suspects (cooling, warming, anoxia, volcanism, impact, methane release) were summarized by White (2002). Even the K/T boundary event is still split into two groups, one favoring an impact, the other flood basalt volcanism.

Overviews on the possible triggering mechanisms, with explanations of the complex feedback interrelations based on case studies can be found, for example, in Ryder et al. (1996), Wignall (2001), Taylor (2004), Hallam (2005) and Twitchett (2006), and some data on the biotic response in the context of mass extinctions can be found in Hart (1996) or Hallam and Wignall (1997). The still large number of newly published papers and debates show that extinction is still a hot topic, and while some theories will turn out to be persistent, other will be volatile.

Bibliography

- Algeo, T. J., and Scheckler, S. E., 1998. Terrestrial-marine teleconnections in the Devonian: links between the evolution of land plants, weathering processes, and marine anoxic event. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **353**, 113–130.
- Alvarez, L. W., Alvarez, W., Asaro, F., and Michel, H. V., 1980. Extraterrestrial cause for the Cretaceous-tertiary extinction. *Science*, **208**, 1095–1108.
- Anbar, A. D., Duan, Y., Lyons, T. W., Arnold, G. L., Kendall, B., Creaser, R. A., Kaufman, A. J., Gordon, G. W., Scott, C., Garvin, J., and Buick, R., 2007. A whiff of oxygen before the Great Oxidation Event? *Science*, **317**, 1903–1906.
- Bailey, J. V., Joye, S., Kalanetra, K. M., Flood, B. E., and Corsetti, F. A., 2007. Evidence of giant sulphur bacteria in Neoproterozoic phosphorites. *Nature*, **445**, 198–201.
- Bekker, A., Holland, H. D., Wang, P.-L., Rumble, III, D., Stein, H. J., Hannah, J. L., Coetzee, L. L., and Beukes, N. J., 2004. Dating the rise of atmospheric oxygen. *Nature*, **427**, 117–120.
- Becker, R. T., and Kirchgasser, A. T., 2007. Devonian events and correlations. *The Geological Society of London, Special Publication*, **278**, 1–280.
- Beerling, D. J., and Berner, D. A., 2005. Feedbacks and the coevolution of plants and atmospheric CO₂. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 1302–1305.
- Berner, R. A., 1998. The carbon cycle and carbon dioxide over Phanerozoic time: the role of land plants. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **353**, 75–82.
- Blair, J. A., and Hedges, B., 2004. Molecular clocks do not support the Cambrian Explosion. *Molecular Biology and Evolution*, **22**, 387–390.
- Bond, D. P. G., and Wignall, P. B., 2008. The role of sea-level change and marine anoxia in the Frasnian–Famennian (Late Devonian) mass extinction. *Paleogeography, Paleoclimatology, Paleogeology*, **263**, 107–118.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Bristow, T. F., and Kennedy, M. J., 2008. Carbon isotope excursions and the oxidant budget of the Ediacaran atmosphere and ocean. *Geology*, **36**, 863–866.
- Buatois, L. A., Mangano, M. G., Genise, J. F., and Taylor, T. N., 1998. The ichnologic record of the continental invertebrate invasion; evolutionary trends in environmental expansion, ecospace utilization, and behavioral complexity. *Palaaios*, **13**, 217–240.
- Buick, R., 2008. When did oxygenic photosynthesis evolve? *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 2731–2743.
- Butterfield, N. J., Knoll, A. H., and Sweet, K., 1990. A bangiophyte red alga from the Proterozoic of Arctic Canada. *Science*, **250**, 104–107.

- Canfield, D. E., 1999. A new model for Proterozoic ocean chemistry. *Nature*, **396**, 450–453.
- Canfield, D. E., Poulton, S. W., and Narbonne, G. M., 2009. Late Neoproterozoic deep-ocean oxygenation and the rise of animal life. *Science*, **315**, 92–95.
- Claeys, P., Kyte, F. T., Herbolch, A., and Casier, J.-G., 1996. Geochemistry of the Frasnian-Famennian boundary in Belgium: Mass extinction, anoxic oceans and microtektite layer, but not much iridium? In Ryder, G., Fastovsky, D., and Gartner, S. (eds.), *The Cretaceous-Tertiary Event and Other Catastrophes in Earth History*. Geological Society of America Special Paper 307, pp. 491–504.
- Debrenne, F., Rozanov, A. Y., and Webers, G. F., 1984. Upper Cambrian Archaeocyatha from Antarctica. *Geological Magazine*, **121**, 291–299.
- DiMichele, W. A., and Hook, A. W., 1992. Paleozoic terrestrial ecosystems. In Behrensmeyer, A. K., Damuth, J. D., DiMichele, W. A., Potts, R., Sues, H. D., and Wing, S. L. (eds.), *Terrestrial Ecosystems through Time. Evolutionary Paleocology of terrestrial Plants and Animals*. Chicago, IL: The University Press of Chicago, pp. 205–326.
- Elewa, A. M. T. (ed.), 2008. *Mass Extinction*. Berlin: Springer.
- Falkowski, P. G., and Godfrey, L. V., 2009. Electrons, life and the evolution of Earth's oxygen cycle. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **363**, 2705–2716.
- Farquhar, J., and Wing, B. A., 2003. Multiple sulfur isotopes and the evolution of the atmosphere. *Earth and Planetary Science Letters*, **213**, 1–13.
- Fraiser, M. L., and Bottjer, D. J., 2007. Elevated atmospheric CO₂ and the delayed biotic recovery from the end-Permian mass extinction. *Paleogeography, Paleoclimatology, Paleocology*, **252**, 164–175.
- Frei, R., Gaucher, C., Poulton, S. W., and Canfield, D. E., 2009. Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. *Nature*, **461**, 250–254.
- Gensel, P. G., 2008. The earliest land plants. *Annual Review of Ecology, Evolution, and Systematics*, **39**, 459–477.
- Gensel, P. G., and Edwards, D., 2001. *Plants Invade the Land – Evolutionary and Environmental Perspectives*. New York: Columbia University Press.
- Goldblatt, C., Lenton, T. M., and Watson, A. J., 2006. Bistability of atmospheric oxygen and the Great Oxidation. *Nature*, **443**, 683–686.
- Gradstein, F. M., Ogg, J. G., and Smith, A. G., 2004. *A Geologic Time Scale 2004*. Cambridge: Cambridge University Press.
- Graham, L. E., Cook, M. E., and Busse, J. S., 2000. The origin of plants: Body plan changes contributing to a major evolutionary radiation. *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 4535–4540.
- Grice, K., Cao, C., Love, G. D., Böttcher, M. E., Twitchett, R. J., Grosjean, E., Summons, R. E., Turgeon, S. C., Dunning, W., and Jin, Y., 2004. Photic zone euxinia during the Permian-Triassic superanoxic event. *Science*, **307**, 706–706.
- Grotzinger, J. P., Watters, W. A., and Knoll, A. H., 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology*, **26**, 334–359.
- Guo, Q., Strauss, H., Kaufman, A. J., Schröder, S., Gutzmer, J., Wing, B., Baker, M. A., Bekker, A., Jin, Q., Kim, S.-T., and Farquhar, J., 2009. Reconstructing Earth's surface oxidation across the Archean-Proterozoic transition. *Geology*, **37**, 399–402.
- Hallam, A., 2005. *Catastrophes and Lesser Calamities: The Causes of Mass Extinctions*. Oxford: Oxford University Press.
- Hallam, A., and Wignall, P. B., 1997. *Mass Extinctions and Their Aftermath*. Oxford: Oxford University Press.
- Hammerschmidt, K., and Engelhardt, W., 1995. ⁴⁰Ar/³⁹Ar dating of the Araguinha impact structure, Mato Grosso, Brazil. *Meteoritics*, **30**, 227–233.
- Haq, B. U., and Schutter, S. R., 2008. A chronology of Paleozoic sea-level changes. *Science*, **322**, 64–68.
- Hardie, L. A., 2003. Secular variations in Precambrian seawater chemistry and the timing of Precambrian aragonite seas and calcite seas. *Geology*, **31**, 785–788.
- Hart, M. B. (ed.), 1996. Biotic recovery from mass extinction events. *Geological Society of London, Special Publication*, **102**, 1–392.
- Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., and Hedges, S. B., 2001. Molecular evidence for the early colonization of land by fungi and plants. *Science*, **293**, 1129–1133.
- Hedges, S. B., Blair, J. E., Venturi, M. L., and Shoe, J. L., 2004. A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evolutionary Biology*, **4**, doi: 10.1186/1471-2148-4-2.
- Hesselbo, S. P., Christopher, A., McRoberts, C. A., and Palfy, J., 2007. Triassic-Jurassic boundary events: problems, progress, possibilities. *Paleogeography, Paleoclimatology, Paleocology*, **244**, 1–424.
- Hinrichs, K.-U., and Boetius, A., 2002. The anaerobic oxidation of methane: new insights in microbial ecology and biogeochemistry. In Wefer, G., Billett, D., Hebbeln, D., Jørgensen, B. B., Schlüter, M., and van Weering, T. (ed.), *Ocean Margin Systems*. Berlin, Heidelberg: Springer, pp. 457–477.
- Hoffman, P. F., and Schrag, D. P., 2002. The snowball Earth hypothesis: testing the limits of global change. *Terra Nova*, **14**, 129–155.
- Holland, H. D., 2006. The oxygenation of the atmosphere and oceans. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **361**, 903–915.
- Hou, X.-G., Aldridge, R. J., Bergström, J., Siveter, D. J., and Feng, X.-H., 2004. *The Cambrian Fossils of Chengjiang, China*: Blackwell Science Ltd, p 233.
- Hua, H., Pratt, B. R., and Zhang, L.-Y., 2003. Borings in *Cloudina* shells: complex predator-prey dynamics in the terminal Neoproterozoic. *Palaio*, **18**, 454–459.
- Jensen, S., 2003. The Proterozoic and earliest Cambrian trace fossil record; patterns, problems and perspectives. *Integrative and Comparative Biology*, **43**, 219–228.
- Jeram, A. J., Selden, P. A., and Edwards, D., 1990. Land animals in the Silurian: arachnids and myriapods from Shropshire, England. *Science*, **250**, 658–661.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, **33**, 865–868.
- Kaufman, A. J., Johnston, D. T., Farquhar, J., Masterson, A. L., Lyons, T. W., Bates, S., Anbar, A. D., Arnold, G. L., Garvin, J., and Buick, R., 2007. Late Archean biospheric oxygenation and atmospheric evolution. *Science*, **317**, 1900–1903.
- Keller, G., Adatte, T., Berner, Z., Harting, M., Baum, G., Prauss, M., Tantawy, A., and Stueben, D., 2007. Chicxulub impact predates K–T boundary: new evidence from Brazos, Texas. *Earth and Planetary Science Letters*, **255**, 339–356.
- Kelley, S., 2007. The geochronology of large igneous provinces, terrestrial impact craters, and their relationship to mass extinctions on Earth. *Journal of the Geological Society*, **164**, 923–936.
- Kerr, R. A., 2005. The story of O₂. *Science*, **308**, 1730–1732.
- Kirschvink, J. L., and Raub, T. D., 2003. A methane fuse for the Cambrian explosion: carbon cycles and true polar wander. *Comptes Rendus Geosciences*, **335**, 65–78.
- Klein, C., 2005. Some Precambrian banded iron-formations (BIFs) from around the world: Their age, geologic setting, mineralogy, metamorphism, geochemistry, and origins. *American Mineralogist*, **90**, 1473–1499.
- Konhauser, K. O., Pecoits, E., Lalonde, S. V., Papineau, D., Nisbet, E. G., Barley, M. E., Arndt, N. T., Zahnle, K., and Kamber, B. S.,

2009. Oceanic nickel depletion and a methanogen famine before the Great Oxidation Event. *Nature*, **458**, 750–753.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A., and Nash, C. Z., 2005. The Paleoproterozoic snowball earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 11131–11136.
- Krug, A. Z., and Patzkowsky, M. E., 2004. Rapid recovery from the Late Ordovician mass extinction. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 17605–17610.
- Kump, L. R., Pavlov, A., and Arthur, M. A., 2005. Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology*, **33**, 397–400.
- Levinton, J. S., 2008. The Cambrian explosion: How do we use the evidence. *BioScience*, **58**, 855–864.
- MacLeod, N., Rawson, P. F., Forey, P. L., Banner, F. T., Boudagher-Fadel, M. K., Bown, R. R., Burnett, J. A., Chambers, P., Culver, S., Evans, S. E., Jeffrey, C., Kaminski, M. A., Lord, A. R., Milner, A. C., Milner, A. R., Morris, N., Owen, E., Rosen, B. R., Smith, A. B., Taylor, P. D., Urquhart, E., and Young, J. R., 1997. The cretaceous–tertiary biotic transition. *Journal of the Geological Society of London*, **154**, 265–292.
- Marriott, S. B., Morrissey, L. B., and Hillier, R. D., 2009. Trace fossil assemblages in Upper Silurian tuff beds: evidence of biodiversity in the Old Red Sandstone of southwest Wales, UK. *Paleogeography, Paleoclimatology, Paleocology*, **274**, 160–172.
- Marshall, C. R., 2006. Explaining the Cambrian “Explosion” of animals. *Annual Review of Earth and Planetary Sciences*, **34**, 355–84.
- McGhee, G. R., 1996. *The Late Devonian Mass Extinction: The Frasnian/Famennian Crisis*. New York: Columbia University Press.
- McMenamin, M. A. S., 1986. *The Garden of Ediacara*. New York: Columbia University Press.
- Melott, A. L., Lieberman, B. S., Laird, C. M., Martin, L. D., Medvedev, M. V., Thomas, B. C., Cannizzo, J. K., Gehrels, N., and Jackman, C. H., 2004. Did a gamma-ray burst initiate the late Ordovician mass extinction? *International Journal of Astrobiology*, **3**, arXiv:astro-ph/0309415v3.
- Meyer, K. M., Kump, L. R., and Ridgwell, A., 2008. Biogeochemical controls on photic-zone euxinia during the end-Permian mass extinction. *Geology*, **36**, 747–750.
- Miller, K. G., Kominz, M. A., Browning, J. V., Wright, J. D., Mountain, G. S., and Katz, M. E., 2005. The phanerozoic record of global sea-level change. *Science*, **310**, 1293–1289.
- Neuweiler, F., Turner, E. C., and Burdige, D. J., 2009. Early Neoproterozoic origin of the metazoan clade recorded in carbonate rock texture. *Geology*, **37**, 475–478.
- Olsen, P. E., Shubin, N. H., and Anders, M. H., 1987. New Early Jurassic tetrapod assemblages constrain Triassic-Jurassic tetrapod extinction event. *Science*, **237**, 1025–1029.
- Over, D. J., Morrow, J. R., and Wignall, P. B., 2005. Understanding Late Devonian and Permian-Triassic biotic and climatic events: towards an integrated approach. *Developments in Paleontology and Stratigraphy*, **20**, 1–337.
- Peters, S. E., 2008. Environmental determinants of extinction selectivity in the fossil record. *Nature*, **454**, 626–629.
- Peterson, K. J., Cotton, J. A., Gehling, J. G., and Pisani, D., 2008. The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philosophical Transactions of the Royal Society B: Biological Sciences*, doi: 10.1098/rstb.2007.2233.
- Petrychenko, O. Y., Peryt, T. M., and Chechel, E. I., 2005. Early Cambrian seawater chemistry from fluid inclusions in halite from Siberian evaporites. *Chemical Geology*, **219**, 149–161.
- Ramezani, J., Bowring, S. A., Pringle, M. S., Winslow III, F. D., and Rasbury, E. T., 2005. The Manicouagan impact melt rock: a proposed standard for the intercalibration of U–Pb and $^{40}\text{Ar}/^{39}\text{Ar}$ isotopic systems. *Geochimica Cosmochimica Acta*, **69**(Suppl), 321.
- Retallack, G. J., 2005. Soils and global change in the carbon cycle. In Drever, J. I. (ed.), *Surface and Ground Water, Weathering, and Soils*. Treatise on Geochemistry, Vol. 5, pp. 581–606.
- Retallack, G. J., and Feakes, C. R., 1987. Trace fossil evidence for Late Ordovician animals on land. *Science*, **235**, 61–63.
- Ryder, G., Fastovsky, D. E., and Gartner, S., 1996. The Cretaceous-Tertiary event and other catastrophes in earth history. *Geological Society of America, Special Papers*, **307**, 1–569.
- Ryskin, G., 2003. Methane-driven oceanic eruptions and mass extinctions. *Geology*, **31**, 741–744.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X., and Anbar, A. D., 2008. Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature*, **452**, 456–459.
- Seilacher, A., and Pflüger, F., 1994. From biotoms to benthic agriculture: a biohistoric revolution. In Krumbein, W. E. (ed.), *Biostabilization of Sediments*. Oldenburg: BIS Verlag, pp. 97–105.
- Şengör, A. M. C., and Atayman, S., 2009. The Permian extinction and the Tethys: an exercise in global geology. *The Geological Society of America, Special Paper*, **448**, 1–85.
- Sheehan, P., 2001. The Late Ordovician mass extinction. *Annual Review of Earth and Planetary Sciences*, **29**, 331–364.
- Shu, D., 2008. Cambrian explosion: birth of tree of animals. *Gondwana Research*, **14**, 219–240.
- Steemans, P., and Wellman, C. H., 2004. Miospores and the emergence of land plants. In Webby, B. D., Paris, F., Droser, M. L., and Percival, I. G. (eds.), *The Great Ordovician Biodiversification Events*. New York: Columbia University Press, pp. 361–366.
- Steiner, M., and Reitner, J., 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. *Geology*, **29**, 1119–1122.
- Tanner, L. H., Lucas, S. G., and Chapman, M. G., 2004. Assessing the record and causes of Late Triassic extinctions. *Earth-Science Reviews*, **65**, 103–139.
- Taylor, P. D. (ed.), 2004. *Extinctions in the History of Life*. New York: Cambridge University Press.
- Taylor, T. N., Kerp, H., and Hass, H., 2005. Life history biology of early land plants: deciphering the gametophyte phase. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 5892–5897.
- Twitchett, R. J., 2006. The paleoclimatology, paleoecology and paleoenvironmental analysis of mass extinction events. *Paleogeography, Paleoclimatology, Paleocology*, **232**, 190–213.
- Valentine, J. W., 2002. Prelude to the Cambrian explosion. *Annual Review of Earth and Planetary Sciences*, **30**, 285–306.
- Weissflog, L., Elansky, N. F., Kotte, K., Keppler, F., Pfennigsdorff, A., Lange, C. A., Putz, E., and Lisitsyna, L. V., 2008. Late Permian changes in condition of the atmosphere and environments caused by halogenated gases. *Doklady Earth Sciences*, **452**, 291–295.
- Wellman, C. H., 2004. Paleoecology and paleophytogeography of the Rhynie chert plants: evidence from integrated analysis of in situ and dispersed spores. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **271**, 985–992.
- Wellman, C. H., Osterloff, P. L., and Mohiuddin, U., 2003. Fragments of the earliest land plants. *Nature*, **425**, 282–285.
- White, R. V., 2002. Earth’s biggest ‘whodunnit’: unravelling the clues in the case of the end-Permian mass extinction. *Philosophical Transactions of the Royal Society London*, **360**, 2963–2985.
- Wignall, P. B., 2001. Large igneous provinces and mass extinctions. *Earth-Science Reviews*, **53**, 1–33.

- Wood, R. A., Grotzinger, J. P., and Dickson, J. A. D., 2002. Proterozoic modular biomineralized metazoan from the Nama Group, Namibia. *Science*, **296**, 2383–2386.
- Wooldbridge, S. A., 2008. Mass extinctions past and present: a unifying hypothesis. *Biogeosciences Discussion*, **5**, 2401–2423.
- Xian-guang, H., Aldridge, R. J., Bergström, J., Sivetter, D. J., Siveter, D. J., and Xiang-hong, F., 2004. *The Cambrian Fossils Of Chengjiang, China. The Flowering Of Early Animal Life*. Oxford: Blackwell.
- Xiao, S., and Kaufman, A. J. (eds.), 2007. Neoproterozoic geobiology and paleobiology. *Topics in Geobiology*, **27**, 300.
- Xiao, S., Yuan, X., and Knoll, A. H., 2000. Eumetazoan fossils in terminal Proterozoic phosphorites? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 13684–13689.
- Young, S. A., Saltzman, M. R., Foland, K. A., Linder, J. J., and Kump, L. R., 2009. A major drop in seawater $^{87}\text{Sr}/^{86}\text{Sr}$ during the Middle Ordovician (Darrivilian): links to volcanism and climate? *Geology*, **37**, 951–954.
- Zhuravlev, A. Y., and Riding, R. (eds.), 2001. *The Ecology of the Cambrian Radiation*. New York: Columbia University Press.
- Zhuravlev, A. Y., and Wood, R. A., 2008. Eve of biomineralization: controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Animal Biocalcification, Evolution
 Animal Skeletons, Advent
 Archaea
 Asteroids and Comet Impacts
 Bacteria
 Banded Iron Formations
 Early Precambrian Eukaryotes
 Ediacaran Biota
 Isotope Fractionation (Metal)
 Mass Extinctions, Phanerozoic
 Mat-Related Sedimentary Structures
 Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Nickel, Biology
 Origin of Life
 Photosynthesis
 Salinity History of the Earth's Ocean
 Snowball Earth
 Soda Ocean Hypothesis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Trace Fossils: Neoproterozoic

CRYOBIOSPHERE

The term “cryobiosphere” refers to organisms living in extremely cold environments. For detailed reading, see entries “*Permafrost Microbiology*” and “*Extreme Environments*.”

CYANOBACTERIA

Kathrin I. Mohr^{1,2}, Nicole Brinkmann¹, Thomas Friedl¹
¹University of Göttingen, Göttingen, Germany
²Helmholtz Centre for Infection Research, Braunschweig, Germany

Synonyms

Blue-green algae; Chloroxybacteria; Cyanophyta; Cyanoprokaryota

Definition

Cyanobacteria are a large and morphologically rather diverse group of photoautotrophic prokaryotes and are classified as a monophyletic phylum within Domain Bacteria. Cyanobacteria share oxygenic photosynthesis with the eukaryotic algae. Cyanobacteria belong to the oldest organisms on earth. Their broad environmental tolerance range makes cyanobacteria well suited to live in habitats of extreme and fluctuating conditions where they can outcompete eukaryotic algae.

Many cyanobacteria have large cells like the eukaryotic algae with which they share the same life-style: Consequently, they are often treated as prokaryotic algae or, formerly, blue-green algae. Some cyanobacteria even form macroscopic colonies from the size of a few millimeters to up to 30 cm (Dodds and Castenholz, 1988). Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony-forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. Photosynthetic cyanobacteria produced earth's first oxygen atmosphere, which fostered the rise of eukaryotes. In addition, ancient cyanobacteria produced massive undersea carbonate deposits, thereby reducing atmospheric carbon dioxide levels and influencing global climate. The first plastids arose from cyanobacteria through a process called primary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003; Reyes-Prieto et al., 2007). Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Nitrogen-fixing cyanobacteria increase soil and water fertility and foster the growth of certain plants and fungi in symbiotic associations. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years (Rasmussen and Willemotte, 2008) and with an evolutionary history stretching over at least 1.5 and possibly 2.7 billion years (Blank, 2004). Cyanobacteria are able to live in some of the most extreme habitats on earth (e.g., Seckbach, 2007 for an overview). The broad environmental tolerance range of cyanobacteria in extreme habitats is in contrast to most other prokaryotic and eukaryotic organisms from extreme environments that occur only within a small window of almost constant conditions. From an evolutionary point of view, it appears that cyanobacteria which are

among the oldest organisms on earth may have been forced by the “modern” eukaryotes to withdraw themselves into habitats of extreme and fluctuating conditions (Whitton, 1992; Bhattacharya et al., 1999).

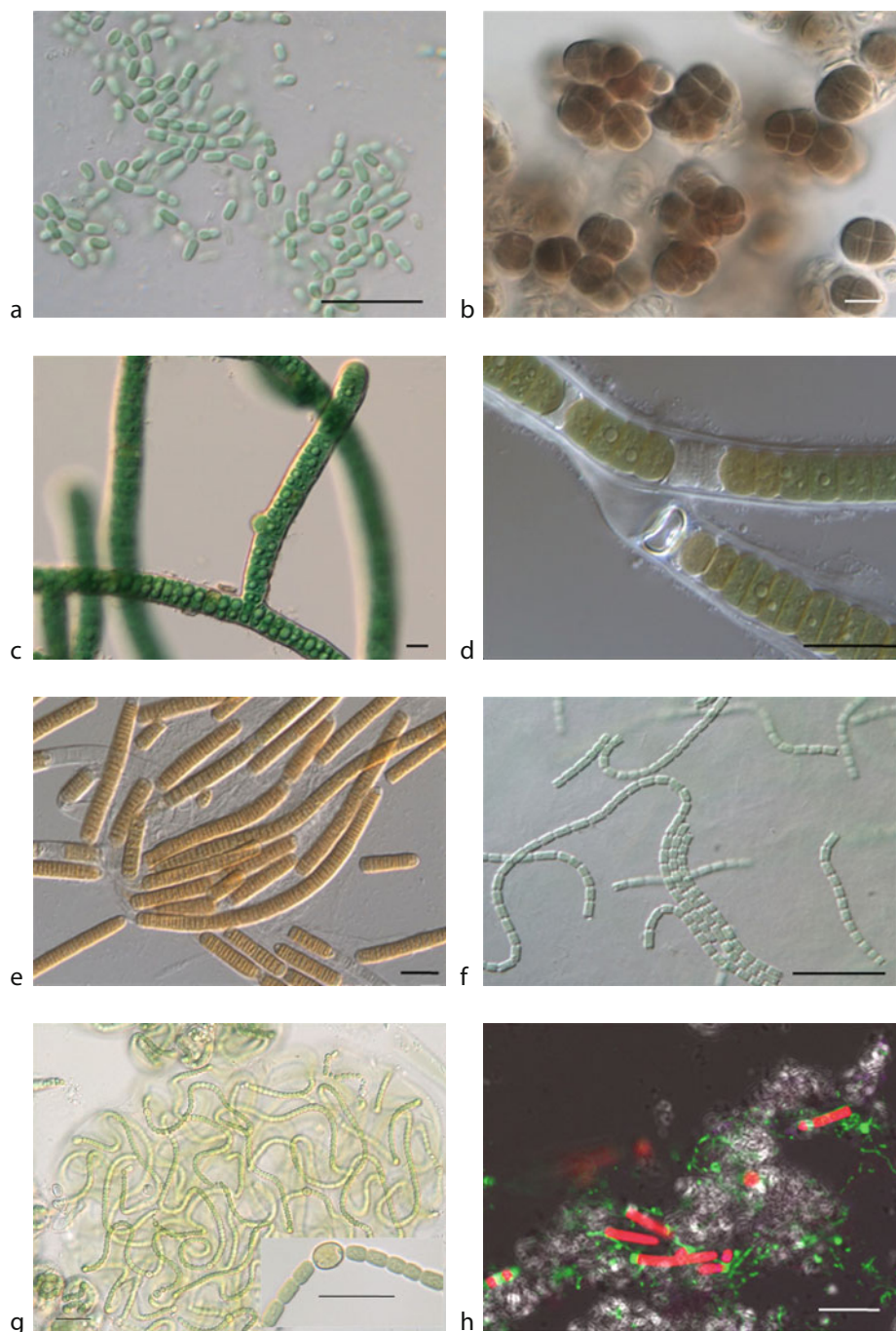
Cell structure

Unlike most groups of prokaryotes, there is substantial morphological and life history variation within the cyanobacteria (Hayes et al., 2007). Although some cyanobacteria occur as solitary unicells, in most species the cells adhere at each other to form at least pairs of cells (representing the products of a recent cell division, *Synechococcus* Figure 1a) or group of cells are held together to form colonies (e.g., in *Myxosarcina*, Figure 1b). Very common growth forms are filaments (trichomes) where the cells are joined end to end through regular divisions into one plane (Figure 1c–g). Such filaments can be even joined into bundles or can be branched (true-branched, Figure 1b or false-branched, Figure 1d). Many filamentous cyanobacteria produce specialized cells known as heterocytes (formerly heterocysts; Figure 1d and g) that function in nitrogen fixation. Such cyanobacteria are usually capable to produce specialized cells for dormancy, known as akinetes, under adverse environmental conditions. Some filamentous cyanobacteria are capable of gliding, a slow uniform motion on a solid substrate. The gliding occurs by an interaction of slime secretion and certain proteinaceous fibers (Hoiczyk, 2000). Cyanobacteria lack sexual reproduction, but gene exchange may be realized through viral transduction by specialized cyanophages (Coleman et al., 2006). The only mode of reproduction is by cell division (binary fission, resulting in two equal-sized cells) or simply by fragmentation of a colony. In unicellular cyanobacteria, baeocytes (endospores) are formed by the division of the cytoplasm several times and in different planes whereby the mother does not increase in size. Filamentous cyanobacteria form hormogonia (Figure 1e), which are short filaments resulting from a breakup of a longer filament, promoted by the controlled death or collapse of certain cells. Hormogonia exhibit gliding motility for several hours, are positively phototactic and lack heterocytes. Light is absorbed by thylakoids, photosynthetic membranes, which are arranged concentrically at the cell periphery, not stacked and not connected to the plasma membrane. They contain the chlorophylls *a* and *d*, in some cyanobacteria also chlorophyll *b*, as well as carotenoids and phycobilins as accessory pigments to capture light energy and convey it to the photosynthetic reaction centers located inside the thylakoid membranes. Phycobilins are blue or red water-soluble pigments and are present in large amounts. They are bound to proteins that form up hemispherical phycobilisomes which occur on the outer thylakoid surfaces. Several particles in the cytoplasm of cyanobacteria may represent storage products, that is, cyanophycin (a polymer of the nitrogen-rich amino acids asparagine and arginine), cyanophycan starch

(a polyglucan), and polyphosphate. In addition, lipid droplets, carboxysomes (polygonal aggregations of *Rubisco*, the key enzyme in CO₂ fixation), and gas vesicles may be present in the cytoplasm. Gas vesicles (assemblies of hollow, pointed cylinders, but not delimited by membranes) occur frequently in aquatic cyanobacteria and enable them for buoyancy and to confer vertical mobility in the water column. The cell wall of cyanobacteria is basically the same as for Gram-negative bacteria, that is, a thin peptidoglycan layer (a polymer composed of sugar derivatives and amino acids) is outside of the cell membrane (cytoplasm membrane). Outside of the peptidoglycan layer is a space surrounded by another membrane, termed outer membrane. A very characteristic feature of cyanobacteria is that they usually are surrounded by a mucilaginous sheath (extracellular polymeric substances [EPS], see entry “*Extracellular Polymeric Substances (EPS)*”), mostly composed of polysaccharides, which protects the cells from drying and enables them to attach to substrates.

Ecology

Cyanobacteria are widely distributed in aquatic and terrestrial environments, including such extreme habitats as hot springs, deserts, and polar regions (Whitton and Potts, 2000). Many cyanobacteria are diazotrophic, that is, they can fix atmospheric molecular nitrogen gas (N₂) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen fixation is an inducible process and is exceptionally energy demanding. The environmental levels of ammonium or nitrate regulate it and it can only be performed if sufficient ATP (produced by photosynthesis) is available. The key enzyme of nitrogen fixation is nitrogenase, which is irreversibly inhibited by oxygen and, therefore, nitrogen can only be fixed under anaerobic conditions. Cyanobacteria that produce heterocytes are capable to fix nitrogen even in aerobic habitats, because several processes within the heterocytes (e.g., intracellular respiration) consume oxygen and the heterocytes’ thick cell walls reduce diffusion of oxygen into the cells. Cyanobacteria without heterocytes may fix nitrogen in the dark. Bloom-forming filamentous cyanobacteria devoid of heterocytes are responsible for fixing one-quarter of the total nitrogen in the oceans (Bergman and Carpenter, 1991). These cyanobacteria fix nitrogen in randomly distributed vegetative cells adjacent to each other within the filaments. Here and inside the colonies, the filaments may be depleted from oxygen. In addition, these species occur in tropical seas that are relatively low in dissolved oxygen. The ability to fix molecular nitrogen makes cyanobacteria attractive as symbionts for many eukaryotes because eukaryotes are not able to fix nitrogen. Very common occurrences of nitrogen-fixing cyanobacteria as extracellular symbionts are lichens, that is, symbioses with certain fungi (Friedl and Büdel, 2008, see entries “*Symbiosis*” and “*Fungi and Lichens*”). In the marine environment, cyanobacterial symbioses are known



Cyanobacteria, Figure 1 Different cyanobacterial morphotypes from pure culture. Figure a, d–g show strains recovered from tufa stromatolite biofilms of hardwater creeks: (a) coccoid unicellular *Synechococcus* sp. The cells adhere at each other to form at pairs representing the products of a recent cell division (Photograph taken by A. Hodačova); (b) *Myxosarcina* sp. (strain SAG 30.84) with adhering groups of cells forming irregular colonies (Photograph taken by A. Hodačova); (c) filamentous, true-branched *Stigonema* sp. (strain SAG 49.90) (Photograph taken by A. Hodačova); (d) filamentous, false-branched *Tolypothrix* sp.; (e) thick unbranched filaments of *Lyngbya* sp. forming hormogonia; (f) thin unbranched filaments of *Pseudanabaena* sp. which are fragmenting (Photograph taken by A. Hodačova); (g) filamentous *Nostoc* sp. forming gelatinous colonies. Insert, filament with a heterocyte located within the filament (intercalary) (Photographs taken by A. Hodačova); (h) Fluorescence in situ hybridization using an oligonucleotide probe specific for bacteria at a cryosection of the top of a tufa-forming biofilm. The red filaments represent cyanobacteria (*Phormidium* sp.). Cyanobacterial autofluorescence is shown in red and calcite in white (Photograph taken by G. Arp, printed with permission of the publisher). Scale bar, 20 μm .

with sponges, ascidians (sea squirts), and echiuroid worms in the benthos and diatoms, as well as dinoflagellates in the plankton. These symbioses can be significant in terms of the biogeochemistry of coastal and open-ocean areas (Carpenter, 2002). Planktonic diatom–cyanobacteria symbioses play a significant role in the ecology and biogeochemistry of the surface ocean (see entry *Algae, Eukaryotic*). Symbiotic associations with cyanobacteria are also known from plants. In the water fern *Azolla* and cycads where filamentous cyanobacteria with heterocytes occur within certain cavities inside the leaf and intercellular spaces inside the root, respectively (Lee, 2008; Graham et al., 2009). N₂-fixing cyanobacteria are common in waterlogged rice fields and the importance of these naturally occurring prokaryotes in the nitrogen economy of rice cultivation has long been known (Whitton, 2000). To support the demand for nitrogen in rice cultivation, input of chemical N fertilizers is a prerequisite, but the inoculation with free-living N₂-fixing cyanobacteria as biofertilizers has been shown to increase growth and crop yield of rice. An example for intracellular associations involving cyanobacteria can be found in certain diatoms (see entry *Diatoms*).

The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles (Hayes et al., 2007). There is an enormous variety of habitats where cyanobacteria play important roles, but to discuss this comprehensively is beyond the scope of this article. Therefore, we confine the discussion of cyanobacteria ecology just to a few selected examples. Other examples may be equally intriguing and important. In the littoral zone of marine environments, cyanobacteria form thin adhering films on porous or soft granular rocks within the spray zone. Cyanobacteria of the littoral zone make a significant contribution to the productivity of rocky shores (Lee, 2008). In the open-ocean minute unicellular cyanobacteria (less than 2 µm in diameter) may occur in high concentrations due to their adaptation to low light and effective nutrient uptake kinetics. Here, cyanobacteria represent the major organisms of the picophytoplankton (Ferris and Palenik, 1998) that dominates biomass and production of these oligotrophic habitats. Massive development of nitrogen-fixing filamentous cyanobacteria occur in certain tropical waters and the large surface area produced by the cyanobacterial filaments forms miniature ecosystems (Carpenter et al., 1992; Lee, 2008). Also in freshwaters blooms of cyanobacteria are common. Bloom-forming cyanobacteria often release significant toxic substances, termed cyanotoxins, with physiologically two types, neurotoxins and hepatotoxins (e.g., Codd et al., 1999). Cyanotoxins may function as anti-herbivore chemicals and can inhibit the growth of other algae. Cyanobacteria are also well known from hot acidic springs throughout the world (e.g., Ward and Castenholz, 2000). Many physiological properties that make the cyanobacteria capable to adapt to a large variety of extreme and environmental conditions may also indicate

their long evolutionary history. Many cyanobacteria are able to tolerate low oxygen conditions and even free sulfide at levels much higher than those tolerated by most eukaryotic algae. Many terrestrial cyanobacteria can tolerate high UVB and -C radiations, which may also be reminiscent of an adaption to early earth's conditions. Some cyanobacteria can even confer anoxygenic photosynthesis where H₂S is utilized as an electron donor. Cyanobacteria are also very successful primary colonizers in terrestrial habitats due to their tolerance of desiccation and to water stress. In biological soil crusts of semideserts, for example, cyanobacteria play a key role in maintaining the stability of the surface (e.g., Büdel et al., 2009). As they bind sand and soil particles, cyanobacteria prevent erosion. They help to maintain moisture in the soil and contribute nitrogen to it through nitrogen fixation. That may even assist higher plant growth by supplying growth substances (Lee, 2008). The cyanobacterial genus *Arthrospira* (formerly *Spirulina*) is used as human food supplement; the *Spirulina* industry is of increasing economic importance. Large filamentous colonies of *Nostoc* are used in China as “hair vegetable” (Facai).

Many species of cyanobacteria have calcium carbonate in the enveloping mucilage of the cells; crystals of calcite may also be trapped in the mucilage excreted by the cells (see entry *Calcite Precipitation, Microbially Induced*). In the marine environment, trapping and binding of the sediments as well as carbonate precipitation by cyanobacteria led to the formation of stromatolites (see entries *Microbialites, Modern*, and *Microbialites, Stromatolites, and Thrombolites*). The production of laminae in the stromatolites depends on fluctuations ultimately derived from movements of the earth, sun, and moon, and requires rhythmicity such as daily photosynthetic cycle of the organisms in the stromatolites. Up to 2,000 million years ago, there were no grazers and boring organisms and, therefore, stromatolites grew uncontested. The occurrence and size of stromatolites declined dramatically after the evolution of grazing and boring organisms. Today stromatolites grow only in warm hypersaline waters inhospitable to grazers and borers (e.g., Shark Bay, Australia). Extant cyanobacteria do not calcify in open-ocean sea water because this water is normally not supersaturated sufficiently with calcium carbonate to allow calcification (Riding, 2000; see entry *Calcified Cyanobacteria*). However, even in extant freshwaters, calcification processes somehow similar to the stromatolite formation in marine environments occur, for example, in tufa-forming biofilms of karstwater streams (Arp et al., 2010). Epilithic cyanobacterial species may enhance calcium carbonate deposition, either by trapping particles or by contributing to communities where calcite and aragonite (in freshwaters) or other carbonates are precipitated. Endolithic species mainly occur in soft and porous limestone (Figure 1h) where they grow actively into the limestone at sites where removal of surface material occurs (Pentecost and Whitton,

2000; see entry *Endoliths*). Whether the cell surface polymers or the degradation of cyanobacterial sheath by bacterial metabolism promote the calcification is still unclear. The calcification seems to be a side effect of the cyanobacteria metabolism related to the alkalization of the surroundings during photosynthesis (Verrecchia et al., 1995). The living cyanobacteria try to limit the mineralization of the surface of their cells by some means. Nevertheless, when the calcification in cyanobacteria occurs, it is connected with their mucilaginous sheath. However, significant influence of cyanobacterial photosynthesis in the regulation of tufa-forming processes has been found recently (Bisset, 2008a, b; Shiraishi et al., 2008).

Fossil history and classification

Fossil evidence indicates that major lineages of cyanobacteria had originated 2.3–2.45 billion years ago (Eigenbrode and Freeman, 2006; Tomitani et al., 2006; Graham et al., 2009), well before the establishment of eukaryotes 1.5 billion years ago (Javaux et al., 2001). Even older fossils resembling cyanobacteria have been found in ancient stromatolites (e.g., Schopf, 2006) and geochemical evidence may also indicate an even earlier origin of cyanobacteria, but this is not left undisputed. Collectively, the available data suggest that oxygenic photosynthesis arose sometime between 3.4 and 2.3 billion years ago (Allan and Martin, 2007). For classification of the cyanobacteria, more recently a combination of phenotypic and genotypic characters has been used (Castenholz, 2001). The fact that cyanobacteria have an algal way of life – cyanobacteria “usually behave like algae” (Wilmotte, 1994) – led to treat them as plants under the botanical code of nomenclature until the late 1970s (e.g., Anagnostidis and Komárek, 1985). However, as cyanobacteria are essentially prokaryotes, their treatment under the bacteriological code of nomenclature seems more appropriate (e.g., Castenholz, 2001). For a review of the difficulties of cyanobacteria classification, see Hayes et al. (2007). Based on the most recent broad-based description of cyanobacterial taxonomy (Castenholz, 2001), cyanobacteria are classified into five subsections (Subsections I–V) which correspond to the five orders Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales which have been erected under the botanical code of nomenclature. Some of the groups described by this current classification scheme are known to be polyphyletic in molecular phylogenies (for reviews see, e.g., Hayes et al., 2007). This shows that taxonomy of cyanobacteria is still in a state of flux. Still more work using multiple gene analyses as well as correction of misidentified strains in DNA sequence databases and culture collections is required to achieve progress toward a more robust taxonomy of the cyanobacteria.

Summary

Cyanobacteria form a large and morphological diverse group of photoautotrophic Gram-negative bacteria. Many cyanobacteria have large cells like the eukaryotic algae with which they share oxygenic photosynthesis. Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years. Cyanobacteria exhibit a broad environmental tolerance range, which makes them well adapted to live under extreme and fluctuating conditions. From an evolutionary point of view, it appears that cyanobacteria may have been forced by the “modern” eukaryotes to withdraw themselves into such habitats. Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Many cyanobacteria can fix atmospheric molecular nitrogen gas (N₂) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen-fixing cyanobacteria increase soil and water fertility and are particularly attractive as symbionts, for example, to form lichens with fungi or being endosymbionts in plants, some metazoa, dinoflagellates and diatoms. The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles.

Bibliography

- Allan, J. G., and Martin, W., 2007. Out of thin air. *Nature*, **445**, 610–612.
- Anagnostidis, K., and Komárek, J., 1985. Modern approach to the classification system of the Cyanophytes. I. Introduction. *Algalological Studies*, **38/39**, 291–302.
- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., De Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. *Tufa-Forming Biofilms of German Karstwater Streams: Microorganisms, Exopolymers, Hydrochemistry and Calcification*. London: Geological Society. Special Publications, Vol. 336, pp. 83–118.
- Bhattacharya, D., Friedl, T., and Schmidt, H., 1999. The phylogeny of thermophiles and hyperthermophiles and the three domains of life. In Seckbach, J. (ed.), *Enigmatic Microorganisms and Life in Extreme Environments*. Dordrecht, The Netherlands: Kluwer, pp. 291–304.
- Blank, C. E., 2004. Evolutionary timing of the origin of mesophilic sulphate reduction and oxygenic photosynthesis: a phylogenetic dating approach. *Geobiology*, **2**, 1–20.
- Bergman, B., and Carpenter, E. J., 1991. Nitrogenase confined to randomly distributed trichomes in the marine cyanobacterium *Trichodesmium thiebautii*. *Journal of Phycology*, **27**, 158–165.
- Bissett, A., de Beer, D., Schoon, R., Shiraishi, F., Reimer, A., and Arp, G., 2008a. Microbial mediation of stromatolite formation in karst-water creeks. *Limnology and Oceanography*, **53**, 1159–1168.

- Bissett, A., Reimer, A., de Beer, D., Shiraishi, F., and Arp, G., 2008b. Metabolic micro-environmental control by photosynthetic biofilms under changing macroenvironmental temperature and pH conditions. *Applied and Environmental Microbiology*, **74**, 6306–6312.
- Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K., Salisch, M., Reisser, W., and Weber, B., 2009. Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. *Microbial Ecology*, **57**, 229–247.
- Carpenter, E. J., 2002. Marine cyanobacterial symbioses. Biology and environment. *Proceedings of the Royal Irish Academy*, **102B1**, 15–18.
- Carpenter, E. J., Capone, D. C., and Reuter, J. (eds.), 1992. *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*, Dordrecht: Kluwer.
- Castenholz, R. W., 2001. Cyanobacteria. In Boone, D. R., and Castenholz, R. W. (eds.), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 1. New York: Springer, pp. 474–487.
- Coleman, M. L., Sullivan, M. B., Martiny, C. A., Steglich, C., Barry, K., DeLong, E., and Chisholm, S. W., 2006. Genomic islands and the ecology and evolution of *Prochlorococcus*. *Science*, **311**, 1768–1770.
- Codd, G. A., Bell, S. G., Kaya, K., Ward, C. J., Beattie, K. A., and Metcalf, J. S., 1999. Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology*, **34**, 405–415.
- Dodds, W. K., and Castenholz, R. W., 1988. The biological effects of nitrate fertilization and water replacement in an oligotrophic cold water pond. *Hydrobiologia*, **162**, 141–146.
- Eigenbrode, J. L., and Freeman, K. H., 2006. Late Archaean rise of aerobic microbial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15759–15764.
- Ferris, M. J., and Palenik, B., 1998. Niche adaptation in ocean cyanobacteria. *Nature*, **396**, 226–228.
- Friedl, T., and Büdel, B., 2008. Photobionts. In Nash, T. III (ed.), *Lichen Biology*. Cambridge: Cambridge University Press, pp. 9–26.
- Graham, L. E., Graham, J. M., and Wilcox, L. W., 2009. *Algae*, 2nd edn. San Francisco: Person Benjamin Cummings.
- Hayes, P. K., El Semary, N. A., and Sánchez-Baracaldo, P., 2007. The taxonomy of cyanobacteria: molecular insights into a difficult problem. In Brodie, J., and Lewis, J. (eds.), *Unravelling the Algae: The Past, Present, and Future of Algal Systematics*. CRC Press/Taylor & Francis, pp. 93–101, ISBN-13: 978-0-8493-7989-5.
- Hoiczky, E., 2000. Gliding motility in cyanobacteria: observation and possible explanations. *Archives of Microbiology*, **174**, 11–17.
- Javaux, E. J., Knoll, A. H., and Walter, M. R., 2001. Morphological and ecological complexity in eukaryotic ecosystems. *Nature*, **412**, 66–69.
- Keeling, P. J., 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany*, **91**, 1481–1493.
- Lee, R. E., 2008. *Phycology*, 4th edn. New York: Cambridge University Press, ISBN 978-0521-68277-0.
- McFadden, G. I., 2001. Primary and secondary endosymbiosis and the origin of plastids. *Journal of Phycology*, **37**, 951–959.
- Palmer, J., 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology*, **39**, 4–12.
- Pentecost, A., and Whitton, B. A., 2000. Limestones. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 233–255.
- Rasmussen, U., and Wilmotte, A., 2008. Diversity and host specificity of *Azolla* cyanobionts. *Journal of Phycology*, **44**, 60–70.
- Reyes-Prieto, A., Weber, A. P. M., and Bhattacharya, D., 2007. The origin and establishment of the plastid in algae and plants. *Annual Review of Genetics*, **41**, 147–168.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Seckbach, J., 2007. *Algae and Cyanobacteria in Extreme Environments*. Berlin: Springer.
- Schopf, J. W., 2006. Fossil evidence of Archaean life. *Philosophical Transactions of the Royal Society B*, **361**, 869–885.
- Shiraishi, F., Bissett, A., de Beer, D., Reimer, A. and Arp, G., 2008. Photosynthesis, respiration and expolymer calcium-binding in biofilm calcification. *Geomicrobiology Journal*, **25**, 83–94. (Westerhöfer and Deinschwanger Creek, Germany) London.
- Tomitani, A., Knoll, A. H., Cavanaugh, C. M., and Ohno, T., 2006. The evolutionary diversification of cyanobacteria: Molecular-phylogenetic and paleontological perspectives. *PNAS*, **103**, 5442–5447.
- Verrecchia, E. P., Freytet, P., Verrecchia, K. E., and Dumont, J. L., 1995. Spherulites in calcrete laminar crusts: biogenic CaCO₃ precipitation as a major contributor to crust formation. *Journal of Sedimental Research*, **65**, 690–700.
- Ward, D. M., and Castenholz, R. W., 2000. Cyanobacteria in geothermal habitats. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 37–59.
- Whitton, B. A., 1992. Diversity, ecology and taxonomy of the cyanobacteria. In Mann, N. H., and Carr, N. G. (eds.), *Photosynthetic Prokaryotes*. New York: Plenum, pp. 1–51.
- Whitton, B. A., 2000. Soils and rice-fields. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 233–255.
- Whitton, B. A., and Potts, M., 2000. Introduction to the Cyanobacteria. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 1–11.
- Wilmotte, A., 1994. Molecular evolution and taxonomy of the cyanobacteria. In Bryant, D. A. (ed.), *The Molecular Biology of the Cyanobacteria*. Dordrecht: Kluwer, pp. 1–25.

Cross-references

Algae (Eukaryotic)
 Biomarkers (Molecular Fossils)
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Chroococcioidiosis
 Critical Intervals in Earth History
 Diatoms
 Endoliths
 Extracellular Polymeric Substances (EPS)
 Fungi and Lichens
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Photosynthesis
 Stromatolites
 Symbiosis

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Bioerosion
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Carbonates
 Cold Seeps
 Cyanobacteria
 Dolomite, Microbial
 Foraminifera
 Hydrothermal Environments, Marine
 Karst Ecosystems
 Microbial Biomineralization
 Microbial Degradation
 Microbial Ecology of Submarine Caves
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Reefs
 Saline Lakes
 Sediment Diagenesis – Biologically Controlled
 Sinter
 Soda Ocean Hypothesis
 Sponges (Porifera) and Sponge Microbes
 Stromatactis
 Stromatolites
 Symbiosis
 Thrombolites
 Tidal Flats
 Tufa, Freshwater
 Waulsortian Mud Mounds

CARBONATES

Martin Dietzel
 Graz University of Technology, Graz, Austria

Synonyms

Carbonate minerals

Definition

Carbonates. The term carbonates comprises solid phases such as minerals and amorphous substances, which basically consist of cations and triangular carbonate groups.

Introduction

The most common carbonate minerals in natural surroundings are calcite (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$). These minerals comprise about 90 wt.% of carbonates within the Earth's crust and 10–15 wt.% of sedimentary rocks. The proportion of dolomite to calcite increases with decreasing geological age, representing the potential for transformation of calcium carbonate to dolomite at elevated temperature and high molar Mg^{2+} to Ca^{2+} ratio during geological history. Additional carbonate

minerals are numerous, but occur in rather special natural surroundings.

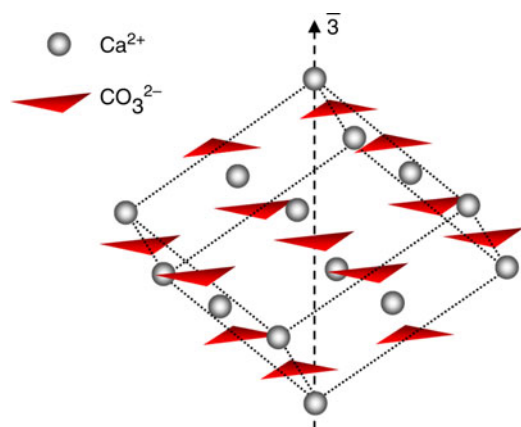
The occurrence and formation of carbonate minerals is mostly related to the precipitation from aqueous solutions and to a lesser extent to the crystallization from carbonate melts. In aquatic systems carbonate formation may be inorganically or biogenically induced (e.g., Morse and Mackenzie, 1990; Dove et al., 2003; Morse et al., 2007). Inorganic precipitation occurs from supersaturated solutions to form evaporites in marine and terrestrial environments, ooids in shallow water, saline lake deposits, speleothems in caves, sinter from spring water, hydrothermal ores, etc. Biogenic precipitation is mostly related to carbonates as skeletal parts and in tissues of marine and non-marine organisms such as foraminifera, coccoliths, corals, bivalves, and prokaryotes. The main mass proportion of carbonates is built from biogenic activity, whereas less arises from inorganic origin.

Once carbonate particles are formed, sedimentation may yield shelf deposits of carbonate sand or mud, deep sea ooze, or mud in lakes. Compaction, cementation, and diagenetic reactions result in lithification to limestone or calcareous marl and sandstone. Under elevated temperatures and pressures, the primary limestone may be transformed to, for example, dolomite rocks or marble.

Crystal structure and chemical composition

The basic units of carbonate minerals are cations and the triangular carbonate groups (CO_3^{2-}). The crystal structure of carbonate minerals is strongly related to the ionic radius of the cation versus the oxygen ion (e.g., Reeder, 1990). As the Ca:O ionic radius ratio in CaCO_3 is close to the upper limit for 6-coordination, CaCO_3 can occur in 6- and 9-coordination of Ca to O yielding calcite and aragonite, respectively. Accordingly, anhydrous carbonate minerals of smaller and larger cations compared to Ca^{2+} crystallize isostructurally to calcite and aragonite, respectively: (1) In the *calcite type* (trigonal carbonates), the carbonate group lies in planes at right angles to the threefold *c*-axis and the metal ions in alternate planes with a sixfold coordination of metal ions to oxygen ions (Figure 1). Metal ions of anhydrous carbonates of the *calcite type* are, e.g., Fe^{2+} , Mg^{2+} , Mn^{2+} , Cd^{2+} , and Zn^{2+} (small cations; see minerals in Table 1). (2) In the *aragonite type* (orthorhombic carbonates), a ninefold coordination of the cation to oxygen ions exists. Respective metal ions of anhydrous carbonates are, e.g., Ba^{2+} , Sr^{2+} , and Pb^{2+} as well as rare earth elements such as Sm, Eu, and Yb (large cations; Table 1).

Beside calcite and aragonite, the third CaCO_3 polymorph vaterite (hexagonal; see Figure 2) and amorphous calcium carbonate phases (ACC) can occur. Hydrus CaCO_3 minerals comprise amorphous $\text{CaCO}_3 \cdot \text{H}_2\text{O}$, monohydrocalciumcarbonate ($\text{CaCO}_3 \cdot \text{H}_2\text{O}$), and ikaite ($\text{CaCO}_3 \cdot 6\text{H}_2\text{O}$). In analogy to calcium, a set of hydrus magnesium carbonates appears: Nesquehonite ($\text{MgCO}_3 \cdot 3\text{H}_2\text{O}$), landsfordite ($\text{MgCO}_3 \cdot 5\text{H}_2\text{O}$), and hydromagnesite ($\text{Mg}_5(\text{OH})_2(\text{CO}_3)_4 \cdot 4\text{H}_2\text{O}$).



Carbonates, Figure 1 Schematic crystal lattice of calcite.

Carbonates, Table 1 Densities and cation radii, and solubility products ($pK_S = -\log(K_S)$ at 25°C and 1 atm) of common carbonate minerals

Mineral	Formula	Density (g cm ⁻³)	Cation radius (pm)	pK_S
<i>Trigonal</i>				
Calcite	CaCO ₃	2.71	100	8.48
Siderite	FeCO ₃	3.80	78	11.03
Magnesite	MgCO ₃	2.96	72	8.10
Rhodocrosite	MnCO ₃	3.13	83	10.50
Otavite	CdCO ₃	4.26	95	12.10
Smithonite	ZnCO ₃	4.40	74	10.00
Dolomite	CaMg(CO ₃) ₂	2.87	-	17.09
Monohydrocalcite	CaCO ₃ ·H ₂ O	2.43	100	7.15
<i>Orthorhombic</i>				
Aragonite	CaCO ₃	2.93	118	8.34
Witherite	BaCO ₃	4.43	147	8.56
Strontianite	SrCO ₃	3.70	131	9.27
Cerussite	PbCO ₃	6.60	135	13.13
<i>Hexagonal</i>				
Vaterite	CaCO ₃	2.54	100	7.91
<i>Monoclinic</i>				
Ikaite	CaCO ₃ ·6H ₂ O	1.77	100	6.62
<i>Amorphous</i>				
Amorphous calcium carbonate (ACC)	CaCO ₃	-	100	6.40

Ordered double carbonate minerals are dolomite, ankerite (CaFe(CO₃)₂), kutnahorite (CaMn(CO₃)₂), huntite (Mg₃Ca(CO₃)₄), and shortite (Na₂Ca₂(CO₃)₃). In the structure of dolomite, distinct Mg²⁺ and Ca²⁺ layers alternate with CO₃²⁻ layers, whereas in the disordered protodolomite (MgCa(CO₃)₂) Mg²⁺ and Ca²⁺ are randomly distributed in the cation layer, as in Mg calcite. Carbonate minerals may contain hydroxide ions, like the copper carbonates malachite (Cu₂(OH)₂CO₃) and azurite (Cu₃(OH)₂(CO₃)₂), where the respective formation is sensitive to the CO₂ partial pressure. Typical alkali carbonate minerals are natron (Na₂CO₃·10H₂O), nahcolite

(NaHCO₃), trona (Na₃(CO₃)(HCO₃)·2H₂O), and kalicinite (KHCO₃).

Solubility and stability

The solubility of carbonate minerals is expressed by the solubility product, $K_S = (Me^{2+}) \cdot (CO_3^{2-})$ for divalent cations. Parentheses denote the activities of aqueous species at thermodynamic equilibrium. In aqueous environments the stability of minerals to each other is given by the individual values of the solubility product. At standard condition, aragonite and vaterite are more soluble than calcite (lowest K_S -value for calcite in Table 1). Accordingly, calcite is the thermodynamically stable CaCO₃ polymorph. At higher pressure, the more dense phase aragonite is the stable polymorph, although aragonite has a larger cation site due to 9-coordination of Ca to O.

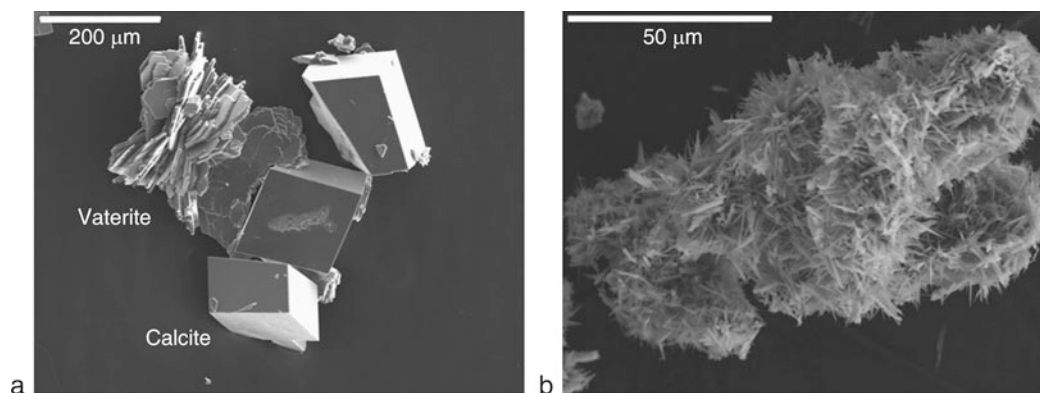
Elevated temperatures cause a decrease of carbonate mineral solubility. Most reliable values of solubility products as a function of temperature are obtained by the empirical equation as compiled by Nordstrom et al. (1990). High pressure favors the dissolution of carbonate minerals as observed in seawater below ≈ 3 –5 km depths, the so-called carbonate compensation depth (CCD, e.g., Morse and Mackenzie, 1990). Deep-sea sediments below the CCD are free of calcite and aragonite.

At elevated pH in alkaline solutions, precipitation of carbonate minerals may occur due to high [CO₃²⁻] to [DIC] ratios ([]: molar concentration, DIC: dissolved inorganic carbon). Components of DIC comprise CO_{2(aq)}, H₂CO₃, HCO₃⁻, and CO₃²⁻, where CO_{2(aq)} is dominant over H₂CO₃ by a factor of 1,000 (see Usdowski, 1982). In acidic solutions the dissolution of carbonates is favored by a high H₂CO₃* content ([H₂CO₃*] = [CO_{2(aq)}] + [H₂CO₃]) and low [CO₃²⁻] to [DIC] ratios.

High ionic solute content generally results in high-dissolution capacities due to a decrease of ion activities versus the concentrations of total dissolved ions. Accordingly, calcite is more soluble in seawater than in freshwater. Seawater is slightly supersaturated with respect to calcite. Supersaturation means that the present ion activity product of the calcium and carbonate ion, $IAP = (Ca^{2+}) \cdot (CO_3^{2-})$, exceeds the K_S value for calcite (saturation degree: $\Omega = IAP/K_S > 1$), and precipitation of calcite may occur. In terrestrial solutions, like drip water in caves and spring water in karst areas, supersaturation with respect to carbonate minerals and sinter formation are mostly related to the degassing of CO₂, which results in an increase of pH.

Precipitation and dissolution

Several carbonate minerals such as calcite, witherite, and strontianite can be easily precipitated from aqueous solutions, whereas others, such as dolomite, are not easily precipitated. In the classical experimental study by Plummer et al. (1978), expressions for the dissolution and precipitation kinetics of calcite are given. Since the Plummer et al. study, various approaches have been used to model the



Carbonates, Figure 2 SEM images of inorganically induced CaCO_3 precipitates with typical shapes. (a) Rhombohedral calcite crystals associated with hexagonal plates of vaterite (CaCl_2 solution without magnesium; 30°C ; pH 9.0). (b) Small aragonite needles (CaCl_2 solution at a molar Mg^{2+} to Ca^{2+} ratio of 2; 20°C ; pH 9.0).

general relationships. Calcite dissolution kinetics is described by empirical rate expressions depending on the reaction rate constant, the reactive surface area, the volume of the solution, and the supersaturation state. More recently, Arakaki and Mucci (1995) interpreted the dissolution of calcite based on the surface complexation theory.

In natural environments, the observed precipitation and dissolution rates (R in $\text{mol m}^{-2} \text{s}^{-1}$) often disagree with the rates estimated from experimental approaches. This may be caused by an incorrect estimation of the reactive mineral surface. Lower rates in natural surroundings may also be due to inhibition by foreign ions or organic matter (e.g., Zaihua et al., 1995).

Gutjahr et al. (1996) showed that in addition to anions like phosphate, the occurrence of divalent ions such as Mg^{2+} , Fe^{2+} , Sr^{2+} , and Ba^{2+} significantly decreases the precipitation rates of calcite. In seawater, the transformation into the more stable phases is hindered by the high Mg^{2+} content. Thus, aragonite and high-Mg calcite are often found in marine sediments, although they are metastable against calcite and low-Mg calcite, respectively (Berner, 1966).

A main aspect of inhibition of carbonate precipitation in natural environments (e.g., biomineralization) and applied systems (e.g., scale formation) is the presence of dissolved organic matter, such as organic acids (e.g., Mann, 2001). For instance, polyaspartic acid can systematically and significantly modify the morphology and growth kinetics of calcite.

Isomorphic substitution

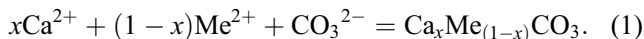
In natural systems, carbonate minerals are generally not pure substances. Impurities can be caused by isomorphic substitution of a host ion by foreign ions in a given crystal lattice. Isomorphic substitution of divalent cations in carbonate minerals depends on the cation radius, which accounts for the crystal structure and the solubility of carbonate minerals. Isomorphic substitution may result

in solid solution formation, as in the irregular but unsymmetrical SrCO_3 – CaCO_3 (aragonite type) solid solution.

Mechanisms of co-precipitation of foreign elements into carbonate minerals are complex and equilibrium thermodynamics may not be appropriate for dealing with many low-temperature reactions. On the other hand, trace element substitution in carbonate minerals can provide information about the environment conditions during formation.

The literature dealing with incorporation of trace elements into carbonate minerals is voluminous, especially with respect to the most abundant calcium and magnesium carbonates. As both the anhydrous magnesium and calcium carbonate crystallize in the *calcite type*, natural skeletal calcite can include up to about 25 mol% MgCO_3 to form so-called Mg calcite, which is isostructural with calcite. Low- and high-Mg calcites are defined by a limit of 4 mol% MgCO_3 with high-Mg calcite being less stable than low-Mg calcite. Only traces of magnesium are incorporated into aragonite as the cation sites are larger due to 9-coordination of Ca to O. Consequently, the larger Sr^{2+} ion is readily incorporated into aragonite, and less Sr^{2+} substitution occurs in calcite.

In the case of a solid CaCO_3 phase, a foreign element is incorporated into the crystal according to the overall expression



The related distribution coefficient is defined by the mole fractions X_{MeCO_3} and X_{CaCO_3} in the solid CaCO_3 and the molar Me^{2+} to Ca^{2+} ratio of the aqueous solution according to $D_{\text{Me}} = (X_{\text{MeCO}_3}/X_{\text{CaCO}_3})/([\text{Me}^{2+}]/[\text{Ca}^{2+}])$. The values of the experimental distribution coefficients for trace elements in carbonate minerals show a systematic behavior, but one that is different from that proposed by a thermodynamic equilibrium approach (e.g., Rimstidt et al., 1998; Lea, 2004; Boettcher and Dietzel, 2010).

The distribution coefficient of Mg^{2+} between calcite and aqueous solution is less than unity ($D_{\text{Mg}} \approx 0.02$ at 25°C). The higher the temperature, the more Mg^{2+} is incorporated into the calcite lattice (e.g., Oomori et al., 1987). In contrast, the distribution coefficients of Sr^{2+} in aragonite is larger than unity, and an increase in temperature results in less incorporation of Sr^{2+} into aragonite (Dietzel et al., 2004). Tesoriero and Pankow (1996) clearly showed that the incorporation of divalent cations into inorganically precipitated calcite is a function of precipitation rate. At low rates, a constant value of the distribution coefficient is reached representing thermodynamic equilibrium. Besides temperature and precipitation rates, the apparent D_{Me} values depend also on the concentrations of additional foreign ions via competitive substitution, the ionic strength of the solution, and the differential partitioning of the foreign cation on nonequivalent crystallographic faces of the carbonate mineral.

Trace element incorporation into carbonate minerals is widely used to estimate conditions of formation. For instance, Mg^{2+} to Ca^{2+} and Sr^{2+} to Ca^{2+} ratios in calcium carbonate minerals of foraminifera, coccoliths, bivalves, or in speleothems are used to estimate paleotemperature and environmental records as well as diagenetic alterations (e.g., Purton et al., 1999; Huang and Fairchild, 2001). The interpretation of datasets for trace element distribution in carbonate minerals is related to saturation states, precipitation rates, temperatures, and metabolisms of the organisms. Accordingly, recent studies of Elderfield et al. (2006) show a carbonate ion saturation state effect on Mg^{2+} incorporation in benthic foraminifera, and Freitas et al. (2006) show that Sr^{2+} to Ca^{2+} ratios in carbonate shell might be controlled by kinetic effects, the latter driven by seasonal variation in shell growth rate that is in turn influenced in part by seawater temperature.

Isotope geochemistry

Stable isotopes of carbon and oxygen of CO_3^{2-} molecules as well as cation isotopes in carbonate minerals are frequently considered in environmental studies to decipher the origin of components, conditions during formation, alteration effects, etc. (e.g., Ripperdan, 2001). For instance, stable oxygen isotopic fractionation between calcite and water has been widely used as a proxy for temperature and environmental changes in paleoceanography, speleology, and applied mineralogy (e.g., Dietzel, 2000; Spötl and Matthey, 2006). The basic concept is referred to the temperature sensitive oxygen isotope fractionation factor between calcite and water, $\alpha_{\text{calcite-water}} = (\delta^{18}\text{O}_{\text{calcite}} + 1,000) / (\delta^{18}\text{O}_{\text{water}} + 1,000)$. For example, Kim and O'Neil (1997) and more recently Coplen (2007) adopted from inorganic precipitation experiments and field studies the $\alpha_{\text{calcite-water}}$ values for equilibrium fractionation. From laboratory experiments and field studies it is widely accepted that the use of $\delta^{18}\text{O}$ as a temperature proxy is complicated by nonequilibrium fractionation (e.g., Kim and O'Neil,

1997; Mickler et al., 2004; Dietzel et al., 2009). In natural biogenic carbonates, the effect of growth rate on $\delta^{18}\text{O}$ values of carbonates is noticeable, with a rapid growth rate resulting in a lower $\delta^{18}\text{O}$ value (e.g., Adkins et al., 2003). Numerous temperature functions are used to estimate $\alpha_{\text{MeCO}_3\text{-water}}$ values for the respective cation content, crystal structure, and organism (e.g., Lea, 2004).

In the case of nontraditional stable isotopes, Mg and Ca isotopic distributions between aqueous cations and carbonate minerals are promising proxies to reconstruct environmental conditions during carbonate formation and to provide insight into geochemical cycles (e.g., Young and Galy, 2004; Tang et al., 2008, respectively). For example, ^{44}Ca is discriminated versus ^{40}Ca during calcium carbonate crystallization. Various models exist for the calcium isotope fractionation during precipitation of calcium carbonate from aqueous solution (e.g., Fantle and DePaolo, 2007). Although, effects of temperature, precipitation rate, crystal structure, and metabolism are identified as main factors for calcium isotope fractionation, future research is required to decipher individual fraction mechanisms.

Biom mineralization

Biom mineralization refers to the process by which living organisms form minerals. The most abundant carbonate biom minerals with respect to quantities and widespread distribution are calcium carbonates such as calcite and Mg calcite, and to a lesser extent aragonite, monohydrocalcite, and ACC (e.g., Weiner and Dove, 2003). Biom mineral phases often have shape, size, crystallinity, isotopic and trace signatures quite unlike their isotopic inorganically formed counterparts as a result of the interaction between organic macromolecules and an amorphous-crystalline phase transformation during early stages of biom mineralization. Sorting out the physiological effects from environmental signals provides a challenge for further scientific research.

Although, ACC is thermodynamically metastable it can be introduced as an important precursor of biom mineralization. Biom mineralization mechanisms and strategies for the biomimetic preparation of functional materials can be followed by transformation of ACC into crystallized calcium carbonates regulated by additives, e.g., functional polymer, proteins, and inorganic ions (Xu et al., 2008).

Han and Aizenberg (2008) showed that the ACC phase can be doped with foreign ions (e.g., Mg^{2+}) and organic molecules (e.g., dyes) and that these compounds later function as growth modifiers of calcite crystals. Such foreign components are incorporated into the crystals during the transformation process of ACC to calcite, which may indicate a vital effect on biogenically induced calcite. Lam et al. (2007) synthesized ACC in the presence of Mg^{2+} and polyaspartic acid. While the first Mg rich ACC precipitates showed short-range structures most similar to aragonite, the initial polyaspartic ACC precipitates possessed short-range structures resembling vaterite. The

influence of these additives on the crystallization of calcium carbonate is apparent even in the precipitation of the first amorphous precursor phase.

Summary

Carbonate minerals have a high diversity and are widely distributed throughout the Earth's crust. The most abundant carbonates contain calcium and magnesium such as calcite, dolomite, Mg calcite, and aragonite. For biogenically formed carbonates, precursor phases such as ACC are of special interest. In aqueous systems the stability of carbonates is related to the solubility product and the kinetics of dissolution and precipitation. Incorporation of foreign cations and isotopes into carbonates is mostly controlled by the composition of the solution, temperature, and precipitation rate. Thus, the chemical and isotopic composition of carbonates can be used as promising proxies to decipher environmental conditions during mineral formation and alteration. Lab experiments will be essential to provide insight about environmental changes throughout the geologic history from field data.

Bibliography

- Adkins, J. F., Boyle, E. A., Curry, W. B., and Lutinger, A., 2003. Stable isotopes in deep-sea corals and a new mechanism for "vital effects". *Geochimica et Cosmochimica Acta*, **67**(6), 1129–1143.
- Arakaki, T., and Mucci, A., 1995. A continuous and mechanistic representation of calcite reaction-controlled kinetics in dilute solutions at 25°C and 1atm total pressure. *Aquatic Geochemistry*, **1**, 105–130.
- Berner, R. A., 1966. Chemical diagenesis of some modern carbonate sediments. *American Journal of Science*, **264**, 1–36.
- Boettcher, M. E., and Dietzel, M., 2010. Metal-ion partitioning during low-temperature precipitation and dissolution of anhydrous carbonates and sulfates. In Prieto, M. (ed.), *Ion partitioning in low temperature aqueous systems: From fundamentals to applications in climate proxies and environmental geochemistry*. EMU Notes in Mineralogy Vol. 10 (in press).
- Coplen, T. B., 2007. Calibration of the calcite-water oxygen-isotope geothermometer at Devils Hole, Nevada, a natural laboratory. *Geochimica et Cosmochimica Acta*, **71**(16), 3948–3957.
- Dietzel, M., 2000. $^{13}\text{C}/^{12}\text{C}$ -signatures of calcite-sinter onto concrete. In Rammlmair, D., et al. (eds.), *Applied Mineralogy*. Rotterdam, The Netherlands: Balkema, pp. 747–748.
- Dietzel, M., Gussone, N., and Eisenhauer, A., 2004. Coprecipitation of Sr^{2+} and Ba^{2+} with aragonite by membrane diffusion of CO_2 between 10 and 50°C. *Chemical Geology*, **203**(1–2), 139–151.
- Dietzel, M., Tang, J., Leis, A., and Köhler, S., 2009. Stable isotopic fractionation during inorganic calcite precipitation – Effects of temperature, precipitation rate and pH. *Chemical Geology*, **268**, 107–115.
- Dove, P. M., De Yoreo, J. J., and Weiner, S., 2003. Biomineralization. *Reviews in Mineralogy and Geochemistry*, **54**, 381 p.
- Elderfield, H., Yu, J., Anand, P., Kiefer, T., and Nyland, B., 2006. Calibrations for benthic foraminiferal Mg/Ca paleothermometry and the carbonate ion hypothesis. *Earth and Planetary Science Letters*, **250**(3–4), 633–649.
- Fantle, M. S., and DePaolo, D. J., 2007. Ca isotopes in carbonate sediment and pore fluid from ODP site 807A: The $\text{Ca}^{2+}_{(\text{aq})}$ -calcite equilibrium fractionation factor and calcite recrystallization rates in Pleistocene sediments. *Geochimica et Cosmochimica Acta*, **71**(10), 2524–2546.
- Freitas, P. S., Clarke, L. J., Kennedy, H., Richardson, C. A., and Abrantes, F., 2006. Environmental and biological controls on elemental (Mg/Ca, Sr/Ca and Mn/Ca) ratios in shells of the king scallop *Pecten maximus*. *Geochimica et Cosmochimica Acta*, **70**(20), 5119–5133.
- Gutjahr, A., Dabringhaus, H., and Lacmann, R., 1996. Studies of the growth and dissolution kinetics of the CaCO_3 polymorphs calcite and aragonite II. The influence of divalent cation additives on the growth and dissolution rates. *Journal of Crystal Growth*, **158**(3), 310–315.
- Han, T. Y. J., and Aizenberg, J., 2008. Calcium carbonate storage in amorphous form and its template-induced crystallization. *Chemistry of Materials*, **20**(3), 1064–1068.
- Huang, Y., and Fairchild, I. J., 2001. Partitioning of Sr^{2+} and Mg^{2+} into calcite under karst-analogue experimental conditions. *Geochimica et Cosmochimica Acta*, **65**(1), 47–62.
- Kim, S. T., and O'Neil, J. R., 1997. Equilibrium and nonequilibrium oxygen isotope effects in synthetic carbonates. *Geochimica et Cosmochimica Acta*, **61**(16), 3461–3474.
- Lam, R. S. K., Charnock, J. M., Lennie, A., and Meldrum, F. C., 2007. Synthesis-dependant structural variations in amorphous calcium carbonate. *Crystengcomm*, **9**(12), 1226–1236.
- Lea, D. W., 2004. Elemental and isotopic proxies of the past ocean temperature. In Elderfield, H. (ed.), *The Oceans and Marine Geochemistry*, Vol. 6. In Holland H. D., and Turekian K. K. (eds.), *Treatise on Geochemistry*, Oxford: Elsevier, pp. 365–390.
- Mann, S., 2001. *Biomineralization*. Oxford: Oxford University Press, p. 198.
- Mickler, P. J., Banner, J. L., Stern, L., Asmerom, Y., Edwards, R. L., and Ito, E., 2004. Stable isotope variations in modern tropical speleothems: evaluating equilibrium vs. kinetic isotope effects. *Geochimica et Cosmochimica Acta*, **68**(21), 4381–4393.
- Morse, J. W., Arvidson, R. S., and Lüttge, A., 2007. Calcium carbonate formation and dissolution. *Chemical Reviews*, **107**, 342–381.
- Morse, J. W., and Mackenzie, F. T., 1990. Geochemistry of sedimentary carbonates. *Developments in Sedimentology*, **48**, 707.
- Nordstrom, D. K., Plummer, N., Langmuir, D. L., Busenberg, E., and May, 1990. Revised chemical-equilibrium data for major water-mineral reactions and their limitations. In Melchior, D. C., and Bassett, R. L. (eds.), *Chemical modeling of aqueous systems II: revised chemical equilibrium*. *American Chemical Society Symposium Series*, **416**, 398–413.
- Oomori, T., Kaneshima, H., Maezato, Y., and Kitano, Y., 1987. Distribution coefficient of Mg^{2+} ions between calcite and solution at 10–50°C. *Marine Chemistry*, **20**(4), 327–336.
- Plummer, L. N., Wigley, T. M. L., and Parkhurst, D. L., 1978. Kinetics of calcite dissolution in CO_2 -water systems at 5°C to 60°C and 0.0 to 1.0atm CO_2 . *American Journal of Science*, **278**(2), 179–216.
- Purton, L. M. A., Shields, G. A., Brasier, M. D., and Grime, G. W., 1999. Metabolism controls Sr/Ca ratios in fossil aragonitic mollusks. *Geology*, **27**(12), 1083–1086.
- Reeder, R. J., 1990. Carbonates: mineralogy and chemistry. *Reviews in Mineralogy*, **11**, 399 p.
- Rimstidt, J. D., Balog, A., and Webb, J., 1998. Distribution of trace elements between carbonate minerals and aqueous solutions. *Geochimica et Cosmochimica Acta*, **62**(11), 1851–1863.
- Ripperdan, R. L., 2001. Stratigraphic variation in marine carbonate carbon isotope ratios. In Valley, J. W., and Cole, D. R. (eds.),

- Stable Isotope Geochemistry*, Chap. 13. Reviews in Mineralogy and Geochemistry, 43, pp. 637–662.
- Spötl, C., and Matthey, D., 2006. Stable isotope microsampling of speleothems for palaeoenvironmental studies: a comparison of microdrill, micromill and laser ablation techniques. *Chemical Geology*, **235**(1–2), 48–58.
- Tang, J., Dietzel, M., Köhler, S. J., Böhm, F., and Eisenhauer, A., (2008) $\text{Sr}^{2+}/\text{Ca}^{2+}$ and $^{44}\text{Ca}/^{40}\text{Ca}$ fractionation during inorganic calcite formation: II. Ca isotopes. *Geochimica et Cosmochimica Acta*, **72**, 3733–3745.
- Tesoriero, A. J., and Pankow, J. F., 1996. Solid solution partitioning of Sr^{2+} , Ba^{2+} , and Cd^{2+} to calcite. *Geochimica et Cosmochimica Acta*, **60**(6), 1053–1063.
- Uzdowski, E., 1982. Reactions and equilibria in the systems $\text{CO}_2\text{-H}_2\text{O}$ and $\text{CaCO}_3\text{-CO}_2\text{-H}_2\text{O}$ ($0^\circ\text{--}50^\circ\text{C}$). *Neues Jahrbuch für Mineralogie, Abhandlungen*, **144**(2), 148–171.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy and Geochemistry*, 54, pp. 1–29.
- Xu, X. R., Cai, A. H., Liu, R., Pan, H. H., and Tang, R. K., 2008. Amorphous calcium carbonate in biomineralization. *Record of Chemical Progress*, **20**(1), 54–59.
- Young, E. D., and Galy, A., 2004. The Isotope geochemistry and cosmochemistry of magnesium. In Johnson, C. M., Beard, B. L., Albarede, F. (eds.), *Geochemistry of Non-Traditional Stable Isotopes*, Chap. 6. Reviews in Mineralogy and Geochemistry, 55, pp. 197–230.
- Zaihua, L., Svensson, U., Dreybrodt, W., Daoxian, Y., and Buhmann, D., 1995. Hydrodynamic control of inorganic calcite precipitation in Huanglong Ravine, China: field measurements and theoretical prediction of deposition rates. *Geochimica et Cosmochimica Acta*, **59**(15), 3087–3097.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Animal Biocalcification, Evolution
 Animal Skeletons, Advent
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Cap Carbonates
 Carbon Cycle
 Carbon (Organic, Cycling)
 Carbonate Environments
 Cold Seeps
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Isotopes and Geobiology
 Karst Ecosystems
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Molar-tooth Structure
 Mud Mounds
 Nanocrystals, Microbially Induced
 Organomineralization
 Pedogenic Carbonates
 Raman Microscopy (Confocal)
 Reefs
 Saline Lakes
 Siderite
 Soda Ocean Hypothesis
 Speleothems

CATHODOLUMINESCENCE MICROSCOPY

Walter Vortisch

Montanuniversität Leoben, Leoben, Austria

Definition

Cathodoluminescence (CL) is the emission of visible light (also ultraviolet [UV] and infrared) of a solid material that is irradiated by an electron beam (in vacuum). During irradiation CL often changes. Besides CL, the emission of element-specific X-rays and backscattered, secondary and Auger electrons is caused (e.g., Walker and Burley, 1991). Moreover, the sample is heated by the electron beam, which represents an important cause for the alteration of CL characteristics during electron bombardment. Cathodoluminescence microscopy (CLM) combines CL with optical microscopy (in geosciences, generally polarizing microscopy). CL microscopic studies are mostly carried out on thin sections (uncovered, preferably polished), sometimes on up to one cm-thick samples, the latter preferably with polished surface as well. Depending on the instrument used (see below), samples can be studied uncoated or with a coating to avoid charging by the electron beam.

Two types of CL are known: intrinsic and extrinsic (for details see discussion by Remond et al., 2000). Intrinsic CL is purely due to lattice features, generally very weak and only visible if there are no activators present in adequate amounts. Extrinsic CL, the generally observable case, is caused by activators, which occur as trace elements. The activators may be supported by sensitizers that transmit energy to the activators. Quenchers are elements that reduce or even inhibit CL. The significance of sensitizers and quenchers is still in discussion. An important example is iron, which is generally considered as a quencher in carbonate minerals, and lead, which seems to be a sensitizer (e.g., Machel, 2000). In contrast to the view of iron as a quencher, Cazenave et al. (2003) showed in a recent study of doped calcite that Mn-activated CL in calcite can be uninfluenced by iron in a relatively wide range of iron concentrations. The question whether Pb and Zn can be sensitizers in calcite was discussed by Budd et al. (2000), who could not find any evidence for sensitizing by these trace elements.

In geosciences, CLM is a powerful tool for detailed analyses of rocks and minerals, including biogenic mineral substances (for a summary of CL data and earlier literature, see for instance Marshall, 1988, Remond et al., 1992 and Pagel et al., 2000). Intensity and wavelengths (colors) of emitted light may vary strongly for the same mineral phase or even within the same mineral grain, depending on genetically determined features. This is especially significant when a mineral substance is growing or recrystallizing under changing ambient conditions, often causing changes of CL intensity or color, sometimes revealing patterns of complex zoning. In most cases, such effects are not visible by optical microscopy. Therefore, for studies of biogenic mineral substances, CLM can

organisms are not capable of using plant litter, e.g., directly and thus entirely dependent on microbial degradation processes to have sufficient minerals available for their nutrition. Properties of microbes for degradation can be useful in bioremediation approaches.

Bibliography

- Abraham, W. R., Nogales, B., Golyshin, P. N., Pieper, D. H., and Timmis, K. N., 2002. Polychlorinated biphenyl-degrading microbial communities in soils and sediments. *Current Opinion in Microbiology*, **5**, 246–253.
- Bumpus, J. A., and Aust, D., 1987. Biodegradation of environmental pollutants by the white rot fungus *Phaenoraete chrysosporium*: involvement of the lignin-degrading system. *Bioessays*, **6**, 166–170.
- Crawford, R. L., 1981. *Lignin Biodegradation and Transformation*. New York: Wiley.
- de Jong, F., and Field, D. A., 1997. Sulfur tuft and turkey tail: biosynthesis and biodegradation of organohalogenes by basidiomycetes. *Annual Review of Microbiology*, **51**, 375–414.
- Demnerova, K., Mackova, M., Specakova, V., Beranova, K., Kochankova, L., Lovecka, P., and Rylslava, E., 2005. Two approaches to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial populations. *International Microbiology*, **8**, 205–211.
- Gold, M. H., and Alic, M., 1993. Molecular biology of the lignin-degrading basidiomycete *Phaenoraete chrysosporium*. *Microbiology Reviews*, **57**, 605–622.
- Hammel, K. E., 1989. Organopollutant degradation by ligninolytic fungi. *Enzyme and Microbial Technology*, **11**, 776–777.
- Holtze, M. S., Sorensen, S. R., Sorensen, J., and Aamand, J., 2008. Microbial degradation of the benzonitrile herbicides dichlobenil, bromoxynil and ioxynil in soil and subsurface environments – insights into degradation pathways, persistent metabolites and involved degrader organisms. *Environmental Pollution*, **154**, 155–168.
- Johnsen, A. R., and Karlson, U., 2007. Diffuse PAH contamination of surface soils: environmental occurrence, bioavailability, and microbial degradation. *Applied Microbiology and Biotechnology*, **76**, 533–543.
- Kim, D. Y., and Rhee, Y. H., 2003. Biodegradation of microbial and synthetic polyesters by fungi. *Applied Microbiology and Biotechnology*, **61**, 300–308.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock: Biology of Microorganisms*. Upper Saddle River: Prentice-Hall.
- Pieper, D. H., 2005. Aerobic degradation of polychlorinated biphenyls. *Applied Microbiology and Biotechnology*, **67**, 170–191.
- Röling, W. F., and van Verseveld, H. W., 2002. Natural attenuation: what does the subsurface have in store? *Biodegradation*, **13**, 53–64.
- Schink, B., 2002. Anaerobic digestion: concepts, limits and perspectives. *Water Science and Technology*, **45**, 1–8.
- Schwitzguebel, J. P., Aubert, S., Grosse, W., and Laturnus, F., 2002. Sulphonated aromatic pollutants. Limits of microbial degradability and potential of phytoremediation. *Environmental Science and Pollution Research International*, **9**, 62–72.
- Smidt, H., and de Vos, W. M., 2004. Anaerobic microbial dehalogenation. *Annual Review of Microbiology*, **58**, 43–73.
- Ueda, K., and Beppu, T., 2007. Lessons from studies of *Symbiobacterium thermophilum*, a unique syntrophic bacterium. *Bioscience Biotechnology and Biochemistry*, **71**, 1115–1121.
- Watanabe, K., Futamata, H., and Harayama, S., 2002. Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. *Antonie van Leeuwenhoek*, **81**, 655–663.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Phosphorus, Phosphorites](#)
[Soils](#)

MICROBIAL ECOLOGY OF SUBMARINE CAVES

Francesco Canganella, Giovanna Bianconi
 University of Tuscia, Viterbo, Italy

Definition

Essential concepts, current investigations, and discussion.

In recent years, the search for the limit of biodiversity in extreme and/or uncommon environments led to the investigation of peculiar ecosystems such as deep-sea, subterranean niches, Antarctic cores, hydrothermal vents, and extraterrestrial sites.

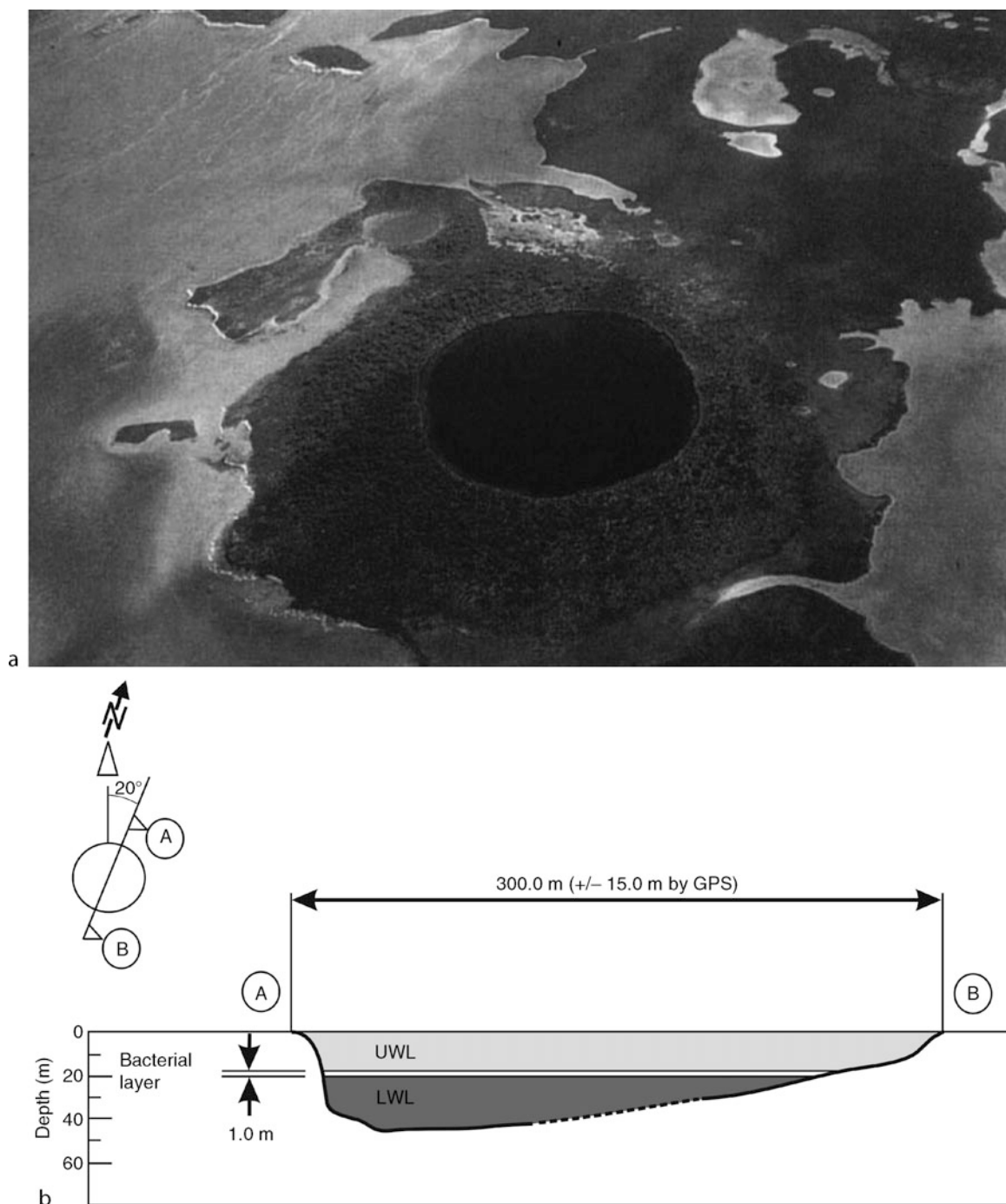
Submarine caves and cavities have also been explored, some were mainly exploited as popular attractions for scuba divers, but some were shown to be of great interest for geologists, biologists, and microbiologists.

Available scientific data on these ecosystems are not abundant, particularly as far as biological disciplines are concerned. Most of the published works have been related to specific submarine cavities known as Blue Holes and Black Holes, as well as to submarine caves showing inner sulfidic emissions. A larger number of data are currently available regarding the microbiology of terrestrial caves, particularly on the microbial ecology of carbonate layers, sediments, bat guano, and flooding waters (Vlasceanu et al., 1997, 2000; Holmes et al., 2001; Engel et al., 2003; Northup et al., 2003; Sugita et al., 2005; Zhou et al., 2007), but these latter ecosystems are not part of the current review.

Blue holes and black holes

The so-called blue holes are underwater karst systems which can develop horizontally in a very extensive way and appear blue due to a combination of blue sky reflection with the white carbonate sand deposited in the cave (Smart et al., 1988; Mylroie et al., 1995; Schwabe et al., 1997; Marano-Briggs, 2000; Colantoni et al., 2003; Canganella et al., 2004). Most of these sites were found and described in Bahamas and Hawaii.

Other cavern systems are known as “black holes,” which are cave systems without known lateral passages and located in the interior regions of the larger Bahamian islands. Most black holes are located in the central to western side of the island of South Andros in the Bahamas (Figure 1), although one has been found on the northern transitional shore of the Grand Bahama Island. They are vertical cave systems which



Microbial Ecology of Submarine Caves, Figure 1 A schematic drawing of the South Andros black hole, (a) aerial view and (b) bathymetry. UWL, upper water layer; LWL, lower water layer; —, floor area not surveyed. (Reprinted from Schwabe and Herbert, 2004 *Black Holes of the Bahamas: what they are and why they are black*, *Quaternary International* 121: 3–11 with permission from Elsevier.)

develop from the surface downward and appear to have no direct link to the sea, except through rock fissures and local porosity (Schwabe, 1998; Schwabe and Herbert, 2004).

In both cave systems, as water exchange is severely restricted, physicochemical gradients are highly stable and even strict anaerobic conditions can develop with remarkable hydrogen sulfide concentrations (from 5 μM to 6 mM).

Submarine caves with sulfidic water springs

A unique marine ecosystem is represented by the submarine caves of Capo Palinuro (Salerno, Italy), of which 13 have inside sulfidic springs exhibiting temperatures up to 25°C that remarkably affect the surrounding ecosystem.

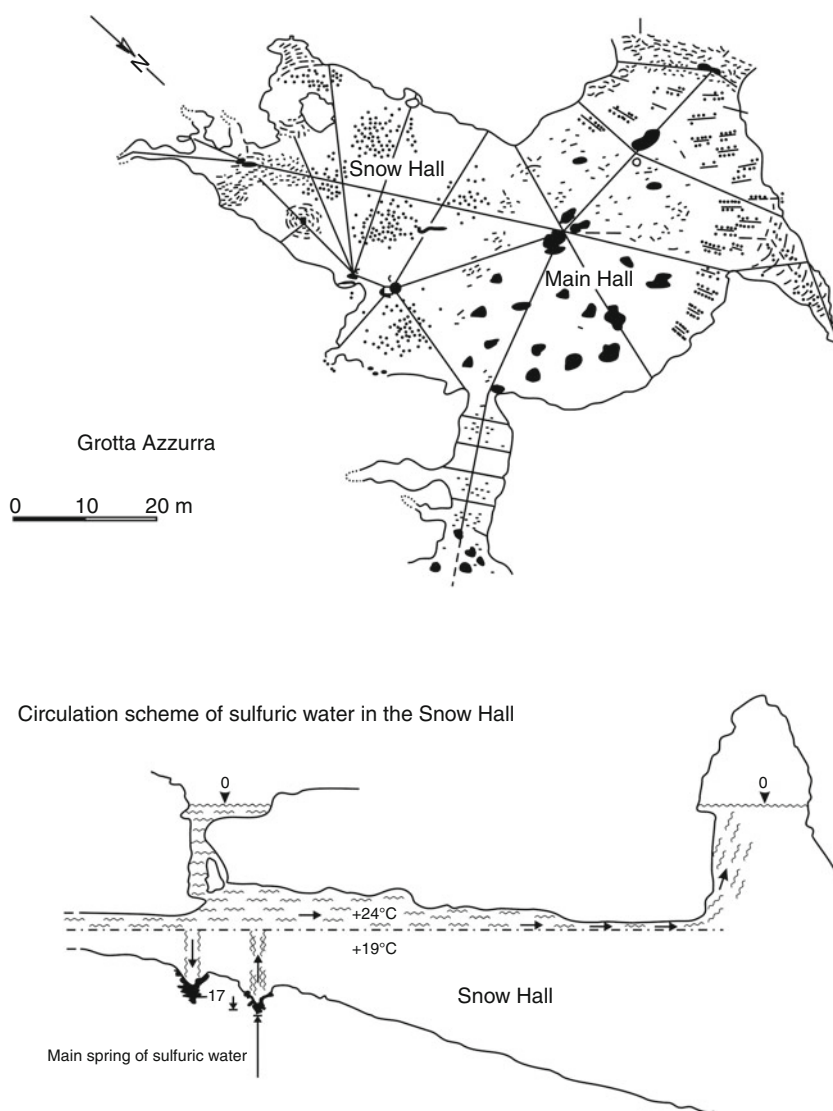
Preliminary investigations concerning the submarine speleology of the site have been carried out since the mid 1980s, but only after 1990 an extensive biological knowledge was achieved. Several ecological and geological studies were performed (Alvisi et al., 1994a, b; Mattison et al., 1998), particularly on the “Grotta Azzurra” (Figure 2). The cave may be separated into two topographically distinct regions: a weakly illuminated outer region and an inmost dark region (Snow Hall). The latter is

characterized by the presence of sulfur springs that arise from fissures in the cave floor. Currently, no other submarine caves with sulfide springs have been reported.

Microbiological studies

The blue holes

Abundant microbial development has been observed in these cave systems, usually linked to temperature anomalies in the range of 36–41°C as recorded by investigators. Marano-Briggs (2000) reported temperatures up to 41°C in the Tarpon Blue Hole, supporting the theory that the mass population of anoxygenic phototrophic bacteria present at a specific water layer may dissipate excess light energy by heat. A dense layer of purple sulfur bacteria



Microbial Ecology of Submarine Caves, Figure 2 A drawing map of the “Grotta Azzurra” showing both entrances, halls, and freshwater circulation.

was indeed found at 4.5–5.5 m depth in the estuarine Tarpon Blue Hole and the dominant population was represented by *Chromatiaceae* species. These bacteria are capable either of photolithoautotrophic growth with sulfide or elemental sulfur under anoxic conditions in the light or of chemo-organotrophic growth under micro-oxygenic conditions in the dark. Their abundance in the site was consistent with their physiological traits, and among them a phototrophic bacterium in particular was identified as a novel strain of *Marichromatium purpuratum* that contained okenone instead of spirilloxanthin as its major carotenoid.

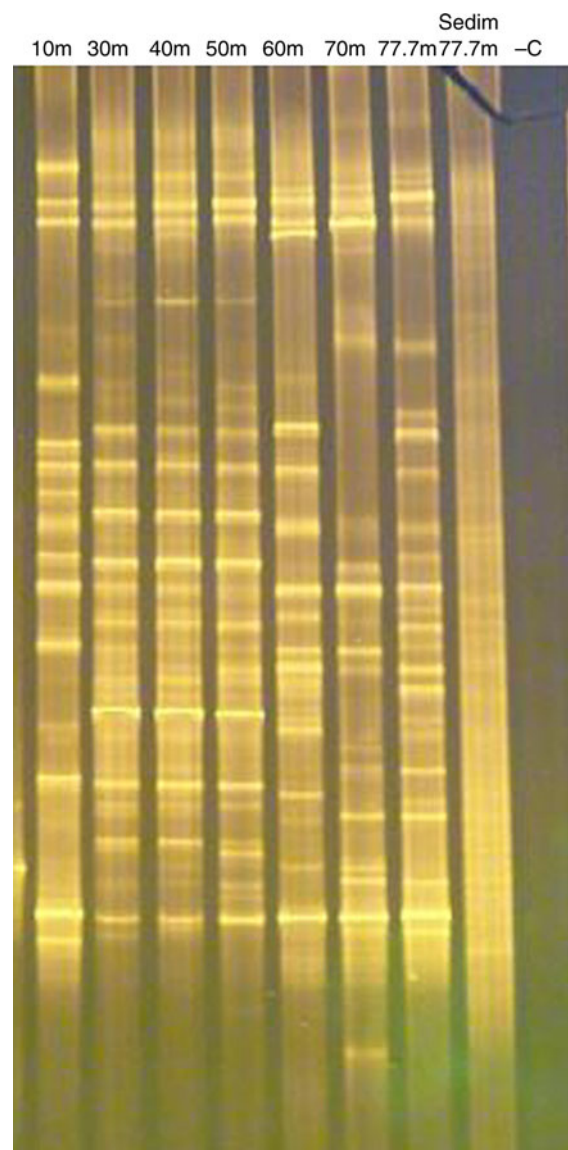
Recently, microbiological studies on a blue hole discovered in the Indian Ocean (Canganella et al., 2004) were preliminarily performed in order to investigate the distribution of microbial populations along the water column and in the bottom sediment of the cave. In Figure 3, the DGGE analysis carried out shows how bacterial populations are distributed among surface layers, mid- and deep-layers, indicating in particular a similar biodiversity between 30 and 50 m depth. The same was investigated by the Biolog system, particularly for anaerobic populations.

The molecular investigation showed that at surface there was a complex microbial community showing several peculiarities with respect to standard pelagic seawater. Characteristic species were *Thioalkalivibrio* and *Thioploca* within the Gamma-Proteobacteria and *Desulfosarcina* and other Delta-Proteobacteria; sequences within the Delta-Proteobacteria related to sponge symbionts were also found. Subsurface bacteria present in the water column appeared dominated by Alpha-Proteobacteria represented by sequences related to photoorganotrophs (*Rhodovibrio*-related), methanotrophs, and coral surface-related bacteria.

Near the bottom, although the Alpha-Proteobacteria were still well represented, the proportion of Delta-Proteobacteria increased. These Delta-Proteobacteria represent sulfate-reducing bacteria mainly belonging to the Desulfobacteriaceae, or related to *Desulfonanticus*, *Syntrophus*, and others related to marine and salt-marsh uncultured bacteria.

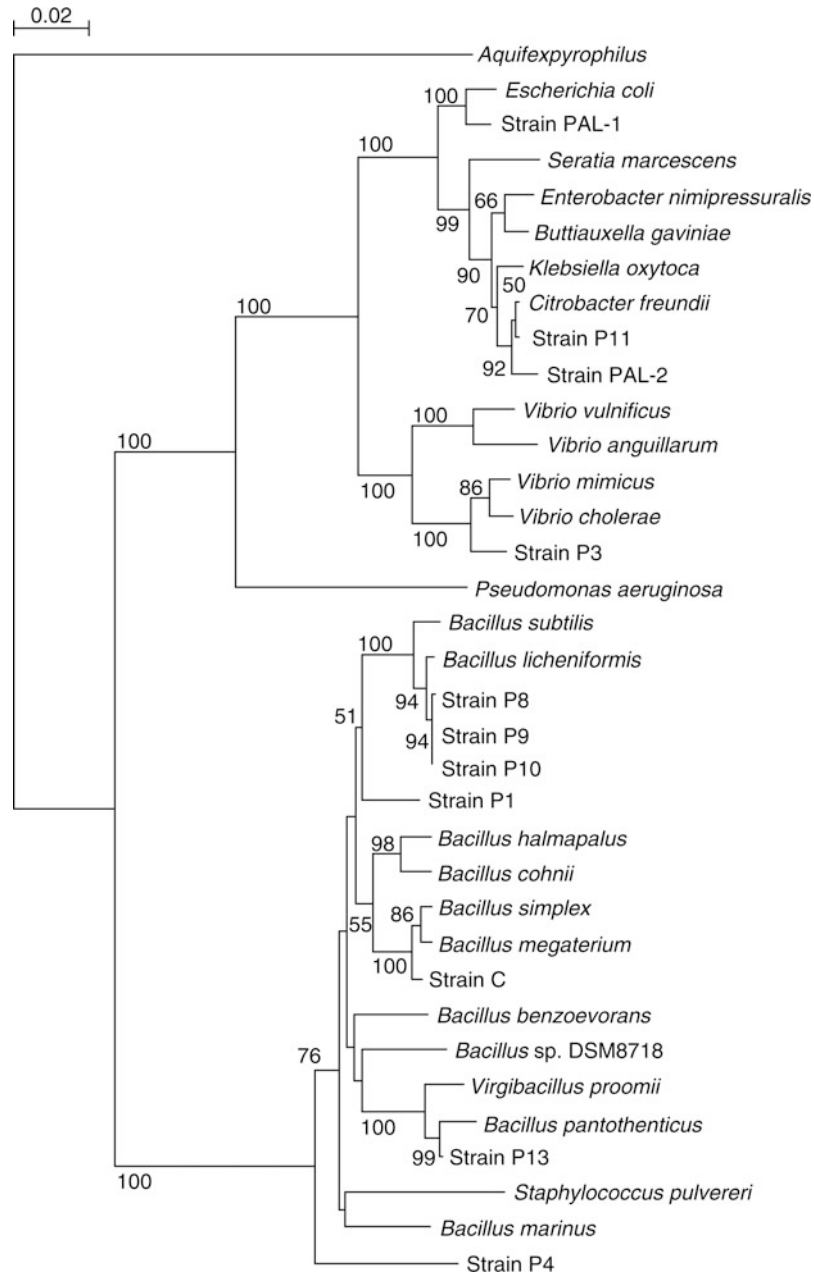
The black holes of the Bahamas

The water in these holes appears black in color due to the presence of a 1 m thick white microbial veil located within the upper third of the water column (18–19 m), at the boundary between the oxygenic low salinity upper water mass and the denser anoxic saline water layer. The boundary between the two water masses is characterized by sharp discontinuities in physicochemical gradients: salinity increased from 12 to 35 psu and temperature from 29°C to 36°C; pH decreased from 8.6 to 6.45 as well as dissolved O₂ from 6 mg/l to <1 mg/l. Morphological microscopic observations of collected samples showed mainly nonmotile spherical and motile rod-shaped purple sulfur bacteria. The dominant members of this warm (36°C), saline, and sulfide-rich layer have been identified



Microbial Ecology of Submarine Caves, Figure 3 DGGE analysis of the water column and bottom sediment of the blue hole in the Indian Ocean.

as anoxygenic phototrophic bacteria belonging to the genera *Allochromatium* and *Thiocapsa* and showed population densities $>10^7$ viable cells/ml. These bacteria grow well in the presence of sulfide and carbon dioxide in the light. During photoautotrophic growth sulfur globules are stored intracellularly as intermediate oxidation products. Moreover, the intracellular photosynthetic membranes are of the vesicular type, and bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series are present (Herbert et al., 2005). Calculations made by these authors revealed that the layer of anoxygenic phototrophic bacteria in the South Andros black hole may have a biomass content of approximately 5.06 ton dry weight.

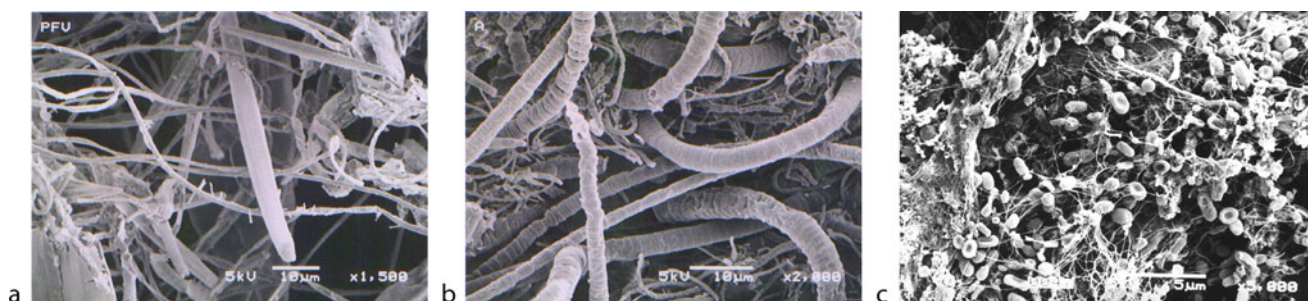


Microbial Ecology of Submarine Caves, Figure 4 A phylogenetic tree based on 16S rRNA gene sequence comparison, including representative heterotrophic isolates from the "Grotta Azzurra."

The marine caves of Capo Palinuro

The emergence of sulfidic water in some of the caves (particularly in the "Grotta Azzurra," "Grotta Sulphurea," and "Grotta di Cala Fetente") gives rise to an unusual situation, characterized by abiotic as well as biotic fluctuations, represented by emissions from sulfidic springs and higher concentration of sulfur-utilizing bacteria following each outflowing event, respectively. Warm sulfidic water of reduced salinity enters the Snow Hall (Grotta Azzurra),

from fissures in the bottom rocks, and rises above the more dense seawater to form a thermocline and chemocline as a visible boundary at a depth of about 9.5 m. The geochemistry of water samples from the Grotta Azzurra was extensively reported by Mattison et al. (1998): above and below the chemocline temperatures were 24.0 ± 0.2 and 22.8 ± 0.3 , respectively; NaCl concentrations (%) were 2.6 ± 0.1 and 3.0 ± 0.1 , respectively; pH values were 7.22 ± 0.02 and 8.15 ± 0.25 , respectively.



Microbial Ecology of Submarine Caves, Figure 5 (a) A SEM picture of a sample collected in the bacterial mat on the vault of the “Grotta Azzurra”; (b) a SEM picture of a sample collected in the bacterial mat on the vault of the “Grotta Azzurra”; (c) A SEM picture of a sediment sample collected nearby a sulfataric spring along the main entrance of the “Grotta Azzurra.”

The present ecosystem is biologically unique particularly because of the following points: (1) the peculiar emissions represent the only available model in shallow waters resembling the deep-sea hydrothermal vents (Canganella, 2001); (2) alike what happens in usual submarine caves, the development of micro- and macro-fauna increases with the decrease of irradiation; (3) the freshwater flow is placed above the seawater and, inside the cave, an interface between the warm/anaerobic upper zone (H_2S -enriched) and the cold, sulfurless lower zone can be observed; (4) giant forms, particularly among *Cnidaria* and *Porifera*, occur mainly along the aerobic/anaerobic interface (Alvisi et al., 1994a, b).

During the last decade both, “Grotta Azzurra” and “Grotta Sulfurea” have been investigated. Some preliminary data on the lithotrophic microflora were published (Mattison et al., 1998), and other studies were performed in order to (1) investigate the microscopic structure of bacterial mat and (2) to understand both the taxonomy and physiology of heterotrophic bacteria inside the “Grotta Azzurra” (Canganella and Bianconi, 1999, 2003). The 16S rRNA genes analysis (Figure 4) showed that heterotrophic isolates were closely related to the genera *Escherichia*, *Citrobacter*, *Vibrio*, and *Bacillus*.

In addition to the analysis of the heterotrophic microflora, recently a preliminary molecular investigation was performed to evaluate the biodiversity of both eubacterial and archaeal populations (Canganella F. and Bianconi G. 2009).

Some bacterial sequences were closely related to the sulfur-oxidizing bacterial group, involving the genus *Beggiatoa* and *Thiothrix*. Showing that these microorganisms may well represent a major microbial community in the bacterial mat of the cave. Other bacterial sequences were phylogenetically linked to the Delta-proteobacterial group, particularly to sulfate-reducing bacteria. Archaeal sequences were partially related to methanogen archaeal group, but the phylogenetic distance was still remarkable; so the involvement of a novel archaeal group can be considered. Other archaeal sequences were related to MG-1,

which is a common uncultivable archaeal group in marine environment.

As regards the microscopic structure of bacterial mat inside the cave and sediments collected nearby a sulfidic spring along the main entrance of the “Grotta Azzurra,” the distribution of various morphological forms is shown in Figures 5a–c. Filamentous microbial forms were abundant and morphologically similar to *Beggiatoa*, *Desulfonema*, *Leucothrix*, or *Thiothrix* species; morphology was, however, not enough to identify such organisms. Several cells showing either coccoid or rod morphologies were also observed in samples collected inside as well as outside the mat.

Conclusions

As regards the blue and black holes, it has been shown that the dominant anoxygenic phototrophic bacteria in the microbial layers are member of the *Chromatiaceae* (Imhoff et al., 1998). The presence of dense phototrophic populations of purple sulfur bacteria particularly explains why the South Andros black hole appears black and not blue. Moreover, the sulfide profile data obtained in this site showed that sulfide concentration was low (30 μM) suggesting that biomass production by the purple sulfur bacteria was sulfide rather than light limited.

Another important point to consider is that the dense microbial phototrophic population probably plays a significant role in enhancing carbonate CO_2 -mediated dissolution operated by heterotrophic bacteria. The effects of microbiological activity on degradation of limestone deposits have been described by several authors (Ehrlich, 1996; Golubic and Schneider, 1979; Paine et al., 1993) and similar observations were reported for the Bahamas cave systems from Smart et al. (1988).

Recent studies have shown that biotic oxidation of sulfide into sulfuric acid can occur in anoxic marine ecosystems due to chemoautotrophic sulfur-oxidizing bacteria such as *Beggiatoa* (Mattison et al., 1998; Vlasceanu et al., 1997, 2000). This biological process may also be involved in carbonate dissolution for the South Andros black hole but the role of sulfur-oxidizing bacteria needs

to be evaluated by further investigations. The same consideration may be valid for the blue hole in the Indian Ocean. In this site the occurrence of highly toxic sulfide and low oxygen content caused the death of the macrobenthos in deep waters, where a chemolithoautotrophic microbial community settled on the cave walls as mucilaginous and hardened short filaments of bacterial origin whose study is still in progress.

In the "Grotta Azzurra" cave of Capo Palinuro, fluids emitted from active springs with maximum temperatures of 25°C and enriched in dissolved sulfur species (H_2S , $\text{S}_2\text{O}_3^{2-}$) allowed the development of a significant micro- and macro-fauna, resembling in some way what occurs around hydrothermal vent sites (Jeanthon, 2000; Canganella, 2001).

Morphological/physiological observations were previously carried out by Mattison et al. (1998) on microbial mats in the "Grotta Azzurra" of Capo Palinuro. In recent studies both sampling and isolation were carried out regardless of the chemocline. Future investigations on the distribution of microbial populations will eventually lead to discrimination between more thermotolerant and more mesophilic species living above or below the chemocline; the same can be true for noncultivable species.

The characterized heterotrophic isolates partially represent the microbial ecology of the site, showing its biological complexity at a phylogenetic level. Moreover, the wide resistance of isolates to temperature, salinity and pH may be associated with the specific ecological system, an hypothesis that certainly deserves further investigations.

In general terms, similar environmental traits can be described for the blue hole in the Indian Ocean and the submarine caves of Palinuro Cape; first of all, the presence of elevated concentrations of hydrogen sulfide, ranging from 5 μM to 200 mM. A chemocline in the water column may be clearly observed in the blue hole of the Indian Ocean or in the black hole of the Bahamas, whereas in the submarine caves of Palinuro Cape the environmental borderline inside the habitat is represented by the threshold between upper anoxic, sulfide-rich freshwater and lower seawater.

As regards the autoctonous microflora, the blue hole of the Indian Ocean showed the presence of filamentous forms as described in the Palinuro caves but mucilaginous forms on the rocky side walls were not found in the latter; moreover sulfate-reducers and sulfide-oxidizers were also detected in both systems.

Some microbiological features of both systems are also shared with other sulfide-rich environments, such as the presence of anaerobic methane oxidizers, filamentous bacteria, anaerobic phototrophs, and archaeal strains. However, the incomplete data set on the inhabitant microflora, the geological diversity of the systems described earlier, and their different geographical locations make a microbiological comparative study difficult. With no

doubts the combination of different approaches and techniques may be a powerful tool to investigate further such astonishing ecosystems by a multiphasic approach.

As a matter of fact, further investigations on the microbial diversity in sulfide-rich, either marine or freshwater, caves will be based on traditional techniques of isolation coupled to simultaneous molecular investigations.

Bibliography

- Alvisi, M., Barbieri, F., and Colantoni, P., 1994a. Le grotte marine di Capo Palinuro. In Alvisi, M., Colantoni, P., Forti, P. (eds.), *Grotte Marine d'Italia*. Bologna: Memorie dell'Istituto Italiano di Speleologia, (pp 143–181).
- Alvisi, M., Barbieri, F., Bruni, R., Cinelli, F., Colantoni, P., Grandi, F., and Maltoni, P., 1994b. La Grotta Azzurra di Capo Palinuro (Salerno). In Alvisi, M., Colantoni, P., Forti, P. (eds.), *Grotte Marine d'Italia*. Bologna: Memorie dell'Istituto Italiano di Speleologia, (pp 51–56).
- Canganella, F., 2001. Hydrothermal vent communities. In *Encyclopedia of Life Sciences*. London: Macmillan.
- Canganella, F., and Bianconi, G., 1999. Microbial investigations on submarine caves in the area of Cape Palinuro (Salerno, ITALY) in Brazilian Microbiology Society (Ed). In *Proceedings of the XX Meeting of the Microbiological Brazilian Society*, Oct 21–25, 2001, p. 223, Salvador, BA.
- Canganella, F., and Bianconi, G., 2003. Microscopic investigations on the cell structure of autoctonous microflora in a sulphidic submarine cave (transl.). In *Fifth Congress FISV*, Oct 10–13, 2003, p 154, Rimini, Italy.
- Canganella, F., Bianconi, G., and Maugeri, T., 2004. A multiphasic approach to investigate the microbial ecology of a Blue Hole in the Indian Ocean. In *Extremophiles Meeting*, September 19–23, 2004, p 83, Cambridge, MA.
- Colantoni, P., Baldelli, G., Bianchi, C. N., Capaccioni, B., Morri, C., Mandrini, M., and Tassi, F., 2003. A cave flooded by marine water with hydrogen sulphide highlights the recent evolution of the Maldives (Indian Ocean): preliminary notes. *Le grotte d'Italia*, 4, 29–37.
- Ehrlich, H. L., 1996. *Geomicrobiology*, 3rd edn. New York: Marcel Dekker, pp 172–215.
- Engel, A. S., Lee, N., Porter, M. L., Stern, L. A., Bennett, P. C., and Wagner, M., 2003. Filamentous "Epsilonproteobacteria" dominate microbial mats from sulfidic cave springs. *Applied and Environmental Microbiology*, 69, 5503–5511.
- Golubic, S. I., and Schneider, J., (1979). Carbonate dissolution. In Trudinger, P. A., Staine, D. J. (eds.), *Biogeochemical Cycling of Mineral-Forming Elements*. Amsterdam: Elsevier, pp. 107–129.
- Herbert, R. A., Ranchou-Peyruse, A., Duran, R., Guyoneaud, R., and Swabe, S., 2005. Characterization of purple sulphur bacteria from the South Andros Black Hole cave system: highlights taxonomic problems for ecological studies among the genera *Allochromatium* and *Thiocapsa*. *Environmental Microbiology*, 7, 1260–1268.
- Holmes, A. J., Tujula, N. A., Holley, M., Contos, A., James, J. M., Rogers, P., and Gillings, M. R., 2001. Phylogenetic structure of unusual aquatic microbial formations in Nullarbor caves, Australia. *Environmental Microbiology*, 3, 256–264.
- Imhoff, J. F., Söling, J., and Petri, R., 1998. Phylogenetic relationships among the *Chromatiaceae*, their taxonomic reclassification and description of the new genera *Allochromatium*, *Halochromatium*, *Isochromatium*, *Marichromatium*, *Thiococcus*, *Thiohalocapsa* and *Thermochromatium*. *International Journal of Systematic Bacteriology*, 48, 1129–1143.

- Jeanthon, C., 2000. Molecular ecology of hydrothermal vent microbial communities. *Antonie van Leeuwen.*, **77**, 117–133.
- Marano-Briggs, K., 2000. *Water Chemistry, Bacterial Abundance and Anoxygenic Photosynthetic Bacteria in an Estuarine Blue Hole, Andros Island, The Bahamas*. PhD Thesis, George Mason University, Fairfax, VA.
- Mattison, R. G., Abbiati, M., Dando, P. R., Fitzsimons, M. F., Pratt, S. M., Southward, A. J., and Southward, E. C., 1998. Chemoautotrophic microbial mats in submarine caves with hydrothermal sulphidic springs at Cape Palinuro, Italy. *Microbial Ecology*, **35**, 58–71.
- Myroie, J. E., Carew, J. L., and Moore, A. I., 1995. Blue holes: definition and genesis. *Carbonate and Evaporites*, **10**, 225–233.
- Northup, D. E., Barns, S. M., Yu, L. E., Spilde, M. N., Schelble, R. T., Dano, K. E., Crossey, L. J., Connolly, C. A., Boston, P. J., Natvig, D. O., and Dahm, C. N., 2003. Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves. *Environmental Microbiology*, **5**, 1071–1086.
- Paine, S. G., Lingood, F. V., Schimmer, F., and Thrupp, T. C., 1993. *The Relationship of Microorganisms to the Decay of Stone*. London: Philosophical Transactions of the Royal Society, pp. 97–127.
- Schwabe, S. J., 1998. The Black Hole. Caves and caving. *British Cave Research Association*, **80**, 1–22.
- Schwabe, S. J., and Herbert, R. A., 2004. Black Holes of the Bahamas: what they are and why they are black. *Quaternary International*, **121**, 3–11.
- Schwabe, S. J., Parkes, R. J., and Smart, P. L., 1997. Biogeochemical investigation of submerged cave systems (Blue Holes) within Bahamian carbonate platforms. In *97th Applied and Environmental Microbiology General Meeting*, May 4–7, 1997, p. 398. Miami, FL.
- Smart, P. L., Dawans, J. M., Whitaker, F., 1988. Carbonate dissolution in a modern mixing zone. *Nature*, **335**, 811–813.
- Sugita, T., Kikuchi, K., Makimura, K., Urata, K., Someya, T., Kamei, K., Niimi, M., and Uehara, Y., 2005. Trichosporon species isolated from guano samples obtained from bat-inhabited caves in Japan. *Applied and Environmental Microbiology*, **71**, 7626–7629.
- Vlasceanu, L., Popa, R., and Kinkle, B., 1997. Characterization of *Thiobacillus thioparus* LV43 and its distribution in a chemoautotrophically based ecosystem. *Applied and Environmental Microbiology*, **63**, 33123–33127.
- Vlasceanu, L., Sarbu, S., Engel, A. S., and Kinkle, B., 2000. Acidic, cave wall biofilms located in the Frasassi George, Italy. *Geomicrobiology*, **17**, 125–139.
- Zhou, J., Gu, Y., Zou, C., and Mo, M., 2007. Phylogenetic diversity of bacteria in an earth-cave in Guizhou province, southwest of China. *Journal of Microbiology*, **45**, 105–112.

Cross-references

Bacteria
 Beggiatoa
 Biofilms
 Hydrothermal Environments, Marine
 Karst Ecosystems
 Metals, Acquisition by Marine Bacteria
 Microbial Communities, Structure, and Function
 Microbial Mats
 Nanocrystals, Microbially Induced
 Siderophores
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Sulfur Cycle

MICROBIAL MATS

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Algal mats; Bacterial mats; Biomats; Thick biofilms

Definition

Microbial mats are often centimeter-thick multilayered structures of microorganisms, mainly bacteria, archaea, fungi, and sometimes these mats are enriched with protozoans. Microbial biofilms are in contrast much thinner (10–100 µm) than microbial mats and have a different architecture (Characklis and Wilderer, 1989). Microbial mats grow at interfaces between different types of material, mostly on moist surfaces, but some of them are also found in dry environments like deserts (e.g., Stal and Caumette, 1994; Characklis and Wilderer, 1989). They colonize environments located in high altitude, they are common in the Deep Biosphere, in subterranean environments with temperatures from –40°C to +120°C. There are also common as endosymbiotic structures, for example, in animals. They are very common in extreme environments like hydrothermal vents, cold seeps, and alkaline and hypersaline lakes (Van Dover, 2000; Pederesen, 2006; Arp et al., 2003; Riding and Awramik, 2000).

Geobiological implications

A microbial mat consists of several layers dominated by specific types of microorganism (Figures 1 and 2). Although the composition of individual mats varies depending on the environment, as a rule the by-products of each group of microorganisms serve as energy source for other groups. Different types of microorganisms dominate different layers based on their comparative advantage (Canfield and Des Marais, 1993; Allison et al., 2000). Ecological relationships between different groups are a combination of competition and cooperation. The metabolic capabilities of bacteria and archaea are generally depend on their phylogeny. In a wet environment where sunlight is available, the upper layers are generally dominated by aerobic consortia like cyanobacteria (Figure 1), while the lowest layers are normally dominated by anaerobic microorganisms such as sulfate-reducing bacteria, methanogenic and/or methanotrophic archaea, or general archaea. Marine mats may grow to a few centimeters in thickness, of which only the top few millimeters are oxygenated. Microbial mats are held together and bound to their substrates by sugar- and protein-rich extracellular polymeric substances (EPS), which they secrete (Decho et al., 2005; Wingender et al., 1999). In many cases, some of these microorganisms form filaments (threads), which tangle and thus increase the microbial

- In Pagel, M., Barbin, V., Blanc, P., and Ohnenstetter, D. (eds.), *Cathodoluminescence in Geosciences*. Berlin: Springer, pp. 59–126.
- Schinagl, W. and Vortisch, W., (in preparation). Sedimentation and diagenesis of an Upper Cretaceous sandstone from eastern Austria.
- Scott, A. C., and Collinson, M. E., 2003. Non-destructive multiple approaches to interpret the preservation of plant fossils: implications for calcium-rich permineralizations. *Journal of the Geological Society*, **160**, 857–862.
- Vortisch, W., 2004. Application of combined cathodoluminescence and energy-dispersive X ray analysis of mineral raw materials. In Pecchio, M., Andrade, F. R. D., D'Agostino, L. Z., Kahn, H., Sant'Agostino, L. M. Tassinari, M. L. (eds.), *Applied Mineralogy – Developments in Science and Technology*. São Paulo, Brazil: International Council for Applied Mineralogy do Brasil, ICAM-BR, Vol. 1, pp. 169–172.
- Vortisch, W., Harding, D., and Morgan, J., 2003. Petrographic analysis using cathodoluminescence microscopy simultaneous with energy-dispersive X-ray spectroscopy. *Mineralogy and Petrology*, **79**, 193–202.
- Walker, G., and Burley, S. D., 1991. Luminescence petrography and spectroscopic studies of diagenetic minerals. In Barker, C. E., and Kopp, O. C. (eds.), *Luminescence Microscopy and Spectroscopy: Qualitative and Quantitative Applications*. Tulsa: Short course 25, SEPM (Society for Sedimentary Research), pp. 83–96.
- Wierzbowski, H., and Joachimski, M., 2007. Reconstruction of late Bajocian-Bathonian marine palaeoenvironments using carbon and oxygen isotope ratios of calcareous fossils from the Polish Jura Chain (central Poland). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 523–540.
- Witkowski, F. W., Blundell, D. J., Gutteridge, P., Horbury, A. D., Oxtoby, N. H., and Quing, H., 2000. Video cathodoluminescence microscopy of diagenetic cements and its applications. *Marine and Petroleum Geology*, **17**, 1085–1093.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Biofilms and Fossilization](#)
[Biogeochemical Cycles](#)
[Biosignatures in Rocks](#)
[Calcareous Algae](#)
[Calcite Precipitation, Microbially Induced](#)
[Calcium Biogeochemistry](#)
[Cap Carbonates](#)
[Carbonate Environments](#)
[Cold Seeps](#)
[Clay Authigenesis, Bacterial](#)
[Deep Biosphere of Sediments](#)

[Dolomite, Microbial](#)
[Foraminifera](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Silicification – Bacteria \(or Passive\)](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Molar-tooth Structure](#)
[Nanocrystals, Microbially Induced](#)
[Pedogenic Carbonates](#)
[Phosphorus, Phosphorites](#)
[Radiolaria](#)
[Reefs](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Silica Biomineralization, Sponges](#)
[Tufa, Freshwater](#)

CHEMOLITHOTROPHY

Volker Thiel
 University of Göttingen, Göttingen, Germany

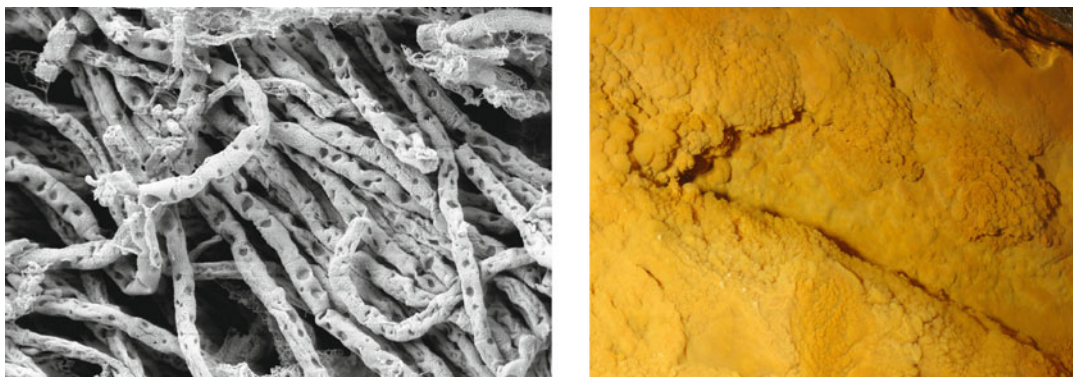
The term “Chemolithotrophy” refers to the gain of energy for cell biosynthesis and maintenance from the oxidation of inorganic compounds (= electron donors), in the absence of light (Kelly and Wood, 2006). The process was first described by the Russian microbiologist Sergej Winogradsky (1887, 1888).

Chemolithotrophy is a strategy unique to some prokaryotes (i.e., Bacteria and Archaea), the so-called chemolithotrophs. Whereas there is no known macrofauna possessing the capability of chemolithotrophy, some animals such as particular tubeworms and bivalves can form symbioses with chemolithotrophs, (e.g., at cold seeps or in hydrothermal environments). There exist many inorganic electron donors that can fuel chemolithotrophs involving geologic, biologic, and anthropogenic sources. Reduced sulfur, nitrogen and iron species and hydrogen are the most common substrates (Table 1). If chemolithotrophs are able to use

Chemolithotrophy, Table 1 Some common reactions performed by chemolithotrophs

Electron donor	Reaction	Organism	$\Delta G_0'$ (kJ/reaction)
Hydrogen	$\text{H}_2 + \frac{1}{2} \text{O}_2 \rightarrow \text{H}_2\text{O}$	Hydrogen bacteria	–237.2
Sulfide	$\text{HS}^- + \text{H}^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{S}^0 + \text{H}_2\text{O}$	Sulfur bacteria	–209.4
Sulfur	$\text{S}^0 + \frac{1}{2} \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$	Sulfur bacteria	–587.1
Ammonium	$\text{NH}_4^+ + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_2^- + 2\text{H}^+ + \text{H}_2\text{O}$	Nitrifying bacteria	–274.7
Nitrite	$\text{NO}_2^- + \frac{1}{2} \text{O}_2 \rightarrow \text{NO}_3^-$	Nitrifying bacteria	–74.1
Iron	$\text{Fe}^{2+} + \text{H}^+ + \frac{1}{4} \text{O}_2 \rightarrow \text{Fe}^{3+} + \frac{1}{2} \text{H}_2\text{O}$	Iron oxidizing bacteria	–32.9

Source: Adapted from Madigan and Martinko (2006)



Chemolithotrophy, Figure 1 *Left:* Filamentous sulfur bacteria forming a bacterial mat in the Tunnel of Äspö, Sweden. These chemoautotrophs oxidize sulfide to elemental sulfur. Holes in the filaments are preparation artifacts that reflect the former location of intracellular sulfur granules. The filaments are approximately 1 μm in diameter (Scanning electron microscope image courtesy of J. Reitner). *Right:* Massive iron-oxidizing microbial mats developing at an underground spring discharging water rich in reduced iron (Fe^{2+}). The chemolithotrophic bacterium *Gallionella ferruginea* is a main component of the microbial community at this site (Tunnel of Äspö, Sweden) and is responsible for the production of large amounts of orange coloured iron oxides and hydroxides. The area displayed is about 1 m wide.

CO_2 as their carbon source (most are) they are referred to as chemolithoautotrophs. Well-known examples of chemolithotrophs relevant in geobiology are sulfur-oxidizing bacteria (e.g., *Beggiatoa*; *Thiomargarita*) and iron-oxidizing bacteria (see entries “*Fe(II)*-Oxidizing Prokaryotes,” “*Gallionella*”) (Figure 1).

Bibliography

- Kelly, D., and Wood, A. P., 2006. The chemolithotrophic prokaryotes. *Prokaryotes*, 2, 441–456.
- Madigan, M., and Martinko, J. (eds.), 2006. *Brock Biology of Microorganisms*, 11th edn. Upper Saddle River, NJ: Prentice Hall.
- Winogradsky, S., 1887. Über Schwefelbakterien. *Botanische Zeitung*, 45, 489–507, 513–523.
- Winogradsky, S., 1888. Über Eisenbakterien. *Botanische Zeitung*, 46, 261–270.

Cross-references

Acetogens
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Bacteria
 Beggiatoa
 Cold Seeps
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Hydrothermal Environments, Marine
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Thiomargarita

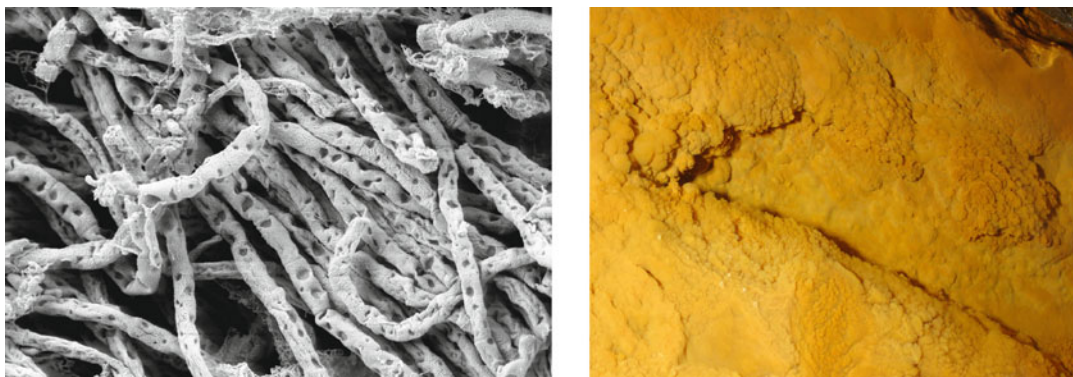
CHERTS

Volker Thiel
 University of Göttingen, Göttingen, Germany

Chert is a sedimentary rock mainly consisting of micro- or cryptocrystalline (=submicroscopic) quartz (SiO_2) varieties.

Cherts often have an “indirect” biogenic origin as they result from re-precipitation of dissolved opal-A shells derived from microorganisms such as diatoms (diatomite, diatomaceous cherts, see also entry “*Diatoms*”) and radiolarians (radiolarite, see also entry “*Protozoa (Heterotroph, Eukaryotic)*”). Postsedimentary dissolution of these shells leads to the accumulation of silica-rich fluids within pore waters, thus, inducing precipitation of metastable cristobalite, also known as opal-CT (Konhauser, 2007). Opal-CT may, under increasing temperatures and pressures, be converted into stable quartz. Chert may also originate from microbial silification, and it may form, with or without microbial mediation, in hydrothermal environments (Konhauser et al., 2004; Jones et al., 2004, see also entries “*Sinter*” and “*Hot Springs and Geysers*”). Depending on the content of clay and other minerals, chert may grade into siliceous shales or -mudstones. The color of cherts may vary greatly with the content of accessory elements. Iron (hematite), for instance, gives rise to red varieties called “jasper.” Banded cherts, with often colorful, rhythmic layers, are called “agate.”

Chert may occur as extensive layers (bedded cherts) within sedimentary formations as well as in concretionary



Chemolithotrophy, Figure 1 *Left:* Filamentous sulfur bacteria forming a bacterial mat in the Tunnel of Äspö, Sweden. These chemoautotrophs oxidize sulfide to elemental sulfur. Holes in the filaments are preparation artifacts that reflect the former location of intracellular sulfur granules. The filaments are approximately 1 μm in diameter (Scanning electron microscope image courtesy of J. Reitner). *Right:* Massive iron-oxidizing microbial mats developing at an underground spring discharging water rich in reduced iron (Fe^{2+}). The chemolithotrophic bacterium *Gallionella ferruginea* is a main component of the microbial community at this site (Tunnel of Äspö, Sweden) and is responsible for the production of large amounts of orange coloured iron oxides and hydroxides. The area displayed is about 1 m wide.

CO_2 as their carbon source (most are) they are referred to as chemolithoautotrophs. Well-known examples of chemolithotrophs relevant in geobiology are sulfur-oxidizing bacteria (e.g., *Beggiatoa*; *Thiomargarita*) and iron-oxidizing bacteria (see entries “*Fe(II)*-Oxidizing Prokaryotes,” “*Gallionella*”) (Figure 1).

Bibliography

- Kelly, D., and Wood, A. P., 2006. The chemolithotrophic prokaryotes. *Prokaryotes*, 2, 441–456.
- Madigan, M., and Martinko, J. (eds.), 2006. *Brock Biology of Microorganisms*, 11th edn. Upper Saddle River, NJ: Prentice Hall.
- Winogradsky, S., 1887. Über Schwefelbakterien. *Botanische Zeitung*, 45, 489–507, 513–523.
- Winogradsky, S., 1888. Über Eisenbakterien. *Botanische Zeitung*, 46, 261–270.

Cross-references

Acetogens
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Bacteria
 Beggiatoa
 Cold Seeps
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Hydrothermal Environments, Marine
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Thiomargarita

CHERTS

Volker Thiel
 University of Göttingen, Göttingen, Germany

Chert is a sedimentary rock mainly consisting of micro- or cryptocrystalline (=submicroscopic) quartz (SiO_2) varieties.

Cherts often have an “indirect” biogenic origin as they result from re-precipitation of dissolved opal-A shells derived from microorganisms such as diatoms (diatomite, diatomaceous cherts, see also entry “*Diatoms*”) and radiolarians (radiolarite, see also entry “*Protozoa (Heterotroph, Eukaryotic)*”). Postsedimentary dissolution of these shells leads to the accumulation of silica-rich fluids within pore waters, thus, inducing precipitation of metastable cristobalite, also known as opal-CT (Konhauser, 2007). Opal-CT may, under increasing temperatures and pressures, be converted into stable quartz. Chert may also originate from microbial silification, and it may form, with or without microbial mediation, in hydrothermal environments (Konhauser et al., 2004; Jones et al., 2004, see also entries “*Sinter*” and “*Hot Springs and Geysers*”). Depending on the content of clay and other minerals, chert may grade into siliceous shales or -mudstones. The color of cherts may vary greatly with the content of accessory elements. Iron (hematite), for instance, gives rise to red varieties called “jasper.” Banded cherts, with often colorful, rhythmic layers, are called “agate.”

Chert may occur as extensive layers (bedded cherts) within sedimentary formations as well as in concretionary

nodules. For example, vast deposits of bedded cherts, alternating with iron minerals, are found within the Precambrian “Banded Iron Formations” (see entry). “Flintstones” on the other hand, are dark-colored chert nodules that precipitated in chalk such as in the Upper Cretaceous Maastrichtian Formation in Middle Europe. Here, the SiO₂ mainly derived from diagenetically dissolved, and re-precipitated, sponge spicules. Deposits with a high content of sponge-derived SiO₂ dispersed in sedimentary rocks are called “spiculites.”

Cherts may provide an excellent matrix for the long-term preservation of macro- and microfossils as well as chemical and isotopic signatures, due to their density and resistance. Examples are the Rhynie Chert that uniquely preserved an early Devonian community of plants, animals, bacteria, and fungi (Taylor et al., 2009), and numerous Precambrian cherts that provide an exciting view on the early dawn of life on Earth (Derenne et al., 2008; Westall et al., 2006, see entry “*Biosignatures in Rocks*”).

Bibliography

- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J.-N., 2008. Molecular evidence for life in the 3.5 billion-year old Warrawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu Geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Taylor, T. N., Taylor, E. L., and Krings, M., 2009. *Paleobotany – The Biology and Evolution of Fossil Plants*. Amsterdam: Elsevier (Academic Press).
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006. The 3.466 Ga Kitty’s Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications, 405, pp. 105–131.

Cross-references

Algae (Eukaryotic)
 Banded Iron Formations
 Biosignatures in Rocks
 Diatoms
 Hot Springs and Geysers
 Hydrothermal Environments, Terrestrial
 Microbial Silicification – Bacteria (or Passive)
 Pore Waters
 Protozoa (Heterotroph, Eukaryotic)
 Sinter
 Silica Biomineralization, Sponges
 Sponges (Porifera) and Sponge Microbes

CHONDRITES

Chondrites are the most common class of stony meteorites. Chondrites largely consist of iron- and magnesium silicate minerals and remain unaffected from melting and differentiation. Therefore, chondrites are considered to reflect the elemental composition of the original solar nebula. *Carbonaceous chondrites* are a rare variety of chondrites containing numerous organic compounds, including amino acids. See “*Meteoritics*” for further reading.

CHROOCOCCIDIOPSIS

Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

The unicellular cyanobacterial genus *Chroococcidiopsis* was first described by Geitler (1933) from Sumatra, where it was found in warm springs.

Definition

The cyanobacterial genus *Chroococcidiopsis* is defined as having more or less spherical cells surrounded by a thin, firm, colorless, sometimes layered extra-cellular polysaccharide sheath (EPS). The cell has an S-layer of a special ribbon-like type, not found in other cyanobacteria so far (Büdel and Rhiel, 1985). It often occurs in large agglomerations of spherical or irregular shape. Fully grown cells (usually 2–6 µm in diameter) divide in two modes: (1) after one or two binary divisions with planes rectangular to each other, resulting daughter cells continue to divide in different planes without intermediate growth (successive multiple divisions); (2) by successive or almost spontaneous irregular cell division (multiple fission) without intermediate growth. In both types, the final daughter cells are very small (1–3 µm in diameter) and are released by rupture of the sheath envelope.

Presently, the genus *Chroococcidiopsis* comprises 14 different species (Komárek and Anagnostidis, 1998). *Chroococcidiopsis* is closely related to the heterocyst-containing, filamentous cyanobacteria (Nostocales, Stigonematales, see entry “*Cyanobacteria*”) and forms their natural sister group (Fewer et al., 2002). The current species concept needs revision.

In terms of ecology, *Chroococcidiopsis* is most interesting because of the wide variety of habitats it colonizes. Among these are different extreme biotopes such as thermal or mineral springs (see entry “*Hydrothermal Environments, Terrestrial*”), and extremely hot and cold deserts (Antarctica), where it is epilithic or endolithic on rocks (Figure 1a–h; see entry “*Endoliths*”). The genus is a common photobiont of the cyanolichens of the

nodules. For example, vast deposits of bedded cherts, alternating with iron minerals, are found within the Precambrian “Banded Iron Formations” (see entry). “Flintstones” on the other hand, are dark-colored chert nodules that precipitated in chalk such as in the Upper Cretaceous Maastrichtian Formation in Middle Europe. Here, the SiO₂ mainly derived from diagenetically dissolved, and re-precipitated, sponge spicules. Deposits with a high content of sponge-derived SiO₂ dispersed in sedimentary rocks are called “spiculites.”

Cherts may provide an excellent matrix for the long-term preservation of macro- and microfossils as well as chemical and isotopic signatures, due to their density and resistance. Examples are the Rhynie Chert that uniquely preserved an early Devonian community of plants, animals, bacteria, and fungi (Taylor et al., 2009), and numerous Precambrian cherts that provide an exciting view on the early dawn of life on Earth (Derenne et al., 2008; Westall et al., 2006, see entry “*Biosignatures in Rocks*”).

Bibliography

- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J.-N., 2008. Molecular evidence for life in the 3.5 billion-year old Warrawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu Geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Taylor, T. N., Taylor, E. L., and Krings, M., 2009. *Paleobotany – The Biology and Evolution of Fossil Plants*. Amsterdam: Elsevier (Academic Press).
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006. The 3.466 Ga Kitty’s Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications, 405, pp. 105–131.

Cross-references

Algae (Eukaryotic)
 Banded Iron Formations
 Biosignatures in Rocks
 Diatoms
 Hot Springs and Geysers
 Hydrothermal Environments, Terrestrial
 Microbial Silicification – Bacteria (or Passive)
 Pore Waters
 Protozoa (Heterotroph, Eukaryotic)
 Sinter
 Silica Biomineralization, Sponges
 Sponges (Porifera) and Sponge Microbes

CHONDRITES

Chondrites are the most common class of stony meteorites. Chondrites largely consist of iron- and magnesium silicate minerals and remain unaffected from melting and differentiation. Therefore, chondrites are considered to reflect the elemental composition of the original solar nebula. *Carbonaceous chondrites* are a rare variety of chondrites containing numerous organic compounds, including amino acids. See “*Meteoritics*” for further reading.

CHROOCOCCIDIOPSIS

Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

The unicellular cyanobacterial genus *Chroococcidiopsis* was first described by Geitler (1933) from Sumatra, where it was found in warm springs.

Definition

The cyanobacterial genus *Chroococcidiopsis* is defined as having more or less spherical cells surrounded by a thin, firm, colorless, sometimes layered extra-cellular polysaccharide sheath (EPS). The cell has an S-layer of a special ribbon-like type, not found in other cyanobacteria so far (Büdel and Rhiel, 1985). It often occurs in large agglomerations of spherical or irregular shape. Fully grown cells (usually 2–6 µm in diameter) divide in two modes: (1) after one or two binary divisions with planes rectangular to each other, resulting daughter cells continue to divide in different planes without intermediate growth (successive multiple divisions); (2) by successive or almost spontaneous irregular cell division (multiple fission) without intermediate growth. In both types, the final daughter cells are very small (1–3 µm in diameter) and are released by rupture of the sheath envelope.

Presently, the genus *Chroococcidiopsis* comprises 14 different species (Komárek and Anagnostidis, 1998). *Chroococcidiopsis* is closely related to the heterocyst-containing, filamentous cyanobacteria (Nostocales, Stigonematales, see entry “*Cyanobacteria*”) and forms their natural sister group (Fewer et al., 2002). The current species concept needs revision.

In terms of ecology, *Chroococcidiopsis* is most interesting because of the wide variety of habitats it colonizes. Among these are different extreme biotopes such as thermal or mineral springs (see entry “*Hydrothermal Environments, Terrestrial*”), and extremely hot and cold deserts (Antarctica), where it is epilithic or endolithic on rocks (Figure 1a–h; see entry “*Endoliths*”). The genus is a common photobiont of the cyanolichens of the

nodules. For example, vast deposits of bedded cherts, alternating with iron minerals, are found within the Precambrian “Banded Iron Formations” (see entry). “Flintstones” on the other hand, are dark-colored chert nodules that precipitated in chalk such as in the Upper Cretaceous Maastrichtian Formation in Middle Europe. Here, the SiO₂ mainly derived from diagenetically dissolved, and re-precipitated, sponge spicules. Deposits with a high content of sponge-derived SiO₂ dispersed in sedimentary rocks are called “spiculites.”

Cherts may provide an excellent matrix for the long-term preservation of macro- and microfossils as well as chemical and isotopic signatures, due to their density and resistance. Examples are the Rhynie Chert that uniquely preserved an early Devonian community of plants, animals, bacteria, and fungi (Taylor et al., 2009), and numerous Precambrian cherts that provide an exciting view on the early dawn of life on Earth (Derenne et al., 2008; Westall et al., 2006, see entry “*Biosignatures in Rocks*”).

Bibliography

- Derenne, S., Robert, F., Skrzypczak-Bonduelle, A., Gourier, D., Binet, L., and Rouzaud, J.-N., 2008. Molecular evidence for life in the 3.5 billion-year old Warrawoona chert. *Earth and Planetary Science Letters*, **272**, 476–480.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu Geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Taylor, T. N., Taylor, E. L., and Krings, M., 2009. *Paleobotany – The Biology and Evolution of Fossil Plants*. Amsterdam: Elsevier (Academic Press).
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006. The 3.466 Ga Kitty’s Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications, 405, pp. 105–131.

Cross-references

Algae (Eukaryotic)
 Banded Iron Formations
 Biosignatures in Rocks
 Diatoms
 Hot Springs and Geysers
 Hydrothermal Environments, Terrestrial
 Microbial Silicification – Bacteria (or Passive)
 Pore Waters
 Protozoa (Heterotroph, Eukaryotic)
 Sinter
 Silica Biomineralization, Sponges
 Sponges (Porifera) and Sponge Microbes

CHONDRITES

Chondrites are the most common class of stony meteorites. Chondrites largely consist of iron- and magnesium silicate minerals and remain unaffected from melting and differentiation. Therefore, chondrites are considered to reflect the elemental composition of the original solar nebula. *Carbonaceous chondrites* are a rare variety of chondrites containing numerous organic compounds, including amino acids. See “*Meteoritics*” for further reading.

CHROOCOCCIDIOPSIS

Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

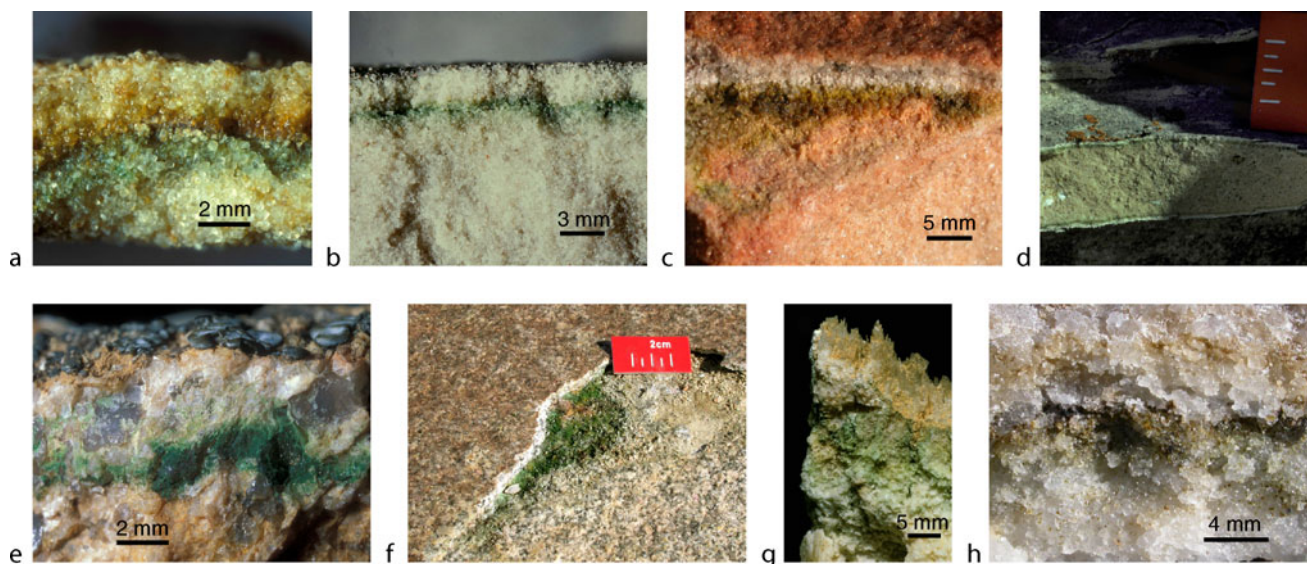
The unicellular cyanobacterial genus *Chroococcidiopsis* was first described by Geitler (1933) from Sumatra, where it was found in warm springs.

Definition

The cyanobacterial genus *Chroococcidiopsis* is defined as having more or less spherical cells surrounded by a thin, firm, colorless, sometimes layered extra-cellular polysaccharide sheath (EPS). The cell has an S-layer of a special ribbon-like type, not found in other cyanobacteria so far (Büdel and Rhiel, 1985). It often occurs in large agglomerations of spherical or irregular shape. Fully grown cells (usually 2–6 µm in diameter) divide in two modes: (1) after one or two binary divisions with planes rectangular to each other, resulting daughter cells continue to divide in different planes without intermediate growth (successive multiple divisions); (2) by successive or almost spontaneous irregular cell division (multiple fission) without intermediate growth. In both types, the final daughter cells are very small (1–3 µm in diameter) and are released by rupture of the sheath envelope.

Presently, the genus *Chroococcidiopsis* comprises 14 different species (Komárek and Anagnostidis, 1998). *Chroococcidiopsis* is closely related to the heterocyst-containing, filamentous cyanobacteria (Nostocales, Stigonematales, see entry “*Cyanobacteria*”) and forms their natural sister group (Fewer et al., 2002). The current species concept needs revision.

In terms of ecology, *Chroococcidiopsis* is most interesting because of the wide variety of habitats it colonizes. Among these are different extreme biotopes such as thermal or mineral springs (see entry “*Hydrothermal Environments, Terrestrial*”), and extremely hot and cold deserts (Antarctica), where it is epilithic or endolithic on rocks (Figure 1a–h; see entry “*Endoliths*”). The genus is a common photobiont of the cyanolichens of the



Chroococcidiopsis, Figure 1 *Chroococcidiopsis* endolithic in rocks. (a) Sandstone of the Clarens formation, Golden Gate Highlands National Park, South Africa. (b) Tshipise Sandstone Plateau, Limpopo Province, South Africa. (c) Beacon Sandstone, Dry Valleys, Antarctica. (d) Cocconino Sandstone, Colorado Plateau, Arizona, USA. (e) Granite inselberg, Limpopo Province, South Africa. (f) Granite rock boulder, Dry Valleys, Antarctica. (g) Gypsum from a sebkah, Tunisia. (h) Calcite rock outcrop, Dry Valleys, Antarctica.

Lichinomycetes group (see entry “*Fungi and Lichens*”), mostly growing on rock surfaces of hot deserts. As an endolith, it contributes to biogenic weathering (see entry “*Bioerosion*”) of silicate rocks by photosynthetic alkalization (Büdel et al., 2004).

Bibliography

- Büdel, B., and Rhiel, E., 1985. A new cell wall structure in a symbiotic and a free-living strain of the blue-green alga genus *Chroococcidiopsis* (Pleurocapsales). *Archiv für Mikrobiologie*, **143**, 117–121
- Büdel, B., Weber, B., Kühl, M., Pfanz, H., Sültemeyer, D., and Wessels, D. C. J., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268
- Fewer, D., Friedl, T., and Büdel, B., 2002. *Chroococcidiopsis* and heterocyst-differentiating cyanobacteria are each others’ closest living relatives. *Molecular Phylogenetics and Evolution*, **41**, 498–506
- Geitler, L., 1933. Diagnosen neuer Blaualgen von den Sunda-Inseln. *Archiv für Hydrobiologie*, **4**(Supplement 12 Tropische Binnengewässer), 622–634
- Komárek, J., and Anagnostidis, K., 1998. *Cyanoprokaryota 1. Teil Chroococcales*. Jena: Gustav Fischer.

Cross-references

[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biological Volcanic Rock Weathering](#)
[Cyanobacteria](#)
[Desert Varnish](#)
[Endoliths](#)
[Extreme Environments](#)
[Fungi and Lichens](#)
[Geomycology](#)

CLAY AUTHIGENESIS, BACTERIAL

Kurt O. Konhauser
 University of Alberta, Edmonton, Alberta, Canada

Definition

The formation of clay minerals in situ through the direct or indirect action of bacteria.

Overview

Clays are one of the largest and most diverse assemblage of minerals known. The vast majority of clay minerals are aluminum silicate in composition, with various other cations substituted into the crystal lattice. The replacement of either Si or Al can lead to an excess of negative charge, which is either neutralized by the adsorption of additional cations onto the outer mineral surfaces or within the interlayer spaces.

Within the past 2 decades, field studies have led to the general recognition that bacteria can mediate the formation of clay minerals. Some clays form as replacement products from the alteration of primary minerals. In this regard, the bacterial community growing on the rock surface potentially function initially as the weathering agents (through the production of organic acids or ligands that facilitate mineral dissolution) and later as reactive surfaces for the nucleation of secondary clay phases. For instance, Konhauser et al. (2002) documented that highly altered rocks found within active steam vents at Kilauea Volcano, Hawaii, contained bacteria with small (<500 nm in diameter) grains of smectite $[(\text{Al}_{1.6}\text{Fe}_{0.4})(\text{Si}_{3.8}\text{Al}_{0.2})\text{O}_{10}(\text{OH})_2\cdot\text{K}_{0.2}]$ attached directly to the outer cell walls.

exist. First, clays provide a good source of exchangeable inorganic nutrients readily available to microbes (Tazaki et al., 1994). Second, clays such as montmorillonite stimulate the respiration of bacteria by maintaining the pH and water content of the environment at a level suitable for growth (Stotzky and Rem, 1966). Third, the formation of an external mineralized matrix may protect the living cells from the detrimental effects of toxin-producing microorganisms (Habte and Barrion, 1984) and predation by grazing protozoans (e.g., Heynen et al., 1988).

Summary

Microorganisms are commonly associated with fine-grained (Fe, Al)-silicates of variable composition. The inorganic phases develop in a predictable manner, beginning with the adsorption of cationic iron to anionic cellular surfaces, supersaturation of the proximal fluid with Fe (III), nucleation and precipitation of a precursor ferric hydroxide phase on the cell surface, followed by reaction with dissolved silica and aluminium, and eventually the growth of an amorphous clay-like phase.

Bibliography

- Davis, C. C., Chen, H. W., and Edwards, M., 2002. Modelling silica sorption to iron hydroxide. *Environmental Science and Technology*, **36**, 582–587.
- Doyle, R. J., 1989. How cell walls of gram-positive bacteria interact with metal ions. In: Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley, pp. 275–293.
- Farmer, V. C., Krishnamurti, G. S. R., and Huang, P. M., 1991. Synthetic allophane and layer-silicate formation in $\text{SiO}_2\text{-Al}_2\text{O}_3\text{-FeO-Fe}_2\text{O}_3\text{-MgO-H}_2\text{O}$ systems at 23°C and 89°C in a calcareous environment. *Clays and Clay Minerals*, **39**, 561–570.
- Ferris, F. G., Fyfe, W. S., and Beveridge, T. J., 1987. Bacteria as nucleation sites for authigenic minerals in a metal-contaminated lake sediment. *Chemical Geology*, **63**, 225–232.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2001. Sorption of Fe Hydr(oxides) to the surface of *Shewanella putrefaciens*: cell-bound fine-grained minerals are not always formed de novo. *Applied and Environmental Microbiology*, **67**, 5544–5550.
- Habte, M., and Barrion, M., 1984. Interaction of *Rhizobium* sp. with toxin producing fungus in culture medium and in a tropical soil. *Applied and Environmental Microbiology*, **47**, 1080–1083.
- Heynen, C. E., van Elsas, J. D., Kuikman, P. J., and van Veen, J. A., 1988. Dynamics of *Rhizobium leguminosarum* biovar *trifolii* introduced in soil; the effect of bentonite clay on predation by protozoa. *Soil Biology and Biochemistry*, **20**, 483–488.
- Konhauser, K. O., and Urrutia, M. M., 1999. Bacterial clay authigenesis: a common biogeochemical process. *Chemical Geology*, **161**, 399–413.
- Konhauser, K. O., Fisher, Q. J., Fyfe, W. S., Longstaffe, F. J., and Powell, M. A., 1998. Authigenic mineralization and detrital clay binding by freshwater biofilms: the Brahmani River, India. *Geomicrobiology Journal*, **15**, 209–222.
- Konhauser, K. O., Schiffman, P., and Fisher, Q. J., 2002. Microbial mediation of authigenic clays during hydrothermal alteration of Basaltic Tephra, Kilauea Volcano. *Geochemistry, Geophysics, and Geosystems*, **3**(12), 1–13.
- Leveille, R. J., Longstaffe, F. J., and Fyfe, W. S., 2002. Kerolite in carbonate-rich speleothems and microbial deposits from Basaltic Sea Caves, Kauai, Hawaii. *Clays and Clay Minerals*, **50**, 514–524.
- Steefel, C. I., and van Cappellen, P., 1990. A new kinetic approach to modeling water–rock interaction: the role of nucleation, precursors, and ostwald ripening. *Geochimica et Cosmochimica Acta*, **54**, 2657–2677.
- Stotzky, G., and Rem, L. T., 1966. Influence of clay minerals on microorganisms. 1. Montmorillonite and kaolinite on bacteria. *Canadian Journal of Microbiology*, **12**, 547–563.
- Taylor, P. D., Jugdaohsingh, R., and Powell, J. J., 1997. Soluble silica and high affinity for aluminium under physiological and natural conditions. *Journal of the American Chemical Society*, **119**, 8852–8856.
- Tazaki, K., Fyfe, W. S., Iizumi, S., Sampei, Y., Watanabe, H., Goto, M., Miyake, Y., and Noda, S., 1994. Clay aerosols and arctic ice algae. *Clays and Clay Minerals*, **42**, 402–408.
- Urrutia, M. M., and Beveridge, T. J., 1994. Formation of fine-grained metal and silicate precipitates on a bacterial surface (*Bacillus subtilis*). *Chemical Geology*, **116**, 261–280.
- Urrutia, M. M., Kemper, M., Doyle, R., and Beveridge, T. J., 1992. The membrane-induced proton motive force influences the metal binding ability of *Bacillus subtilis* cell walls. *Applied and Environmental Microbiology*, **58**, 3837–3844.
- Wada, S. I., and Wada, K., 1980. Formation, composition and structure of hydroxy-aluminosilicate ions. *Journal of Soil Science*, **31**, 457–467.
- Warren, L. A., and Ferris, F. G., 1998. continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Cross-references

Black Shales
Extracellular Polymeric Substances (EPS)
Microbial Biomineralization
Microbial Silicification – Bacteria (or Passive)
Microbial Surface Reactivity
Soils

COCCOLITHOPHORES

Volker Thiel

University of Göttingen, Göttingen, Germany

Coccolithophores (coccolithophorids) are a group of unicellular, marine, planktonic algae belonging to the haptophytes (Prymnesiophyta). The coccolithophores are capable of controlling the intracellular precipitation of calcite onto organic plates and the assembly of the mature carbonate scales at the cell surface. These scales, the coccoliths, are spherical or oval, often intricately patterned and generally less than 20 µm in diameter. Given the abundance of coccoliths in sediments, coccolithophorids have been playing a major role in the global carbon budget. They were very abundant in the Mesozoic era, particularly in the Cretaceous period, in which they became a major component of the “Chalk” lithology (e.g., White Cliffs of Dover, England). Today, coccolithophorids are accounting for about a third of the total marine CaCO_3 production, and coccoliths are a major component of the

modern deep-sea calcareous oozes. The most widespread modern species is *Emiliania huxleyi*. Recent studies showed that calcification and net primary production in *E. huxleyi* have increased by ~40% over the last 220 years due to ocean acidification in response to rising atmospheric CO₂ partial pressures (Iglesias-Rodriguez et al., 2008). Contributions of sedimentary organic matter from coccolithophorids, and other haptophyte algae, can be tracked by particular lipid biomarkers, the long-chain alkenones. These compounds are often used as a “paleothermometer” for the estimation of past sea surface temperatures (see entry “*Biomarkers (Organic, Compound-Specific Isotopes)*”). For detailed reading and further references please see entry “*Algae (Eukaryotic)*.”

Bibliography

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, V., and Boessenkool, K. P., 2008. Phytoplankton calcification in a high-CO₂ world. *Science*, **320**, 336–340.

COLD SEEPS

Robert G. Jenkins
Yokohama National University, Yokohama City, Japan

Synonyms

Diffusive seep; Ground water seep; Hydrocarbon seep; Methane seep; Oil seep. The terms “seep” and “seepage” can be used as alternates

Definition

Fluid flow containing reduced compounds, e.g., methane or hydrogen sulfide (see entry *Sulfur Cycle*), with almost no temperature anomalies compared with ambient seawater gushing out from or reaching very close proximity to the seafloor.

Introduction

Active water circulation systems can be found at seafloor level and beneath the seafloor along the plate boundaries such as mid-ocean ridges and trenches. It is well known that at ocean ridges, hydrothermal fluid containing reduced compounds and minerals vent out from the seafloor. In contrast to the hydrothermal vents, focused flow containing reduced compounds, e.g., methane and hydrogen sulfide, gushing out from the seafloor can also be found along active and passive continental margins and around diapirs of serpentine and salt rock (halite) with no or less temperature anomalies compared to the ambient seawater. This fluid flow is called a cold seep.

The first cold seep was found at the Florida Escarpment off Oregon in 1984 by the submersible Alvin (Paull et al., 1984). The Alvin found a large number of alleged tube

worms and white clams which were thought to be highly endemic to hydrothermal vents. It turned out that there are fluid flows which contain reduced compounds and which support chemosynthesis-based life, similar to hydrothermal vents (Kulm et al., 1986).

Examples of the reduced compounds contained in cold seeps are methane and hydrogen sulfide. These compounds lead to extensive biogeochemical reaction when the fluid encounters oxic interstitial water which has penetrated into sediments from seawater. The reaction is mainly mediated by microbes as a way of obtaining chemosynthetic energy to synthesize their organic compounds. Primary production based on the chemosynthesis found in cold seeps results in the formation of characteristic life forms similar to those which can also be found in hydrothermal vents.

The important role played by methane in global warming is due to the fact that the greenhouse effect that results from it is more than 20 times that of carbon dioxide (Kvenvolden, 1988). Hence if we think of cold seeps as a pipe connecting the deep subsurface and the oceans, we can understand their importance in terms of global climate change and biogeochemical cycles at the sediment–water interface.

Distribution

Cold seeps are widely distributed along the active and passive continental margins of the world’s oceans (Figure 1; Sibuet and Olu, 1998; Levin, 2005). The depth range of a cold seep varies from shallow water less than 15 m (Montagna and Spies, 1985) to deep water more than 7,400 m in the Japan Trench (Fujikura et al., 1999).

The total number of cold seeps reported to date represent a minimum, because finding a cold seep is significantly more difficult than finding a hydrothermal vent. The difficulties of identification result from the natural characteristics of cold seeps which are difficult to observe by the use of a submersible or remote operated vehicle (ROV).

Natural characteristics of cold seeps

Cold seeps vary in accordance with their natural characteristics. Most cold seeps are very calm, i.e., the low seepage intensity and low fluid flow rate has no effect on the sea floor. The almost total absence of any temperature anomaly in relation to the ambient seawater also makes it difficult to distinguish the flow by visual observation. The occurrence of chemical anomalies is found over too narrow an area and the anomalies usually occur only in the sediment. Thus, this kind of calm cold seep, called a diffusive seep, is rather difficult to find on the basis of geological and geochemical signatures which exist only beneath the sea floor. One good indicator pointing to the existence of a cold seep is the occurrence of chemosynthesis-based life obtaining energy from the reduced compounds contained in the seep fluids (Sibuet and Olu, 1998). Such biota often comprises large bivalves and/or

modern deep-sea calcareous oozes. The most widespread modern species is *Emiliania huxleyi*. Recent studies showed that calcification and net primary production in *E. huxleyi* have increased by ~40% over the last 220 years due to ocean acidification in response to rising atmospheric CO₂ partial pressures (Iglesias-Rodriguez et al., 2008). Contributions of sedimentary organic matter from coccolithophorids, and other haptophyte algae, can be by tracked by particular lipid biomarkers, the long-chain alkenones. These compounds are often used as a “paleothermometer” for the estimation of past sea surface temperatures (see entry “*Biomarkers (Organic, Compound-Specific Isotopes)*”). For detailed reading and further references please see entry “*Algae (Eukaryotic)*.”

Bibliography

Iglesias-Rodriguez, M. D., Halloran, P. R., Rickaby, R. E. M., Hall, I. R., Colmenero-Hidalgo, E., Gittins, J. R., Green, D. R. H., Tyrrell, T., Gibbs, S. J., von Dassow, P., Rehm, E., Armbrust, V., and Boessenkool, K. P., 2008. Phytoplankton calcification in a high-CO₂ world. *Science*, **320**, 336–340.

COLD SEEPS

Robert G. Jenkins
Yokohama National University, Yokohama City, Japan

Synonyms

Diffusive seep; Ground water seep; Hydrocarbon seep; Methane seep; Oil seep. The terms “seep” and “seepage” can be used as alternates

Definition

Fluid flow containing reduced compounds, e.g., methane or hydrogen sulfide (see entry *Sulfur Cycle*), with almost no temperature anomalies compared with ambient seawater gushing out from or reaching very close proximity to the seafloor.

Introduction

Active water circulation systems can be found at seafloor level and beneath the seafloor along the plate boundaries such as mid-ocean ridges and trenches. It is well known that at ocean ridges, hydrothermal fluid containing reduced compounds and minerals vent out from the seafloor. In contrast to the hydrothermal vents, focused flow containing reduced compounds, e.g., methane and hydrogen sulfide, gushing out from the seafloor can also be found along active and passive continental margins and around diapirs of serpentine and salt rock (halite) with no or less temperature anomalies compared to the ambient seawater. This fluid flow is called a cold seep.

The first cold seep was found at the Florida Escarpment off Oregon in 1984 by the submersible Alvin (Paull et al., 1984). The Alvin found a large number of alleged tube

worms and white clams which were thought to be highly endemic to hydrothermal vents. It turned out that there are fluid flows which contain reduced compounds and which support chemosynthesis-based life, similar to hydrothermal vents (Kulm et al., 1986).

Examples of the reduced compounds contained in cold seeps are methane and hydrogen sulfide. These compounds lead to extensive biogeochemical reaction when the fluid encounters oxic interstitial water which has penetrated into sediments from seawater. The reaction is mainly mediated by microbes as a way of obtaining chemosynthetic energy to synthesize their organic compounds. Primary production based on the chemosynthesis found in cold seeps results in the formation of characteristic life forms similar to those which can also be found in hydrothermal vents.

The important role played by methane in global warming is due to the fact that the greenhouse effect that results from it is more than 20 times that of carbon dioxide (Kvenvolden, 1988). Hence if we think of cold seeps as a pipe connecting the deep subsurface and the oceans, we can understand their importance in terms of global climate change and biogeochemical cycles at the sediment–water interface.

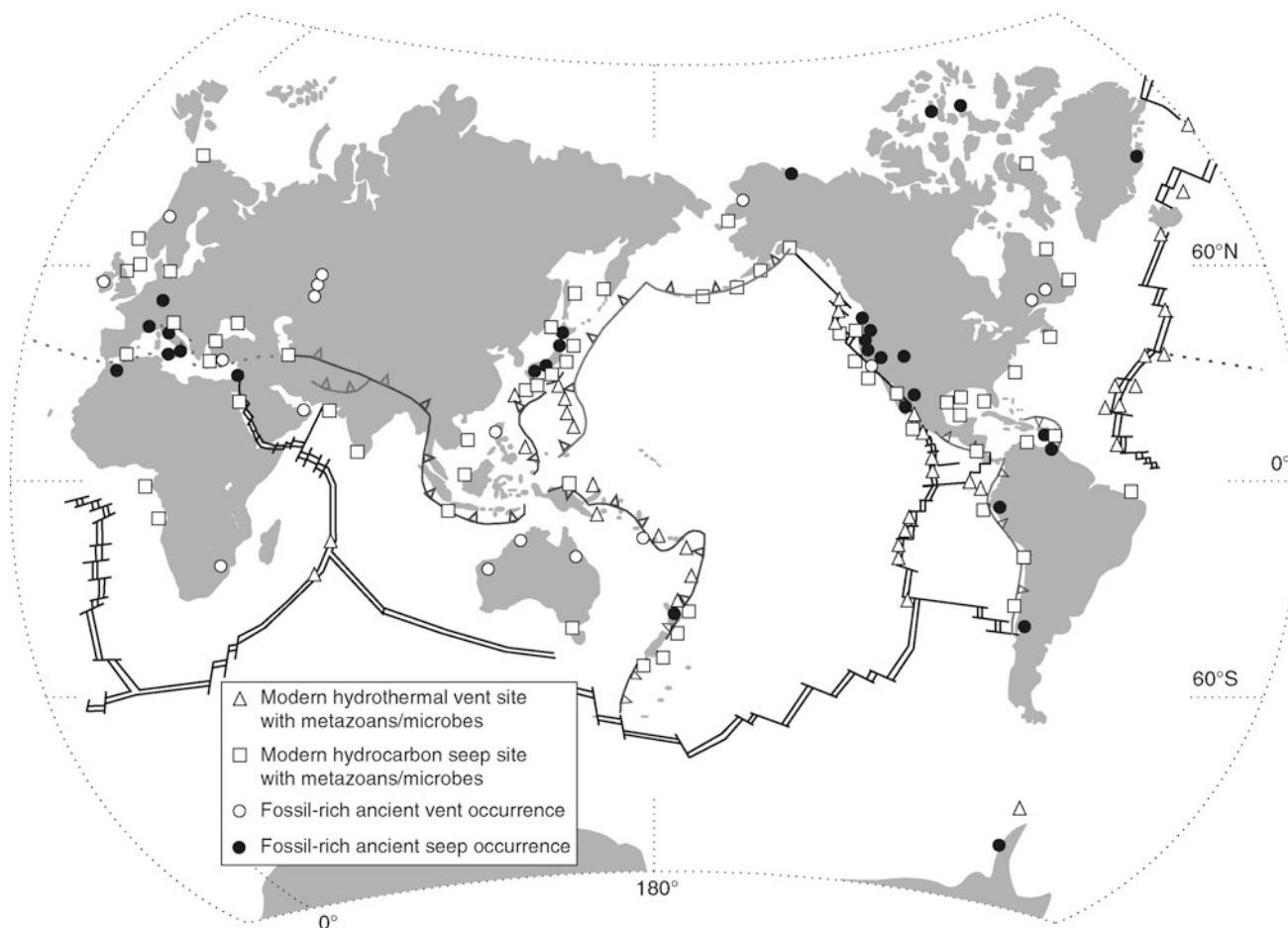
Distribution

Cold seeps are widely distributed along the active and passive continental margins of the world's oceans (Figure 1; Sibuet and Olu, 1998; Levin, 2005). The depth range of a cold seep varies from shallow water less than 15 m (Montagna and Spies, 1985) to deep water more than 7,400 m in the Japan Trench (Fujikura et al., 1999).

The total number of cold seeps reported to date represent a minimum, because finding a cold seep is significantly more difficult than finding a hydrothermal vent. The difficulties of identification result from the natural characteristics of cold seeps which are difficult to observe by the use of a submersible or remote operated vehicle (ROV).

Natural characteristics of cold seeps

Cold seeps vary in accordance with their natural characteristics. Most cold seeps are very calm, i.e., the low seepage intensity and low fluid flow rate has no effect on the sea floor. The almost total absence of any temperature anomaly in relation to the ambient seawater also makes it difficult to distinguish the flow by visual observation. The occurrence of chemical anomalies is found over too narrow an area and the anomalies usually occur only in the sediment. Thus, this kind of calm cold seep, called a diffusive seep, is rather difficult to find on the basis of geological and geochemical signatures which exist only beneath the sea floor. One good indicator pointing to the existence of a cold seep is the occurrence of chemosynthesis-based life obtaining energy from the reduced compounds contained in the seep fluids (Sibuet and Olu, 1998). Such biota often comprises large bivalves and/or



Cold Seeps, Figure 1 Distribution of modern and ancient cold seeps and hydrothermal vents. (After Campbell, 2006.)

worm tubes which are clearly visible (Figure 2). However, seep-associated infaunal animals (i.e., animals living only in the sediments) and microorganisms are potentially difficult to detect.

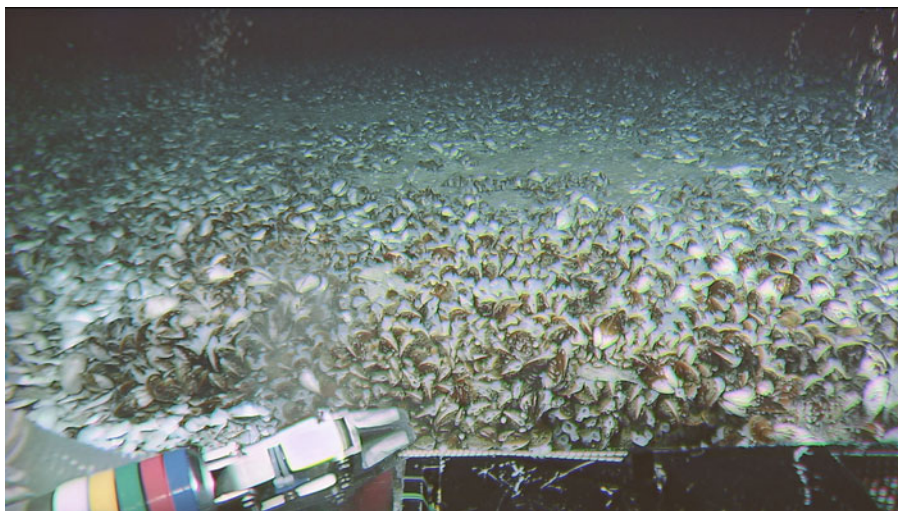
Direct measurements of cold seep fluid chemistry started around the end of the last century (e.g., Tryon and Brown, 2001, 2004). The results show that the fluid pattern and rate of flow are more complex than was previously thought to be the case. Although most cold seeps are calm, there are some effusive and explosive cold seeps. When the saturation level of dissolved methane in sea water is exceeded, the excess methane forms gas bubbles (Figure 3) or gas hydrate (Figure 4) depending on the physical conditions. The bubbly gaseous methane can be found by visual observation (Figure 2) and also by hydroacoustic sounders, like a fisheries sonar (Figure 5; Paull et al., 2007; Aoyama and Matsumoto, 2009). The acoustic sounder makes it possible to see the plume due to the relatively low density of the methane bubbles compared to the ambient sea water. In waters off Niigata, Sea of Japan, methane bubble streams venting out from the sea floor at about 900 m water depth produce a plume

reaching to about 300 m below the sea surface (Figure 5; Aoyama and Matsumoto, 2009).

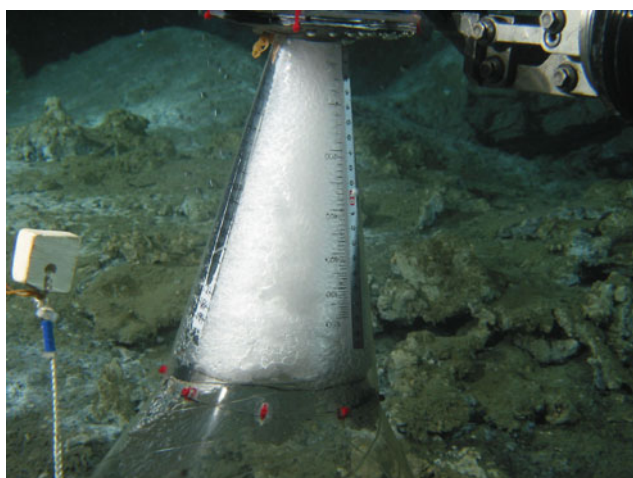
Cold seeps associated with a mud volcano or serpentine diapir show that the flow gushes out together with muddy sediment or brecciated serpentine rocks. It may also happen that methane erupts from the seafloor due to a pressure increase in the sediments caused by an accumulation of methane gas. Such an eruption forms chaotic structures of strata, like landslides, and crater-like topographically depressed areas, so-called pock marks. The size of a pock mark may vary from a few meters to a few hundred meters in diameter and from 1 m to 80 m in depth (Gay et al., 2006).

Pathway and migration of fluid flow

Generally, reduced compounds increase with depth in marine sediments. These reduced compounds can be oxidized by oxidants such as oxygen, nitrate, and sulfate and usually occur at a shallow depth under the sea floor. Although these reactions do also occur elsewhere below the sea floor, substantial reaction occurs in cold seeps



Cold Seeps, Figure 2 Cold seep in the Kuroshima Knoll, Okinawa, southwestern Japan. A dense colony of bathymodiolian bivalves, housing chemosynthetic microbes, covered the sea floor. Gushing out of gaseous bubbly methane can be seen. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).



Cold Seeps, Figure 3 Collecting methane gas bubbles at a cold seep. ROV Hyper-dolphin collected the bubbly methane gas venting out from the cold seep in the waters off Joetsu, Sea of Japan. Putative shell-like gas hydrate covers each gas bubble. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).



Cold Seeps, Figure 4 Outcrop observation of gas hydrate in the waters off Joetsu, Sea of Japan. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).

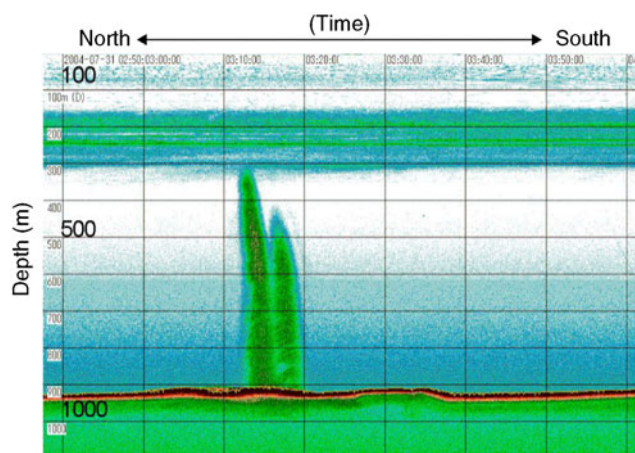
because of the high amounts of reduced compounds brought up to the sea floor from the deep subsurface. Accumulation and migration of reduced compounds are required to form a cold seep.

The pore water containing reduced compounds favors migration through physically weak paths, e.g., faults, and permeable sediments. Tectonic stresses, e.g., plate subduction or diapirs, induce the formation of such pathways. Thus, occurrences of cold seeps are largely dependent on the tectonic background of the area concerned.

Cold seep pathways take the form of a fault or faulted anticline, salt or serpentine diapirs, or a cut bank of strata. These structures can be revealed by seismic profiles, direct observations of the outcrops in the ocean, and drill core samples with chemical profiles of the pore water (Figure 6; Moore et al., 1990, 2001).

Iodine radioisotopes (see entry *Isotopes, Radiogenic*) have recently been identified as a useful tool to determine the age of the strata from which the methane originated, as the iodine will be released at the same time as methane is generated from organic matter, and the diffusion rates of methane and iodine are very similar (Fehn et al., 2003).

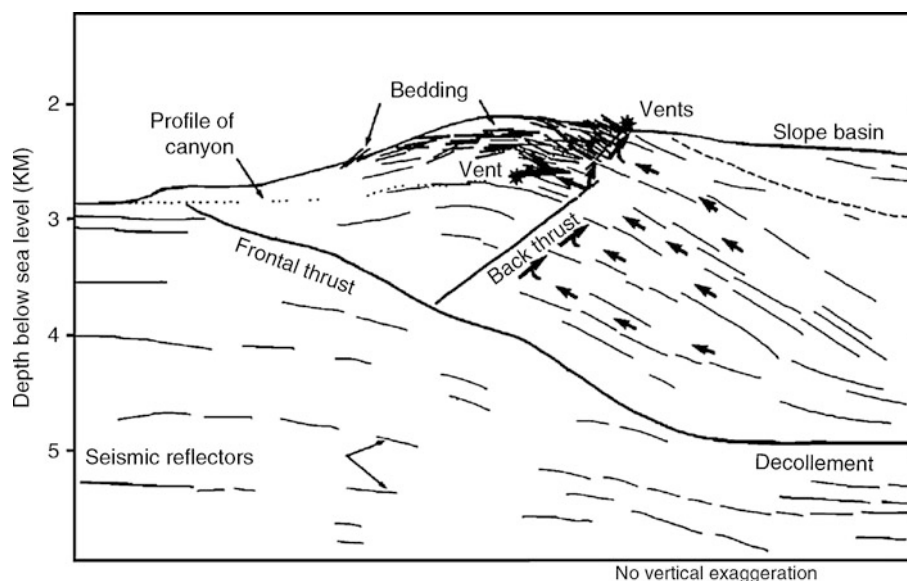
For example, pore water from a cold seep in the Japan Sea passed through and probably originated from strata which sedimented during 30–20 Ma (Tomaru et al., 2007). Faults associated with cold seeps occur particularly on the landward wedge of subduction zones, e.g., the Barbados accretionary wedge, the Cascadia margin and the Japan Trench. Faults created by tectonic stress due to subduction of



Cold Seeps, Figure 5 Methane plumes observed by acoustic sounder at the Umitaka spur, off Joetsu, Sea of Japan. The plumes rose up from the sea floor at depths ranging from ca. 900 m to ca. 300 m. The horizontal axis indicates time. The vehicle obtaining the echogram ran from north to south. (Modified from Aoyama and Matusmoto (2009) (Patent no. US 7539081 B2, Seabed resource exploration system and seabed exploration method by Chiharu Aoyama).

plates play the role of physically weak pathways for the fluid flow. It may also happen that faults cut through a methane reservoir formed under impermeable strata, and the methane then migrates upward through the fault. Indeed, the direction formed by *Calymene* (bivalve) colonies, which mostly live in cold seeps and/or hydrothermal vents, is coincident with a theoretical stress fracture pattern caused by subduction tectonics (Ogawa et al., 1996).

The main driving forces of the migration of pore water containing reduced compounds are density differences and the compression of pore water by increasing pressure within the sediments. The buoyancy of gaseous and dissolved methane and light hydrocarbons in pore water is the main driving force for upward migration. Gaseous methane, the main hydrocarbon gas in the fluid flow, has a strong buoyancy, for example, the density of gaseous methane beneath 3–4 km of water is $200\text{--}300\text{ kg m}^{-3}$ compared with $1,024\text{ kg m}^{-3}$ for seawater (Clennell et al., 2000). Water in which methane is dissolved is also lighter than methane-free water. Thus, the pore water containing gaseous and dissolved methane potentially tends to migrate upward within permeable sediments. Entrapment of the fluid usually occurs where impermeable sediments are found. When strata composed of impermeable sediment are tilted, the fluid migrates within the permeable sediment in a lateral-upward direction directly beneath the impermeable strata. Thus, the hinge zone of an anticline is suitable for the accumulation of methane contained in pore water. Methane-rich fluids may further migrate upwards through faults that cut through the impermeable strata. The fluid also gushes out from outcrops where there is a break in the strata caused by erosion.



Cold Seeps, Figure 6 Interpreted seismic reflection obtained from accretionary prism, off Oregon. Rigid arrows indicate inferred flow paths for fluids. Direct measurements of bedding from the submersible Alvin correlate well with the seismic reflection lines. (After Moore et al., 1990.)

Increasing pressure arising from compaction due to continuous loading of sediments or tectonic stress squeezes out pore water containing reduced compounds into overlying strata. The increasing pressure causes not only pore water migration but also mud diapirs. This is why mud diapirs (or mud volcanoes) are often associated with methane or other reduced compounds.

Heating of the pore water is the other factor causing upward fluid migration. Heating occurs as a result of geothermal activities in the deep subsurface. After heating by geothermal activity in the deep subsurface, the temperature of the fluid drops during upward migration. Thus, the fluid temperature at sea floor level shows no or fewer anomalies in contrast to hydrothermal vents.

Migration of reduced compounds is also caused by diffusion. Although diffusion can be considered as a factor much less important than buoyancy and pressure increase, it is important for the penetration of oxidants from the sea water into sediments.

Chemistry of fluid flow

Major reduced compounds in the fluid flow in cold seeps are methane and other light hydrocarbons, hydrogen sulfide, ammonia, reduced iron, barium, and hydrogen (e.g., Tunnicliffe et al., 2003; Tryon and Brown, 2004). Methane is the most important by virtue of the fact that it is the most common hydrocarbon gas in marine sediments. Methane has a pronounced greenhouse effect and plays an important role both as an energy source and as a carbon source for microbes; furthermore, the anaerobic oxidation of methane (AOM) coupled with sulfate reduction produces hydrogen sulfide which acts as a major energy source for chemosynthesis-based ecosystems.

Elements contained in the fluid flow change with the depth of sediments, because the generation and consumption occur at different depths. Concentrations of reduced compounds generally increase in line with the depth of the sediments. Drastic changes in the chemical profile occur at shallower depths where the reduced compounds encounter oxidants. These reactions take place mainly within sediments, but it sometimes happens that the reduced compounds discharge directly into the sea water column. In such a case, the oxidation reactions take place within the water column.

The drastic chemical changes in cold seeps are generally responsible for substantial biological activities. Methane, the major reduced compound in a cold seep, is generated at a relatively deep subsurface level under anaerobic conditions. When a fluid flow containing methane migrates toward the sea floor and encounters oxidants such as oxygen and sulfate (see entry *Sulfur Cycle*) which penetrated into the sediment from oxic sea water, intensive biogeochemical reactions occur. The most important and extensive of these is AOM which occurs where the methane and sulfate coexist in anaerobic conditions. Following the AOM, oxidation of hydrogen sulfide, formation of pyrite (see entry *Iron Sulfide Formation*), and

precipitation of carbonate minerals occur. When the fluid flow gushes out into well-oxygenated conditions, aerobic oxidation of methane (*Methane Oxidation (Aerobic)*) occurs.

The origin of methane

There are several pathways for the generation of methane of which biogenic and thermogenic processes are the most important. In the case of the former, the methane is produced directly by microbes, and in the case of the latter, the methane is produced through the thermal degradation of organic compounds in the deep subsurface. Methane that is produced neither from organic material nor through organic processes can be found mainly along the edge of hydrothermal vent fields and above serpentine diapirs.

Biogenic methane

Biogenic methane is produced by microbes. The methanogenic microbes (methanogens) belong to the phylum Euryarchaeota within the domain Archaea. Methanogens are obligatory anaerobes, meaning that they live in an anoxic environment which usually occurred below the zone of sulfate reduction. The main substrates linked to the generation of methane are simple carbon-containing molecules such as carbon dioxide, formate, acetate, methanol, and methylamine (Whiticar, 1999). The two simple pathways of methanogenesis, i.e., carbonate reduction and acetate fermentation, are expressed as $\text{CO}_2 + 8\text{H}^+ \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ and $\text{CH}_3\text{COOH} \rightarrow \text{CH}_4 + \text{CO}_2$, respectively. Archaeal methanogenesis promotes large fractionations of carbon isotopes. The stable carbon isotopic composition of methane produced by carbonate reduction generally ranges from -60 to -80‰ (vs. PDB), while the methane produced by the fermentation process ranges from -50 to -60‰ (vs. PDB; Whiticar, 1999). The methane is mostly a unitary hydrocarbon product of the biological process, whereas the thermogenic process explained below produces methane as well as other hydrocarbons such as ethane and propane. However, the recent suggestion that ethane and propane are also produced biologically (Hinrichs et al., 2006) upset the long-held concept of the origin of light hydrocarbons larger than methane, which were thought to be generated by the thermal degradation of organic matter only. The biogenic process is carried out at a relatively shallow depth compared to where thermogenic methane is produced. This is due to the presence of appropriate thermodynamic conditions, i.e., not too high a temperature and pressure, to permit microbial activities.

Thermogenic methane

The methane generated through the degradation of sedimentary organic matter as a result of thermodynamic conditions is called thermogenic methane (Kotelnikova, 2002). The rising temperature and pressure of sediments that accompany the increasing depth accelerates the

degradation of organic compounds. Thus, the production of thermogenic methane generally increases in proportion to the depth of the sediments, usually more than a few hundred meters below the seafloor. Contrarily, a high temperature inhibits microbial activities at a deep subsurface level, thus it follows that the dominant methane in the deep subsurface is thermogenically generated. This process produces not only methane but also ethane, propane, and other light hydrocarbons. There are fewer isotopic effects on carbon and hydrogen from methane produced by thermal maturation compared to biogenic methane. Thus, the carbon isotopic composition of thermogenic methane ranges from -20 to -50‰ (vs. PDB; Whiticar, 1999).

Abiogenic methane

Methane produced from inorganic matter through an inorganic process is called abiogenic methane. Such methane is thought to be generated through the processes of cooling and degassing of mafic igneous rocks and magma and the process of serpentinization of ultramafic rocks. Although the exact mechanism of abiogenic methane formation is still not fully understood, some abiogenic methane formation processes have been revealed (e.g., Horita and Berndt, 1999; Proskurowski et al., 2008). We can now say that abiogenic methane is probably the dominant methane source in hydrothermal vent systems especially along mid-ocean ridges and above serpentine diapirs.

Identification of the origin of methane

Looking specifically at the methane in cold seeps, it has been thought that this originated from the processes of biogenesis and/or thermogenesis. Most abiogenic methane will be found at hydrothermal vents and not at cold seeps except in the case of cold seeps associated with serpentine diapirs. Means of allowing us to estimate the origin of methane are a combination of analyses of the carbon isotopic compositions of the methane and the relative abundance of ethane and propane compared to the amount of methane included in fluid seepage (Schoell, 1988). Specifically, extremely negative carbon isotope values of methane, generally less than -50‰ (vs. PDB), with a high methane/(ethane + propane) ratio indicate that the methane was generated by a biogenic process. In contrast, less negative carbon isotopic composition, generally -20‰ to -50‰ (vs. PDB), with a relatively low methane/(ethane + propane) ratio indicates that the methane was generated by a thermogenic process.

Anaerobic oxidation of methane (AOM)

AOM is a key reaction at cold seeps. Although several probable reactions have been proposed (Valentine, 2002), the details of the AOM reaction are not yet clearly understood. What we can say is that the net reaction of the AOM is expressed as $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$. Thus, the relative amounts of both sulfate and methane regulate the activity of AOM. This reaction occurs

widely in oceans because large amounts of sulfate are contained in sea water whereas the reaction will not occur in freshwater environments such as ponds, lakes, rivers, and rice fields because freshwater lacks sulfate (Whiticar, 1999). It has recently been recognized that AOM by sulfate reduction is carried out by consortia of anaerobic methane-oxidizing archaea and sulfate-reducing bacteria (Elvert et al., 1999; Boetius et al., 2000; Orphan et al., 2001, 2004). The anaerobic methane-oxidizing archaea (ANME) are found in three different phylogenetic clusters belonging to the Euryarchaeota, i.e., ANME-1, -2, and -3. It has also been revealed by genome analysis that methane is oxidized under anoxic conditions by means of a “reverse methanogenesis” process (Hallum et al., 2004). The ecology of each of these archaeal lineages (ANME-1, -2, and -3) differs from that of the others. Thus, the differences reflect their distribution pattern in cold seeps (Elvert et al., 2005; Knittel et al., 2005).

An important function of the AOM reaction is that it prevents the escape of methane, which has a pronounced greenhouse effect, from sediments into the ocean and the atmosphere. The carbon derived from methane is transformed into bicarbonate or carbonate ions. An increase in the ions leads to an increase in alkalinity and thus to the subsequent precipitation of carbonate minerals given the existence of calcium or other appropriate cations (explained below).

Oxidation of sulfides

The hydrogen sulfide (see entry *Sulfide Mineral Oxidation*), a byproduct of the AOM, is used for subsequent reactions. One is the oxidation of sulfides mediated by sulfur-oxidizing bacteria under (sub)oxic conditions, and the other is the formation of pyrite (see entry *Iron Sulfide Formation*) in anoxic conditions. Sulfur-oxidizing (thiotrophic) bacteria, such as *Beggiatoa* (qv), *Thiothrix*, and *Thioploca*, use the hydrogen sulfide as an electron donor and oxidize it by oxygen. The final product of the reaction is sulfate. Most sulfur-oxidizing bacteria are chemolithoautotrophs, but some can use organic compounds for their cell synthesis. The thiotrophic bacteria sometimes form white mats that cover the sea floor and some may live as symbiotic bacteria in animal tissues (e.g., in gills).

Aerobic oxidation of methane (qv)

The aerobic oxidation of methane (see entry *Methane Oxidation (Aerobic)*) is performed by methane-oxidizing (methanotrophic) bacteria, whereas archaea are responsible for AOM. When the methane is released directly into an oxic water column, it will be aerobically oxidized by these methanotrophs. However, in normal marine environments, methane is mostly oxidized anaerobically by AOM within the sediments. Aerobic oxidation of methane becomes important when methane vents out vigorously into oxic sea water.

Authigenic mineral precipitation in cold seeps (qv “microbial mineralization” and “microbially induced sedimentary structures”)

One result of the substantial biological reactions referred to above is the precipitation of several minerals. The minerals most commonly found in cold seeps are carbonate such as aragonite, high magnesian calcite, and low magnesian calcite. The precipitation of these carbonates occurred because of increasing alkalinity resulting from AOM. When AOM occurs at sea-floor level or above the sea floor in euxinic waters, reef-like carbonate build-ups may be formed on the sea floor, as in the Black Sea (Michaelis et al., 2002). Under an oxic water column, the reaction generally occurred within the sediment, thus the carbonate formed beneath the sea floor. At a shallower depth below the sea floor, aragonite and high magnesian calcite can be formed, whereas low magnesian calcite and dolomite are frequently found relatively deeper within the sediments (e.g., Takeuchi et al., 2007). Methane-derived carbonate bodies are sometimes exposed on the sea floor because of surface erosion. Then the carbonate may function as a hard substrate for sessile organisms or carbonate borers.

Pyrite is another common authigenic mineral found in cold seeps. Hydrogen sulfide produced as a result of AOM may combine with reduced iron to form monosulfide, i.e., a precursor of pyrite. Subsequent reaction of the precursor with elemental sulfur or sulfides promotes the formation of pyrite. It is commonly the case that the pyrite crystal aggregation forms a framboidal shape, a so-called framboidal pyrite (for details see Chapter *Iron Sulfide Formation*). The sulfur isotopic composition of pyrite inherits the composition of the hydrogen sulfide source and indicates whether or not the hydrogen sulfide derived from bacterial sulfate reduction (Peckmann and Thiel, 2004).

Relationship to methane gas hydrate

Cold seeps are frequently associated with gas hydrate (Figure 4) where the environment has appropriate physical conditions for gas hydrate formation. The main component of the gas hydrate is methane but carbon dioxide, ethane, and other gaseous molecules are also present. Gas hydrates form in low temperature – high pressure environments where the appropriate concentrations of methane exist (Zhang, 2003). Such environments are usually distributed in the deep sea around continental margins and in permafrost areas on land. The hydrate is one of the largest methane reservoirs in the world. It has been estimated that more than 2,100 Gt of methane are stored in gas hydrates.

Formation of a mass of methane gas hydrate usually occurs within sediments whereas methane rapidly escapes and does not readily accumulate in water columns. If a substantial amount of methane, e.g., bubbly methane gas, is released into a water column, gas hydrate may form as a thin shell on the surface of a gas bubble (Figure 3).

The depth limit of gas hydrate formation and occurrence in the sediments is usually determined by the local geothermal gradient. The gas hydrate itself may act as an impermeable barrier within the sediment. In this case, the methane gas, which has migrated from the deep subsurface, is trapped and accumulates beneath the gas hydrate layer.

The importance of the gas hydrate for cold seeps is that (1) pore water surrounding the methane gas hydrate has an oversaturated level of methane, and (2) the rapid dissociation of methane gas hydrate may result in an episodic increase of cold seeps and sometimes an eruption of methane. When methane-undersaturated water penetrates the sediment and comes into proximity with the gas hydrate, the gas hydrate dissociates so as to create a physical equilibrium between the pore water and the gas hydrate. When the pore water, after taking up a substantial amount of methane, migrates further upward, a continuous methane supply is established between the methane gas hydrate reservoir and the sea floor.

Episodic emission of methane because of the dissociation of methane gas hydrate occurs, for example, in cases where the sea level decreases at the beginning of a glacial period or increasing sea water temperature because of global warming (e.g., Judd et al., 2002). The dissociation of gas hydrates may cause instability of the sea floor inducing landslides (Buffett, 2000). Such an underwater landslide results in additional lowering of pressure and may produce further dissociation of gas hydrate. It should be noted that the meaning of the existence of methane gas hydrate is that the area has large potential for the generation and/or accumulation of methane.

Because the density of gas hydrate is lower than that of sea water, the gas hydrate itself tends to rise upwards. Thus, an insufficient mixture of sediment in the gas hydrate and/or insufficient loading by overlain sediments causes the release of a mass of gas hydrate into a water column.

Ecosystem established at cold seeps – chemosynthesis-based ecosystems

Cold seeps can be considered as chemosynthesis-based ecosystems that support a large amount of life in deep seas (Figures 2 and 7; Sibuet and Olu, 1998). The main primary producers of the ecosystem found in cold seeps are chemoautotrophic microbes, such as sulfur-oxidizing bacteria. For the sulfur-oxidizing bacteria, hydrogen sulfide has the role of an electron acceptor, oxygen that of an electron donor, and carbon dioxide that of a carbon source. Such microbes that metabolize via the chemical reaction of inorganic compounds with an inorganic carbon source are called chemolithoautotrophs. An ecosystem based on chemoautotrophy is called a chemosynthesis-based ecosystem. This kind of ecosystem is a counterpart to the photosynthetic ecosystem found on the surface of the Earth. Chemosynthetic ecosystems establish themselves especially in areas where light does not penetrate such as the



Cold Seeps, Figure 7 Chemosynthesis-based community composed of a dense accumulation of *Bathymodiolus* (mytilid bivalves) from Kuroshima Knoll, off Okinawa, southwest of Japan. (by courtesy of the Japan Agency for Marine-Earth Science and Technology).

deep sea and the subsurface below the sea floor. Within these settings, chemosynthesis-based ecosystems particularly flourish at cold seeps and hydrothermal vents because these are environments where large amounts of reduced compounds encounter oxic waters.

With chemosynthetic microbes as a basis, heterotrophic microbes and animals flourish in cold seeps as members of the chemosynthetic ecosystem. In order to flourish in a oxygen-poor cold seep environment which is instead rich in toxic substances such as hydrogen sulfide, the animals found there often have a detoxification system (Felbeck et al., 1985). Most animals living in cold seeps house chemosynthetic bacteria, such as sulfur-oxidizing bacteria and methane-oxidizing bacteria, and obtain organic matter from those symbionts. These symbiotic chemosynthetic bacteria are called as chemosymbiotic bacteria. The animals living in cold seeps are generally endemic at a higher taxon level and diverge from the animals belonging to photosynthetic ecosystems. Major animals living in a cold seep environment are vesicomyid, mytilid, thyasirid, lucinid, and solemyid bivalves, provannid, acmaeid and neomphalid gastropods, and vestimentiferan tube worms. Crabs, sea urchins, sponges, and barnacles are occasionally found as minor faunal elements. Nectobenthic animals, i.e., animals that can live near the sea floor, such as hydrothermal vent shrimps, are relatively fewer in number at cold seeps than in hydrothermal vent fields. This is because the reduced compounds, their energy source, are often consumed already within the sediments, and only occasionally appear above the sea floor. In contrast, infaunal animals, i.e., animals that live in the sediment, are more abundant in cold seeps than in hydrothermal vents, because the cold seeps are usually covered by rich soft sediments whereas fresh

(recently formed) hard igneous rocks are exposed at hydrothermal vent fields.

The biotic composition of cold seeps is controlled by physicochemical factors including the quantity of reduced compounds such as methane and hydrogen sulfides. One good example of the biotic elements controlled by methane accumulations is seen at Hydrate Ridge (Cascadia Margin, off Oregon). *Beggiatoa* (sulfur-oxidizing bacteria) mats, *Calyptogena* (vesicomyid bivalve), and *Acharax* (solemyid bivalve) all flourish in this cold seep area in a zoned distribution. A large concentration of hydrogen sulfide was detected in the *Beggiatoa* zone, decreasing toward the *Acharax* zone through the *Calyptogena* zone (Sahling et al., 2002). A similar regulation of distribution caused by the concentration of hydrogen sulfide occurred at the species level. Two species of *Calyptogena* (bivalve), *C. kilmeri* and *C. pacifica*, coexisted in a cold seep but they were roughly segregated. The *C. kilmeri* lived where there was a much higher concentration of hydrogen sulfide and the other lived where the concentration of hydrogen sulfide was much lower (Barry et al., 1997).

The biotic component that lives in cold seep environments looks similar to those found in other reduced environments such as hydrothermal vents, sunken vertebrate carcasses, and driftwood (see Chapter *Whale and Wood Falls*). Actually, the components are mostly the same at a higher taxonomic level (i.e., genus or family level), although they are restricted to living in a single type of habitat at the species level (Peck et al., 1997), except for vertebrate carcasses (Smith and Baco, 2003). Twenty species found in a cold seep environment also live on/around vertebrate carcasses. Thus, vertebrate carcasses may play a role as a dispersal stepping stone prior to living in a cold seep at least for some species. It is generally thought that the macro organisms in hydrothermal vent environments have evolved from cold seep species (Hecker, 1985; McLean 1985; Craddock et al., 1995). In addition, molecular data indicate that mytilid bivalves living in a cold seep environment adapt to such a reduced environment via whale carcasses as an evolutionary stepping stone (Distel et al., 2000; Smith and Baco, 2003).

Ancient cold seeps

Recognition of ancient cold seeps

Numerous ancient cold seeps dating from various ages have been found in a variety of tectonic settings (Figures 1 and 8; see review in Campbell, 2006). The identification of ancient cold seeps depends on revealing features which indicate the presence of methane and other hydrocarbons which escaped from the sea floor. In order to make these features evident, a comprehensive approach based on geological, geochemical, and paleontological techniques is a primary requirement.

It is well known that carbonate minerals are forming in modern cold seep environments as a result of increased alkalinity due to AOM (Ritger et al., 1987; Aharon,



Cold Seeps, Figure 8 Late Cretaceous cold seep deposit (*above*) and dense accumulation of lucinid bivalves associated with the carbonate (*below*) from South Dakota, USA. The carbonate rock was induced by anaerobic oxidation of methane at a late Cretaceous active cold seep in the Western Interior Seaway, located in the eastern part of what is today the Rocky Mountains in northwestern America.

2000). Methane-derived carbonates are potentially imprinted with environmental information concerning the location where they were formed. Shapes, textures, and imprinted chemical signatures of modern cold seep

deposits can be seen as a good analog facilitating the recognition of ancient cold seeps. A conduit pipe such as a carbonate chimney (Takeuchi et al., 2007), although they are generally formed under the sea floor in contrast to chimneys at hydrothermal vents, is a good example indicating the route of fluid. Cold seep carbonates often have chaotic fractured structures. The fracture is usually ductile and/or accompanied by brittle sediment deformations. The deformations are probably due to active fluid venting. Microbially induced carbonate cements, e.g., clotted fabrics and isopachous fibrous cements, are frequently found in the carbonate. The influence of methane and microbial activity can be detected by further biogeochemical analysis (Peckmann and Thiel, 2004).

The carbon isotopic compositions of the carbonate reflect the values of the carbon source. Methane, the main gaseous component of cold seeps, generally shows negative carbon isotopic compositions in the case of thermogenic and biogenic methane (see above). The carbon isotopic compositions of thermogenic and biogenic methane commonly range from -30 to -50‰ and -50 to -80‰ (vs. PDB), respectively, and are thus distinguished from other carbon sources in the marine environment. These values will be imprinted at roughly the same level in the carbon isotopic compositions of the methane-derived carbonate. Considering that the $\delta^{13}\text{C}$ -values of carbonate are often somewhat higher than the values of the source methane because of the admixture of other carbon sources (Peckmann and Thiel, 2004), values ca. lower than -30‰ (vs. PDB) suggest that a major portion of the carbonate carbon is derived from methane.

Lipid biomarkers provide information on ancient microbial activities. It has been revealed that AOM is mediated by microbial consortia composed of archaea and sulfate-reducing bacteria (Orphan et al., 2002). Cell membrane lipids produced by those microbes have a strong propensity to be preserved in sediments (Peckmann and Thiel, 2004). Analyzing the carbon isotopic composition of each of these biomarkers (see entry *Biomarkers (Organic, Compound-Specific Isotopes)*) tells us whether or not the microbes used methane as the carbon source for their biosynthesis. The carbon isotopic compositions of lipids derived from methanotrophic microbes sometimes reach as low as -120‰ or even lower. On the basis of this information, the presence of isotopically depleted lipid biomarkers derived from archaea will be a good indicator for the occurrence of AOM (Elvert et al., 2005).

Oldest cold seep

Ancient cold seeps with chemosymbiotic macrofossils have been traced back to at least Silurian (Barbieri et al., 2004). As it is difficult to find and recognize ancient cold seeps without macrofossils, there is practically no record of older cold seeps. However, a possible cold seep occurring after the severe glacial period around 600 Ma, i.e., the period known as “Snowball Earth,” has recently been

proposed. The evidence for a cold seep related to the dissociation of methane gas hydrate is the strongly negative carbon isotopic composition of the “cap carbonate” from south China (Jiang et al., 2003). However, cap carbonates on a glacial deposit formed around 600 Ma are widely distributed throughout the world. If all the cap carbonates were formed under the influence of dissociating methane gas hydrate, numerous cold seeps would have appeared at that time. It is easy to imagine that widespread cold seeps existed in the Precambrian because biogenic methane already existed at 3.5 Ga (Ueno et al., 2006). The apparent lack of cold seeps in the Precambrian, apart from the cap carbonate, is probably due to their having been overlooked because of a lack of associated macro fossils.

Changes in cold seeps through time

The number of cold seeps varies through the Phanerozoic. In the Paleozoic, there are only five ancient cold seep localities. In spite of the fact that a stratigraphic change in carbon isotope ratios shows a global negative shift at the Permian/Triassic boundary, probably caused by the release of a large amount of methane into the ocean and the atmosphere, probably from methane gas hydrate, there are no records of ancient cold seeps from the Permian and Triassic. However, the number of records of ancient cold seeps in post-Triassic marine sediments has increased. More than 15 localities have been found in the Mesozoic and in an abundance of localities, more than a dozen, from the Cenozoic (Campbell, 2006). The cause of the drastic increase in cold seeps since the late Mesozoic might be due to an increase of organic flux into sediments resulting from increasing planktonic productivity and diversity during the Mesozoic Era (Tappan and Loeblich, 1973; COSOD II, 1987) leading to increasing generation of methane in marine sediments.

The biotic components of cold seeps changed through the Phanerozoic (Campbell and Bottjer, 1995; Little and Vrijenhoek, 2003). Mollusks are the most dominant organism in modern cold seeps, despite the dominance of brachiopods in the Paleozoic and mid-Mesozoic. The brachiopods were replaced by mollusks, mainly by bivalves, in the late Mesozoic (Campbell and Bottjer, 1995; Kiel and Peckmann, 2008). The last abundant occurrence of brachiopods in cold seeps is found from the Campanian, late Cretaceous (ca. 80 Ma), from the Omagari, Hokkaido, Japan (Hikida et al., 2003; Kaim et al., 2010). Vesicomyid and bathymodiolian bivalves, which flourished in modern cold seeps, entered the cold seep environment during the Eocene (Ca. 40 Ma; Kiel, 2006; Amano and Kiel, 2007; Amano et al., 2008). It should be noted that the fossil record of vesicomyid bivalves has recently been reexamined and its origin changed from Cretaceous to Eocene (Amano and Kiel, 2007; Amano et al., 2008; Kiel et al., 2008). Most living molluscan genera which lived in ancient cold seeps originated in the late Mesozoic to Paleogene (see basic data in Kiel and Little, 2006 and updated data in Amano et al.,

2007; Jenkins et al., 2007; Campbell et al., 2008; Kaim et al., 2008; Kiel et al., 2008; Kaim et al., 2009).

Impact on climate change

Judd et al. (2002), indicate that the input of greenhouse gases from the sea floor into the world's oceanic and atmospheric systems may be an important component of the atmospheric carbon budget, because the greenhouse potential of methane is more than 20 times that of carbon dioxide. A catastrophic release of methane may therefore produce global climate changes. Such an event due to the rapid escape of methane into the atmosphere happened at the Paleocene–Eocene boundary (ca. 55.5 Ma). At that time, the rapid dissociation of methane hydrate and subsequent release of methane into the atmosphere resulted in global warming and an increase of 6°C in the sea surface temperature (Dickens et al., 1995). A similar methane release outburst is thought to have occurred at the end-Permian forming one of the biggest known biodiversity crises (see entry *Critical Intervals in Earth History*) (Ryskin, 2003; Retallack and Krull, 2006). Kennet et al. (2002) proposed that the periodic glacial-interglacial cycle during Quaternary was controlled by the decomposition of methane gas hydrate and the subsequent release of methane gas into the atmosphere. Evidence of these methane releases comes mostly from carbon isotopic anomalies which were recorded in carbonate and organic material. The release of isotopically depleted biogenic methane induces negative carbon isotope excursions because of the transfer of ¹³C-depleted carbon from methane to carbon dioxide.

Summary

Cold seeps have been the object of considerable attention because of their role as a connecting pipe between the lithosphere, hydrosphere, and biosphere on the Earth. Reduced compounds contained in cold seep fluid result in substantial biogeochemical reactions. The establishment of a cold seep requires specific geological and biological background conditions, such as tectonic stress to make a route for the fluid, sedimentary organic matter supply to provide a source of methane and other hydrocarbons, microbial and/or geothermal activities to form the hydrocarbons, and microbial activities accounting for the oxidation of reduced compounds. Revealing the processes and mechanisms has largely progressed during the last 2 decades. For example, it was revealed at the time of the transition from the twentieth to the twenty-first century that anaerobic methane-oxidizing archaea and sulfate-reducing bacteria are responsible for the anaerobic oxidation of methane. However, there are still many questions about cold seeps that remain unresolved, e.g., the long-term behavior of cold seeps, response to climate change and details of various biological processes and mechanisms. Gaining an understanding of cold seeps is a primary requirement because a cold seep provides a condensed version of

geobiochemical processes related to methane. Thus, the knowledge that we can obtain from the research on cold seep may help us to understand a much wider range of geobiological phenomena taking place on Earth.

Furthermore, methane, the main component contained in cold seep fluids, has a potentially large effect on global warming. It is evident from several geological records through the Phanerozoic that dissociation of methane gas hydrate has drastic effects in terms of global climate change. Methane is also attractive to human beings because of its large potential as a future energy source. In fact, the methane amount stored in gas hydrate beneath the sea floor and permafrost is more than 2,100 Gt. However, as noted above, methane has a tremendous potential to bring about global climate change on the Earth. Thus, any form of intervention in the natural gases in marine sediments should be carefully carried out on the basis of sufficient knowledge of what will happen when the methane is released from the sea floor. Adopting this stance implies a very strong requirement to reveal the entire range of processes and mechanisms within a cold seep.

Bibliography

- Aharon, P., 2000. Microbial processes and products fueled by hydrocarbons at submarine seeps. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 270–281.
- Amano, K., Jenkins, R. G., and Hikida, Y., 2007. A new gigantic *Nucinella* (Bivalvia: Solemyoidea) from the Cretaceous cold-seep deposit in Hokkaido, northern Japan. *Veliger*, **49**, 84–90.
- Amano, K., Jenkins, R. G., Kurihara, Y., and Kiel, S., 2008. A new genus for *Vesicomya inflata* Kanie & Nishida, a lucinid shell convergent with that of Vesicomys, from Cretaceous strata of Hokkaido, Japan. *Veliger*, **50**, 255–262.
- Amano, K., and Kiel, S., 2007. Fossil vesicomyid bivalves from the North Pacific region. *Veliger*, **49**, 270–293.
- Aoyama, C., and Matsumoto, R., 2009. Acoustic surveys of methane plume by quantitative echo sounder in Japan Sea and the estimate of the seeping amount of the methane hydrate bubbles. *Journal of Geography*, **118**, 156–174 (Japanese with English abstract).
- Barbieri, R., Ori, G. G., and Cavalazzi, B., 2004. A Silurian cold-seep ecosystem from the Middle Atlas, Morocco. *Palaio*, **19**, 527–542.
- Barry, J. P., Kochevar, R. E., and Baxter, C. H., 1997. The influence of pore-water chemistry and physiology on the distribution of vesicomyid clams at cold seeps in Monterey Bay: implications for patterns of chemosynthetic community organization. *Limnology and Oceanography*, **42**, 318–328.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Buffett, B. A., 2000. Clathrate hydrates. *Annual Reviews in Earth and Planetary Sciences*, **28**, 477–507.
- Campbell, K. A., 2006. Hydrocarbon seep and hydrothermal vent paleoenvironments and paleontology: past developments and future research directions. *Palaeogeography Palaeoclimatology Palaeoecology*, **232**, 362–407.
- Campbell, K. A., and Bottjer, D. J., 1995. Brachiopods and chemosymbiotic bivalves in Phanerozoic hydrothermal vent and cold seep environments. *Geology*, **23**, 321–324.
- Campbell, K. A., Peterson, D. E., and Alfaro, A. C., 2008. Two new species of *Retiskenea* (Gastropoda: Neomphalidae) from Lower Cretaceous hydrocarbon seepcarbonates of northern California. *Journal of Paleontology*, **82**, 140–153.
- Clennell, M. B., Judd, A., and Hovland, M., 2000. Movement and accumulation of methane in marine sediments: relation to gas hydrate systems. In Max, M. D. (ed.), *Natural Gas Hydrate in Ocean and Permafrost Environments*. The Netherlands: Kluwer, pp. 105–122.
- COSOD II, 1987. *Report of the Second Conference on Scientific Ocean Drilling*. European Science Foundation, Strasbourg, France, 142 pp.
- Craddock, C., Hoeh, W. R., Gustafson, R. G., Lutz, R. A., Hashimoto, J., and Vrijenhoek, R. J., 1995. Evolutionary relationships among deep-sea mytilids (Bivalvia: Mytilidae) from hydrothermal vents and cold-water methane/sulfide seeps. *Marine Biology*, **121**, 477–485.
- Dickens, G. R., O'Neil, J. R., Rea, D. K., and Owen, R. M., 1995. Dissociation of oceanic methane hydrate as a cause of the carbon isotope excursion at the end of the Paleocene. *Paleoceanography*, **10**, 965–971.
- Distel, D. L., Baco, A. R., Chuang, E., Morrill, W., Cavanaugh, C., and Smith, C. R., 2000. Do mussels take wooden steps to deep-sea vents? *Nature*, **403**, 725–726.
- Elvert, M., Hopmans, E. C., Treude, T., Boetius, A., and Suess, E., 2005. Spatial variations of methanotrophic consortia at cold methane seeps: implications from a high-resolution molecular and isotopic approach. *Geobiology*, **3**, 195–206.
- Elvert, M., Suess, E., and Whiticar, M. J., 1999. Anaerobic methane oxidizing associated with marine gas hydrates: superlight C-isotopes from saturated and unsaturated C₂₀ and C₂₅ irregular isoprenoids. *Naturwissenschaften*, **86**, 295–300.
- Fehn, U., Snyder, G. T., Matsumoto, R., Muramatsu, Y., and Tomaru, H., 2003. Iodine dating of pore waters associated with gas hydrates in the Nankai area, Japan. *Geology*, **31**, 521–524.
- Felbeck, H., Powell, M. A., Hand, S. C., and Somero, G. N., 1985. Metabolic adaptations of hydrothermal vents animals. *Bulletin of the Biological Society of Washington*, **6**, 261–272.
- Fujikura, K., Kojima, S., Tamaki, K., Maki, Y., Hunt, J., and Okutani, T., 1999. The deepest chemosynthesis-based community yet discovered from the hadal zone, 7326 m deep, in the Japan Trench. *Marine Ecology Progress Series*, **190**, 17–26.
- Gay, A., Lopez, M., Ondreas, H., Charlou, J. L., Sermondadaz, G., and Cochonat, P., 2006. Seafloor facies related to upward methane flux within a Giant Pockmark of the Lower Congo Basin. *Marine Geology*, **226**, 81–95.
- Hallum, S. J., Putnam, N., Prestion, C. M., Detter, J. C., Rockhsar, D., Richardson, P. M., and DeLong, E. F., 2004. Reverse methanogenesis: testing the hypothesis with environmental genomics. *Science*, **305**, 1457–1462.
- Hecker, B., 1985. Fauna from a cold sulfur-seep in the Gulf of Mexico: comparison with hydrothermal vent communities and evolutionary implications. *Bulletin of the Biological Society of Washington*, **6**, 465–473.
- Hikida, Y., Suzuki, S., Togo, Y., and Ijiri, A., 2003. An exceptionally well-preserved fossil seep community from the Cretaceous Yezo Group in the Nakagawa area, Hokkaido. *Paleontological Research*, **7**, 329–342.
- Hinrichs, K. U., Hayes, J. M., Bach, W., Spivack, A. J., Hmelo, L. R., Holm, N. G., Johnson, C. G., and Sylva, S. P., 2006. Biological formation of ethane and propane in the deep marine subsurface. *Proceedings of the National Academy of Sciences*, **103**, 14684–14689.
- Horita, J., and Berndt, M. E., 1999. Abiogenic methane formation and isotopic fractionation under hydrothermal conditions. *Science*, **285**, 1055–1057.

- Jenkins, R. G., Kaim, A., Hikida, Y., and Tanabe, K., 2007. Methane-flux-dependent lateral faunal changes in a Late Cretaceous chemosymbiotic assemblage from the Nakagawa area of Hokkaido, Japan. *Geobiology*, **5**, 127–139.
- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopic evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Judd, A. G., Hovland, M., Dimitrov, L. I., Gil, S. G., and Jukes, V., 2002. The geological methane budget at Continental Margins and its influence on climate change. *Geofluids*, **2**, 109–126.
- Kaim, A., Jenkins, R. G., and Waren, A., 2008. Provannid and provannid-like gastropods from the Late Cretaceous cold seeps of Hokkaido (Japan) and the fossil record of the Provannidae (Gastropoda: Abysochrysoidea). *Zoological Journal of the Linnean Society*, **154**, 421–436.
- Kaim, A., Jenkins, R. G., and Hikida, Y., 2009. Gastropods from Late Cretaceous Omagari and Yasukawa hydrocarbon seep deposits in the Nakagawa area, Hokkaido, Japan. *Acta Palaeontologica Polonica*, **54**, 463–490.
- Kaim, A., Bitner, M. A., Jenkins, R. G., and Hikida, Y., 2010. A monospecific assemblage of terebratulide brachiopods in the Upper Cretaceous seep deposits of Omagari, Hokkaido, Japan. *Acta Palaeontologica Polonica*, **55**, 73–84.
- Kennet, J. P., Cannariato, K. G., Hendy, I. L., and Behl, R. J., 2002. *Methane Hydrates in Quaternary Climate Change*. Washington: American Geophysical Union.
- Kiel, S., 2006. New records and species of molluscs from Tertiary cold-seep carbonates in Washington State, USA. *Journal of Paleontology*, **80**, 121–137.
- Kiel, S., Amano, K., and Jenkins, R. G., 2008. Bivalves from Cretaceous cold seep deposits on Hokkaido, Japan. *Acta Palaeontologica Polonica*, **53**, 525–537.
- Kiel, S., and Little, C. T. S., 2006. Cold-seep mollusks are older than the general marine mollusk fauna. *Science*, **313**, 1429–1431.
- Kiel, S., and Peckmann, J., 2008. Paleoecology and evolutionary significance of an Early Cretaceous *Peregrinella*-dominated hydrocarbon-seep deposit on the Crimean Peninsula. *Palaios*, **23**, 751.
- Knittel, K., Losekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Kotelnikova, S., 2002. Microbial production and oxidation of methane in deep subsurface. *Earth-Science Reviews*, **58**, 367–395.
- Kulm, L. D., Suess, E., Moore, J. C., Carson, B., Lewis, B. T., Ritger, S. D., Kadko, D. C., Thornburg, T. M., Embley, R. W., Rugh, W. D., Massoth, G. J., Langseth, M. G., Cochrane, G. R., and Scamman, R. L., 1986. Oregon subduction zone - venting, fauna, and carbonates. *Science*, **231**, 561–566.
- Kvenvolden, K. A., 1988. Methane hydrate – a major reservoir of carbon in the shallow geosphere? *Chemical Geology*, **71**, 41–51.
- Levin, L. A., 2005. Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. *Oceanography and Marine Biology – An Annual Review*, **43**, 1–46.
- Little, C. T. S., and Vrijenhoek, R. C., 2003. Are hydrothermal vent animals living fossils? *Trends in Ecology and Evolution*, **18**, 582–588.
- McLean, J. H., 1985. Preliminary report on the limpets at hydrothermal vents. *Bulletin of the Biological Society of Washington*, **6**, 159–166.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jorgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., and Gulin, M. B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Montagna, P. A., and Spies, R. B., 1985. Meiofauna and chlorophyll associated with *Beggiatoa* mats of a natural submarine petroleum seep. *Marine Environmental Research London*, **16**, 231–242.
- Moore, G. F., Taira, A., Klaus, A., Becker, L., Boeckel, B., Cragg, B. A., Dean, A., Fergusson, C. L., Henry, P., and Hirano, S., 2001. New insights into deformation and fluid flow processes in the Nankai Trough accretionary prism: results of Ocean Drilling Program Leg 190. *Geochemistry, Geophysics, Geosystems*, **2**, 1058.
- Moore, J. C., Orange, D., and Kulm, L. D., 1990. Interrelationship of fluid venting and structural evolution: Alvin observations from the frontal accretionary prism, Oregon. *Journal of Geophysical Research*, **95**, 8795–8808.
- Ogawa, Y., Fujioka, K., Fujikura, K., and Iwabuchi, Y., 1996. En echelon patterns of *Calypotgena* colonies in the Japan Trench. *Geology*, **24**, 807–810.
- Orphan, V., House, C., Hinrichs, K., McKeegan, K., and DeLong, E., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 7663–7668.
- Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D., and DeLong, E. F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., Ussler, W., Naehr, T. H., House, C. H., Hinrichs, K. U., and Paull, C. K., 2004. Geological, geochemical, and microbiological heterogeneity of the seafloor around methane vents in the Eel River Basin, offshore California. *Chemical Geology*, **205**, 265–289.
- Paull, C. K., Hecker, B., Commeau, R., Freeman-Lynde, R. P., Neumann, C., Corso, W. P., Golubic, S., Hook, J. E., Sikes, E., and Curran, J., 1984. Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. *Science*, **226**, 965–967.
- Paull, C. K., Ussler III, W., Peltzer, E. T., Brewer, P. G., Keaten, R., Mitts, P. J., Nealon, J. W., Greinert, J., Herguera, J.-C., and Elena Perez, M., 2007. Authigenic carbon entombed in methane-soaked sediments from the northeastern transform margin of the Guaymas Basin, Gulf of California. *Deep-Sea Research Part II*, **54**, 1240–1267.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Peck, A. S., Gustafson, R. G., Lutz, R. A., and Vrijenhoek, R. C., 1997. Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalve: Vesicomyidae): results from the mitochondrial cytochrome oxidase subunit 1. *Marine Biology*, **130**, 151–161.
- Proskurowski, G., Lilley, M. D., Seewald, J. S., Fruh-Green, G. L., Olson, E. J., Lupton, J. E., Sylva, S. P., and Kelley, D. S., 2008. Abiogenic hydrocarbon production at Lost City Hydrothermal Field. *Science*, **319**, 604–607.
- Retallack, G. J., and Krull, E. S., 2006. Carbon isotopic evidence for terminal-Permian methane outbursts and their role in extinctions of animals, plants, coral reefs, and peat swamps. In Greb, S. F., and DiMichele, W. A. (eds.), *Wetlands Through Time*. Geological Society of America Special Paper, pp. 249–268.
- Ritger, S., Carson, B., and Suess, E., 1987. Methane-derived authigenic carbonates formed by subduction-induced pore-water expulsion along the Oregon/Washington margin. *Geological Society of America Bulletin*, **98**, 147–156.
- Ryskin, G., 2003. Methane-driven oceanic eruptions and mass extinctions. *Geology*, **31**, 741–744.

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of microorganisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of microorganisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of microorganisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

and its release to the environment has resulted in Cu pollution in soils and surface waters.

Properties: Copper has two stable isotopes ^{63}Cu and ^{65}Cu with a natural abundance of 69.17 and 30.83, respectively, and a standard atomic weight of 63.546 (Coplen et al., 2002; Wieser, 2007). The copper concentration in the bulk continental crust, in the oceanic crust, and in soils is estimated to be 27 ppm (Rudnick and Gao, 2003), 78 ppm (Wedepohl and Hartmann, 1994), and 39 ppm (Han et al., 2002), respectively. The concentrations of copper in unpolluted seawater is typically around 0.3 ppb and in river water, 1.5 ppb (Gaillardet et al., 2003). The world average concentration of copper in rivers has been estimated as 23.6 nM Cu dissolved load and 100 ppm Cu suspended particulate load (Martin et al., 1993; Martin and Meybeck, 1979; Martin and Thomas, 1994). In natural systems, it occurs in the Cu(I) (cuprous) or Cu(II) (cupric) redox states and rarely as elemental copper. The standard redox potential of the aqueous Cu(I)/Cu(II) couple is at 0.34 V, but the actual redox potential depends strongly on its coordinative environment. This is exploited by organisms using Cu as reactive center of reductases with high redox potentials between +0.2 and +0.8 V.

Copper toxicity: Due to its toxicity to humans, the US-EPA maximum contaminant level goals and EU standards for copper in drinking water has been set at 1.3 (USEPA, 1991) and 2 mg/l (EU, 1998), respectively. Copper is also toxic to marine and terrestrial prokaryotic and eukaryotic organisms (Flemming and Trevors, 1989; Gledhill et al., 1997). Its toxicity derives from its redox reactions producing radical ions similar to Fenton chemistry causing oxidative stress. Phytochelatins (plants) and metallothioneins (animals, fungi) are intracellular metal binding proteins that play an important role in detoxification and homeostasis of Cu and other metal ions.

Copper as a nutrient: Copper-containing oxidoreductase enzymes are widespread among prokaryotic and eukaryotic organisms and mediate important biogeochemical processes, including nitrification, denitrification, and methane oxidation. Examples are particulate methane monooxygenase, ammonia monooxygenase, nitrite reductase, cytochrome *c* oxidase, and superoxide dismutase. It has been suggested that Cu-bearing reductases evolved only after the oxygenation of the Earth atmosphere, considering the low solubility of Cu in sulfidic environments and the high redox potential of organically bound Cu(I)/Cu(II) up to the limit of the stability field of water (Frausto da Silva and Williams, 2001). Copper is part of a metalloenzyme involved in the uptake of iron by diatoms so that a Cu/Fe co-limitation of primary productivity in the ocean is conceivable (Maldonado et al., 2006; Peers et al., 2005). Recently, it was discovered that some methanotrophic bacteria possess a high-affinity Cu uptake system involving methanobactin, a structurally distinct chromopeptide metallophore analogous to iron-specific siderophores (DiSpirito et al., 1998). This highlights the potential role of copper availability and copper uptake in regulating biogeochemical cycles.

Bibliography

- Coplen, T. B., Böhlke, J. K., De Bièvre, P., Ding, T., Holden, N. E., Hopple, J. A., Krouse, H. R., Lamberty, A., Peiser, H. S., Revesz, K., Rieder, S. E., Rosman, K. J. R., Rothe, E., Taylor, P. D. P., Vocke, R. D., and Xiao, Y. K., 2002. Isotope-abundance variations of selected elements (IUPAC Technical Report). *Pure and Applied Chemistry*, **74**, 1987–2017.
- DiSpirito, A. A., Zahn, J. A., Graham, D. W., Kim, H. J., Larive, C. K., Derrick, T. S., Cox, C. D., and Taylor, A., 1998. Copper-binding compounds from *Methylosinus trichosporium* OB3b. *Journal of Bacteriology*, **180**, 3606–3613.
- EU, 1998. The quality of water intended for human consumption. *Council Directive 98/83/EC*.
- Flemming, C. A., and Trevors, J. T., 1989. Copper toxicity and chemistry in the environment – a review. *Water, Air, and Soil Pollution*, **44**, 143–158.
- Frausto da Silva, J. J. R., and Williams, R. J. P., 2001. *The Biological Chemistry of the Elements – The Inorganic Chemistry of life*. Oxford: Oxford University Press.
- Gaillardet, J., Viers, J., and Dupre, B., 2003. Trace elements in river waters. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry*. Oxford: Pergamon.
- Gledhill, M., Nimmo, M., Hill, S. J., and Brown, M. T., 1997. The toxicity of copper(II) species to marine Algae, with particular reference to macroalgae. *Journal of Phycology*, **33**, 2–11.
- Han, F. X., Banin, A., Su, Y., Monts, D. L., Plodinec, M. J., Kingery, W. L., and Triplett, G. E., 2002. Industrial age anthropogenic inputs of heavy metals into the pedosphere. *Naturwissenschaften*, **89**, 497–504.
- Maldonado, M. T., Allen, A. E., Chong, J. S., Lin, K., Leus, D., Karpenko, N., and Harris, S., 2006. Copper-dependent iron transport in coastal and oceanic diatoms. *Limnology and Oceanography*, **51**, 1729–1743.
- Martin, J. M., and Meybeck, M., 1979. Elemental mass-balance of material carried by major world rivers. *Marine chemistry*, **7**, 173–206.
- Martin, J. M., and Thomas, A. J., 1994. The global insignificance of telluric input of dissolved trace metals (Cd, Cu, Ni and Zn) to ocean margins. *Marine chemistry*, **46**, 165–178.
- Martin, J. M., Guan, D. M., Elbazpoulchiet, F., Thomas, A. J., and Gordeev, V. V., 1993. Preliminary assessment of the distributions of some trace elements (As, Cd, Cu, Fe, Ni, Pb, and Zn) in a pristine aquatic environment-The Lena River estuary (Russia). *Marine Chemistry*, **43**, 185–199.
- Peers, G., Quesnel, S. A., and Price, N. M., 2005. Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. *Limnology and Oceanography*, **50**, 1149–1158.
- Rudnick, R. L., and Gao, S., 2003. The composition of the continental crust. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry*. New York: Elsevier.
- USEPA, 1991. Maximum contaminant level goals and national primary drinking water regulation for lead and copper; final rule (40 CFR Parts 141 and 143). *Federal Register*, **56**, 110.
- Wedepohl, K. H., and Hartmann, G., 1994. The composition of the primitive upper earth's mantle. In Meyer, H. O. A., and Leonardos, O. H. (eds.), *Kimberlites, Related Rocks and Mantle Xenoliths*. Rio de Janeiro, Brazil: Companhia de Pesquisa de Recursos Minerais.
- Wieser, M. E., 2007. Atomic weights of the elements 2005. *Journal of Physical and Chemical Reference Data*, **36**, 485–496.

Cross-references

Metals, Acquisition by Marine Bacteria
Siderophores

C

CALCAREOUS ALGAE

The term “calcareous algae” refers to various kinds of benthic and planktonic algae whose thalli contain biochemically precipitated calcium carbonate (CaCO_3) as skeletal material (Wray, 1977; Braga and Riding, 2005). Precipitation of CaCO_3 (as calcite and/or aragonite) may occur within or on the algal bodies. The term may also include mechanically accreted deposits of calcium carbonate caused by algae, usually as an interaction of biological and physical processes. Calcareous algae are a highly artificial group that constitutes calcifying members of the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) and is sometimes also used for Cyanobacteria. At present, calcareous algae are one of the most important reef builders (see “[Carbonate Environments](#)”). For a detailed reading, please refer to “[Algae \(Eukaryotic\)](#).”

Bibliography

- Braga, J. C., and Riding, R., 2005. Calcareous algae. In Selley, R., Cocks, L. R. M., and Plimer, I. R. (eds.), *Encyclopedia of Geology*. Amsterdam: Elsevier, pp. 428–436.
- Wray, J. L., 1977. *Calcareous Algae. Developments in Palaeontology and Stratigraphy*. Amsterdam: Elsevier, Vol. 4, 185 pp.

CALCIFICATION

See entries “[Animal Biocalcification, Evolution](#),” “[Biofilms and Fossilization](#),” “[Calcified Cyanobacteria](#),” “[Calcite Precipitation, Microbially Induced](#),” “[Calcium Biogeochemistry](#),” “[Carbonate Environments](#),” “[Dolomite, Microbial](#),” “[Microbialites, Modern](#),” “[Microbialites, Stromatolites, and Thrombolites](#),” and “[Pedogenic Carbonates](#).”

CALCIFIED CYANOBACTERIA

Robert Riding
University of Tennessee, Knoxville, TN, USA

Definition

Cyanobacteria are alga-like bacteria that can perform oxygenic photosynthesis and nitrogen fixation (Whitton and Potts, 2000; Herrero and Flores, 2008). They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments at the present-day, where they are key primary producers in both microbial mat and planktic ecosystems. As the initiators of plastids, they have played a fundamental role in algal and plant evolution (Raven, 2002). Cyanobacteria occupy benthic substrates and can also drift in the water column. Their photosynthetic uptake of inorganic carbon can stimulate CaCO_3 precipitation. This calcification can produce filamentous microfossils in benthic mats that are preserved as stromatolites and thrombolites, and can also cause water column precipitation of carbonate mud that settles to lake and sea floors.

Introduction

However, cyanobacterial calcification is not obligatory and is directly dependent on environmental conditions. This accounts for apparent discrepancies between the geological ranges of organic-walled and calcified cyanobacterial fossils. Calcified cyanobacteria have not been recognized in marine sediments older than ~1200 Ma ago (Kah and Riding, 2007), whereas there is evidence that cyanobacteria appeared in the late Archaean or Palaeoproterozoic, in the range ~2900–2150 Ma ago (Cavalier-Smith et al., 2006; Hofmann, 1976). This mid-Proterozoic appearance of sheath-calcified cyanobacteria is thought to reflect the development of CO_2 -concentrating

mechanisms (CCMs) as atmospheric CO₂ levels declined (Riding, 2006). Calcified cyanobacterial fossils remained conspicuous components of marine stromatolites and thrombolites through much of the Neoproterozoic, Palaeozoic, and Mesozoic, but became vanishingly scarce in the Cenozoic, including present-day seas (Riding, 1982), probably due to decline in seawater saturation for CaCO₃ minerals (Riding, 1993, p. 514; 2000, p. 200; Kempe and Kazmierczak, 1994). Cyanobacterial calcification is thus a good example of “induced,” as opposed to “controlled” biocalcification. Its close environmental dependence can be used to interpret changes in past conditions such as carbonate saturation state and the availability of inorganic carbon for photosynthesis. In addition to their sedimentological importance, calcified cyanobacteria can therefore assist in the reconstruction of seawater chemistry and atmospheric composition over long geological time scales.

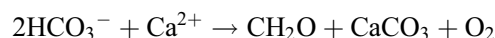
Controls on calcification

The ability of cyanobacteria to grow and reproduce whether they are calcified or uncalcified illustrates the non-obligate nature of their calcification (Pentecost and Riding, 1986). Two factors that directly influence cyanobacterial calcification are the saturation state of ambient waters and the mechanism of photosynthetic uptake of inorganic carbon (Riding, 2006).

Carbonate saturation state: Cyanobacterial calcification requires waters in which CaCO₃ precipitation is thermodynamically favored (Pentecost, 1981; Kempe and Kazmierczak, 1994; Merz-Preiß and Riding, 1999). It has been very common in marine environments at times since the Neoproterozoic, but these episodes are markedly episodic and can be interspersed by long periods when cyanobacterial calcification is scarce (Riding, 1992). Cyanobacterial sheath calcification has not been confidently recognized in present-day marine environments, and is rare to absent throughout the Cenozoic (Riding, 1982; Arp et al., 2001). In contrast, cyanobacterial calcification is locally well-developed in present-day calcareous streams and lakes, and is often significantly involved in the formation of tufa and oncoids (Golubic, 1973; Pedley, 1990; Pentecost, 2005). In streams, calcification reflects increased saturation state that results from warming and degassing of spring waters, especially in turbulent zones, together with the stimulus of photosynthetic carbon removal (Merz-Preiß and Riding, 1999; Bissett et al., 2008). In lakes, precipitation is stimulated by seasonal warming as well as the activity of phytoplankton blooms that include cyanobacteria (Kelts et al., 1978; Thompson and Ferris, 1990). These present-day environments are vulnerable to pollutants such as agricultural fertilizers. Cyanobacterial calcification has declined over the past century in temperate climate hardwater streams and lakes of Europe and North America, largely in response to these anthropogenic changes (Pentecost, 2005, pp. 283–287),

among which phosphate inhibition of CaCO₃ precipitation (Raistrick, 1949) may be important (Hägele et al., 2006).

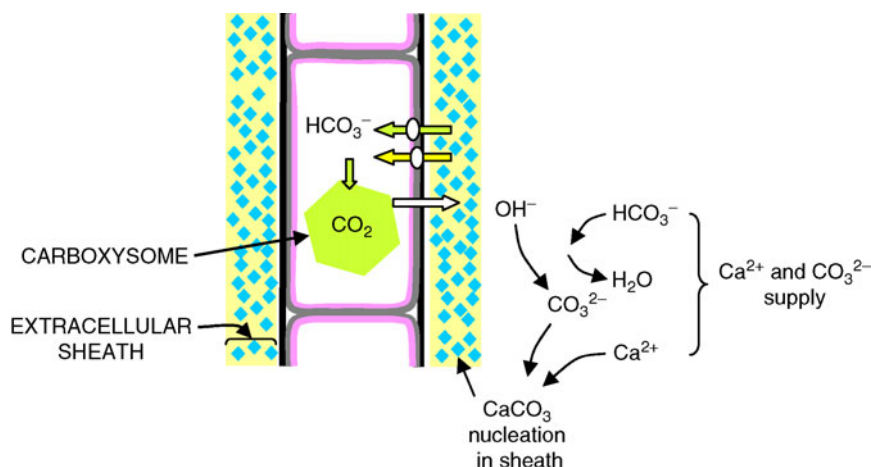
CO₂-concentrating mechanisms: Photosynthetic carbon uptake can directly influence cyanobacterial calcification. Diffusive entry of CO₂ into the cell may not significantly affect ambient pH, but active bicarbonate uptake increases pH near the cell (Miller and Colman, 1980) that promotes calcification (Thompson and Ferris, 1990). Bicarbonate (HCO₃[−]) is actively transported into the cell and intracellularly converted to CO₂ for photosynthesis. These processes lead to increased pH in the immediate vicinity of the cell. Where ambient waters are sufficiently saturated for CaCO₃ minerals then this localized pH increase can trigger the nucleation of CaCO₃ crystallites on or near the cell surface or in the enveloping mucilaginous sheath (Figures 1 and 2):



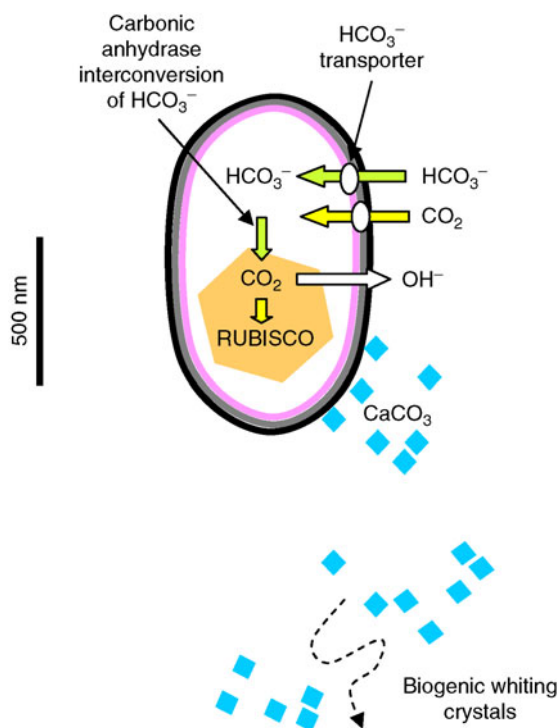
Active bicarbonate uptake and its conversion within the cell to CO₂ by carbonic anhydrase are adaptations to reduced availability of CO₂. They constitute CCMs (Kaplan and Reinhold, 1999). CCM induction can be triggered by localized carbon limitation, e.g., within microbial mats or phytoplankton blooms, and also by fall in global atmospheric CO₂ levels. Modeled estimates suggest that atmospheric CO₂ has fluctuated widely during the Phanerozoic, up to levels that are 25 or more times higher than present atmospheric level (PAL) (Berner and Kothavala, 2001). CCMs are well-developed in cyanobacteria (Kaplan et al., 1980; Giordano et al., 2005) and experiments suggest that they are induced when ambient CO₂ falls below a critical threshold that is roughly equivalent to 10 PAL (Badger et al., 2002). It therefore seems likely that CCM induction plays a significant role in cyanobacterial calcification (Thompson and Ferris, 1990; Merz, 1992), especially at times in the geological past when CO₂ levels have been below 10 PAL (Riding, 2006, p. 302).

Sites of calcification

Calcification in cyanobacteria is close, but external, to the cell. CaCO₃ crystals nucleate either within the protective mucilaginous sheath, or on or close to the cell surface (Thompson and Ferris, 1990; Merz, 1992). Sheath impregnation by crystallites (Figure 1) can produce coherent tubular and shrub-like calcified structures that preserve the sheath morphology and can be preserved as microfossils for hundreds of millions of years (Riding, 1991). In contrast, if isolated crystals form near the cell surface, they do not form a preservable shape but can be released as allochthonous particles (“whittings”) (Figure 2). These can accumulate as masses of micron-size carbonate mud sediment on lake and sea floors and can also survive as ancient geological deposits. However, they are not known to possess features that distinguish them as



Calcified Cyanobacteria, Figure 1 Model of in vivo sheath calcification in a filamentous cyanobacterium related to CO_2 -concentrating mechanism (CCM)-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CCMs involve active carbon transport into the cell and conversions that liberate OH^- ions. Calcification is stimulated by photosynthetic carbon uptake and OH^- release which elevates sheath pH. If ambient carbonate saturation is already elevated, with raised pH, extracellular HCO_3^- converts into CO_3^{2-} , further increasing saturation state that promotes CaCO_3 nucleation in the sheath.

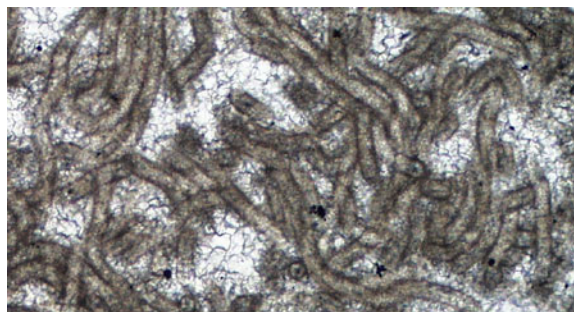


Calcified Cyanobacteria, Figure 2 Model of in vivo sheath calcification in a picoplanktic coccoid cyanobacterium related to CCM-enhanced photosynthesis (based on information in Riding, 2006, Fig. 3). The CaCO_3 nucleation (blue diamonds) occurs on and near the cell surface. The crystals can ultimately be sedimented to the lake or sea floor as carbonate mud.

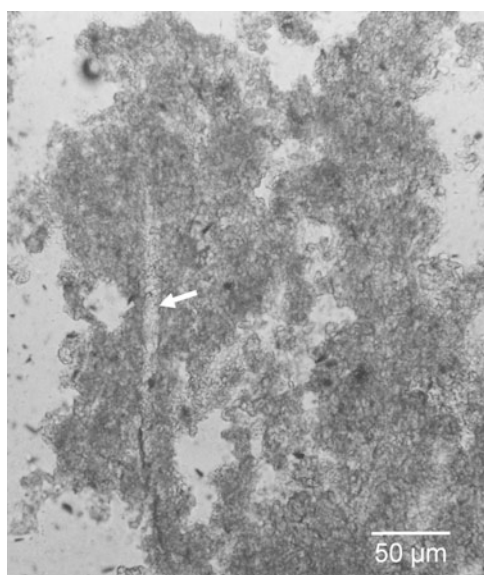
cyanobacterially induced precipitates, and cannot at present be differentiated from carbonate mud of other origins.

Sheath calcification: The protective mucilaginous sheath that envelops benthic calcified cyanobacteria provides a diffusion limited site that enhances the pH rise resulting from carbon uptake (Figures 3 and 4). The sheath is a structured form of EPS (Drews and Weckesser, 1982) providing support, stability, and protection against physical damage, dehydration, and grazers (Dudman, 1977). It can contain the pigment scytonemin that acts as a barrier to ultraviolet radiation (Garcia-Pichel and Castenholz, 1991; Proteau et al., 1993; Dillon and Castenholz, 1999), binds nutrients and metals (Yee et al., 2004), and facilitates gliding motility (Stal, 1995, p. 4; Hoiczky, 1998). Sheath calcification can be partial or complete, and can be limited to the sheath interior (sheath impregnation) or form an external crust (sheath encrustation) (Riding, 1977). For example, it can occur as isolated crystals (Pentecost, 1987, Fig. 1d), form a crystalline network (e.g., Friedmann, 1979, Fig. 9), or create a relatively solid tube of closely juxtaposed crystals (e.g., Couté and Bury, 1988, pl. 2).

Although saturation state with respect to carbonate minerals and CCM induction appear to be key controls on cyanobacterial sheath calcification over geological timescales (Riding, 2006), differences in degree of calcification between different species/strains of cyanobacteria in the same environment also indicate taxonomic specificity for calcification (Golubic, 1973; Merz, 1992; Défarge et al., 1994). Further research is required to elucidate the extent to which such specificity may reflect differences in sheath structure and sheath development, CCM induction, or other factors.



Calcified Cyanobacteria, Figure 3 *Girvanella*, calcified cyanobacterial sheath, early mid-Ordovician, Lunnan, Tarim, China. Width of view 1 mm. Thin-section courtesy Jia-Song Fan.



Calcified Cyanobacteria, Figure 4 Thin-section of present-day oncoid microfabric showing calcified shrub-like sheath surrounding space left by the strand of cells (trichome, arrowed). The cyanobacterium is thought to be the oscillatoriacean *Schizothrix calcicola*, and the calcified sheath closely resembles the microfossil *Angulocellularia* (also *Angusticellularia*), which is locally common in Cambro-Ordovician reefs (Riding and Voronova, 1982). Squaw Island, Canandaigua Lake, New York State, USA.

In addition to in vivo sheath calcification, postmortem sheath degradation by heterotrophic bacteria can result in partial external calcification (Chafetz and Buczynski, 1992), although in culture experiments this may in part be related to the growth medium used (Arp et al., 2002). This incomplete and irregular calcification of decomposing sheaths contrasts with sheath impregnation that produces well-defined tubiform fossils such as *Girvanella* in which wall-thickness remains constant in individual specimens (Riding, 1977, 2006).

Biogenic whiting precipitation: In contrast with relatively large and distinct calcified fossils, such as *Girvanella*, that result from sheath calcification, CaCO_3 precipitates associated with coccoid cyanobacterial blooms are most noticeable as “whittings”. These are ephemeral milk-white patches in freshwater calcareous lakes and shallow tropical seas formed by dense masses of suspended small CaCO_3 crystals (Cloud, 1962). Whiting is a descriptive term, and all whittings are not necessarily biogenic. In addition to the triggering effect of phytoplankton photosynthesis, they could reflect essentially abiogenic CaCO_3 crystal nucleation in the water column, and also – in very shallow water – bottom mud re-suspended by currents or fish (Shinn et al., 1989).

Biogenic cyanobacterial whittings are documented by studies of seasonal blooms of unicellular picoplanktic (cell size in range 0.2–2 μm) cyanobacteria, such as *Synechococcus*. These benefit from efficient CCMs (Badger and Price, 2003) where dissolved inorganic carbon (DIC) availability is limited, as under bloom conditions (Rost et al., 2003). Together with diatoms and other planktic algae, *Synechococcus* is implicated in stimulating biogenic whiting precipitation in present-day freshwater calcareous oligotrophic lakes (Thompson and Ferris, 1990; Dittrich et al., 2004; Lee et al., 2004). Since a sheath is lacking in picoplankton such as *Synechococcus*, calcification is instead localized on a paracrystalline surface layer that provides a binding site (Thompson, 2000, p. 253). This surface layer can be shed, producing biogenic whiting crystals that are deposited from suspension, either individually or as poorly structured aggregates along with organic cells, on lake beds.

In addition to lakes, picoplanktic cyanobacteria are widespread in the open ocean and in nearshore marine environments. They form blooms in Florida Bay, and marine strains of *Synechococcus* calcify under experimental conditions (Lee et al., 2004). There has been much debate concerning whether marine whittings in shallow tropical seas, such as the Bahama Banks, have a similar origin to those in temperate calcareous lakes, and could therefore potentially account for abundant lime mud production on ancient carbonate platforms. If marine whittings on the Bahama Banks are water column precipitates, then they would be important sources of carbonate mud and could be triggered by planktic cyanobacteria (Robbins and Blackwelder, 1992; Robbins et al., 1997). However, CaCO_3 precipitation in freshwater lakes is favored by low pH buffering, whereas in present-day seawater buffering limits pH fluctuation, thereby reducing the response to photosynthetic removal of CO_2 and HCO_3^- . Several studies have suggested that Great Bahama Bank whittings are not due to water column precipitation (Broecker and Takahashi, 1966; Morse et al., 1984; Broecker et al., 2000). For example, whiting CaCO_3 has a $^{14}\text{C}/\text{C}$ ratio that differs from that of inorganic carbon in the whiting water, but is similar to that of the seafloor sediment. In addition, the saturation state of Bahama Bank waters appears to be too low for pseudohomogeneous precipitation of CaCO_3 .

(Broecker et al., 2001, p. 591; Morse et al., 2003). Broecker et al. (2000) concluded that resuspension of sediment is the dominant process involved in marine whittings on the Bahama Banks. Further work is required to resolve this question, but present-day seawater saturation state in general may be too low to permit CaCO_3 nucleation even within blooms of picoplankton that are operating CCMs.

Nonetheless, biogenic whittings may have occurred under different conditions of seawater chemistry in the geological past, and could therefore have been important sources of fine-grained carbonate. Within plankton blooms, photosynthetic uptake can significantly deplete pCO_2 (Riebesell et al., 1993; Rost et al., 2003), and it is likely that selective pressure for picoplankton to induce CCMs first developed under bloom conditions.

Fossil calcified cyanobacteria

Calcified cyanobacterial fossils have long been noticed in limestones, although they have often been confused with other organisms. Sheath calcified cyanobacteria are morphologically simple fossils, mainly with the appearance of shrub-like dendritic masses (Figure 4) and tangled or erect, sometimes radially arranged, tubes (Figure 3). Densely micritic branched filaments such as *Epiphyton*, and chambered clusters such as *Renalcis* have also often been regarded as possible calcified cyanobacteria. At the same time, because of their general lack of distinguishing features, many of these fossils have often also been compared with calcified green and red algae, and also with foraminifers and sponges. For example, when Nicholson and Etheridge (1878) named *Girvanella* they compared it with foraminifers, and it has subsequently variously been regarded as a calcareous sponge (Seely, 1885), green alga (Rothpletz, 1891), and red alga (Korde, 1973). Bornemann (1886) recognized its cyanobacterial nature, and Pollock (1918) identified it as a calcified sheath. Individual calcified cyanobacteria are normally microscopic, but they commonly form much larger aggregates that are significant components of stromatolites, oncoids, thrombolites, dendrolites, reef crusts, and freshwater tufa deposits that range up to decametric in size.

Three main morphological groups can be distinguished (Riding, 1991): tubes, dendritic shrubs and filaments, and hollow chambers.

Tubes: *Girvanella*, originally described from the Middle Ordovician of Scotland, is very simple: unbranched, often irregularly tangled, tubular filaments of uniform diameter, usually <50 microns, and a thin wall of even thickness (Figure 3). Similar tubes, but often organized into cable-like strands, include *Batinivia*, *Botominella*, *Cladogirvanella*, *Razumovskia*, and *Subtifloria*. *Obruchevella* is coiled. *Ortonella* was described from the Mississippian of England (Garwood, 1914) where it forms oncoids (spherical stromatolite nodules) and stromatolitic crusts. It consists of tubes similar in size to those of *Girvanella*, but distinguished by being erect and branched. Somewhat similar forms include *Bajanophyton*,

Bija, *Botomaella*, *Cayeuxia*, and *Hedstroemia*. Pia (1927, pp. 36–40) recognized that very small tubiform fossils such as *Girvanella* and *Ortonella* were cyanobacteria when he placed them in a new group, the Porostromata.

Dendritic shrubs and filaments: *Angusticellularia* (also named *Angulocellularia*) described from the Cambrian of Siberia by Vologdin (1962) is a submillimetric vertically orientated and irregularly dendritic shrub-like mass of dense micrite (Figure 4). In contrast, narrow branched micritic filaments, usually not tubiform but in some cases with light-colored transverse bands, comprise a group that includes *Epiphyton*, originally described from the Cambrian of Sardinia by Bornemann (1886), and among others, *Epiphytonoides*, *Gordonophyton*, and *Tubomorphophyton*.

Hollow chambers: *Renalcis*, described from the Cambrian of southern Siberia by Vologdin (1932), is a hollow thick-walled chambered microfossil that typically forms irregular submillimetric clusters. Broadly similar fossils, such as *Izhella* and *Shuguria*, are distinguished by wall thickness and number of chambers. Some form millimetric dendritic growths. *Chabakovia* filaments consist of thin-walled chambers. In contrast, *Gemma* and *Tarthina* have few chambers with very thick walls.

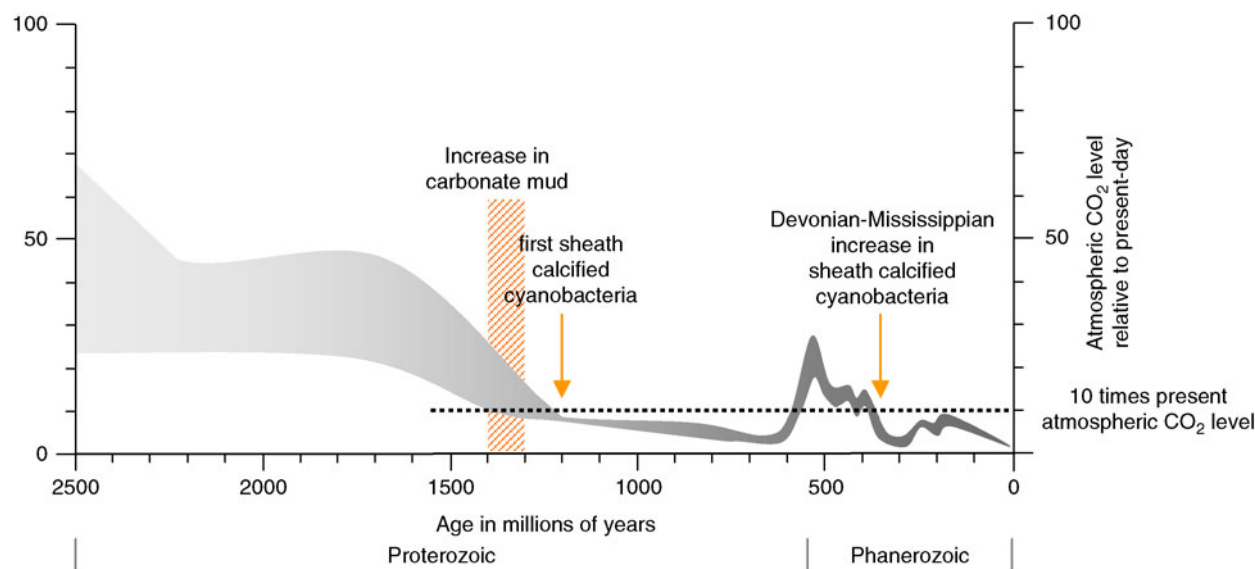
There is considerable mingling of characters among some of these generally simply organized fossils that can make them difficult to distinguish within these broad groups (Pratt, 1984; Riding and Voronova, 1985; Woo et al., 2008). Dense filaments and chambered forms are much more problematic, whereas tubiform and shrub-like forms can often be compared with present-day sheath calcified cyanobacteria. These include sedimentologically important Cambrian and late Devonian fossils such as *Epiphyton* and *Renalcis* that have some resemblances to cyanobacteria but are not identical to modern examples, and are generally referred to as calcimicrobes. Some *Epiphyton* closely resemble red algae (Luchinina and Terleev, 2008).

Proterozoic record and secular controls

The possibilities that marine carbonate saturation state and changes in atmospheric CO_2 level could be reflected in the geological record of cyanobacterial calcified microfossils and carbonate mud (Riding, 2006, p. 309) focuses attention on the mid-Proterozoic interval when calcified sheaths first appeared (Kah and Riding, 2007) and carbonate mud became abundant (Sherman et al., 2000, p. 290) (Figure 5).

CCM induction

Badger et al. (2002) suggested that cyanobacterial CCMs developed in the late Palaeozoic as CO_2 declined and O_2 increased. However, both modeling and palaeosol proxies (Kasting, 1987; Sheldon, 2006) suggest that similar decline in CO_2 occurred in the mid-Proterozoic. It can therefore be suggested that the Mesoproterozoic appearance of calcified sheath microfossils reflects development of CCMs as CO_2 fell below a critical threshold that may



Calcified Cyanobacteria, Figure 5 Increase in marine carbonate mud accumulation ~1400–1300 Ma ago (Sherman et al., 2000) and first appearance of sheath-calcified cyanobacteria ~1200 Ma ago (Kah and Riding, 2007) compared with decline in estimated atmospheric CO₂ values. It is suggested that decline in CO₂ to ~10× present atmospheric level favored development of CCMs that promoted cyanobacterial sheath calcification and whiting production (Riding, 2006). Incorporation of calcified filaments in microbial carbonates gave rise to calcified microbe thrombolites and dendrolites. Inferred Proterozoic CO₂ trend based on Sheldon (2006), Kah and Riding (2007), Hyde et al. (2000), and Ridgwell et al. (2003). Phanerozoic CO₂ trend from Berner and Kothavala (2001, Fig. 13). Threshold for CCMs based on Badger et al. (2002). Decline in CO₂ level below 10× present-level was repeated ~350 Ma ago and coincides with marked Devonian-Mississippian increase in calcified cyanobacteria (see Arp et al., 2001, Figs. 3d and 8b).

have been ~10× PAL (Badger et al., 2002; Riding, 2006; Kah and Riding, 2007). Under high early Proterozoic CO₂ levels, cyanobacterial photosynthesis may have relied on CO₂ diffusion that may not have significantly altered pH near the cell surface. Falling CO₂ levels would have encouraged active carbon uptake to maintain photosynthesis and this, in turn, promoted calcification (Figures 1 and 5).

Carbonate mud: Carbonate mud appears to have been scarce in the late Archaean (Sumner and Grotzinger, 2004), but it increased in abundance during the Proterozoic (Kah and Grotzinger, 1992) and was widespread in the Neoproterozoic (Herrington and Fairchild, 1989). Much of it is thought to have been produced by whittings stimulated by phytoplankton photosynthesis (Grotzinger, 1989, 1990). A significant increase in carbonate mud occurred ~1400–1300 Ma ago (Sherman et al., 2000, p. 290), prior to the appearance of sheath calcification (Kah and Riding, 2007). This “water column factory” transformed carbonate platform sedimentation by creating extensive micrite-rich subtidal deposits (Sherman et al., 2000, p. 290). The timing of this increase in carbonate mud therefore broadly coincided with the first appearance of *Girvanella*-like and other sheath-calcified fossils (see below), and may be linked to CCM development in cyanobacteria, but in this case to planktic forms, possibly similar to present-day *Synechococcus* (Riding, 2006). Muddy carbonate platforms were the deposits in which distinctive molar tooth facies developed (James et al.,

1998; Sherman et al., 2000, p. 290). It can therefore be speculated that mid-late Proterozoic increase in micrites and molar tooth facies, together with *Girvanella* and thrombolites, all reflect decline in atmospheric CO₂ that stimulated CCM induction and calcification in cyanobacteria (Riding, 2006) (Figure 5).

Cyanobacterial sheath calcification: The first appearance of calcified cyanobacteria in the mid-Proterozoic – interpreted to reflect the first development of CCMs in photosynthetic organisms (Riding, 2006) – marked a radical change from stromatolitic to thrombolitic fabrics. Mesoproterozoic examples reported from the ~1200 Ma Society Cliffs Fm are currently the oldest known *Girvanella*-like calcified filaments, and are associated with micritic bush-like structures also interpreted as calcified cyanobacteria (Kah and Riding, 2007). The presence of filamentous fabrics had long been recognized in late Proterozoic and early Palaeozoic stromatolites. Microstructure with filament moulds was termed Canaliphorida and Filiformita by Komar (1976, 1989, see Bertrand-Sarfati et al., 1994, p. 182, Fig. 18). Aitken (1989, pp. 15–16) described “dendriform” and “lamelliform elements” as important components of Little Dal reefs in the Mackenzie Mountains. He regarded both as stromatolites with “unusual” or “unique” characteristics: thin-walled tubes and *Renalcis*-like objects in dendriform element, and a reticulate “ladder-rung” arrangement that “may be formed by a meshwork of tubes” in lamelliform element. He remarked that these fabrics “are not typically

stromatolitic” and that “sediment trapping may not have been the dominant process in their formation” (p. 15). Dendriiform and lamelliform elements look quite similar in two of his illustrations (Figs. 10 and 13). Subsequently, Turner et al. (1993, 2000a, Fig. 10b, 2000b) compared the tubules with *Girvanella* and noted that the lamelliform fabric consists of alternating dark layers of “calcimicrobial filaments” and lighter “more cement-rich” areas. Little Dal “hollow tubules with micritic walls” are figured by Batten et al. (2004, Fig. 9b). Knoll and Semikhatov (1998, p. 410, Figs. 3 and 4) described well-preserved “filmy or platy” microstructure in early Neoproterozoic *Baicalia lacera* stromatolites from the Chernaya Rechka Fm, Igarka, Siberia. They found it to be associated with “a distinctly filamentous microstructure” in which “laminae comprising densely interwoven to scattered, vertically, or subhorizontally oriented filaments are interspersed with layers of spongy or dense microspar.” They interpreted the 8–10 μm tubes as “sheaths of LPP-type (*Lyngbya*, *Phormidium*, *Plectonema*) cyanobacteria and preserved as drusy microspar encrustations” (Knoll and Semikhatov, 1998, p. 411). Similar *Baicalia lacera* fabrics in the $\sim 1\text{Ga}$ Burovaya Formation of west-central Siberia locally contain calcified tubes resembling *Siphonophycus* (Petrov and Semikhatov, 2001, p. 270). The lamelliform element shown by Turner et al. (2000a, Fig. 10b) resembles the distinctive “filmy” microstructure of similar age *Baicalia lacera* (Petrov and Semikhatov, 2001, Figs. 5b and 6a) which also has steeply dipping laminae and, as noted above, quite possibly filamentous microstructure too. In these examples, layers of filamentous fabric are generally interleaved with lighter, sparry, layers. If the sparry layers were lacking, the deposit would be indistinguishable from “skeletal stromatolite” (Riding, 1977) and “porostromate stromatolite” (Monty, 1981). In addition to the Little Dal and Chernaya Rechka examples, calcified filaments reminiscent of *Girvanella* are relatively widespread elsewhere

in the Neoproterozoic, e.g., in the ~ 750 – 700 Ma Draken Fm, ~ 725 – 675 Ma Svanbergfjellet Fm, and $\sim 700\text{ Ma}$ Upper Eleonore Bay Supergroup, Greenland (refs in Knoll and Semikhatov, 1998, p. 413). Throughout the Neoproterozoic, these thrombolites with calcified microbial fabrics (Kennard and James, 1986) are intimately associated with stromatolites but typically occur in deeper subtidal environments (Aitken, 1967).

Marine carbonate saturation

Inception of cyanobacterial calcification would have required overall seawater carbonate saturation to be relatively high. Grotzinger (1990) has argued that seawater saturation state progressively reduced during the Proterozoic and that this could account for concomitant decline in stromatolite abundance. Nonetheless, the widespread continued development of microbial carbonates into the Neoproterozoic, together with calcified sheaths themselves, indicates that saturation state was not low. This may have changed during widespread glaciations that occurred in the late Proterozoic ~ 700 – 570 Ma ago (Walter et al., 2000). Sheath-calcified cyanobacteria appear to have been scarce during “Snowball” glaciations. Lower temperature and $p\text{CO}_2$ levels would have decreased seawater saturation state, hindering microbial calcification generally. Shields (2002) attributed decline in Molar Tooth facies $\sim 750\text{ Ma}$ ago to reduction in carbonate saturation. Since cooling would also have favored diffusive entry of CO_2 into cells (Raven et al., 2002) it could have slowed CCM development, further reducing cyanobacterial calcification. As Earth emerged from these snowball glaciations, global warming and O_2 rise could have reactivated CCM development, and rising temperature, calcium, and $p\text{CO}_2$ levels would have increased seawater saturation state, stimulating calcification (Riding, 2006). Calcified cyanobacteria became relatively diverse



Calcified Cyanobacteria, Figure 6 Early Cambrian (Botomian) dendrolite-thrombolite reef, $\sim 70\text{ m}$ thick, near Tafraoute, Anti-Atlas Mountains, Morocco. Figures in foreground for scale.

in the earliest Cambrian (Nemakit-Daldynian) (Riding and Voronova, 1984) and were important components of Cambrian reefs (Riding, 2000; Rowland and Shapiro, 2002).

Calcmicrobial reefs and the Cambrian radiation

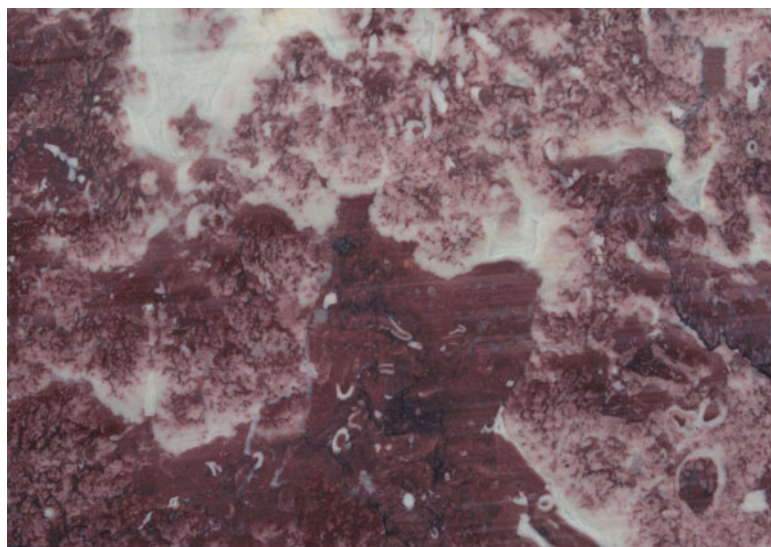
Calcified microfossils such as *Epiphyton*, *Renalcis*, and *Angusticellularia* are major components of Cambrian reefs (Figures 6 and 7) and similar forms reappeared in the late Devonian. The widespread development of microbial reefs in the early Cambrian may have directly contributed to marine invertebrate diversification. Reefs constructed by calcified microbes appeared in the latest Neoproterozoic, ~550 Ma ago (Grotzinger et al., 2000) and were common in Siberia, Altai Sayan, and Mongolia during the Nemakit-Daldynian (543–526 Ma ago) (Riding, 2002). In the Tommotian, 526–522 Ma ago, this reef-building association was augmented by archaeocyath sponges, with calcified microbes generally being volumetrically dominant (Rowland and Shapiro, 2002), and the reefs became large and cavernous. The exceptional biodiversity that characterizes reefs in general is largely due to the process of reef bioconstruction itself (Cocito, 2004). Rapid accretion of framework with synoptic relief and internal growth cavities creates substrates and spaces that differ in incident light, water movement, and sedimentation, as well as accessibility. This increased habitat size and partitioning generates heterogeneity that promotes biodiversity. For example, Cambrian trilobites show peak diversity in reefal environments (Westrop and Adrain, 2001), and comparison of level-bottom and reef communities in general shows that early Cambrian reefs were centers of diversity (Burzin et al., 2001, Fig. 10.2) with number of reef-building species comparable to that of late

Neogene fossil reefs (Kiessling, 2002, Fig. 20). The expansion of these globally distributed reef communities corresponded with rapid diversification of the shallow marine biota, especially during the Tommotian-early Botomian (~526–517 Ma ago) (Zhuravlev 2001, Fig. 8.1), suggesting a direct link between microbial carbonate development and metazoan evolution.

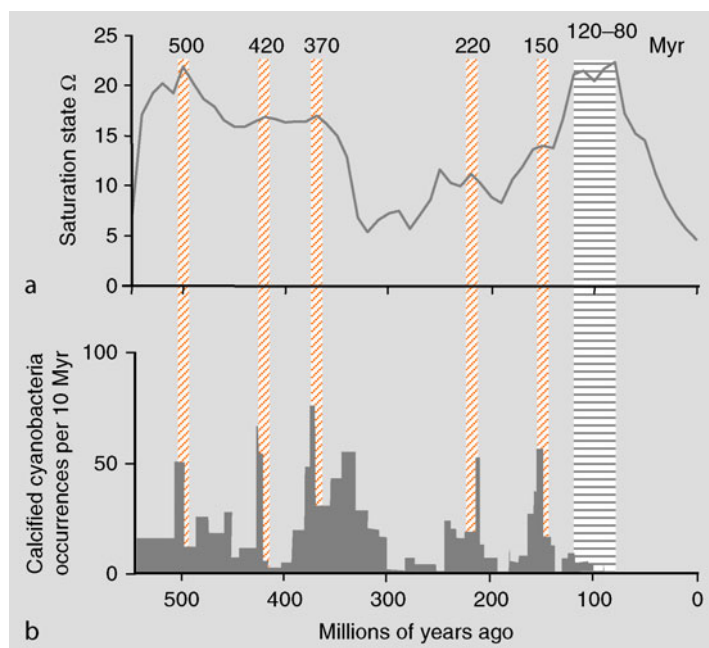
Phanerozoic record and secular controls

Cyanobacterial sheath calcification: Following their Mesoproterozoic inception, sheath-calcified cyanobacteria remained generally common in shallow-water marine carbonates during much of the Palaeozoic and until the late Jurassic (Arp et al., 2001) (Figure 8b). The abundance of sheath-calcified cyanobacteria during the early Palaeozoic (when CO₂ levels are thought to have been high, see Berner and Kothavala, 2001), suggests that CCMs continued to be induced even when pCO₂ substantially exceeded 10 PAL. This may reflect calcification in habitats subject to carbon limitation that induced CCMs, such as microbial mats and reefs (Riding, 2006). Nonetheless, enhancement of CCMs by overall decline in atmospheric CO₂ could be reflected by increased calcified-sheath abundance in the Mississippian, ~335 Ma ago (Riding, 2006) (Figure 5). However, it seems that this increase in calcified-sheath abundance promoted by falling CO₂ was itself terminated by subsequent steep decline in seawater saturation state ~325 Ma ago, before the end of the Mississippian (Figure 8a).

Broad similarities between calcified-sheath abundance and carbonate saturation are suggested by peaks in the late Cambrian (~500 Ma), late Silurian (~420 Ma), late Devonian (~370 Ma), late Triassic (~220 Ma), and late



Calcified Cyanobacteria, Figure 7 Early Cambrian calcimicrobial reef framework, showing clusters of mainly pendant filaments, early cement and geopetal cavity fill. Near Taroudannt, Anti-Atlas Mountains, Morocco. Width of view ~35 cm.



Calcified Cyanobacteria, Figure 8 Comparison of (a) Marine saturation ratio (Ω) for calcite, calculated from estimates of seawater and atmospheric composition, and (b) abundance of marine sheath-calcified cyanobacteria (Arp et al. 2001, Fig. 3d) (from Riding and Liang, 2005, Fig. 5). Obliquely ruled areas indicate five intervals approximately 500, 420, 370, 220, and 150 millions of years (Myr) ago where peaks of calculated saturation ratio broadly coincide with increased abundances of calcified cyanobacteria. This correspondence supports the view that cyanobacterial calcification has been increased at times of generally elevated seawater saturation for CaCO_3 minerals. The horizontally ruled area indicates the interval ~120–80 Myr ago in which high calculated saturation contrasts with low abundance of calcified cyanobacteria. This anomaly could reflect lowered Cretaceous carbonate saturation due to widespread deposition of pelagic carbonate by coccolithophore algae and globigerine foraminifers, since biological removal of CaCO_3 is not incorporated into the calculation of saturation values.

Jurassic (~150 Ma) (Figure 8), supporting the view that saturation state has significantly influenced the degree of cyanobacterial sheath calcification over geological time (Riding and Liang, 2005).

Reduced abundance of sheath-calcified cyanobacteria ~120–80 Ma ago contrasts with calculated saturation state which is elevated during this interval. This anomaly is thought to reflect reduction in actual (as opposed to calculated) saturation state due to burial of CaCO_3 precipitated as calcified skeletons by plankton such as coccolithophore algae and globigerine foraminifers (Riding and Liang, 2005). Such biological removal of calcium is not included in the input used to calculate the saturation values shown. This effect of planktic calcifiers on the seawater system may have continued into the Palaeogene. During the past 50 Ma, from the early Eocene onward calculated saturation has declined steeply in response to low values of Ca^{2+} ions and pCO_2 , and this is consistent with scarcity of marine calcified cyanobacteria. Consequently, although low pCO_2 values during the Cenozoic (see Berner and Kothavala, 2001) are expected to induce cyanobacterial CCMs, the absence of sheath calcification in marine cyanobacteria presumably reflects the overriding effect of low carbonate saturation.

Biogenic whittings: It can be inferred that, following the development of CCMs in the mid-Proterozoic, picoplanktic cyanobacterial activity could have been an important source of marine carbonate mud whenever inorganic carbon could be a limiting resource, as in plankton blooms (Rost et al., 2003), even when overall atmospheric CO_2 levels were high. If this is correct, then the principal factor determining biogenic whiting occurrence would have been carbonate saturation state. Calculations suggest that saturation was relatively elevated during much of the early-mid Palaeozoic and parts of the Mesozoic (Riding and Liang, 2005; and Figure 8a).

The origins of micrite are notoriously difficult to interpret even in present-day seas, and it is known that calcified green algae can also be significant producers (Stockman and Ginsburg, 1967). How can the possible contribution of biogenic whittings therefore be assessed in the geological past? One line of reasoning is to assume that sheath-calcified cyanobacteria reflect generally similar conditions to those that favor picoplanktic whiting precipitation. The geological record of sheath-calcified fossils such as *Girvanella* is relatively well-documented, and they show marked fluctuations in abundance (Arp et al., 2001, Fig. 3d) (Figure 8b). It could therefore be predicted that picoplanktic mud should show a similar pattern of

abundance, with peaks for example in the late Cambrian, Devonian-Mississippian, and late Jurassic. It should be possible to test whether carbonate mud was relatively abundant at these times. It is also possible that biogenic whittings – and sheath calcification – should have been further intensified whenever $p\text{CO}_2$ levels fell to levels near or below ~ 10 PAL, as for example ~ 350 Ma ago (Figure 5).

Cenozoic decline in seawater carbonate saturation, suggested by calculations based on modeled values (Riding and Liang, 2005, Figs. 5a and 8a), would be expected to have reduced biogenic whiting precipitation. Certainly, sheath calcification is scarce to absent during most of the past 100 Ma (Arp et al., 2001, Fig. 3d) (Figure 8b). In this context, it is not surprising that there is uncertainty whether present-day marine whittings on the Bahaman Banks are biogenic or mainly reflect sediment resuspension (Broecker et al., 2000). From a geobiological perspective, however, even if present-day marine conditions do not greatly favor phytoplankton-stimulated precipitation of whittings, these may have been very important at times in the geologic past environment when carbonate saturation state was sufficiently high. If this is correct, biogenic whittings could account for a substantial part of the carbonate mud that accumulated on shallow shelves between the late Mesoproterozoic and Mesozoic.

Summary

Calcified cyanobacteria are easily overlooked fossils, yet their long history coupled with their sensitivity to environmental controls on their calcification, make them potentially significant indices of changes in seawater chemistry and atmospheric CO_2 level throughout the Proterozoic and Phanerozoic. Locally they are also sedimentologically important reef components, and they have played a major role in the formation of calcimicrobial thrombolites that are especially widespread in the Neoproterozoic and early Palaeozoic. At the present day their vanishing scarcity in marine environments is thought to reflect relatively low seawater carbonate saturation that has persisted throughout the Cenozoic. However, their local abundance in oncoids and tufas of freshwater calcareous streams and lakes, and their involvement in lacustrine whittings, are reminders of the ability of cyanobacteria to promote intense CaCO_3 deposition when conditions that include ambient saturation state, are favorable.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Aitken, J. D., 1989. Giant “algal” reefs, middle/upper Proterozoic Little Dal group (>770 , <1200 Ma), Mackenzie Mountains, N. W.T., Canada. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Area*, Canadian Society of Petroleum Geologists, Memoir, Vol. 13, pp. 13–23.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2002. Calcification of cyanobacterial filaments: Girvanella and the origin of lower Paleozoic lime mud: comment. *Geology*, **30**, 579–580.
- Badger, M., and Price, D., 2003. CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany*, **54**, 609–622.
- Badger, M. R., Hanson, D., and Price, G. D., 2002. Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Functional Plant Biology*, **29**, 161–173.
- Batten, K. L., Narbonne, G. M., and James, N. P., 2004. Paleoenvironments and growth of early Neoproterozoic calcimicrobial reefs: platform Little Dal Group, northwestern Canada. *Precambrian Research*, **133**, 249–269.
- Berner, R. A., and Kothavala, Z., 2001. GEOCARB III. A revised model of atmospheric CO_2 over Phanerozoic time. *American Journal of Science*, **301**, 182–204.
- Bertrand-Sarfati, J., Freytet, P., and Plaziat, J. C., 1994. Microstructures in tertiary nonmarine stromatolites (France). Comparison with Proterozoic. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 155–191.
- Bissett, A., de Beer, D., Schoon, R., Shiraishi, F., Reimer, A., and Arp, G., 2008. Microbial mediation of stromatolite formation in karst-water creeks. *Limnology and Oceanography*, **53**(3), 1159–1168.
- Bornemann, J., 1886. Die Versteinerungen des cambrischen Schichtensystems der Insel Sardinien nebst vergleichenden Untersuchungen über analoge Vorkommnisse aus anderen Ländern 1. *Nova Acta der Kaiserslichen Leopoldinisch-Carolinischen Deutschen Akademie der Naturforscher*, **51**(1), 1–147.
- Broecker, W. S., and Takahashi, T., 1966. Calcium carbonate precipitation on the Bahama Banks. *Journal of Geophysical Research*, **71**, 1575–1602.
- Broecker, W. S., Sanyal, A., and Takahashi, T., 2000. The origin of Bahamian whittings. *Geophysical Research Letters*, **27**, 3759–3760.
- Broecker, W. S., Langdon, C., Takahashi, T., and Peng, T. H., 2001. Factors controlling the rate of CaCO_3 precipitation on the Great Bahama Bank. *Global Biogeochemical Cycles*, **15**, 589–596.
- Burzin, M. B., Debrenne, F., and Zhuravlev, A. Yu., 2001. Evolution of shallow-water level-bottom communities. In Zhuravlev, A. Yu., and Riding, R. (eds.), *The Ecology of the Cambrian Radiation*. New York: Columbia University Press, pp. 217–237.
- Cavalier-Smith, T., Brasier, M., and Embley, T. M., 2006. Introduction: how and when did microbes change the world? *Philosophical Transactions of the Royal Society, B*, **361**, 845–850.
- Chafetz, H. S., and Buczynski, C., 1992. Bacterially induced lithification of microbial mats. *Palaios*, **7**, 277–293.
- Cloud, P. E., Jr., 1962. Environment of calcium carbonate deposition west of Andros Island, Bahamas. *US Geological Survey Professional Paper*, **350**, 138.
- Cocito, S., 2004. Bioconstruction and biodiversity: their mutual influence. *Scientia Marina*, **68**(Supplement 1), 137–144.
- Couté, A., and Bury, E., 1988. Ultrastructure d'une Cyanophycée aérienne calcifère cavernicole: *Scytonema julianum* Frank (Richter) (Hormogonophicidae, Nostocales, Scytonemataceae). *Hydrobiologia*, **160**, 219–239.
- Défarge, C., Trichet, J., and Couté, A., 1994. On the appearance of cyanobacterial calcification in modern stromatolites. *Sedimentary Geology*, **94**, 11–19.

- Dillon, J. G., and Castenholz, R. W., 1999. Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *Journal of Phycology*, **35**, 673–681.
- Dittrich, M., Kurz, P., and Wehrli, B., 2004. The role of autotrophic picocyanobacteria in calcite precipitation in an oligotrophic lake. *Geomicrobiology Journal*, **21**, 45–53.
- Drews, G., and Weckesser, J., 1982. Function, structure and composition of cell walls and external layers. In Carr, N. G., and Whitton, B. A. (eds.), *The Biology of Cyanobacteria*. Oxford: Blackwell, pp. 333–357.
- Dudman, W. F., 1977. The role of surface polysaccharides in natural environments. In Sutherland, I. (ed.), *Surface Carbohydrates of the Prokaryotic Cell*. London: Academic Press, pp. 357–414.
- Friedmann, E. I., 1979. The genus *Geitleria* (Cyanophyceae or Cyanobacteria): distribution of *G. calcarea* and *G. floridana* n. sp. *Plant Systematics and Evolution*, **131**, 169–178.
- Garcia-Pichel, F., and Castenholz, R. W., 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *Journal of Phycology*, **27**, 395–409.
- Garwood, E. J., 1914. Some new rock-building organisms from the Lower Carboniferous beds of Westmorland. *Geological Magazine*, 6th decade, **1**, 265–271, pls. 20–21.
- Giordano, M., Beardall, J., and Raven, J. A., 2005. CO₂ concentrating mechanisms in algae: mechanisms, environmental modulation, and evolution. *Annual Review of Plant Biology*, **56**, 99–131.
- Golubic, S., 1973. The relationship between blue-green algae and carbonate deposits. In Carr, N., and Whitton, B. A. (eds.), *The Biology of Blue-Green Algae*. Oxford: Blackwell, pp. 434–472.
- Grotzinger, J. P., 1989. Facies and evolution of Precambrian carbonate depositional systems. The emergence of the modern platform archetype. In Crevello, P. D., Wilson, J. L., Sarg, J. F., and Read, J. F. (eds.), *Controls on Carbonate Platform and Basin Development*. Tulsa, OK: SEPM special publication number, 44, pp. 79–106.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., Watters, W. A., and Knoll, A. H., 2000. Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology*, **26**, 334–359.
- Hägele, D., Leinfelder, R., Grau, J., Burmeister, E.-G., and Struck, U., 2006. Oncoids from the river Alz (southern Germany): tiny ecosystems in a phosphorus-limited environment. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 378–395.
- Herrero, A., and Flores, E. (eds.), 2008. *The Cyanobacteria: Molecular Biology, Genomics and Evolution*. UK: Caister Academic Press, 484 pp.
- Herrington, P. M., and Fairchild, I. J., 1989. Carbonate shelf and slope facies evolution prior to Vendian glaciation, central East Greenland. In Gayer, R. A. (ed.), *The Caledonide Geology of Scandinavia*. London: Graham and Trotman, pp. 263–273.
- Hofmann, H. J., 1976. Precambrian microflora, Belcher Islands, Canada: significance and systematics. *Journal of Paleontology*, **50**, 1040–1073.
- Hoiczky, E., 1998. Structural and biochemical analysis of the sheath of *Phormidium uncinatum*. *Journal of Bacteriology*, **180**, 3923–3932.
- Hyde, W. T., Crowley, T. J., Baum, S. K., and Peltier, W. R., 2000. Neoproterozoic ‘snowball Earth’ simulations with a coupled climate/ice-sheet model. *Nature*, **405**, 425–429.
- James, N. P., Narbonne, G. M., and Sherman, A. G., 1998. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic. *Journal of Sedimentary Research*, **68**, 716–722.
- Kah, L. C., and Grotzinger, J. P., 1992. Early Proterozoic (1.9 Ga) thrombolites of the Rocknest Formation, Northwest Territories, Canada. *Palaaios*, **7**, 305–315.
- Kah, L. C., and Riding, R., 2007. Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. *Geology*, **35**, 799–802.
- Kaplan, A., and Reinhold, L., 1999. The CO₂-concentrating mechanism of photosynthetic microorganisms. *Annual Review of Plant Physiology and Plant Molecular Biology*, **50**, 539–570.
- Kaplan, A., Badger, M. R., and Berry, J. A., 1980. Photosynthesis and the intracellular inorganic carbon-pool in the blue green algae *Anabaena variabilis*. Response to external CO₂ concentration. *Planta*, **149**, 219–226.
- Kasting, J. F., 1987. Theoretical constraints on oxygen and carbon dioxide concentrations in the Precambrian atmosphere. *Precambrian Research*, **34**, 205–228.
- Kelts, K., Hsü, K. J., and Lerman, A., 1978. Freshwater carbonate sedimentation. In Lerman, A. (ed.), *Lakes; Chemistry, Geology, Physics*. New York, NY: Springer-Verlag, pp. 295–323.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. *Bulletin de la Institut Océanographique (Monaco)*, **13**, 61–117.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaaios*, **1**, 492–503.
- Kiessling, W., 2002. Secular variations in the Phanerozoic reef ecosystem. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*, Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 72, pp. 625–690.
- Knoll, A. H., and Semikhatov, M. A., 1998. The genesis and time distribution of two distinctive Proterozoic stromatolite microstructures. *Palaaios*, **13**, 408–422.
- Komar, V. A., 1976. Classification of stromatolites according to microstructure. In ‘*Palaeontology of the Precambrian and Early Cambrian*’, Novosibirsk, USSR: All Union Symposium, pp. 41–43 (in Russian).
- Komar, V. A., 1989. Classification of the microstructures of the Upper Precambrian stromatolites. *Himalayan Geology*, **13**, 229–238.
- Korde, K. B., 1973. *Vodorosli kembriya (Cambrian Algae)*. Nauka, Moscow: Academy of Sciences of the U.S.S.R., Transactions of the Palaeontological Institute, Vol. 139, pp. 1–350 (in Russian).
- Lee, B. D., Apel, W. A., and Walton, M. R., 2004. Screening of cyanobacterial species for calcification. *Biotechnology Progress*, **20**, 1345–1351.
- Luchinina, V. A., and Terleev, A. A., 2008. The morphology of the genus *Epiphyton* Bornemann. *Geologia Croatica*, **61**(2–3), 105–111.
- Merz, M. U. E., 1992. The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**, 81–102.
- Merz-Preiß, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams: ambient environment, chemical thresholds and biological processes. *Sedimentary Geology*, **126**, 103–124.
- Miller, A. G., and Colman, B., 1980. Evidence for HCO₃-transport in the blue-green alga (cyanobacterium) *Coccochloris penicostis*. *Plant Physiology*, **65**, 397–402.
- Monty, C. L. V., 1981. Spongiostromate vs. porostromate stromatolites and oncolites. In Monty, C. (ed.), *Phanerozoic Stromatolites*. Berlin: Springer-Verlag, pp. 1–4.
- Morse, J. W., Millero, F. J., Thurmond, V., Brown, E., and Ostlund, H. G., 1984. The carbonate chemistry of Grand Bahama Bank waters – after 18 years another look. *Journal of Geophysical Research – Oceans*, **89**, 3604–3614.

- Morse J. W., Gledhill D. K., and Millerog, F.J., 2003. CaCO_3 precipitation kinetics in waters from the Great Bahama Bank; implications for the relationship between bank hydrochemistry and whittings. *Geochimica et Cosmochimica Acta*, **67**, 2819–2826.
- Nicholson, H. A., and Etheridge, R. Jr., 1878. *A Monograph of the Silurian Fossils of the Girvan District in Ayrshire with Special Reference to those Contained in the "Gray Collection": Volume I, Fasciculus I (Rhizopoda, Actinozoa, Trilobita)*. Edinburgh, London: W. Blackwood and Sons, 1–135, pls 1–9.
- Pedley, H. M., 1990. Classification and environmental models of cool freshwater tufas. *Sedimentary Geology*, **68**, 143–154.
- Pentecost, A., 1981. The tufa deposits of the Malham district, north Yorkshire. *Field Studies* **5**, 365–387.
- Pentecost, A., 1987. Growth and calcification of the freshwater cyanobacterium *Rivularia haematites*. *Proceedings of the Royal Society of London, Series B*, **232**, 125–136.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer, p. 445.
- Pentecost, A., and Riding, R., 1986. Calcification in cyanobacteria. In Leadbeater, B. S. C., and Riding, R. *Biomining in Lower Plants and Animals*. Oxford: Clarendon Press. Systematics Association Special Volume 30, pp. 73–90.
- Petrov, P. Yu., and Semikhatov, M. A., 2001. Sequence organization and growth patterns of late Mesoproterozoic stromatolite reefs: an example from the Burovaya Formation, Turukhansk Uplift, Siberia. *Precambrian Research*, **111**, 257–281.
- Pia, J., 1927. Thallophtya. In Hirmer, M. (ed.), *Handbuch der Paläobotanik*. Munich: Oldenbourg, Vol. 1, pp. 31–136.
- Pollock, J. B., 1918. Blue-green algae as agents in the deposition of marl in Michigan lakes. *Report of the Michigan Academy of Science*, **20**, 247–260.
- Pratt, B. R., 1984. *Epiphyton and Renalcis* – diagenetic microfossils from calcification of coccoid blue-green algae. *Journal of Sedimentary Research*, **54**, 948–971.
- Proteau, P. J., Gerwick, W. H. et al., 1993. The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria. *Experientia*, **49**, 825–829.
- Raistrick, B., 1949. The stabilisation of the supersaturation of calcium carbonate solutions by anions possessing O-P-O-P-O chains. *Faraday Discussion Society*, **5**, 234–237.
- Raven, J. A., 2002. Evolution of cyanobacterial symbioses. In Rai, A. N., Bergman, B., and Rasmussen, U. (eds.), *Cyanobacteria in Symbiosis*. Dordrecht: Kluwer, pp. 329–346.
- Raven, J. A., Johnston, A. M., Kübler, J. E., Korb, R., McInroy, S. G., Handley, L. L., Scrimgeour, C. M., Walker, D. I., Beardall, J., Clayton, M. N., Vanderklift, M., Fredriksen, S., and Dunton, K. H., 2002. Seaweeds in cold seas: evolution and carbon acquisition. *Annals of Botany*, **90**, 525–536.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Riding, R., 1977. Calcified *Plectonema* (blue-green algae), a Recent example of *Girvanella* from Aldabra Atoll. *Palaeontology*, **20**, 33–46.
- Riding, R., 1982. Cyanophyte calcification and changes in ocean chemistry. *Nature*, **299**, 814–815.
- Riding, R., 1991. Calcified cyanobacteria. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 55–87.
- Riding, R., 1992. Temporal variation in calcification in marine cyanobacteria. *Journal of the Geological Society (London)*, **149**, 979–989.
- Riding, R., 1993. Phanerozoic patterns of marine CaCO_3 precipitation. *Naturwissenschaften* **80**, 513–516.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**(Supplement 1), 179–214.
- Riding, R., 2002. Structure and composition of organic reefs and carbonate mud mounds: concepts and categories. *Earth-Science Reviews*, **58**, 163–231.
- Riding, R., 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology*, **4**, 299–316.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Riding, R., and Voronova, L., 1982. Recent freshwater oscillatorian analogue of the Lower Palaeozoic calcareous alga *Angulocellularia*. *Lethaia*, **15**, 105–114.
- Riding, R., and Voronova, L., 1984. Assemblages of calcareous algae near the Precambrian/Cambrian boundary in Siberia and Mongolia. *Geological Magazine*, **121**, 205–210.
- Riding, R., and Voronova, L., 1985. Morphological groups and series in Cambrian calcareous algae. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology: Contemporary Research and Applications*. Berlin: Springer-Verlag, pp. 56–78.
- Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V., 1993. Carbon dioxide limitation of marine phytoplankton growth rates. *Nature*, **361**, 249–251.
- Robbins, L. L., and Blackwelder, P., 1992. Biochemical and ultrastructural evidence for the origin of whittings. A biologically induced calcium carbonate precipitation mechanism. *Geology*, **20**, 464–468.
- Robbins, L. L., Tao, Y., and Evans, C. A., 1997. Temporal and spatial distribution of whittings on Great Bahama Bank and a new lime mud budget. *Geology*, **25**, 947–950.
- Rost, B., Riebesell, U., Burkhardt, S., and Sültemeyer, D., 2003. Carbon acquisition of bloom-forming marine phytoplankton. *Limnology and Oceanography*, **48**, 55–67.
- Rothpletz, A., 1891. Fossile Kalkalgen aus den Familien der Codiaceen und der Corallineen. *Zeitschrift der Deutschen geologischen Gesellschaft*, **43**(2), 295–322, Berlin.
- Rowland, S. M., and Shapiro, R. S., 2002. Reef patterns and environmental influences in the Cambrian and earliest Ordovician. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 72, pp. 95–128.
- Seely, H. M., 1885. A new genus of Chazy sponge, *Streptochoetus*. *American Journal of Science*, Series 3, **30**, 355–357, 3 text-figs.
- Sheldon, N. D., 2006. Precambrian paleosols and atmospheric CO_2 levels. *Precambrian Research*, **147**, 148–155.
- Sherman, A. G., James, N. P., and Narbonne, G. M., 2000. Sedimentology of a late Mesoproterozoic muddy carbonate ramp, northern Baffin Island, Arctic Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. Special publication, Vol. 67, pp. 275–294.
- Shields, G. A., 2002. 'Molar-tooth microspar': a chemical explanation for its disappearance ~750 Ma. *Terra Nova*, **14**, 108–113.
- Shinn, E. A., Steinen, R. A., Lidz, B. H., and Swart, P. K., 1989. Whittings, a sedimentologic dilemma. *Journal of Sedimentary Research*, **59**, 147–161.
- Stal, L. J., 1995. Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist*, **131**, 1–32.
- Stockman, K. W., and Ginsburg, R. N. 1967. The production of lime mud by algae in south Florida. *Journal of Sedimentary Research*, **37**, 633–648.
- Sumner, D. Y., and Grotzinger, J. P., 2004. Implications for Neoproterozoic ocean chemistry from primary carbonate mineralogy of the Campbellrand-Malmani platform, South Africa. *Sedimentology*, **51**, 1–27.

- Thompson, J. B., 2000. Microbial whittings. In Riding, R., and Agramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer-Verlag, pp. 250–260.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N. W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. SEPM special publication number, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaios*, **15**, 87–111.
- Vologdin, A. G., 1932. *Arkheotsiaty Sibiri. Vypusk 2: Fauna kembriyskikh izvestnykh Altaya*. Moscow; Leningrad: State Sci.Tech. Geol. Explor. Publishing House p. 106 (in Russian with English resume).
- Vologdin, A. G., 1962. *Drevneyshie vodorosli SSSR*. Moscow: U.S.S.R. Academy of Sciences Publishing House, 656 pp. (in Russian).
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research* **100**, 371–433.
- Westrop, S. R., and Adrain, J. M. 2001. Sampling at the species level: impact of spatial biases on diversity gradients. *Geology*, **29**, 903–906.
- Whitton, B. A., and Potts, M. (eds.), 2000. *The Ecology of Cyanobacteria, their Diversity in Time and Space*. Dordrecht: Kluwer, 669 pp.
- Woo, J., Chough, S. K., and Han, Z. 2008. Chambers of epiphyton thalli in microbial buildups, zhangxia formation (Middle Cambrian), Shandong Province, China. *Palaios* **23**, 55–64.
- Yee, N., Benning, L. G., Phoenix, V. R., and Ferris, F. G. 2004. Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environmental Science and Technology*, **38**, 775–782.
- Zhuravlev, A. Yu., 2001. Biotic diversity and structure during the Neoproterozoic-Ordovician transition. In Zhuravlev, A. Yu., and Riding, R. (eds.), *The Ecology of the Cambrian Radiation*, New York: Columbia University Press, pp.173–199.

Cross-references

Alkalinity
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Carbonate Environments
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Organomineralization
 Soda Ocean Hypothesis
 Stromatolites
 Tufa, Freshwater

CALCITE PRECIPITATION, MICROBially INDUCED

Tanja Bosak

Massachusetts Institute of Technology, Cambridge, MA, USA

Definition

Microbially induced calcite precipitation describes the formation of calcium carbonate minerals from a solution due to the presence of microbial cells, biosynthetic products, or metabolic activity.

Calcium carbonate precipitation

The most basic requirement for the precipitation of calcium carbonate (CaCO_3) minerals, calcite, and aragonite is that the product of the concentrations of calcium $[\text{Ca}^{2+}]$ and carbonate ions $[\text{CO}_3^{2-}]$ exceeds the solubility product of calcite (Equation 1) and aragonite, respectively.

$$[\text{Ca}^{2+}][\text{CO}_3^{2-}] > 10^{-8.35} \quad (1)$$

The solubility of carbonate minerals depends on the temperature and pressure, decreasing with increasing temperatures and increasing with the increasing pressure. When a solution is in equilibrium with carbon dioxide, $[\text{CO}_3^{2-}]$ is determined by pH. In solutions that are undersaturated or not highly saturated, such as modern seawater, the biological activity can strongly control the precipitation of CaCO_3 . Biological processes exert considerably less control on the precipitation of CaCO_3 in environments characterized by high temperature, high pH, and high evaporation and degassing rates such as soda lakes, hot springs, caves, and freshwater tufas.

Microbial processes that promote the precipitation of CaCO_3

Culture-dependent and culture-independent studies have shown that microbes (Bacteria and Archaea) can induce extracellular precipitation of calcium carbonate minerals by:

- Increasing the local pH and the concentration of carbonate ion
- Promoting the nucleation of calcium carbonate minerals and removing the kinetic inhibitors of CaCO_3 precipitation.

Increase in local pH and the concentration of carbonate ion

Because the concentration of carbonate ion increases with the increasing pH, the precipitation of calcium carbonate minerals will also increase with the increasing pH. Many microbial metabolic processes can increase the pH and/or the concentration of carbonate ions: sulfate reduction in marine sediments (Dupraz and Visscher, 2005; Van Lith et al., 2003; Visscher et al., 2000), oxygenic

T

TERRESTRIAL DEEP BIOSPHERE

Christine Heim
University of Göttingen, Göttingen, Germany

Synonyms

Continental deep biosphere; Subterranean biosphere

Definition

The terrestrial deep biosphere comprises ecosystems in the marine (e.g., shelf-sediments) and continental subsurface, beneath the rhizosphere or bioturbated zone. The habitable depth is limited by space (rock porosity), the availability of water and in particular the maximum viable temperature depending on the local geothermal gradient (Gold, 1992; Pedersen, 1993, 2000, 2001, 2002; Stevens, 2002).

Introduction

Microbial life exists in, and seems to be adapted to all kinds of ecological niches on earth. From the early 1930s, increasing numbers of publications report on the occurrence of microorganisms in deep terrestrial settings, such as coal mines (Lipman, 1931, 1937; Lieske, 1932) and deep formation waters from oil drilling, as deep as 2,000 m (Ginsburg-Karagitscheva, 1933; Issatchenko, 1940). During the following decades the scientific interest in subsurface microbiology focused on the effects of microorganisms on oil and gas exploration, such as corrosion of drilling devices, oil transformation, but also the role of microorganisms in the biological origin of natural gas resources (Bailey et al., 1973; Belyaev et al., 1983; Colwell et al., 2004; Lollar et al., 2006; Zobell, 1945). Deep subsurface microorganisms have a potential impact not only on oil and gas exploration, but also on

groundwater (Chapelle, 2000), long-term storage of nuclear waste (Boivin-Jahns et al., 1996; Christofi and Philip, 1997; Hersman, 1997; Pedersen, 1993, 1997; Pedersen et al., 2008), and finally, implications for the origin of life and the search for extraterrestrial life (Cockell, 2004; Faison, 2003; Stevens, 1997a).

Scientific continental drilling (ICDP), and also site investigations for the storage of nuclear waste for a better understanding of geological, hydrological, and biogeochemical processes in the deep continental crust, supported the development of contamination minimizing, drilling, sampling, and analytical techniques (Abyzov et al., 2001; Griffin et al., 1997; Pedersen, 1993 (and references therein); Pedersen et al., 2008; Russel, 1997). Using enhanced drilling and sampling techniques, a broad diversity of microorganisms living under extreme conditions (extremophiles), i.e., under elevated temperatures and high pressure conditions, was observed (Gold, 1992; Boone et al., 1995; Bonch-Osmolovskaya et al., 2003; Kotelnikova et al., 1998; Lin et al., 2006; Szewzyk et al., 1994; Sahl et al., 2008). Whether the organisms observed in the deep terrestrial subsurface are indigenous or derive from surface contamination has been a major issue since the first decades of exploration and is still (even though more advanced sampling techniques are now available), an important question to be considered (Farrell and Turner, 1932; Griffin et al., 1997; Haldemann, 1997; Lieske, 1932; Lipman, 1937; Pedersen, 1993; Phelps et al., 1989; Russel, 1997; Stevens, 2002). Furthermore comprehensive sampling (contamination controlled sampling) and careful data interpretation is matter of major importance (Hallbeck and Pedersen 2008; Lehman et al., 1995; Zhang et al., 2006).

The deep terrestrial biosphere

Numerous reports about diverse, well adapted, active microorganisms, being most likely indigenous, and

occurring down to a depth of several km, rise the questions of (a) how the organisms got down there, (b) how are they able to maintain, i.e., life and growth, (c) what are the limiting factors for life in the deep biosphere.

There are different theories about microbial migration or transport into the deep subsurface can happen. One possibility is the transport of microorganism within groundwater aquifers as underground rivers (Karst systems) or percolating through the sediment or fracture systems. Groundwater may reach depths of several hundreds of meters and may have intervals from hundreds to thousands of years (Moser et al., 1988; Seiler and Lindner, 1995; Simpkins and Bradbury, 1992). Further, it is assumed that living microbial matter is buried with the sediments, thus migrating vertically as a part of the rock cycle. As a consequence, some microorganisms must be capable to survive for millions of years. Reports on the detection and isolation of bacteria from the Taylorsville Triassic Basin from 2.7 km depth (Boone et al., 1995), and from the Piceance Basin of western Colorado from 858 m depth (Colwell et al., 1997) support this scenario.

General requirements for life are the availability of water, space, nutrients (O, C, H, N, Ca, P, and S), and trace elements (Fe, Ni, Mn, W, Mo, V, Zn, Cu, Co, Se, and Cr). Being independent from photosynthesis, subterranean organisms have to adapt their metabolism to local resources or form dormant stages, in order to survive at great depth (Amy, 1997; Dobretsov et al., 2006; Kieft and Phelps, 1997; Pedersen, 1993; Stevens, 2002). Since the discovery of the first subsurface microorganisms, and the subsequent identification of a broad variety of aerobic, anaerobic, autotrophic, and heterotrophic microbes, the potential carbon and energy sources available, and depth, were intensively debated and approved. One major theory is the deep hydrogen-driven biosphere hypothesis (Pedersen, 1997), where acetogenic bacteria, acetoclastic methanogens, and autotrophic methanogens consume abiotic, deep crustal H_2 as an energy source and CO_2 as carbon source. These organisms could be considered as the primary producers in the deep terrestrial biosphere. Experimental studies, calculating the production rates of radiolytic H_2 , support the potential microbial consumption of H_2 (Lin et al., 2005). Beside H_2 as electron donor, chemotrophic organisms can use, for example, methane (CH_4), elemental sulfur (S^0), sulfite (SO_3^{2-}), thiosulfate ($S_2O_3^{2-}$) with water H_2O , nitrate (NO_3^-), sulfate (SO_4^{2-}) or iron (Fe (III)), thereby forming nitrogen gas, hydrogen sulfide (H_2S) and ferrous iron, respectively (Amend and Teske, 2005 and references therein; Kieft and Phelps, 1997; Stevens, 2002).

Numerous microorganisms observed in the deep subsurface are not only metabolically well adapted, they also manage to survive and reproduce under extreme conditions, such as hot or freezing temperatures, high pressure, alkaline or acidic conditions, and high salinity. Examples of these extremophiles and references are listed below in the section of the respective deep terrestrial environment. Moreover, some microorganisms are able to get into

a dormancy stage by forming spores, cysts, or other types of resting cells and survive starvation, desiccation periods, exposure to extreme temperatures, and elevated background radiation (Amy, 1997; Burke and Wiley, 1937; Johnson et al., 2007; Ponder et al., 2005; Suzina et al., 2004).

Regarding these adaptation mechanisms and considering the ongoing discovery of unknown microorganisms, new metabolic pathways and adaptation mechanisms, it is difficult to define the limits of life in the terrestrial deep biosphere. These actual limits may be due to a combination of several factors: with increasing depth, the growing load of the overlying rocks or ice masses progressively decreases the pore space available, temperature rises due to the geothermal gradients and the water activity changes with increasing temperatures and pressures (Gold, 1992; Pedersen, 1993, 2000; Stevens, 2002).

Terrestrial deep biosphere environments

Pedersen (2000) divided the terrestrial deep biosphere into continental sedimentary rocks, ancient salt deposits, aquifers in igneous rocks, and caves, whereas Stevens (2002) subdivided the deep biosphere of the continental crust into sedimentary environments, permafrost, ice sheets and glaciers, and bedrock environments. Salt deposits and permafrost soils are sediments by definition, and therefore, in this chapter, they are discussed in the section sedimentary environments. Caves exhibit a great variety of different ecosystems that might serve as models for the formation of special adaptations or symbioses and may therefore represent a link between surface and subsurface environments (Dattagupta et al., 2009; Pedersen, 2000).

Sedimentary environments

Since the beginning of the last century, microorganisms in deep sedimentary environments have been frequently found and described from exploration sites, for example in coal (Burke and Wiley, 1937; Lieske, 1932; Lipman, 1931, 1937). Later when advanced drilling techniques and modern sequencing methods became available, a more precise characterization of these microorganisms is possible. A strict anaerobe, *Bacillus* species (*Bacillus infernus*) was isolated from drill core samples obtained from 2,700 m depth, from the Triassic Taylorsville basin (Boone et al., 1995). Several sediment core samples from 856 to 2,096 m from the Piceance Basin of Colorado contained Fe(III)-reducing and -fermenting bacteria (Colwell et al., 1997). Krumholz (2000) reported microbial communities within Cretaceous rocks in New Mexico, where living sulfate-reducing bacteria (SRB) and acetogens were found at the interface of porous sandstone and dense organic-rich shale. Later studies of the same site revealed high numbers of Fe(III) and S-reducing bacteria (Kovacik et al., 2006). Sass and Cypionka (2004) isolated moderately thermophilic SRB in porous sandstones from 600 to 1,060 m depth. In coal seam groundwater within 843 to 907 m depth microbial communities of

methanogenic archaea, denitrifying, acetogenic, and SRB where detected (Shimizu et al., 2007). Thermophilic archaea and bacteria, including SRB, are common in oil reservoirs and geothermal fluids (Bonch-Osmolovskaya et al., 2003; Kimura et al., 2007; Zavarzina et al., 2007; Zobell, 1945). Culturing experiments and biomarker studies of 170 million years old claystone indicate the presence of active SRB within the rock (Mauclaire et al., 2007).

A special form of sedimentary environment is salt deposits: massive salt deposits have formed mainly due to marine transgression and regression cycles within epicontinental seas. Halophilic bacteria were described by Vreeland et al. (1998) from salt deposits in New Mexico and Gruber et al. (2004) isolated halophilic archaea in alpine salt deposits. Whether the halophilic microorganisms are indigenous and survived for millions of years in the salt, is still unclear (McGenity et al., 2000). However, the isolation of viable *Halobacterium salinarum* from brine inclusion within 9,600-years-old halite crystals (Mormile et al., 2003) indicates long-term survival of the microorganisms enclosed in salt deposits.

Permafrost soils are composed of silt, loam, peat, organic material from plants, top soil, and ice. Although temperatures in permafrost regions range between -10 and -30°C viable microorganisms have been detected in frozen sediment samples from Siberia, Alaska, Canada, and Antarctica (Vorobyova et al., 1997). Methanogenic archaea are presumably responsible for the production of high amounts of methane in permafrost soils (Rivkina et al., 2000, 2004). Gilichinsky et al. (1992) observed not only prokaryotes in Pliocene and Pleistocene sediments, even eukaryotic organisms were found in Holocene sediments.

Bedrock environments

Igneous and metamorphic rocks contain considerably less organic matter and pore space compared to sediments. Sampling of microorganisms is a problematic issue, as drilled rock material is often contaminated by drilling fluids (Pedersen, 2000; Hallbeck and Pedersen, 2008). However, investigations of a deep biosphere in igneous rocks were performed using the fracture and well water, drilling fluids, and groundwater aquifers. In deep granitic aquifers of the Fennoscandian shield, anaerobic thermophilic fermenting bacteria and SRB (Szewzyk et al., 1994), methanogens, homoacetogens (Kotelnikova and Pedersen, 1998; Pedersen, 2000; Kotelnikova, 2002), yeasts (Ekendahl et al., 2003), viruses (Kyle et al., 2008), and bacteriophages (Eydal et al., 2009) were observed. The detection of fungi in deep biosphere environments was also reported by Reitner et al. (2005), describing hyphae from unknown fungal mycelia in the Triberg Granite (Germany). In deep granitic fracture water and rock cores from the Henderson mine (Colorado), iron-oxidizing bacteria and the "Henderson candidate division" were detected (Sahl et al., 2008). In deep anaerobic and alkaline aquifers within the Columbia River Basalt Group,

SRB and metal-reducing bacteria were described by Fry et al. (1997). Drilling fluids from boreholes at 2,290–3,350 m depth, from the Chinese Continental Drilling Program, exhibited a huge diversity of extreme thermophilic, anaerobic chemoorganotrophs, anaerobic Fe(III) reducers, halotolerant, alkaliphilic microorganisms (Zhang et al., 2006). Lin et al. (2006) report about thermophilic SRB in deep alkaline saline groundwaters in Archaean metabasalt. A remarkable finding was a star shaped bacterial morphotype at 1,700 m depth in a South African platinum mine, observed by Wanger et al. (2008).

Ice sheets and glaciers

Microbiological investigations of ice cores obtained above the subglacial lake Vostok, Antarctica, exhibited various microorganisms from moraine material, from shallow regions of the glacier, but also presumably from the lake water (Abyzov et al., 2001). Poglazova et al. (2001) described Cyanobacteria and microalgae occurring in accreted ice of the subglacial lake Vostok. Highly diverse microbial communities in 120,000-years-old deep glacier ice cores from Greenland were described and isolated by Miteva and Brenchley (2005), Miteva et al. (2004).

General remarks

Fossil deep biosphere, evolutionary aspects, and implications for extraterrestrial life

As research on the deep biosphere continues, novel organisms, metabolic pathways, and adaption mechanisms will certainly be discovered. A look at the different subsurface environments shows that a generalization of the microbial diversity and adaption mechanisms is rather impossible as they all host their own specialized ecosystems.

Learning more about life in deep subterranean environments, may help to understand how life might have evolved and survived during meteoric impacts, increased rates of volcanic activity, or a potential global glaciation (Snowball Earth). In fact, geothermal waters, H_2 and CH_4 degassing volcanic or plutonic rocks may have served as local nutrient and energy sources for chemotrophic ecosystems (Dobretsov et al., 2006). Models about the habitability of the Hadean earth indicate that even the late heavy meteoric bombardment, around 3.9 Ga would not have been able to sterilize the primeval biosphere of the earth crust, considering the existence of a near and subsurface biosphere (Abramov and Mojzsis, 2009). Traces of a fossil subterranean biosphere were observed in metasedimentary rocks from Timmins (Canada) from the late Archaean (Ventura et al., 2007) and Bons et al. (2009) report about fossilized microbes within 585 Ma old calcite veins from Oppaminda Creek (Australia).

Increased interest for research in deep terrestrial, means also extreme environments, is its implication for extraterrestrial life. A better understanding of the earth's deep biosphere allows the development of models to understand

general biogeochemical processes not only for our earth but maybe for biogeochemical processes on other planets. For example, permafrost soils on Mars may comprise recent or ancient life (Gilichinsky et al., 1992; Rivkina et al., 2000, 2004) or Jupiter's satellites may have ice-covered lakes similar to lake Vostok (Abyzov et al., 2001) serving as a potential deep biosphere environments.

Bibliography

- Abramov, O., and Mojzis, S., 2009. Microbial habitability of the Hadean earth during late heavy bombardment. *Nature*, **459**, 419–422.
- Abyzov, S. S., Mitskevich, I. N., Poglazova, M. N., Barkov, N. I., Lipenkov, V. Ya., Bobin, N. E., Koudryashov, B. B., Pashkevich, V. M., and Ivanov, M. V., 2001. Microflora in the basal strata at an antarctic ice core above the Vostok lake. *Advances in Space Research*, **28**, 701–706.
- Amend, J. P., and Teske, A., 2005. Expanding frontiers in deep subsurface microbiology. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 131–155.
- Amy, P. S., 1997. Microbial dormancy and survival in the subsurface. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 185–203.
- Bailey, N. J. L., Jobson, A. M., and Rogers, M. A., 1973. Bacterial degradation of crude oil: Comparison of field and experimental data. *Chemical Geology*, **11**, 203–221.
- Belyaev, S. S., Wolkin, R., Kenealy, M., DeNiro, M. J., Epstein, S., and Zeikus, J. G., 1983. Methanogenic bacteria from the Bondyushskoe oil field: General characterization and analysis of stable-carbon isotopic fractionation. *Applied environmental microbiology*, **45**, 691–697.
- Boivin-Jahns, V., Ruimy, R., Bianchi, A., Daumas, S., and Christen, R., 1996. Bacterial diversity in a deep-subsurface clay environment. *Applied and Environmental Microbiology*, **62**, 3405–3412.
- Bonch-Osmolovskaya, E. A., Miroshnichenko, M. L., Lebedinsky, A. V., Chernyh, N. A., Nazina, T. N., Ivoilov, V. S., and Belyaev, S. S., 2003. Radioisotopic, culture-based, and oligonucleotide microchip analyses of thermophilic microbial communities in a continental high-temperature petroleum reservoir. *Applied and Environmental Microbiology*, **69**, 6143–6151.
- Bons, P. D., Montenari, M., Bakker, R. J., and Elburg, M., 2009. Potential evidence of fossilised Neoproterozoic deep life: SEM observations on calcite veins from Oppaminda Creek, Arkoola, South Australia. *International Journal of Earth Sciences*, **98**, 327–343.
- Boone, D. R., Liu, Y., Zhao, Z.-J., Balkwill, D. L., Drake, G. R., Stevens, T. O., and Aldrich, H. C., 1995. *Bacillus infernus* sp. nov., an Fe(III)- and Mn(IV)-reducing anaerobe from the deep terrestrial subsurface. *International Journal of Systematic Bacteriology*, **45**, 441–448.
- Burke, V., and Wiley, A. J., 1937. Bacteria in coal. *Journal of Bacteriology*, **34**, 475–481.
- Chapelle, F. H., 2000. *Ground-Water Microbiology and Geochemistry*. New York: Wiley, 496 p.
- Christofi, N., and Philip, J. C., 1997. European microbiology related to the subsurface disposal of nuclear waste. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 267–297.
- Cockell, C. S., 2004. Impact-shocked rocks – insights into archaean and extraterrestrial habitats (and sites for prebiotic chemistry?). *Advances in Space Research*, **33**, 1231–1235.
- Colwell, F. S., Onstott, T. C., Delwiche, M. E., Chandler, D., Fredrickson, J. K., Yao, Q.-J., McKinley, J. P., Boone, D. R., Griffiths, R., Phelps, T. J., Ringelberg, D., White, D. C., LaFreniere, L., Balkwill, D., Lehman, R. M., Konisky, J., and Long, P. E., 1997. Microorganisms from deep high temperature sand stones: constraints on microbial colonization. *FEMS Microbiology Reviews*, **20**, 425–435.
- Colwell, F., Matsumoto, R., and Reed, D., 2004. A review of the gas hydrates, geology, and biology of the Nankai Trough. *Chemical Geology*, **205**, 391–404.
- Dattagupta, S., Schaperdorth, I., Monanary, A., Mariani, S., Kita, N., Valley, J. W., and Macalady, J. L., 2009. A novel symbiosis between chemoautotrophic bacteria and a freshwater cave amphipod. *The ISME Journal*, **3**, 935–943.
- Dobretsov, N. L., Kolchanov, N. A., and Suslov, V. V., 2006. On the early stages of the evolution of the geosphere and biosphere. *Paleontological Journal*, **40**, 407–424.
- Ekendahl, S., O'Neill, A. H., Thomson, E., and Pedersen, K., 2003. Characterisation of yeasts isolated from deep igneous rock aquifers of the Fennoscandian shield. *Microbial Ecology*, **46**, 416–428.
- Eydal, H. S. C., Jägevall, S., Hermansson, M., and Pedersen, K., 2009. Bacteriophage lytic to *Desulfovibrio aesopensis* isolated from deep groundwater. *The ISME Journal*, **3**, 1139–1147.
- Faison, B. D., 2003. Microbial contributions to the search for extraterrestrial life. *Advances in Applied Microbiology*, **52**, 397–432.
- Farrell, M. A., and Turner, H. G., 1932. Bacteria in anthracite coal. *Journal of Bacteriology*, **23**, 155–162.
- Fry, N. K., Fredrickson, J. K., Fishbain, S., Wagner, M., and Stahl, D., 1997. Population structure of microbial communities associated with two deep, anaerobic, alkaline aquifers. *Applied and Environmental Microbiology*, **63**, 1498–1504.
- Gilichinsky, D. A., Vorobyova, E. A., Erokhina, L. G., Fyodorov-Davydov, D. G., and Chaikovskaya, N. R., 1992. Long-term preservation of microbial ecosystems in permafrost. *Advances in Space Research*, **12**, 4255–4263.
- Ginsburg-Karagitscheva, T. L., 1933. Microflora of oil waters and oil-bearing formations and biochemical processes caused by it. *Bulletin of the American Association of Petroleum Geologists*, **17**, 52–65.
- Gold, T., 1992. The deep hot biosphere. *Proceedings of the National Academy of Sciences USA*, **89**, 6045–6049.
- Griffin, W. T., Phelps, T. J., Colwell, F. S., and Fredrickson, J. K., 1997. Methods for obtaining deep subsurface microbiological samples. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 23–44.
- Gruber, C., Legat, A., Pfaffenhuemer, M., Radax, C., Weidler, G., Busse, H.-J., and Stan-Lotter, H., 2004. *Halobacterium noricense* sp. nov., an archaeal isolate from a bore core of an alpine Permian salt deposit, classification of *Halobacterium* sp. NRC-1 as a strain of *H. salinarum* and emended description of *H. salinarum*. *Extremophiles*, **8**, 431–439.
- Haldemann, D. L., 1997. The storage related phenomenon: Implications for handling and analysis of subsurface samples by drilling. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 61–74.
- Hallbeck, L., and Pedersen, K., 2008. Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. *Applied Geochemistry*, **23**, 1796–1819.
- Hersman, L. E., 1997. Subsurface Microbiology: effects on the transport of radioactive waste in the Vadose Zone. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 299–323.
- Issatchenko, V., 1940. On the microorganisms of the lower limits of the biosphere. *Journal of Bacteriology*, **40**, 379–381.
- Johnson, S. S., Hebsgaard, M. B., Christensen, T. R., Mastepanov, M., Nielsen, R., Munch, K., Brand, T., Gilbert, T. P., Zuber, M. T., Bunce, M., Rønn, R., Gilichinsky, D., Froese, D., and Willerslev, E., 2007. *PNAS*, **104**, 14401–14405.

- Kieft, T. L., and Phelps, T. J., 1997. Life in the slow lane: activities of microorganisms in the subsurface. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 137–163.
- Kimura, H., Ishibashi, J.-I., Masuda, H., Kato, K., and Hanada, S., 2007. Selective phylogenetic analysis targeting 16S rRNA genes of hyperthermophilic archaea in the deep-subsurface hot biosphere. *Applied and Environmental Microbiology*, **73**, 2110–2117.
- Kotelnikova, S., 2002. Microbial production and oxidation of methane in deep subsurface. *Earth-Science Reviews*, **58**, 367–395.
- Kotelnikova, S., and Pedersen, K., 1998. Distribution and activity of methanogenes and homacetogenes on deep granitic aquifers at Äspö Hard Rock Laboratory, Sweden. *FEMS Microbiology Ecology*, **26**, 121–134.
- Kotelnikova, S., Macario, A. J. L., and Pedersen, K., 1998. *Methanobacterium subterraneum* sp. nov., a new alkaliphilic, eurythermic and halotolerant methanogen isolated from deep granitic groundwater. *International Journal of Systematic Bacteriology*, **48**, 357–367.
- Kovacic, W. P., Takai, K., Mormile, M. R., McKinley, J. P., Brockman, F. J., Fredrickson, J. K., and Holben, W., 2006. Loecular analysis of deep subsurface Cretaceous rock indicates abundant Fe(III)- and S⁰-reducing bacteria in a sulfate-rich environment. *Environmental Microbiology*, **8**, 141–155.
- Krumholz, L. R., 2000. Microbial communities in the deep subsurface. *Hydrogeology Journal*, **8**, 4–10.
- Kyle, J. E., Eydal, H. S. C., Ferris, F. G., and Pedersen, K., 2008. Viruses in granitic groundwater from 69 to 450 m depth of the Äspö Hard Rock Laboratory, Sweden. *The ISME Journal*, **2**, 571–574.
- Lehman, R. M., Colwell, F. S., Ringelberg, D. B., and White, D. C., 1995. Combined microbial community-level analysis for quality assurance of terrestrial subsurface cores. *Journal of Microbiological Methods*, **22**, 263–281.
- Lieske, R., 1932. Über das Vorkommen von Bakterien in Kohlenflözen. *Biochemische Zeitschrift*, **250**, 339–351.
- Lin, L.-H., Wang, P.-L., Rumble, D., Lippmann-Pipke, J., Boice, E., Pratt, L. M., Lollar, B. S., Brodie, E. L., Hazen, T. C., Andersen, G. L., DeSantis, T. Z., Moser, D. P., Kershaw, D., and Onstott, T. C., 2006. Long-term sustainability of a high-energy, low-diversity crustal biome. *Science*, **314**, 479–482.
- Lin, L.-H., Slater, G. F., Lollar, B. S., Lacrampe-Couloume, G., and Onstott, T. C., 2005. The yield and isotopic composition of radiolytic H₂, a potential energy source for the deep subsurface biosphere. *Geochimica et Cosmochimica Acta*, **694**, 893–903.
- Lipman, C. B., 1931. Living microorganisms in ancient rocks. *Journal of Bacteriology*, **22**, 183–198.
- Lipman, C. B., 1937. Bacteria in coal. *Journal of Bacteriology*, **34**, 483–488.
- Lollar, B. S., Lacrampe-Couloume, G., Slater, G. F., Ward, J., Moser, D. P., Gihring, T. M., Lin, L.-H., and Onstott, T. C., 2006. Unravelling abiogenic and biogenic sources of methane in the earth's deep subsurface. *Chemical Geology*, **226**, 328–339.
- Mauclaire, L., McKenzie, J. A., Schwyn, B., and Bossart, P., 2007. Detection and cultivation of indigenous microorganisms in Mesozoic claystone core sample from the Opalinus Clay Formation (Mont Terri Rock Laboratory). *Physics and Chemistry of the Earth*, **32**, 232–240.
- McGenity, T. J., Gemmell, R. T., Grant, W. D., and Stan-Lotter, H., 2000. Origins of halophilic microorganisms in ancient salt deposits. *Environmental Microbiology*, **2**(3), 243–250.
- Miteva, V. I., and Brenchley, J. E., 2005. Detection and isolation of ultra small microorganisms from a 120,000 year-old Greenland glacier ice core. *Applied and Environmental Microbiology*, **71**, 7806–7818.
- Miteva, V. I., Sheridan, P. P., and Brenchley, J. E., 2004. Phylogenetic and physiological diversity of microorganism from a deep Greenland glacier ice core. *Applied and Environmental Microbiology*, **70**, 202–213.
- Mormile, M. R., Biesen, M. A., Gutierrez, M. C., Ventosa, A., Pavlovich, J. B., Onstott, T. C., and Fredrickson, J. K., 2003. Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions. *Environmental Microbiology*, **5**(11), 1094–1102.
- Moser, H., Wolf, M., Fritz, P., Fontes, J.-Ch., Florkowski, T., and Payne, B. R., 1988. Deuterium, oxygen-18, and tritium in Stripa groundwater. *Geochimica et Cosmochimica Acta*, **53**, 1757–1763.
- Pedersen, K., 1997. Microbial life in deep granitic rock. *FEMS Microbiology Reviews*, **20**, 399–414.
- Pedersen, K., 1993. The deep subterranean biosphere. *Earth-Science Reviews*, **34**, 243–260.
- Pedersen, K., 2000. Exploration of deep intraterrestrial microbial life: current perspectives. *FEMS Microbiology Letters*, **185**, 9–16.
- Pedersen, K., 2001. Diversity and activity of microorganisms in deep igneous rock aquifers of the Fennoscandian Shield. In Fredrickson, J. K., and Fletcher, M. (eds.), *Subsurface Microbiology and Biogeochemistry*. New York: Wiley, pp. 97–139.
- Pedersen, K., 2002. Microbial processes in the disposal of high level radioactive waste 500 m underground in Fennoscandian shield rocks. In Keith-Roach, M. J., and Livens, F. R. (eds.), *Interactions of Microorganisms with Radionuclides*. Amsterdam: Elsevier, pp. 279–311.
- Pedersen, K., Arlinger, J., Hallbeck, A., Hallbeck, L., Eriksson, S., and Johansson, J., 2008. Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4 to 450 m in Olkiluoto, Finland. *The ISME Journal*, **2**, 760–775.
- Phelps, T. J., Fliermans, C. B., Garland, T. R., Pfiffner, S. M., and White, D. C., 1989. Methods for recovery of deep terrestrial subsurface sediments for microbiological studies. *Journal of Microbiological Methods*, **9**, 267–279.
- Poglavzova, M. N., Mitskevich, I. N., Abyzov, S. S., and Ivanov, M. V., 2001. Microbiological characterization of the accreted ice of subglacial Lake Vostok, Antarctica. *Microbiology*, **70**, 723–730.
- Ponder, M. A., Gilour, S. J., Bergholz, P. W., Mindock, C. A., Hollingsworth, R., Thomashow, M. F., and Tiedje, J. M., 2005. Characterization of potential stress responses in ancient Siberian permafrost psychrotolerant bacteria. *FEMS Microbiology Ecology*, **53**, 103–115.
- Reitner, J., Schumann, G. A., and Pedersen, K., 2005. Fungi in subterranean environments. In Gadd, G. J. (ed.), *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, pp. 788–1002.
- Rivkina, E. M., Friedmann, E. I., McKay, C. P., and Gilichinsky, D. A., 2000. Metabolic activity of permafrost bacteria below the freezing point. *Applied and Environmental Microbiology*, **66**(8), 3230–3233.
- Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V., and Gilichinsky, D., 2004. Microbial life in permafrost. *Advances in Space Research*, **33**, 1215–1221.
- Russel, C. E., 1997. The collection of subsurface samples by mining. In Amy, P., and Haldeman, D. (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 45–60.
- Sahl, J. W., Schmidt, R., Swanner, E. D., Mandernack, K. W., Templeton, A. S., Kieft, T. L., Smith, R. L., Sandford, W. E., Callaghan, R. L., Mitton, J. B., and Spear, J. R., 2008. Subsurface microbial diversity in deep-granitic fracture water in Colorado. *Applied and Environmental Microbiology*, **74**, 143–152.

- Sass, H., and Cypionka, H., 2004. Isolation of sulfate-reducing bacteria from the terrestrial deep subsurface and description of *Desulfovibrio cavernae* sp. nov. *Systematic and Applied Microbiology*, **27**, 541–548.
- Seiler, K.-P., and Lindner, W., 1995. Near-surface and deep groundwaters. *Journal of Hydrology*, **165**, 33–44.
- Shimizu, S., Akiyama, M., Naganuma, T., Fujioka, M., Nako, M., and Ishijima, Y., 2007. Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. *Geobiology*, **5**, 423–433.
- Simpkins, W. W., and Bradbury, K. R., 1992. Groundwater flow, velocity, and age in a thick, fine-grained till unit in southeastern Wisconsin. *Journal of Hydrogeology*, **132**, 283–319.
- Stevens, T. O., 2002. The deep subsurface biosphere. In Staley, J. T., and Reysenbach, A.-L. (eds.), *Biodiversity of Microbial Life. Foundation of the Earth's Biosphere*. New York: Wiley, 552 p.
- Stevens, T. O., 1997a. Subsurface microbiology and the evolution of the biosphere. In Amy, P., and Haldeman, D., (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 203–221.
- Stevens, T. O., 1997b. Lithoautotrophy in the subsurface. *FEMS Microbiology Reviews*, **20**, 327–337.
- Suzina, N. E., Mulyukin, A. L., Kozlova, A. N., Shorokova, A. P., Dmitriev, V. V., Barinova, E. S., Mokhova, O. N., El'-Registan, G. I., and Duda, V. I., 2004. Ultrastructure of resting cells of some non-spore-forming bacteria. *Microbiology*, **73**, 516–529.
- Szewzyk, U., Szewzyk, R., and Stenström, T.-A., 1994. Thermophilic, anaerobic bacteria isolated from a deep borehole in granite, Sweden. *Proceedings of the National Academy of Sciences, USA*, **91**, 1810–1813.
- Ventura, G. T., Kenig, F., Reddy, C. M., Schieber, J., Frysinger, G. S., Nelson, R. K., Dinel, E., Gaines, R. B., and Schaeffer, P., 2007. Molecular evidence of late Archaean archaea and the presence of a subsurface hydrothermal biosphere. *PNAS*, **104**, 14260–14625.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkma, E., and Vishnivetskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Vreeland, R. H., Piselli, A. F., McDonnough, S., and Meyers, S. S., 1998. Distribution and diversity of halophilic bacteria in a subsurface salt formation. *Extremophiles*, **2**, 321–331.
- Wanger, G., Onstott, T. C., and Southam, G., 2008. Stars of the terrestrial deep subsurface: a novel 'star-shaped' bacterial morphotype from a South African platinum mine. *Geobiology*, **6**, 325–330.
- Zavarzina, D., Sokolova, T. G., Tourova, T. P., Chernyh, N. A., Kostrikina, N. A., and Bonch-Osmolovskaya, E. A., 2007. *Thermincola ferriacetica* sp. Nov., a new anaerobic, thermophilic facultatively chemolithoautotrophic bacterium capable of dissimilatory Fe(III) reduction. *Extremophiles*, **11**, 1–7.
- Zhang, G., Dong, H., Jiang, H., Xu, Z., and Eberl, D. D., 2006. Unique microbial community in drilling fluids from Chinese Continental Scientific Drilling. *Geomicrobiology Journal*, **23**, 499–514.
- Zobell, C. E., 1945. The role of bacteria in the formation and transformation of petroleum hydrocarbons. *Science*, **102**, 364–369.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Biofilms and Fossilization](#)
[Biosignatures in Rocks](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Halobacteria – Halophiles](#)
[Hydrothermal Environments, Terrestrial](#)
[Origin of Life](#)
[Permafrost Microbiology](#)
[Snowball Earth](#)
[Sulfate-Reducing Bacteria](#)

THIOESTER WORLD

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition

Christian de Duve (1991, 1994, 1995) proposed a Hadean “Thioester World” which should have preceded the succeeding RNA world. Thioester molecules were probably very common in the proposed “primordial organic soup”. Thioesters are formed when a ‘thiol’ joins a carboxylic acid (R'–COOH) under acidic and high temperature environmental conditions. The general form is written as an organic group (R) bonded with sulfur and hydrogen, hence R-SH. H₂O is released in this process, and forms finally a thioester: R–S–CO–R'. The “Thioester World” represents a hypothetical very early stage in the origin of life that could have provided the energetic and catalytic framework of a protometabolic set of primitive chemical reactions, like in the transaction of molecular groups, and in Redox-reactions. Thioesters were probably involved in the formation of energy-rich phosphates via acylphosphates and pyrophosphates as precursor compounds. The thioester bond is a high-energy bond and probably thioesters had the same function as ATP as an energy supplier. With the help of thioesters amino acids (AS) could also spontaneously polymerise and form first simple proteins. The “primordial organic soup” was enriched with AS may be provided by carbonaceous chondrites and comets which collided with the early earth and the early earth was also characterized by an intense volcanic activity. This volcanic activity generated H₂S and thiols are expected to arise readily in these H₂S-rich fluids. In laboratory experiments, imitating the Hadean conditions on Earth, it was possible to obtain cysteamine, b-alanine and pantonic acid known as ‘pantetheine’ which is a precursor of coenzyme A (Keefe et al., 1995). The thioester world is an important hypothesis in the understanding of early life processes which may explain essential reactions expected in the prebiotic world.

Bibliography

- De Duve, C., 1991. *Blueprint for a Cell: The Nature and Origin of Life*. Burlington, NC: Carolina Biological Supply Company (Neil Patterson Publisher).

Halobacteria – Halophiles
 Saline Lakes
 Salinity History of the Earth's Ocean
 Terrestrial Deep Biosphere

DEEP BIOSPHERE OF SEDIMENTS

See entries “*Terrestrial Deep Biosphere*,” “*Deep Biosphere of Salt Deposits*,” “*Deep Biosphere of the Oceanic Deep Sea*,” and “*Basalt (Glass, Endoliths)*.”

DEEP BIOSPHERE OF THE OCEANIC DEEP SEA

Kristina Rathsack, Nadia-Valérie Quéric, Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition and overview

Although used in many different ways, the term “biosphere” is principally defined either as zone in which life occurs, thereby overlapping the atmosphere, the hydrosphere, and the lithosphere, or as the entity of living organisms on Planet Earth. Both perceptions commonly focus on the Earth's near-surface environment, with all domains sharing solar energy used in the process of photosynthesis. The deep-sea realm takes a special position in this context, as deep-sea pelagic and the majority of benthic organisms live in the ocean's aphotic zone and inhabit the widespread abyssal plains, respectively. For a long time, their main food source has been considered to be based on particulate organic matter (POM) from the ocean's surface primary production and its sedimentation to abyssal depths (Gage and Tyler, 1991 and references therein, D'Hondt et al., 2002, 2004). With the discovery of “ocean vents” in the late 1970s (Corliss et al., 1979), this general perspective was broadened by the perception of the enormous potential of chemical energy through the reaction of seawater, rock material, and fluids rising from the Earth's interior. According to this concept of energy for life, the term ‘surface biosphere’ has been opposed to ‘sub-surface biosphere’ (also commonly found in literature as ‘deep biosphere’). Following this definition, the deep-seafloor with its highly diverse topography from heterotrophic to pure chemotrophic habitats has to be treated as a transition zone between both biospheres. Opposed to the “*deep hot biosphere*” (Gold, 1992), occurring by definition in oceanic as well as terrestrial subsurface environments, stands the “*deep cold biosphere*” as defined for permafrost sediments (Vorobyova et al., 1997) and ice cores from the depths of Lake Vostoc (Venter, 2001).

Life in the deep sea

Comprising approximately 65% of the Earth's surface, the deep-sea environment is characterized by hyperbaric, aphotic, and low-temperature conditions and highly

diverse seascapes. Canyons, seamounts, ridges, fractures, and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems, and deep-water coral reefs provide ample niches for a highly diverse pelagic and benthic deep-sea community (Tyler, 2003). It was only during the construction of the transoceanic telegraphic communication network that people realized the ocean's topographic alterations and astonishing depths. In 1861, the repair of an overgrown cable from 1,800 m water depth in the Mediterranean finally aroused the scientific community which by then adhered to Edward Forbes' theory on a completely ‘azotic’ zone below a water depth of 550 m. Though, 11 years had elapsed before the first global, scientific expedition onboard the “*Challenger*” (1872–1876) finally convinced people that a flourishing life in fact exists in the deep-sea realm. Numerous, further expeditions and a rushing development of technical facilities allowed deep-sea researches in 1960 to reach even the ocean's deepest surveyed point, the Challenger Deep at 10,911 meters below sea level (mbsl), located at the southern end of the Mariana Trench within the western Pacific Ocean (Piccard and Dietz, 1961). Since then, several studies on large-scale patterns and the zoogeographical origins of deep-sea organisms evidenced a high macrobenthic diversity (Gage and Tyler, 1991 and references therein). These organisms display a depth-dependent zonation as a result of basin age, deep currents (as barriers or dispersal), topographic boundaries, disturbance processes, and sedimentation in connection with depth-related environmental patterns (for review see Levin et al., 2001; Stuart et al., 2003). Macro- and meiofauna are losing importance with increasing water and sediment depth, whereas microorganisms like bacteria, archaea, and fungi account for up to 90% of the deep-sea benthic biomass (Pfannkuche, 1992). Sinking particles may carry large numbers of microorganisms from upper zones (10^8 – 10^{10} cells $m^{-2} d^{-1}$), inoculating deep marine surface sediments with an autotrophic and heterotrophic microbial community, as demonstrated by results from sediment traps (Turley and Mackie, 1995; Danovaro et al., 2000; Vanucci et al., 2001) or the deep-seafloor (Lochte and Turley, 1988).

Particulate organic matter (POM)

Due to the fact that most deep-sea benthic species are deposit feeders (Sanders and Hessler, 1969), the locally qualitatively and quantitatively, variable import of POM from the ocean's surface waters plays a crucial role for macro-, meio-, and microorganisms living in deep surface sediments (Goody and Turley, 1990). Mainly consisting of phytoplankton, marine snow, fecal pellets, (dead) zooplankton and molts, this material undergoes different steps of degradation during its passage from the photic, epipelagic (0–200 mbsl), through the mesopelagic (200–1,000 mbsl) to the actual deep-sea zones, in particular the bathypelagic (1,000–4,000 mbsl), the abyssal

(4,000–6,000 mbsl), and the hadal zone (6,000–11,000 mbsl). Depending on the residence time in the water column, the bioavailable part of POM finally reaching the deep-seafloor may be small (De La Rocha and Passow, 2007 and references therein). The refractory remainders such as animal skeletons are continuously accumulating at the seafloor and turn into deeply buried sediment over time, thereby representing the largest global reservoir organic carbon (Parkes et al., 2000 and references therein).

Deep-sea sediment types

Grain size (Gray, 1974) and sediment heterogeneity (Etter and Grassle, 1992) may additionally govern community composition and distribution of macro-, meio-, and micro-organisms in deep-sea benthic environments. In relation to their basic sources, deep-sea sediments may be biogenic (POM from pelagic primary production, benthic in-situ production), lithogenous (terrestrial weathering and transport by wind and rivers), hydrogenous (precipitation from seawater or pore water), volcanic, or cosmic (Seibold and Berger, 1996). According to grain size and settling velocity, lithogenous gravel and sandy fractions usually are deposited along the coast, while silt and clay are transported farther offshore through waves and currents, hence dominating the basically biogenous deep-sea sediments. Regional deviations may be linked to currents, downslope slides, submarine canyon dynamics, or to a release of ice-trapped rock material in polar waters (e.g., Ramseier et al., 2001). Covering almost one-half of the shelves and more than half of the deep ocean bottom, biogenous sediments mainly consist of calcite, aragonite, opal, and calcium phosphate, originating from foraminifera, diatoms, and radiolarians (Hay et al., 1988).

Deep biosphere of deep-sea sediments

Microbiological studies on sediment cores collected during several cruises of the Deep Sea Drilling Project (DSDP), the Ocean Drilling Program (ODP), and the Integrated Ocean Drilling Program (IODP) gave evidence for the presence of complex microbial communities in deeply buried marine sediments down to several hundred meters below seafloor (e.g., Whelan et al., 1986; Parkes et al., 1994; Roussel et al., 2008). Most striking, new insights into subsurface microbiology were gained during the ODP cruise Leg 201 to the equatorial Pacific Ocean and the continental margin of Peru, including sites recognized as most typical for oceanic subsurface environments (D'Hondt et al., 2002). A large fraction of the sub-seafloor bacteria has been proven to be alive and culturable, displaying turnover rates (based on sulfate reduction as dominating mineral process at these sites) comparable to surface sediment communities (D'Hondt et al., 2004; Schippers et al., 2005).

After a logarithmic decline within the uppermost 6 meters below seafloor (mbsf) (Parkes et al., 1994) to about 40 mbsf (Schippers et al., 2005), bacterial cells have proven to be more or less evenly distributed down to

several hundred mbsf. Local peaks within these deeply buried sediments seem to mirror sulfate (diffusing from crustal fluids) and methane (from in-situ production) concentration shifts (Engelen et al., 2008). However, published variations of absolute cell numbers (by a factor of up to 3) have to be treated with caution: varying estimations not only depend on the geochemical conditions at the respective sampling sites, but also on the enumeration techniques applied. Calculations based on early results revealed that sub-seafloor sediments comprise – at least – half of all prokaryotic cells and up to one-third of the living biomass on Earth pointing to a slow-growing strategy of high biomass in areas of low-energy flux (Whitman et al., 1998).

The prokaryotic community in deeply buried sediments can not exclusively be traced back to contaminations from biologically active surface layers or reactivation of spores and dormant cells (Parkes et al., 2000 and references therein).

Porewater chemistry data obtained from sites throughout the world's oceans (ODP, DSDP) showed that sulfate reduction, methanogenesis, and fermentation are the principal degradative metabolic processes in subsurface sediments. These results give evidence for significant lower metabolic rates for the subsurface compared to the surface biosphere and for methanogenesis becoming more important the more sulfate gets depleted with increasing sediment depth (D'Hondt et al., 2002 and references therein).

Windows to the subsurface biosphere

Hydrothermal vents

The discovery of the “ocean vents” near Galapagos Island (Corliss et al., 1979) was the first proof for the active movement of the gigantic oceanic plates of the Earth's crust creating series of cracks in the ocean floor, teeming with life. At these discharge areas, hydrothermal fluids with temperatures of more than 400°C (Haase et al., 2007) mix up with the cold ocean seawater, resulting in a precipitation of dissolved metals and in the formation of characteristic chimneys over time. Iron and sulfide precipitates turn the smokers black (“black smokers,” Figure 1), while barium, calcium, and silicon minerals result in “white smokers.” Thermal precipitation and/or direct magma degassing of H₂, H₂S, CH₄, CO, and CO₂ in combination with oxygen as electron acceptor provide enough energy to support a highly productive and physiologically diverse chemoautotrophic microbial community (Reysenbach and Shock, 2002).

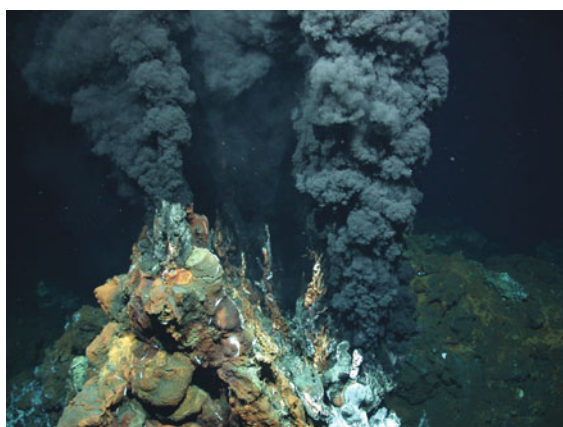
As the highly diverse and dense hot vent macrofauna (e.g., vestimentiferan tubeworms, bivalve mollusks, provannid gastropods, alvinellid polychaete, and bresiliid shrimps) cannot feed on the released chemicals themselves, they either feed on chemoautotrophic microbes or host them as symbionts. The predominant endosymbionts are mesophilic to moderately thermophilic chemoautotrophs (mostly Gammaproteobacteria), whereas most

episymbionts belong to the Epsilonproteobacteria, which can oxidize H_2 and sulfur compounds while reducing oxygen, nitrate, and sulfur compounds (for review, see Nakagawa and Takai, 2008). The vent habitat proved to harbor methanogens (*Methanococcus*), sulfate-reducers (*Archaeoglobus*), and facultative autotrophs and heterotrophs such as the thermophilic aerobic *Thermus* and *Bacillus* (Harmsen et al., 1997) or, for example, *Thermococcus*, *Pyrococcus*, *Desulfurococcus* (Prieur et al., 1995; Teske et al., 2000; Nercessian et al., 2003; Schrenk et al., 2003). Generally detected archaeal phylotypes were affiliated with hyperthermophilic *Crenarchaeota*, *Euryarchaeota* Group I, II, III (Takai and Horikoshi, 1999) and the “Deep-sea Hydrothermal Vent Euryarchaeotal Group” (Hoek et al., 2003).

Cold seeps and mud volcanoes

Just a few years after the discovery of the hydrothermal vent systems, cold seep ecosystems were reported from active and passive continental margins and subduction zones all over the world (Aharon, 1994 and references therein).

High-pressure, low oxygen and low-temperature conditions favour the formation of marine gas hydrates. In the subsurface realm, such gas reservoirs are stored in a crystalline form, whereas they get dissolved in pore waters and finally leave the sediment surface in gaseous form. High fluxes of methane, sulfide, and other reduced elements characterize these ecosystems such as cold seeps, hydrocarbon vents and mud volcanoes, often leaving mineral precipitation in their immediate surroundings. Coupled to sulfate reduction, rich bacterial and archaeal communities perform anaerobic oxidation of hydrocarbons, but predominately of methane (Boetius et al., 2000; Borowski et al., 2000; Treude et al., 2005).

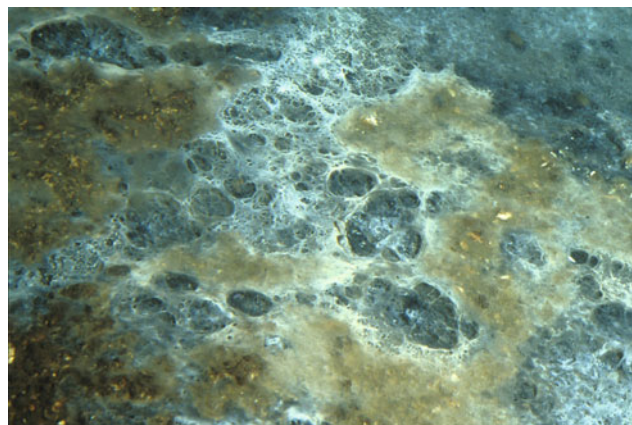


Deep Biosphere of the Oceanic Deep Sea, Figure 1 Black smoker “Candelabra,” Logachev hydrothermal field, Mid-Atlantic Ridge (3,000 m water depth). (By courtesy of MARUM, Center for Marine Environmental Sciences, Bremen, Germany.)

Conversion of methane is mainly mediated by two different groups of anaerobic methanotrophic archaea (ANME-I and ANME-II) (Nauhaus et al., 2005), forming syntrophic consortia with the sulfate-reducing bacteria (SRB) *Desulfosarcina* and *Desulfococcus* (Hinrichs et al., 1999; Boetius et al., 2000; Michaelis et al., 2002; Knittel et al., 2003).

The methane-emitting Haakon Mosby Mud Volcano (HMMV, Barents Sea) has shown to harbor three key communities in methane conversion such as aerobic, methanotrophic bacteria (Methylococcales), anaerobic methanotrophic archaea (ANME-2) thriving below siboglinid tubeworms, and a previously undescribed clade of archaea (ANME-3) associated with bacterial mats (Niemann et al., 2006). Similarly, some cold seeps on the deeper Black Sea shelf are characterized by intense methane bubble discharge, mainly related to microbial methanogenesis (Pape et al., 2008 and references therein). Diffuse gas seeps in more shallow, oxic Black Sea waters often exhibit a netlike coverage of microbial mats similar to *Beggiatoa*-mats observed at HMMV (Figure 2). *Beggiatoa* spp. are discussed as keystone members of seep communities owing to their ability to (directly and indirectly) influence the metabolic activity of δ -Proteobacteria, Planctomycetales, and ANME archaea by providing sulfate and ammonia as reactants (Mills et al., 2004).

The question remains, to which extent such seep systems influence the global methane cycle, as the quantification of bubble dissolution and/or the release of methane-rich pore fluids from the sediment into the hydrosphere is difficult to achieve (Vogt et al., 1999; Reeburgh, 2007). Niemann et al. (2006) estimated that methanotrophy at active marine mud volcanoes consumes less than 40% of the total methane flux, due to limitations



Deep Biosphere of the Oceanic Deep Sea, Figure 2 Shallow-water cold seep area, Ukrainian shelf, Black Sea. White, net-like microbial mats are constructed of sulfide oxidizing bacteria (“*Beggiatoa*”). (By courtesy of Karin Hissmann and Jürgen Schauer, Jago Team, Leibniz-Institut für Meereswissenschaften (IFM-GEOMAR) Kiel, Germany.)

of the relevant electron acceptors in the upward flowing, sulphate- and oxygen-free fluids.

Deep biosphere of the oceanic crust

The fact that microorganisms are present in the subsurface realm had been reported decades ago in terrestrial subsurface environments (Farrell and Turner, 1931; Lipman, 1931). Early drilling operations performed for commercial purposes such as mining, oil and hot water recovery, and the search for underground waste repositories reported on the existence of a large community of microorganisms obviously involved in geochemical processes in the deep biosphere (Gold, 1992; Pedersen, 1993 and references therein). Hence, it was only in the early 1990s that scientists started to focus on the investigation of prospering life beneath the Earth's crust, thanks to a chance encounter of a deep oceanic, volcanic eruption during a dive onboard the submersible *Alvin*, releasing white microbial bulk mats (Haymon et al., 1993). The upper layers of the oceanic crust are characterized by high basaltic porosity, hosting a vast hydrothermal reservoir (Johnson and Pruis, 2003) inhabited by a microbial community composed of species that are also found in deep-sea waters, sediments, and the deep oceanic crust (Thorseth et al., 2001; Huber et al., 2006). Among the most prominent anaerobic thermophiles indigenous for the oceanic crust, the *Ammonifex* group of bacteria (Nakagawa et al., 2006) or groups within *Crenarchaeota*, *Euryarchaeota*, and *Korarchaeota* (Ehrhardt et al., 2007). Since 3.5 billion years, basalt-associated glass textures and vesicular cavities within the basaltic matrix provide niches for microbial colonization (Furnes et al., 2004; Peckmann et al., 2008). For instance, the fossil record of the oceanic crust even gives evidence for a previous fungal life in deep ocean basaltic rocks (Schumann et al., 2004).

Much effort has been put into the investigation of the deep biosphere of the deep sea during the past 20 years. However, we still are neither aware of the final composition of the living subsurface community, nor of its interrelationship to, for example, crustal fluid-derived compounds, nor of its global impact.

Bibliography

- Aharon, P., 1994. Geology and biology of modern and ancient submarine hydrocarbon seeps and vents: an introduction. *Geomarine Letters*, **14**(2), 69–73.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jorgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**(6804), 623–626.
- Borowski, W. S., Cagatay, N., Ternois, Y., and Paull, C. K., 2000. Data report: carbon isotopic composition of dissolved CO₂, CO₂ gas, and methane, Blake-Bahama Ridge and northeast Bermuda Rise, ODP Leg 172. *Proceedings of the Ocean Drilling Program*, Scientific Results 172. http://www.odp.tamu.edu/publications/172_SR/chap_03/chap_03.htm.
- Corliss, J. B., Dymond, J., Gordon, L. I., Edmond, J. M., von Herzen, R. P., Ballard, R. D., Green, K., Williams, D., Bainbridge, A., Crane, K., and VanAndel, T. H., 1979. Submarine thermal springs on the Galápagos rift. *Science*, **203**, 1073–1083.
- Danovaro, R., Della Croce, N., Dell'Anno, A., Mauro F., Marrale, D., and Matorano, D., 2000. Seasonal changes and biochemical composition of the labile organic matter flux in the Cretan Sea. *Progress in Oceanography*, **46**, 259–278.
- De La Rocha, C., and Passow, U., 2007. Factors influencing the sinking of POC and the efficiency of the biological carbon pump. *Deep Sea Research II*, **54**, 639–658.
- D'Hondt, S., Rutherford, S., and Spivack, A. J., 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science*, **295**(5562), 2067–2070.
- D'Hondt, S., Jorgensen, B. B., Miller, D. J., Batzke, A., Blake, R., Cragg, B. A., Cypionka, H., Dickens, G. R., Ferdelman, T., Hinrichs, K.-U., Holm, N. G., Mitterer, R., Spivack, A., Wang, G., Bekins, B., Engelen, B., Ford, K., Gettemy, G., Rutherford, S. D., Sass, H., Skilbeck, C. G., Aiello, I. W., Guerin, G., House, C. H., Inagaki, F., Meister, P., Naehr, T., Niitsuma, S., Parkes, R. J., Schippers, A., Smith, D. C., Teske, A., Wiegel, J., Padilla, C. N., and Acosta, J. L. S., 2004. Distributions of microbial activities in deep seafloor sediments. *Science*, **306**(5705), 2216–2221.
- Engelen, B., Ziegmüller, K., Wolf, L., Köpke, B., Gittel, A., Cypionka, H., Treude, T., Nakagawa, S., Inagaki, F., Lever, M. A., and Steinsbu, B. O., 2008. Fluids from the oceanic crust support microbial activities within the deep biosphere. *Geomicrobiology Journal*, **25**(1), 56–66.
- Ehrhardt, C. J., Haymon, R. M., Lamontagne, M. G., and Holden, P. A., 2007. Evidence for hydrothermal Archaea within the basaltic flanks of the East Pacific Rise. *Environmental Microbiology*, **9**, 900–912.
- Etter, R. J., and Grassle, J. F., 1992. Patterns of species diversity in the deep sea as a function of sediment particle size diversity. *Nature*, **360**, 576–578.
- Farrell, M. A., and Turner, H. G., 1931. Bacteria in anthracite coal. *Journal of Bacteriology*, **23**, 155pp.
- Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- Gage, J. D., and Tyler, P. A., 1991. *Deep-sea Biology – A Natural History of Organisms at the Deep-Sea Floor*. Cambridge: Cambridge University Press.
- Gold, T., 1992. The deep, hot biosphere. *Proceedings of the National Academy of Sciences of the United States of America*, **89**(13), 6045–6049.
- Gooday, A. J., and Turley, C. M., 1990. Responses by benthic organisms to inputs of organic material to the ocean floor: a review. *Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences*, **331**(1616), 119–137.
- Gray, J. S., 1974. Animal–sediment relationships. *Oceanography and Marine Biology: An Annual Review*, **12**, 223–261.
- Haase, K. M., Petersen, S., Koschinsky, A., Seifert, R., Devey, C. W., Keir, R., Lackschewitz, K. S., Melchert, B., Perner, M., Schmale, O., Söling, J., Dubilier, N., Zielinski, F., Fretzdorff, S., Garbe-Schönberg, D., Westernströer, U., German, C. R., Shank, T. M., Yoerger, D., Giere, O., Kuever, J., Marbler, H., Mawick, J., Mertens, C., Stöber, U., and Walter, M., 2007. Young volcanism and related hydrothermal activity at 5°S on the slow-spreading southern Mid-Atlantic ridge. *Geochemistry Geophysics Geosystems*, **8**(11), 1–17.
- Harmsen, H. J. M., Prieur, D., and Jeanthoni, C., 1997. Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture of thermophilic subpopulations. *Applied and Environmental Microbiology*, **63**(7), 2876–2883.

- Hay, W. W., Sloan II, J. L., and Wold, C. N., 1988. Mass/age distribution and composition of sediments on the ocean floor and the global rate of sediment subduction. *Journal of Geophysical research*, **93**(B12), 14,933–14,940.
- Haymon, R., Fornari, D., Von Damm, K., Lilley, M., Perfit, M., Edmond, J., Shanks III, W. C., Lutz, R., Grebmeier, J., Carbotte, S., Wright, D., McLaughlin, E., Smith, M., Beedle, N., and Olson, E., 1993. Volcanic eruption of the mid-ocean ridge along the East Pacific Rise at 9°45'–52°N: direct submersible observation of seafloor phenomena associated with an eruption event in April, 1991. *Earth and Planetary Science Letters*, **119**, 85–101.
- Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. G., DeLong, E. F., 1999. Methane-consuming archaeobacteria in marine sediments. *Nature* **398**, 802–805.
- Hoek, J., Banta, A., Hubler, F., and Reysenbach, A. L., 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. *Geobiology*, **1**, 119–127.
- Huber, J. A., Johnson, H. P., Butterfield, D. A., and Baross, J. A., 2006. Microbial life in ridge flank crustal fluids. *Environmental Microbiology*, **8**, 88–99.
- Johnson, H. P., and Pruis, M. J., 2003. Fluxes of fluid and heat from the oceanic crustal reservoir. *Earth and Planetary Science Letters*, **216**, 565–574.
- Knittel, K., Boetius, A., Lemke, A., Eilers, H., Lochte, K., Pfannkuche, O., and Linke, K., 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria in sediments above gas hydrate (Cascadia Margin, Oregon). *Geomicrobiology Journal*, **20**, 269–294, Kulm et al., 1986.
- Levin, L. A., Etter, R. J., Rex, M. A., Gooday, A. J., Smith, C. R., Pineda, J., Stuart, C. T., Hessler, R. R., and Pawson, D. L., 2001. Environmental influences on regional deep-sea species diversity. *Annual Review of Ecology and Systematics*, **32**, 51–93.
- Lipman, C. B., 1931. Living micro-organisms in ancient rocks. *Journal of Bacteriology*, **22**, 183pp.
- Lochte, K., and Turley, C. M., 1988. Bacteria and cyanobacteria associated with phytodetritus in the deep sea. *Nature*, **333**, 67–68.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., Knittel, K., Gieseke, A., Peterknecht, K., Pape, T., Boetius, A., Amann, R., Jørgensen, B. B., Widdel, F., Peckmann, J., Pimenov, N. V., and Gulin, M. B., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**(5583), 1013–1015.
- Mills, H. J., Martinez, R. J., Story, S., and Sobecky, P. A., 2004. Identification of members of the metabolically active microbial populations associated with *Beggiatoa* species mat communities from Gulf of Mexico cold-seep sediments. *Applied and Environmental Microbiology*, **70**(9), 5447–5458.
- Nakagawa, S., and Takai, K., 2008. Deep-sea vent chemoautotrophs: diversity, biochemistry and ecological significance. *FEMS Microbiology Ecology*, **65**(1), 1–14.
- Nakagawa, S., Inagaki, F., Suzuki, Y., Steinsbu, B. O., Lever, M. A., Takai, K., Engelen, B., Sako, Y., Wheat, C. G., and Horikoshi, K., 2006. Microbial community in black rust exposed to hot ridge flank crustal fluids. *Applied and Environmental Microbiology*, **72**(10), 6789–6799.
- Nauhaus, K., Treude, T., Boetius, A., and Krüger, M., 2005. Environmental regulation of the anaerobic oxidation of methane: a comparison of ANME-I and ANME-II communities. *Environmental Microbiology*, **7**(1): 98–106.
- Nercessian, O., Reysenbach, A. L., Prieur, D., and Jeanthon, C., 2003. Archaeal diversity associated with *in situ* samplers deployed on hydrothermal vents on the East Pacific Rise (13°N). *Environmental Microbiology*, **5**(6), 492–502.
- Niemann, H., Lösekann, T., de Beer, D., Elvert, M., Nadalig, T., Knittel, K., Amann, R., Sauter, E. J., Schlüter, M., Klages, M., Foucher, J. P., and Boetius, A., 2006. Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature*, **443**, 854–858.
- Pape, T., Blumenberg, M., Seifert, R., Bohrmann, G., and Michaelis, W., 2008. Marine methane biogeochemistry of the Black Sea: a review. In: Dilek, Y., Furnes, H., and Muehlenbachs, K. (eds.), *Links Between Geological Processes, Microbial Activities & Evolution of Life*. Berlin: Springer, pp. 281–311.
- Parkes, R. J., Cragg, B. A., Bale, S. J., Getliff, J. M., Goodman, K., Rochelle, P. A., Fry, J. C., Weightman, A. J., and Harvey, S. M., 1994. Deep bacterial biosphere in Pacific Ocean sediments. *Nature*, **371**(6496), 410–413.
- Parkes, J. R., Cragg, B. A., and Wellsbury, P., 2000. Recent studies on bacterial populations and processes in subseafloor sediments: a review. *Hydrogeology Journal*, **8**, 11–28.
- Peckmann, J., Bach, W., Behrens, K., and Reitner, J., 2008. Putative cryptoendolithic life in Devonian pillow basalt, Rheinisches Schiefergebirge, Germany. *Geobiology*, **6**, 125–135.
- Pedersen, K., 1993. The deep subterranean biosphere. *Earth-Science Reviews*, **34**(4), 243–260.
- Pfannkuche, O., 1992. Organic carbon flux through the benthic community in the temperate abyssal northeast Atlantic. In: Rowe, G. T., and Pariente, V. (eds.), *Deep-Sea Food Chains and the Global Carbon Cycle*. The Netherlands: Kluwer, pp. 183–198.
- Piccard, J., and Dietz, R. S., 1961. *Seven Miles Down*. New York: Putnam.
- Prieur, D., Erauso, G., and Jeanthon, C., 1995. Hyperthermophilic life at deep-sea hydrothermal vents. *Planetary and Space Science*, **43**(1–2), 115–122.
- Ramseier, R. O., Garrity, C., and Martin, T., 2001. An overview of sea-ice conditions in the Greenland Sea and the relationship of oceanic sedimentation to the ice regime. In: Schäfer, P., Ritzrau, W., Schlüter, M., and Thiede, J. (eds.), *The Northern North Atlantic – A Changing Environment*. Berlin: Springer, pp. 19–38.
- Reeburgh, W. S., 2007. Oceanic methane biogeochemistry. *Chemical Reviews*, **107**(2), 486–513.
- Reysenbach, A. L., and Shock, E., 2002. merging genomes with geochemistry in hydrothermal ecosystems. *Science*, **296**(5570), 1077–1082.
- Roussel, E. G., Cambon-Bonavita, M. A., Querellou, J., Cragg, B. A., Webster, G., Prieur, D., and Parkes, R. J., 2008. Extending the sub-sea-floor biosphere. *Science*, **320**(5879), 1046.
- Sanders, H. L., and Hessler, R. R., 1969. Ecology of the deep-sea benthos. *Science*, **163**(3874), 1419–1424.
- Schippers, A., Neretin, L. N., Kallmeyer, J., Ferdelman, T. G., Cragg, B. A., Parkes, R. J., and Jørgensen, B. B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature*, **433**(7028), 861–864.
- Schrenk, M. O., Kelley, D. S., Delaney, J. R., and Baross, J. A., 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology*, **69**, 3580–3592.
- Schumann, G., Manz, W., Reitner, J., and Lustrino, M., 2004. Ancient fungal life in North Pacific Eocene Oceanic Crust. *Geomicrobiology Journal*, **21**, 241–246.
- Seibold, E., and Berger, W. H., 1996. *The Sea Floor – An Introduction to Marine Geology*, 3rd edn. Berlin/Heidelberg/New York: Springer.
- Stuart, C. T., Rex, M. A., and Etter, R. J., 2003. Large-scale spatial and temporal patterns of deep-sea benthic species diversity. In: Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 295–311.

- Takai, K., and Horikoshi, K., 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Brinkhoff, T., Muyzer, G., Moser, D. P., Rethmeier, J., and Jannasch, H. W., 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. *Applied and Environmental Microbiology*, **66**(8), 3125–3133.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daae, F. L., and Pedersen, R. B., Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**(10), 6375–6378.
- Turley, C. M., and Mackie, P. J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep-Sea Research I*, **42**, 1453–1474.
- Tyler, P. A., 2003. *Ecosystems of the Deep Oceans. Ecosystems of the World*, 28. Elsevier: Amsterdam.
- Vanucci, S., Dell'Anno, A., Pusceddu, A., Fabiano, M., Lampitt, R. S., and Danovaro, R., 2001. Microbial assemblages associated with sinking particles in the Porcupine Abyssal Plain (NE Atlantic Ocean). *Progress in Oceanography*, **50**, 105–121.
- Venter, A., 2001. Deep cold biosphere. *Trends in Microbiology*, **9**(7), 313–313.
- Vogt, P. R., Gardner, J., and Crane, K., 1999. The Norwegian-Barents-Svalbard (NBS) continental margin: Introducing a natural laboratory of mass wasting, hydrates, and ascent of sediment, pore water, and methane. *Geo-Marine Letters*, **19**, 2–21.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivatskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., Lee, C., 1986. Evidence for sulfate-reducing and methane-producing micro-organisms in sediments from Site 618, Site 619, and Site 622. *Initial Reports of the Deep Sea Drilling Project*, **96**, 767–775.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Basalt \(Glass, Endoliths\)](#)
[Beggiatoa](#)
[Biofilms](#)
[Carbon Cycle](#)
[Carbon \(Organic, Cycling\)](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Mud Mounds](#)

[Piezophilic Bacteria](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)

DEEP FLUIDS

See entries “[Terrestrial Deep Biosphere](#),” “[Deep Biosphere of Salt Deposits](#),” and “[Deep Biosphere of the Oceanic Deep Sea](#).”

DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed **biodegradation**. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#),” and “[Microbial degradation](#) (of organic matter)” for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO_3^-) to N_2 and, to a minor extent, other gaseous species such as N_2O . See entry “[Nitrogen](#)” for further reading.

DESERT VARNISH

Randall S. Perry
 Imperial College, London, UK

Synonyms

Rock Varnish, *Rock Glaze*, *Silica Glaze*, and *Schutzrinde* were introduced in 1891, from a translation of the French term *manteau protecteur*: “*Schutzrinde*” for all dark coatings, including “*Wustenlack*” a thin, polished coating that may be an eolian polished patina; “*Dunkle Rinden*” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “*Schweinfurth*,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish ([Figure 1](#)). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama

D

DEEP BIOSPHERE OF SALT DEPOSITS

Helga Stan-Lotter¹, Sergiu Fendrihan²

¹University of Salzburg, Salzburg, Austria

²Bioresource Center and Advanced Research Association, Bucharest, Romania

Definition

The *biosphere* is defined either as “the place on Earth’s surface where life dwells” (Eduard Suess’ original definition, 1875, in *The Origin of the Alps*), meaning the zone on Earth where life naturally occurs, or in a more narrow sense as just the organisms themselves. Recent advances in microbiology have demonstrated that microorganisms live deep beneath the Earth’s terrestrial surface and that the total mass of microbial life may, in biomass, exceed all animal and plant life on the surface (Whitman et al., 1998). Microorganisms live at such extremes that the “thickness” of the biosphere extends from 5,400 m above sea level to at least 9,000 m below sea level.

Salt deposits are accumulations of solid materials and are usually derived from evaporation of seawater. There are >70 elements dissolved in extant seawater, but only 6 ions make up >99% of all the dissolved salts (wt% in brackets): Na⁺ (30.51), Cl[−] (55.08), SO₄^{2−} (7.69), Mg²⁺ (3.67), Ca²⁺ (1.17), K⁺ (1.10) (Hay et al., 2006).

Introduction

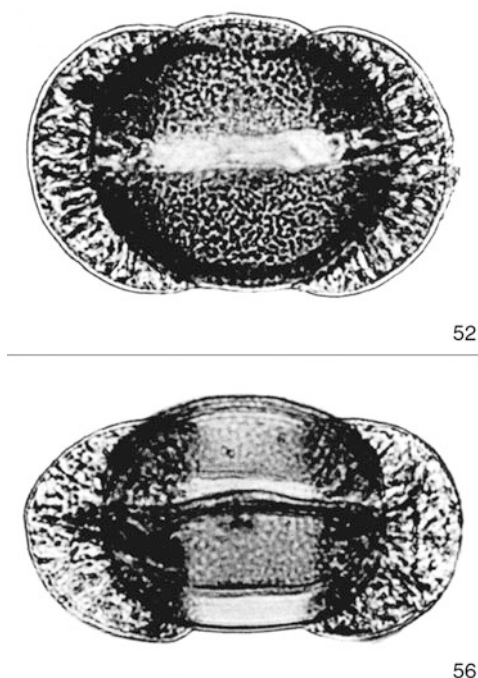
About 250 million years ago the continents on Earth were close together and formed Pangaea, a supercontinent, which persisted for about 100 million years and then fragmented. The land masses at that time were located predominantly in the southern hemisphere. The climate was arid and dry, and the average temperature is thought to have been several degrees higher than at present. This was one of the time periods in the history of the Earth, when

huge salt sediments formed. A total of about 1.3 million cubic kilometers of salt were estimated to have been deposited during the late Permian and early Triassic period alone (250–192 million years ago; Zharkov, 1981); newer research has discovered additional vast salt deposits, which were previously unknown – especially deposits below the Gulf of Mexico, and extensive Miocene salt (about 20 million years old) underlying the Mediterranean Sea, the Red Sea, and the Persian Gulf (Hay et al., 2006).

The thickness of the ancient salt sediments can reach 1,000–2,000 m. When Pangaea broke up, land masses were drifting in latitudinal and northern direction. Mountain ranges such as the Alps, the Carpathians, and the Himalayas were pushed up by the forces of plate tectonics. In the Alpine basin and in the region of the Zechstein Sea, which covered northern Europe, no more salt sedimentation took place after the Triassic period. Dating of the salt deposits by sulfur-isotope analysis (ratio of ³²S/³⁴S as measured by mass spectrometry), in connection with information from stratigraphy, indicated a Permo–Triassic age for the Alpine and Zechstein deposits (Holser and Kaplan, 1966). This age was independently confirmed by the identification of pollen grains in the sediments (Klaus, 1974). Figure 1 shows pollen grains from extinct coniferous trees, which were found in Alpine Permian salt and also in Zechstein salt (Klaus, 1963).

Microorganisms and signature sequences from salt deposits

As recently as 1981, Larsen (1981) described mined rock salts as free from bacteria, although isolations of halophilic microorganisms from ancient salt sediments had occasionally been reported since the early decades of the twentieth century (see Grant et al., 1998; McGenity et al., 2000). From Alpine Permian rock salt, which was collected from the salt mine in Bad Ischl, Austria, a haloarchaeon (see “*Halobacteria – Halophiles*”) was



52

56

Deep Biosphere of Salt Deposits, Figure 1 Photomicrograph of winged pollen grains of the genus *Lueckisporites*, found in Alpine Permian salt deposits and in Zechstein salt. *Upper panel: L. virkkiae*, dimensions $60 \times 40 \mu\text{m}$; *lower panel: L. microgranulatus*, dimensions $64 \times 35 \mu\text{m}$ (From Klaus, 1963 with permission.)

isolated, which was recognized as a novel species and named *Halococcus salifodinae* strain BIp (Denner et al., 1994). This was the first isolate from ancient rock salt, which was formally classified and deposited in international culture collections. Two independently isolated strains, Br3 (from solution-mined brine in Cheshire, England) and BG2/2 (from a bore core from the mine of Berchtesgaden, Germany) resembled *Hc. salifodinae* strain BIp in numerous properties, including the characteristic morphology of coccoid cells arranged in large clusters (see Figure 2, right panel, in “*Halobacteria – Halophiles*”); in addition, rock salt samples were obtained 8 years later from the same site and several halococci were recovered from these samples, which proved to be identical to strain BIp (Stan-Lotter et al., 1999). The data suggested that viable halophilic archaea, which belong to the same species, occur in geographically separated evaporites of similar geological age. Another halococcal isolate from the Bad Ischl salt formation, which differed from the previously described strains, was identified as a novel species and named *Halococcus dombrowskii* (Stan-Lotter et al., 2002). *Hc. salifodinae* and *Hc. dombrowskii* have so far not been found in any hypersaline surface waters, or any location other than salt mines. Recently, a non-coccoid novel haloarchaeon, *Halobacterium noricense* was obtained from a freshly



Deep Biosphere of Salt Deposits, Figure 2 Drilled bore cores, stored in a wooden box, from the Alpine salt mine in Altaussee, Austria obtained from about 500 m below surface. Pink portions represent halite (NaCl), containing traces of hematite; grayish portions contain mostly anhydrite (CaSO_4).

drilled bore core (Figure 2) at the salt mine in Altaussee, Austria (Gruber et al., 2004). Other isolates from ancient salt deposits include a single rod-shaped *Halobacterium* from 97 000 year old rock salt in the USA (Mormile et al., 2003), which was deemed to resemble the well-characterized *Halobacterium salinarum* NRC-1 (see “*Halobacteria – Halophiles*”). From the Permian Salado formation in New Mexico, a novel strain, *Halosimplex carlsbadense*, was isolated (Vreeland et al., 2002).

Although the microbial content of ancient rock salt is low – estimates range from 1 to 2 cells/kg of salt from a British mine (Norton et al., 1993) to 1.3×10^5 colony forming units (CFUs) per kg of Alpine rock salt (Stan-Lotter et al., 2000), and up to 10^4 CFUs per g of Permian salt of the Salado formation (Vreeland et al., 1998), equivalent to a range of 1 pg to 10 μg of biomass per kg of salt – the reports showed that viable haloarchaeal isolates were obtained reproducibly by several groups around the world. The data support the hypothesis that the halophilic isolates from subterranean salt deposits could be the remnants of populations which inhabited once ancient hypersaline

seas; in addition, they provide strong evidence against the notion that the recovered strains could be the result of laboratory contamination, since the isolates were obtained independently from different locations.

The amplification of diagnostic molecules, such as the 16S rRNA genes of bacteria and archaea, by the polymerase chain reaction is now the standard method for obtaining material for subsequent nucleotide sequencing. For this technique it is not necessary to cultivate the microorganisms; the genes can be amplified by using DNA prepared from the material of interest. Analysis of dissolved Alpine rock salt with molecular methods was performed by extracting DNA and sequencing of 16S rRNA genes. The results provided evidence for the occurrence of numerous haloarchaea, which have not yet been cultured (Radax et al., 2001). Similarities of the 16S rDNA gene sequences were less than 90–95% to known sequences in about 37% of approximately 170 analyzed clones (Radax et al., 2001; Stan-Lotter et al., 2004); the remaining clone sequences were 98–99% similar to isolates from rock salts of various ages (McGenity et al., 2000) and to known haloarchaeal genera. In a similar experimental approach, using halite samples ranging in age from 11 to 425 millions of years, Fish et al. (2002) found haloarchaeal sequences and, in the older samples, also evidence for bacterial 16S rRNA genes which were related to the genera *Aquabacterium*, *Leptothrix*, *Pseudomonas*, and others. These data suggested the presence of a probably very diverse microbial community in ancient rock salt.

Long-term survival of cells

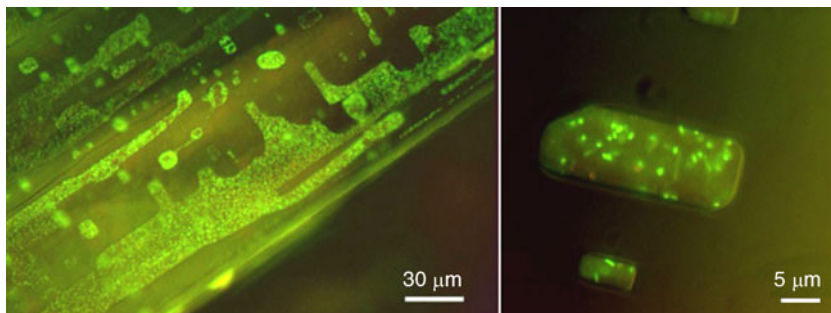
The reports cited above provide evidence that viable extremely halophilic archaea were isolated from salt sediments, which are thought to have been deposited about tens of thousands or even millions of years ago. The fluid inclusions in Permian rock salt were reported to contain cations and anions in a similar composition as today's seawater (Horita et al., 1991; Hay et al., 2006). While there is no direct proof that haloarchaea or other microorganisms have been entrapped in rock salt since its sedimentation, it would also be difficult to prove the opposite, namely that

masses of diverse microorganisms entered the evaporites in recent times (see also McGenity et al., 2000, for further discussion).

If a Permo–Triassic age is postulated for some of the haloarchaeal isolates and DNA signatures, then it becomes necessary to explain the biological mechanisms for such extreme longevity. Grant et al. (1998) discussed several possibilities, such as the formation of resting stages other than spores – since archaea are not known to form spores – or the maintenance of cellular functions with traces of carbon and energy sources within the salt sediments, which would imply an almost infinitely slow metabolism. At this time, there are no methods available to prove directly a great microbial age, whether it be a bacterium or a haloarchaeon. However, it can be shown, when simulating the formation of halite in the laboratory by drying salty solutions, which contained microorganisms that the cells accumulate within small fluid inclusions (Figure 3). The cells can be pre-stained with the fluorescent dyes of the LIVE/DEAD kit (Fendrihan et al., 2006), which provides information on the viability status of a cell (green fluorescence indicating viable cells); the procedure improves also the visualization of cells within crystals. The fluid inclusions were square or rectangular, as is common in the rectangular mineral halite, and the cells were rather densely packed within the fluid-filled spaces. From such experiments it appeared that the cells accumulated always in the fluid inclusions; there were no stained cells within the mineralic halite (Figure 3; Fendrihan et al., 2006). Suggestions have been made that fluid inclusions migrate within evaporites and thus, new nutrients might become accessible for the entrapped cells (McGenity et al., 2000).

Extraterrestrial halite and conclusion

Traces of halite were found in the SCN meteorites (named after the locations where they were found – Shergotty in India, Nakhla in Egypt, and Chassigny in France), which stem from Mars (Treiman et al., 2000). The Monahans meteorite, which fell in Texas in 1998, contained macroscopic crystals of halite, in addition to potassium chloride and water inclusions (Zolensky et al., 1999). Recently,



Deep Biosphere of Salt Deposits, Figure 3 Localization of pre-stained haloarchaea in fluid inclusions of artificial halite. Cells were stained with the LIVE/DEAD BacLight kit prior to embedding. Low (left panel) and high (right panel) magnification of *Halobacterium salinarum* NRC-1 cells. Cells were observed with a Zeiss Axioskope fluorescence microscope.

evidence for salt pools on the Martian surface was obtained (Osterloo et al., 2008). These results are intriguing – they suggest that the formation of halite with liquid inclusions could date back billions of years and occurred probably early in the formation of the solar system (Whitby et al., 2000). Could halophilic life have originated in outer space and perhaps traveled with meteorites, could haloarchaea have persisted in environments as they are found today on Mars?

Viable extremely halophilic archaea, representing novel strains, were isolated repeatedly from Permo–Triassic and other ancient salt sediments, suggesting their capacity for long-term survival under dry conditions. Together with the discovery of extraterrestrial halite it appears thus feasible to include into the search for life on other planets or moons specifically a search for halophilic microorganisms.

Bibliography

- Denner, E. B. M., McGenity, T. J., Busse, H. J., Wanner, G., Grant, W. D., and Stan-Lotter, H., 1994. *Halococcus salifodinae* sp. nov., an archaeal isolate from an Austrian salt mine. *International Journal of Systematic Bacteriology*, **44**, 774–780.
- Fendrihan, S., Legat, A., Gruber, C., Pfaffenhuemer, M., Weidler, G., Gerbl, F., and Stan-Lotter, H., 2006. Extremely halophilic archaea and the issue of long term microbial survival. *Reviews in Environmental Science and Bio/technology*, **5**, 1569–1605.
- Fish, S. A., Shepherd, T. J., McGenity, T. J., and Grant, W. D., 2002. Recovery of 16S ribosomal RNA gene fragments from ancient halite. *Nature*, **417**, 432–436. Erratum in: 2002. *Nature*, **420**, 202.
- Gooding, J. L., 1992. Soil mineralogy and chemistry on Mars: possible clues from salts and clays in SNC meteorites. *Icarus*, **99**, 28–41.
- Grant, W. D., Gemmell, R. T. and McGenity, T. J., 1998. Halobacteria: the evidence for longevity. *Extremophiles*, **2**, 279–287.
- Gruber, C., Legat, A., Pfaffenhuemer, M., Radax, C., Weidler, G., Busse, H. J., and Stan-Lotter, H., 2004. *Halobacterium noricense* sp. nov., an archaeal isolate from a bore core of an alpine Permo-Triassic salt deposit, classification of *Halobacterium* sp. NRC-1 as a strain of *Halobacterium salinarum* and emended description of *Halobacterium salinarum*. *Extremophiles*, **8**, 431–439.
- Hay, W. W., Migdisov, A., Balukhovskiy, A. N., Wold, C. N., Flögel, S., and Söding, E., 2006. Evaporites and the salinity of the ocean during the Phanerozoic: implications for climate, ocean circulation and life. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **240**, 3–46.
- Holser, W. T., and Kaplan, I. R., 1966. Isotope geochemistry of sedimentary sulfates. *Chemical Geology*, **1**, 93–135.
- Horita, J., Friedman, T. J., Lazar, B., and Holland, H. D., 1991. The composition of Permian seawater. *Geochimica et Cosmochimica Acta*, **55**, 417–432.
- Klaus, W., 1963. Sporen aus dem südalpinem Perm (Vergleichsstudie für die Gliederung nordalpiner Salzserien). *Jahrbuch der Geologischen Bundesanstalt Wien, Band*, **106**, 229–361.
- Klaus, W., 1974. Neue Beiträge zur Datierung von Evaporiten des Oberperm. *Carinthia II*, **164**, Jahrgang 84: 79–85.
- Larsen, H., 1981. The family *Halobacteriaceae*. In Starr, M. P., Stolp, H., Trüper, H. G., Balows, A., and Schlegel, H. G. (eds.). *The Prokaryotes. A Handbook on Habitat, Isolation and Identification of Bacteria*. Berlin: Springer, vol. I, pp. 985–994.
- McGenity, T. J., Gemmell, R. T., Grant, W. D., and Stan-Lotter, H., 2000. Origins of halophilic microorganisms in ancient salt deposits (MiniReview). *Environmental Microbiology*, **2**, 243–250.
- Mormile, M. R., Biesen, M. A., Gutierrez, M. C., Ventosa, A., Pavlovich, J. B., Onstott, T. C., and Fredrickson, J. K., 2003. Isolation of *Halobacterium salinarum* retrieved directly from halite brine inclusions. *Environmental Microbiology*, **5**, 1094–1102.
- Norton, C. F., McGenity, T. J., and Grant, W. D., 1993. Archaeal halophiles (halobacteria) from two British salt mines. *Journal of General Microbiology*, **139**, 1077–1081.
- Osterloo, M. M., Hamilton, V. E., Bandfield, J. L., Glotch, T. D., Baldrige, A. M., Christensen, P. R., Tornabene, L. L., and Anderson, F. S., 2008. Chloride-bearing materials in the southern highlands of Mars. *Science*, **319**, 1651–1654.
- Radax, C., Gruber, C., and Stan-Lotter, H., 2001. Novel haloarchaeal 16S rRNA gene sequences from Alpine Permo-Triassic rock salt. *Extremophiles*, **5**, 221–228.
- Stan-Lotter, H., McGenity, T. J., Legat, A., Denner, E. B. M., Glaser, K., Stetter, K. O., and Wanner, G., 1999. Very similar strains of *Halococcus salifodinae* are found in geographically separated Permo-Triassic salt deposits. *Microbiology*, **145**, 3565–3574.
- Stan-Lotter, H., Radax, C., Gruber, C., McGenity, T. J., Legat, A., Wanner, G., and Denner, E. B. M., 2000. The distribution of viable microorganisms in Permo-Triassic rock salt. In Geertman, R. M. (ed.), *SALT 2000. 8th World Salt Symposium*, Amsterdam: Elsevier Science BV, vol. 2, pp. 921–926.
- Stan-Lotter, H., Pfaffenhuemer, M., Legat, A., Busse, H. J., Radax, C., and Gruber, C., 2002. *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permo-Triassic alpine salt deposit. *International Journal of Systematic and Evolutionary Microbiology*, **52**, 1807–1814.
- Stan-Lotter, H., Radax, C., McGenity, T. J., Legat, A., Pfaffenhuemer, M., Wieland, H., Gruber, C., and Denner, E. B. M., 2004. From intraterrestrials to extraterrestrials – viable haloarchaea in ancient salt deposits. In Ventosa A. (ed.), *Halophilic Microorganisms*. Berlin: Springer, pp. 89–102.
- Treiman, A. H., Gleason, J. D., and Bogard, D. D., 2000. The SNC meteorites are from Mars. *Planetary and Space Science*, **48**, 1213–1230.
- Vreeland, R. H., Piselli, A. F., Mc-Donnough, S., and Meyers, S. S., 1998. Distribution and diversity of halophilic bacteria in a subsurface salt formation. *Extremophiles*, **2**, 321–331.
- Vreeland, R. H., Straight, S., Krammes, J., Dougherty, K., Rosenzweig, W. D., and Kamekura, M., 2002. *Halosimplex carlsbadense* gen. nov., sp. nov., a unique halophilic archaeon, with three 16S rRNA genes, that grows only in defined medium with glycerol and acetate or pyruvate. *Extremophiles*, **6**, 445–452.
- Whitby, J., Burgess, R., Turner, G., Gilmour, J. and Bridges, J., 2000. Extinct ¹²⁹I halite from a primitive meteorite: evidence for evaporite formation in the early solar system. *Science*, **288**, 1819–1821.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.
- Zharkov, M. A., 1981. *History of Paleozoic Salt Accumulation*. Berlin: Springer.
- Zolensky, M. E., Bodnar, R. J., Gibson, E. K., Nyquist, L. E., Reese, Y., Shih, C. Y., and Wiesman, H., 1999. Asteroidal water within fluid inclusion-bearing halite in an H5 chondrite, Monahans (1998). *Science*, **285**, 1377–1379.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Deep Biosphere of the Oceanic Deep Sea
 Extreme Environments

Halobacteria – Halophiles
 Saline Lakes
 Salinity History of the Earth's Ocean
 Terrestrial Deep Biosphere

DEEP BIOSPHERE OF SEDIMENTS

See entries “*Terrestrial Deep Biosphere*,” “*Deep Biosphere of Salt Deposits*,” “*Deep Biosphere of the Oceanic Deep Sea*,” and “*Basalt (Glass, Endoliths)*.”

DEEP BIOSPHERE OF THE OCEANIC DEEP SEA

Kristina Rathsack, Nadia-Valérie Quéric, Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition and overview

Although used in many different ways, the term “biosphere” is principally defined either as zone in which life occurs, thereby overlapping the atmosphere, the hydrosphere, and the lithosphere, or as the entity of living organisms on Planet Earth. Both perceptions commonly focus on the Earth's near-surface environment, with all domains sharing solar energy used in the process of photosynthesis. The deep-sea realm takes a special position in this context, as deep-sea pelagic and the majority of benthic organisms live in the ocean's aphotic zone and inhabit the widespread abyssal plains, respectively. For a long time, their main food source has been considered to be based on particulate organic matter (POM) from the ocean's surface primary production and its sedimentation to abyssal depths (Gage and Tyler, 1991 and references therein, D'Hondt et al., 2002, 2004). With the discovery of “ocean vents” in the late 1970s (Corliss et al., 1979), this general perspective was broadened by the perception of the enormous potential of chemical energy through the reaction of seawater, rock material, and fluids rising from the Earth's interior. According to this concept of energy for life, the term ‘surface biosphere’ has been opposed to ‘sub-surface biosphere’ (also commonly found in literature as ‘deep biosphere’). Following this definition, the deep-seafloor with its highly diverse topography from heterotrophic to pure chemotrophic habitats has to be treated as a transition zone between both biospheres. Opposed to the “*deep hot biosphere*” (Gold, 1992), occurring by definition in oceanic as well as terrestrial subsurface environments, stands the “*deep cold biosphere*” as defined for permafrost sediments (Vorobyova et al., 1997) and ice cores from the depths of Lake Vostoc (Venter, 2001).

Life in the deep sea

Comprising approximately 65% of the Earth's surface, the deep-sea environment is characterized by hyperbaric, aphotic, and low-temperature conditions and highly

diverse seascapes. Canyons, seamounts, ridges, fractures, and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems, and deep-water coral reefs provide ample niches for a highly diverse pelagic and benthic deep-sea community (Tyler, 2003). It was only during the construction of the transoceanic telegraphic communication network that people realized the ocean's topographic alterations and astonishing depths. In 1861, the repair of an overgrown cable from 1,800 m water depth in the Mediterranean finally aroused the scientific community which by then adhered to Edward Forbes' theory on a completely ‘azotic’ zone below a water depth of 550 m. Though, 11 years had elapsed before the first global, scientific expedition onboard the “*Challenger*” (1872–1876) finally convinced people that a flourishing life in fact exists in the deep-sea realm. Numerous, further expeditions and a rushing development of technical facilities allowed deep-sea researches in 1960 to reach even the ocean's deepest surveyed point, the Challenger Deep at 10,911 meters below sea level (mbsl), located at the southern end of the Mariana Trench within the western Pacific Ocean (Piccard and Dietz, 1961). Since then, several studies on large-scale patterns and the zoogeographical origins of deep-sea organisms evidenced a high macrobenthic diversity (Gage and Tyler, 1991 and references therein). These organisms display a depth-dependent zonation as a result of basin age, deep currents (as barriers or dispersal), topographic boundaries, disturbance processes, and sedimentation in connection with depth-related environmental patterns (for review see Levin et al., 2001; Stuart et al., 2003). Macro- and meiofauna are losing importance with increasing water and sediment depth, whereas microorganisms like bacteria, archaea, and fungi account for up to 90% of the deep-sea benthic biomass (Pfannkuche, 1992). Sinking particles may carry large numbers of microorganisms from upper zones (10^8 – 10^{10} cells $m^{-2} d^{-1}$), inoculating deep marine surface sediments with an autotrophic and heterotrophic microbial community, as demonstrated by results from sediment traps (Turley and Mackie, 1995; Danovaro et al., 2000; Vanucci et al., 2001) or the deep-seafloor (Lochte and Turley, 1988).

Particulate organic matter (POM)

Due to the fact that most deep-sea benthic species are deposit feeders (Sanders and Hessler, 1969), the locally qualitatively and quantitatively, variable import of POM from the ocean's surface waters plays a crucial role for macro-, meio-, and microorganisms living in deep surface sediments (Goody and Turley, 1990). Mainly consisting of phytoplankton, marine snow, fecal pellets, (dead) zooplankton and molts, this material undergoes different steps of degradation during its passage from the photic, epipelagic (0–200 mbsl), through the mesopelagic (200–1,000 mbsl) to the actual deep-sea zones, in particular the bathypelagic (1,000–4,000 mbsl), the abyssal

Halobacteria – Halophiles
 Saline Lakes
 Salinity History of the Earth's Ocean
 Terrestrial Deep Biosphere

DEEP BIOSPHERE OF SEDIMENTS

See entries “*Terrestrial Deep Biosphere*,” “*Deep Biosphere of Salt Deposits*,” “*Deep Biosphere of the Oceanic Deep Sea*,” and “*Basalt (Glass, Endoliths)*.”

DEEP BIOSPHERE OF THE OCEANIC DEEP SEA

Kristina Rathsack, Nadia-Valérie Quéric, Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition and overview

Although used in many different ways, the term “biosphere” is principally defined either as zone in which life occurs, thereby overlapping the atmosphere, the hydrosphere, and the lithosphere, or as the entity of living organisms on Planet Earth. Both perceptions commonly focus on the Earth's near-surface environment, with all domains sharing solar energy used in the process of photosynthesis. The deep-sea realm takes a special position in this context, as deep-sea pelagic and the majority of benthic organisms live in the ocean's aphotic zone and inhabit the widespread abyssal plains, respectively. For a long time, their main food source has been considered to be based on particulate organic matter (POM) from the ocean's surface primary production and its sedimentation to abyssal depths (Gage and Tyler, 1991 and references therein, D'Hondt et al., 2002, 2004). With the discovery of “ocean vents” in the late 1970s (Corliss et al., 1979), this general perspective was broadened by the perception of the enormous potential of chemical energy through the reaction of seawater, rock material, and fluids rising from the Earth's interior. According to this concept of energy for life, the term ‘surface biosphere’ has been opposed to ‘sub-surface biosphere’ (also commonly found in literature as ‘deep biosphere’). Following this definition, the deep-seafloor with its highly diverse topography from heterotrophic to pure chemotrophic habitats has to be treated as a transition zone between both biospheres. Opposed to the “*deep hot biosphere*” (Gold, 1992), occurring by definition in oceanic as well as terrestrial subsurface environments, stands the “*deep cold biosphere*” as defined for permafrost sediments (Vorobyova et al., 1997) and ice cores from the depths of Lake Vostoc (Venter, 2001).

Life in the deep sea

Comprising approximately 65% of the Earth's surface, the deep-sea environment is characterized by hyperbaric, aphotic, and low-temperature conditions and highly

diverse seascapes. Canyons, seamounts, ridges, fractures, and trenches, but also biogeochemical oases such as cold seeps, mud volcanoes, carbonate mounds, brine pools, gas hydrates, hot vent systems, and deep-water coral reefs provide ample niches for a highly diverse pelagic and benthic deep-sea community (Tyler, 2003). It was only during the construction of the transoceanic telegraphic communication network that people realized the ocean's topographic alterations and astonishing depths. In 1861, the repair of an overgrown cable from 1,800 m water depth in the Mediterranean finally aroused the scientific community which by then adhered to Edward Forbes' theory on a completely ‘azotic’ zone below a water depth of 550 m. Though, 11 years had elapsed before the first global, scientific expedition onboard the “*Challenger*” (1872–1876) finally convinced people that a flourishing life in fact exists in the deep-sea realm. Numerous, further expeditions and a rushing development of technical facilities allowed deep-sea researches in 1960 to reach even the ocean's deepest surveyed point, the Challenger Deep at 10,911 meters below sea level (mbsl), located at the southern end of the Mariana Trench within the western Pacific Ocean (Piccard and Dietz, 1961). Since then, several studies on large-scale patterns and the zoogeographical origins of deep-sea organisms evidenced a high macrobenthic diversity (Gage and Tyler, 1991 and references therein). These organisms display a depth-dependent zonation as a result of basin age, deep currents (as barriers or dispersal), topographic boundaries, disturbance processes, and sedimentation in connection with depth-related environmental patterns (for review see Levin et al., 2001; Stuart et al., 2003). Macro- and meiofauna are losing importance with increasing water and sediment depth, whereas microorganisms like bacteria, archaea, and fungi account for up to 90% of the deep-sea benthic biomass (Pfannkuche, 1992). Sinking particles may carry large numbers of microorganisms from upper zones (10^8 – 10^{10} cells $m^{-2} d^{-1}$), inoculating deep marine surface sediments with an autotrophic and heterotrophic microbial community, as demonstrated by results from sediment traps (Turley and Mackie, 1995; Danovaro et al., 2000; Vanucci et al., 2001) or the deep-seafloor (Lochte and Turley, 1988).

Particulate organic matter (POM)

Due to the fact that most deep-sea benthic species are deposit feeders (Sanders and Hessler, 1969), the locally qualitatively and quantitatively, variable import of POM from the ocean's surface waters plays a crucial role for macro-, meio-, and microorganisms living in deep surface sediments (Goody and Turley, 1990). Mainly consisting of phytoplankton, marine snow, fecal pellets, (dead) zooplankton and molts, this material undergoes different steps of degradation during its passage from the photic, epipelagic (0–200 mbsl), through the mesopelagic (200–1,000 mbsl) to the actual deep-sea zones, in particular the bathypelagic (1,000–4,000 mbsl), the abyssal

- Takai, K., and Horikoshi, K., 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Brinkhoff, T., Muyzer, G., Moser, D. P., Rethmeier, J., and Jannasch, H. W., 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. *Applied and Environmental Microbiology*, **66**(8), 3125–3133.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daae, F. L., and Pedersen, R. B., Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**(10), 6375–6378.
- Turley, C. M., and Mackie, P. J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep-Sea Research I*, **42**, 1453–1474.
- Tyler, P. A., 2003. *Ecosystems of the Deep Oceans. Ecosystems of the World*, 28. Elsevier: Amsterdam.
- Vanucci, S., Dell'Anno, A., Pusceddu, A., Fabiano, M., Lampitt, R. S., and Danovaro, R., 2001. Microbial assemblages associated with sinking particles in the Porcupine Abyssal Plain (NE Atlantic Ocean). *Progress in Oceanography*, **50**, 105–121.
- Venter, A., 2001. Deep cold biosphere. *Trends in Microbiology*, **9**(7), 313–313.
- Vogt, P. R., Gardner, J., and Crane, K., 1999. The Norwegian-Barents-Svalbard (NBS) continental margin: Introducing a natural laboratory of mass wasting, hydrates, and ascent of sediment, pore water, and methane. *Geo-Marine Letters*, **19**, 2–21.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivatskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., Lee, C., 1986. Evidence for sulfate-reducing and methane-producing micro-organisms in sediments from Site 618, Site 619, and Site 622. *Initial Reports of the Deep Sea Drilling Project*, **96**, 767–775.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Basalt \(Glass, Endoliths\)](#)
[Beggiatoa](#)
[Biofilms](#)
[Carbon Cycle](#)
[Carbon \(Organic, Cycling\)](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Mud Mounds](#)

[Piezophilic Bacteria](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)

DEEP FLUIDS

See entries “[Terrestrial Deep Biosphere](#),” “[Deep Biosphere of Salt Deposits](#),” and “[Deep Biosphere of the Oceanic Deep Sea](#).”

DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed **biodegradation**. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#),” and “[Microbial degradation](#) (of organic matter)” for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO_3^-) to N_2 and, to a minor extent, other gaseous species such as N_2O . See entry “[Nitrogen](#)” for further reading.

DESERT VARNISH

Randall S. Perry
 Imperial College, London, UK

Synonyms

Rock Varnish, *Rock Glaze*, *Silica Glaze*, and *Schutzrinde* were introduced in 1891, from a translation of the French term *manteau protecteur*. “*Schutzrinde*” for all dark coatings, including “*Wustenlack*” a thin, polished coating that may be an eolian polished patina; “*Dunkle Rinden*” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “*Schweinfurth*,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish ([Figure 1](#)). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama

- Takai, K., and Horikoshi, K., 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Brinkhoff, T., Muyzer, G., Moser, D. P., Rethmeier, J., and Jannasch, H. W., 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. *Applied and Environmental Microbiology*, **66**(8), 3125–3133.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daae, F. L., and Pedersen, R. B., Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**(10), 6375–6378.
- Turley, C. M., and Mackie, P. J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep-Sea Research I*, **42**, 1453–1474.
- Tyler, P. A., 2003. *Ecosystems of the Deep Oceans. Ecosystems of the World*, 28. Elsevier: Amsterdam.
- Vanucci, S., Dell'Anno, A., Pusceddu, A., Fabiano, M., Lampitt, R. S., and Danovaro, R., 2001. Microbial assemblages associated with sinking particles in the Porcupine Abyssal Plain (NE Atlantic Ocean). *Progress in Oceanography*, **50**, 105–121.
- Venter, A., 2001. Deep cold biosphere. *Trends in Microbiology*, **9**(7), 313–313.
- Vogt, P. R., Gardner, J., and Crane, K., 1999. The Norwegian-Barents-Svalbard (NBS) continental margin: Introducing a natural laboratory of mass wasting, hydrates, and ascent of sediment, pore water, and methane. *Geo-Marine Letters*, **19**, 2–21.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivatskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., Lee, C., 1986. Evidence for sulfate-reducing and methane-producing micro-organisms in sediments from Site 618, Site 619, and Site 622. *Initial Reports of the Deep Sea Drilling Project*, **96**, 767–775.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Basalt \(Glass, Endoliths\)](#)
[Beggiatoa](#)
[Biofilms](#)
[Carbon Cycle](#)
[Carbon \(Organic, Cycling\)](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Mud Mounds](#)

[Piezophilic Bacteria](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)

DEEP FLUIDS

See entries “[Terrestrial Deep Biosphere](#),” “[Deep Biosphere of Salt Deposits](#),” and “[Deep Biosphere of the Oceanic Deep Sea](#).”

DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed **biodegradation**. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#),” and “[Microbial degradation](#) (of organic matter)” for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO_3^-) to N_2 and, to a minor extent, other gaseous species such as N_2O . See entry “[Nitrogen](#)” for further reading.

DESERT VARNISH

Randall S. Perry
 Imperial College, London, UK

Synonyms

Rock Varnish, *Rock Glaze*, *Silica Glaze*, and *Schutzrinde* were introduced in 1891, from a translation of the French term *manteau protecteur*. “*Schutzrinde*” for all dark coatings, including “*Wustenlack*” a thin, polished coating that may be an eolian polished patina; “*Dunkle Rinden*” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “*Schweinfurth*,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish ([Figure 1](#)). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama



Desert Varnish, Figure 1 California desert varnish.

Deserts. The dark, lustrous coatings have attracted the interest of scientists for centuries. The German naturalist and explorer, Alexander Humboldt, observed desert varnish on a transatlantic expedition and questioned how this enigmatic feature would have formed. His contemporary, Darwin (1887), also engaged in the search for explanations for this unusual rock coating. To date, many other noteworthy scientists have examined desert varnish and have commented on its bulk chemistry, the arid conditions in which it forms and the concentration of manganese that makes it opaque and causes it to be black (cf. Perry et al., 2006, 2007; Perry, 1979; Staley et al., 1992).

Geochemists, planetary geologists, and microbiologists as well as archeologists are interested in desert varnish, as petroglyphs are incised in varnish coatings all over the world.

Yet, despite years of scientific effort, the origin of desert varnish is still shrouded in controversy. Most investigators have looked into biological causes where microbes create varnish and preferentially concentrate manganese relative to the local soils and rocks. However, more modern theories propose a nonbiological origin with sequential episodes of inorganic chemical reactions dominating formation processes.

“Desert varnish” is used here to define coatings that appear dark (reddish brown, chocolate, black, and blue-black). Reasons for not abandoning desert varnish as a term are both because of its historical use and because

it seems a most apt description for dark surfaces formed in subaerial conditions in hot and cold, and high and low elevation deserts. It must be emphatically stated that this definition of darkness is a macrovisual phenomenon. Close inspection of varnish coatings exposes the heterogeneity of the surface composition.

Desert varnish is a thin sedimentary deposit ($\sim <200\ \mu\text{m}$ thick) and under the microscope, its most notable feature is the presence of micron-sized layers. In typical geological thin sections ($30\ \mu\text{m}$ thickness) cut normal to the varnish surface the coatings are opaque. The making of a special ultra-thin section revealed the structure within the coating. This heterogeneity was first observed by Perry and Adams (1978) who showed that desert varnish coatings are composed of alternating light and dark layers. Silicon and oxygen are the primary elements in desert varnish, but oxides including aluminum, manganese, iron, titanium and magnesium are also important, and it is the variability in abundance of these oxides that creates the layers. Dark layers within varnishes are enhanced in manganese. Less laterally uniform variations in composition are also evident, and whole detrital grains can be embedded in varnish coatings. The thickness of layers is also variable and in three dimensions the growth of layers can lead to botryoidal (mounded) structures (Perry and Adams, 1978).

The black desert varnish is not the only type of rock coating draped in controversy; other coating types have also been observed in nature. Silica glazes are also found throughout the world and have been investigated in Hawaii (Curtiss et al., 1985; Farr and Adams, 1984), Morocco (Smith and Whalley, 1988), and Oregon (Farr, 1981). Their clear color is a result of not containing enough oxides to tint them black (manganese) or red (iron).

Recent investigations

It is now widely agreed that desert varnish and silica glazes are true coatings rather than weathering products of an underlying material. Consequently, some source materials for desert varnish must originate from external environments and are introduced by atmospheric transport. Dust can land on rock surfaces and its constituents take part in chemical reactions with some components becoming concentrated and adhered or “glued” together. Limited amount of water is essential for this process. Atmospheric deposits of trace elements are also deposited and have become part of varnish coatings (Thiagarajan and Lee, 2004). Importantly, the chemical composition of desert varnish necessitates that unused elements and materials need to be removed in a never-ending interchange that allows for the concentration and formation of mineral components.

In the past, this enigmatic coating has been ascribed to the action of bacteria or fungi on desert rock surfaces. Microbes are thought to be pervasive in all environments on Earth, and researchers have actively sought a causative link to desert varnish. However, ongoing Martian analog studies in extreme arid conditions such

as the Atacama Desert show that bacteria are rarely present on rock surfaces (Jones, 1991). The black top coatings were attributed to biological action, and early investigators speculated that the manganese oxidizing bacteria concentrated the manganese oxides on the top but did not grow as readily underneath rocks. Primarily, these investigations used culture-based studies of microbes, both bacterial and fungal (Hungate et al., 1987). The emphasis was on an exhaustive search for manganese and iron oxidizing microbes. The manganese and iron were thought to have been absorbed from dust by the bacteria and oxidized during biochemical reactions over thousands of years (Dorn and Oberlander, 1981). According to conventional thought, the silicate particles were also obtained from the atmosphere and cemented together by oxides produced by bacteria. Yet, biological mechanisms remain unsatisfactory, and no viable hypothesis has been presented that provides an explanation for key varnish features such as its hardness, a mechanism for binding the heterogeneous components together, a means of producing the lamellar and botryoidal morphologies, and its slow rates of formation (Liu and Broecker, 2000).

While cultures of bacteria were obtainable from the surfaces of varnish-coated rocks, a direct look at those surfaces using electron microscopes, after fixing the surfaces in order to preserve any biology present, rarely revealed the presence of bacteria. This was noted by Jones (1991) in the Atacama and Smith and Whalley (1988) in the Atlas Mountains of Morocco and Perry et al. (2007) in the Mojave Desert.

Incomplete remains of organisms are, however, entombed in desert varnish coatings. The first examination of organic molecules being trapped or complexed in varnish coatings was published by Perry et al. (2003). Other organic compounds may also make a C–O bond or be entombed in the disordered silica matrix. Following the discovery of amino acids, DNA was found using molecular techniques by Perry et al. (2004) and Kuhlman et al. (2006); also several polymorphic compounds are present in varnishes (Perry et al., 2007) and lipids (Schelble et al., 2001). These findings suggest that varnish coatings preserve past biology and as discussed later, may be an important recorder of contemporaneous life in the local environment.

Several investigative techniques including, high resolution electron microscopy has revealed that silica as opal, not clays or metal oxides, is probably the most important mineral present in desert varnish. When there is moisture, depressions on rock surfaces form small dimples to larger indentations in which silicic acid may form, leaving varnish structures in the depressions. Evaporation of water eventually causes concentrations to increase and, eventually, condensation of the silicic acid occurs to produce a gel. During gel formation, surface detrital material and organic compounds are incorporated, and when the gels are dried and dehydrated the components are entombed in a lustrous rock coating (Perry et al., 2006, 2007). Evidence of the occasional action of water is forthcoming from analyses which indicate that despite being formed

in arid environments, the coatings have been shown to contain up to 9% water (Perry, 1979). This in itself represented a conundrum until the hypothesis of water-rich silica was put forth.

We can then conclude that silica in desert varnish and silica glazes and even hot-spring silica-rich sinters (see Chapter *Sinter*) are made by similar processes (Phoenix et al., 2006; Perry and Lynne, 2006). Desert varnish then can be thought of as a silica glaze enriched in oxides.

Hypotheses of formation must stand up to scientific testing, and it has often been said that if we could only make a synthetic coating in the laboratory that we must inevitably understand how coatings are made in nature. Laboratory experiments support the role of silica polymerization. Surprisingly, it turns out to be a relatively easy process to make coatings in the laboratory (Perry et al., 2005). Conversely, several attempts to make coatings using microbes produced no coatings further supporting nonbiological explanations for desert varnish. It cannot be ignored, however, that biological organic compounds may effect the chemical formation of coatings when they are present.

Conclusions

Recent work then indicates that biology is not required for desert varnish formation and the source of the organic components is from outside sources landing on the surface. Importantly, however, the accumulation of environmental products in desert coatings preserves a biological, climatological, and environmental record. Keeping a chronicle of past life is not only of interest on Earth, but similar process may also be in portent on other planets such as Mars.

Recently, camera images from the Mars Pathfinder landing site have strongly suggested the presence of desert varnish on many Martian rocks (Murchie et al., 2004). Angular, equant, and tabular rocks and large boulders display spectral characteristics suggesting varying levels of ferric minerals. These minerals form part of a desert varnish-like coating that may have formed in the presence of thin films of water when Mars had a moist climate than the present-day climate.

If silica exists in desert varnish-like coatings on Mars, as on Earth, then it may contain chemical signatures of previous life (Perry and Hartmann, 2006). It is possible that on Mars the earliest formed layers in any stromatolite-like sequence may have recorded a wetter and more biologically amenable Martian environment. Moreover, it is likely that Martian desert varnish would be a better preserver of organic matter than its Earth counterpart (Perry and Sephton, 2006). The current Martian environment is much colder and drier than that on Earth. Martian desert varnish records may also extend further back in time. On Earth, desert varnish is a relatively recent rock coating generated in time periods commonly less than 100,000 years; older examples are removed by physical and chemical weathering. Evidence of ancient surfaces and events on Mars suggests that both physical and chemical weathering are less aggressive than on our planet.

Bibliography

- Curtiss, B., Adams, J. B., and Ghiorso, M. S., 1985. Origin, development and chemistry of silica-alumina rock coatings from the semi-arid regions of the Island of Hawaii. *Geochimica et Cosmochimica Acta*, **49**, 49–56.
- Darwin, C., 1887. Bahaia-Brazil. Habits of a diodon. *Journal of Researches into the Natural History and Geology of the Countries Visited During the Voyage of H.M.S. Beagle Round the World*, 12–13.
- Dorn, R. I., and Oberlander, T. M., 1981. Microbial origin of desert varnish. *Science*, **213**, 1245–1247.
- Farr, T. G., 1981. *Surface Weathering of Rocks in Semiarid Regions and its Importance for Geologic Remote Sensing*. Unpublished PhD Thesis, University of Washington, Washington, DC.
- Farr, T. G., and Adams, J. B., 1984. Rock coatings in Hawaii. *Geological Society of America Bulletin*, **95**, 1077–1083.
- Hungate, B., Danin, A., Pellerin, N. B., Stemmler, J., Kjellander, P., Adams, J. B., and Staley, J. T., 1987. Characterization of manganese-oxidizing (MnII-MnIV) bacteria from Negev desert rock varnish: implications in desert varnish formation. *Canadian Journal of Microbiology*, **33**, 939–943.
- Jones, C. E., 1991. Characteristics and origin of rock varnish from the hyperarid coastal deserts of Northern Peru. *Quaternary Research*, **35**, 116–129.
- Kuhlman, K. R., Fusco, W. G., La Duc, M. T., Allenbach, L. B., Ball, C. L., Kuhlman, G. M., Anderson, R. C., Erickson, I. K., Struecker, T., Benardini, J., Strap, J. L., and Crawford, R. L., 2006. Diversity of microorganisms within rock varnish in the Whipple Mountains, California. *Applied and Environmental Microbiology*, **72**, 1708–1715.
- Liu, T., and Broecker, W. S., 2000. How fast does rock varnish grow? *Geology*, **28**, 183–186.
- Murchie, S., Barnouin-Jha, O., Barnouin-Jha, K., Bishop, J., Johnson, J., McSween, H., and Morris, R., 2004. Old desert varnish-like coatings and young breccias at the mars pathfinder landing site. *LPSC*, **35**, Abstract 1740.
- Perry, R. S., 1979. *Chemistry and Structure of Desert Varnish* 62. Unpublished Masters Thesis, University of Washington, Washington, DC.
- Perry, R. S., and Adams, J. B., 1978. Desert varnish: evidence for cyclic deposition of manganese. *Nature*, **276**, 489–491.
- Perry, R. S., and Hartmann W. K., 2006. Mars primordial crust: unique sites for investigating proto-biologic properties. *Origin of Life and Evolution of Biospheres*, **36**(5–6), 533–540.
- Perry, R. S., and Lynne, B. Y., 2006. New insights into natural recorders of planetary surface environments: the role of silica in the formation and diagenesis of desert varnish and siliceous sinter. *Lunar and Planetary Science*, **XXXVII**, 1272.
- Perry, R. S., and Sephton, M. A., 2006. Desert varnish - an environmental recorder on earth and a target material for Mars sample return. *Astronomy and Geophysics*, **47**(4), 34–35.
- Perry, R. S., Engel, M. H., Botta, O., and Staley, J. T., 2003. Amino acid analyses of desert varnish from the Sonoran and Mojave deserts. *Geomicrobiology Journal*, **20**, 427–438.
- Perry, R. S., Dodsworth, J., Staley, J. T., and Engel, M. H., 2004. Bacterial diversity in desert varnish. In *Third European Workshop on Exo/Astrobiology, Mars: The Search for Life ESA Publications Netherlands*. Noordwijk: ESA, SP-545, pp. 259–260.
- Perry, R. S., Kolb, V. M., Ajish, P. I., Lynne, B. Y., McLoughlin, N., Sephton, M. A., Wacey, D., and Green, O. R., 2005. Making silica rock coatings in the lab: synthetic Desert varnish. In Hoover, R. B., Levin, G. V., Rosanov, A. Y., and Gladstone, R. G. (eds.), *Astrobiology IX*. Bellingham: SPIE, pp. 265–275.
- Perry, R. S., Lynne, B. Y., Sephton, M., Kolb, V. M., Perry, C. C., and Staley, J. T., 2006. Baking black opal in the desert sun: the importance of silica in desert varnish. *Geology*, **34**, 537–540.
- Perry, R. S., McLoughlin, N., Lynne, B. Y., Sephton, M. A., Oliver, J., Perry, C. C., Campbell, K. A., Engel, M. H., Farmer, J. D., Brasier, M. D., and Staley, J. T., 2007. Defining biominerals and organominerals: direct and indirect indicators of life. *Sedimentary Geology*, **201**, 157–179.
- Phoenix, V. R. C. B. P., Engel, A. S., Tyler, S. W., and Ferris, F. G., 2006. Chilean high-altitude hot-spring sinters: a model system for UV screening mechanisms by early precambrian cyanobacteria. *Geobiology*, **4**, 15–28.
- Schelble, R. T., Westall, F., Allen, C. C., and Brearley, A. T., 2001. Hematite mineralized bacterial remains: Implications for Martian hematite deposits (Abstract). Abstracts of Papers, 32nd Lunar and Planetary Science Conference Abstracts (Online). Abstract #1438. (<http://www.lpi.usra.edu/publications/meetingpubs.html>)
- Smith, B. J., and Whalley, W. B., 1988. A note on the characteristics and possible origins of desert varnishes for southeast Morocco. *Earth Science Processes and Landforms*, **13**, 251–258.
- Staley, J. T., Adams, J. B., and Palmer, F. E., 1992. Desert varnish: a biological perspective. In Stotsky, G., and Bollag, J.-M. (eds.), *Soil Biochemistry*. New York: Marcel Dekker, pp. 173–195.
- Thiagarajan, N., and Lee, C.-T. A., 2004. Trace-element evidence for the origin of desert varnish by direct aqueous atmospheric deposition. *Earth and Planetary Science Letters*, **224**, 131–141.

Cross-references

Astrobiology
Endoliths
Extreme Environments
Microbial Silicification – Bacteria (or Passive)
Sinter

DETACHMENT

Definition

Detachment refers to the release and transport of microbial cells and their associated extracellular polymeric substances from an attached microbial biofilm to the fluid compartment bathing the film (Stewart, 1993).

Together with microbial attachment and growth, detachment is a primary process that balances biofilm accumulation and activity. Detachment processes control the (re-)dispersal of cells from biofilms into the planktonic community and hence are crucial for bacterial expansion and the exploration and colonization of new niches (Toutain et al., 2004). Bryers (1988) distinguished several categories of detachment processes: (i) *Erosion* is a more or less continuous removal of individual cells or small groups of cells resulting from the moving fluid in contact with the surface of the biofilm, whereas (ii) *abrasion* is caused by the collision of particles. (iii) *Sloughing* refers to the release of relatively large particles of biomass, whose size is comparable to or greater than the thickness of the biofilm itself. Furthermore, (iv) *predator grazing* may be considered as another form of detachment process.

Bibliography

- Curtiss, B., Adams, J. B., and Ghiorso, M. S., 1985. Origin, development and chemistry of silica-alumina rock coatings from the semi-arid regions of the Island of Hawaii. *Geochimica et Cosmochimica Acta*, **49**, 49–56.
- Darwin, C., 1887. Bahaia-Brazil. Habits of a diodon. *Journal of Researches into the Natural History and Geology of the Countries Visited During the Voyage of H.M.S. Beagle Round the World*, 12–13.
- Dorn, R. I., and Oberlander, T. M., 1981. Microbial origin of desert varnish. *Science*, **213**, 1245–1247.
- Farr, T. G., 1981. *Surface Weathering of Rocks in Semiarid Regions and its Importance for Geologic Remote Sensing*. Unpublished PhD Thesis, University of Washington, Washington, DC.
- Farr, T. G., and Adams, J. B., 1984. Rock coatings in Hawaii. *Geological Society of America Bulletin*, **95**, 1077–1083.
- Hungate, B., Danin, A., Pellerin, N. B., Stemmler, J., Kjellander, P., Adams, J. B., and Staley, J. T., 1987. Characterization of manganese-oxidizing (MnII-MnIV) bacteria from Negev desert rock varnish: implications in desert varnish formation. *Canadian Journal of Microbiology*, **33**, 939–943.
- Jones, C. E., 1991. Characteristics and origin of rock varnish from the hyperarid coastal deserts of Northern Peru. *Quaternary Research*, **35**, 116–129.
- Kuhlman, K. R., Fusco, W. G., La Duc, M. T., Allenbach, L. B., Ball, C. L., Kuhlman, G. M., Anderson, R. C., Erickson, I. K., Struecker, T., Benardini, J., Strap, J. L., and Crawford, R. L., 2006. Diversity of microorganisms within rock varnish in the Whipple Mountains, California. *Applied and Environmental Microbiology*, **72**, 1708–1715.
- Liu, T., and Broecker, W. S., 2000. How fast does rock varnish grow? *Geology*, **28**, 183–186.
- Murchie, S., Barnouin-Jha, O., Barnouin-Jha, K., Bishop, J., Johnson, J., McSween, H., and Morris, R., 2004. Old desert varnish-like coatings and young breccias at the mars pathfinder landing site. *LPSC*, **35**, Abstract 1740.
- Perry, R. S., 1979. *Chemistry and Structure of Desert Varnish* 62. Unpublished Masters Thesis, University of Washington, Washington, DC.
- Perry, R. S., and Adams, J. B., 1978. Desert varnish: evidence for cyclic deposition of manganese. *Nature*, **276**, 489–491.
- Perry, R. S., and Hartmann W. K., 2006. Mars primordial crust: unique sites for investigating proto-biologic properties. *Origin of Life and Evolution of Biospheres*, **36**(5–6), 533–540.
- Perry, R. S., and Lynne, B. Y., 2006. New insights into natural recorders of planetary surface environments: the role of silica in the formation and diagenesis of desert varnish and siliceous sinter. *Lunar and Planetary Science*, **XXXVII**, 1272.
- Perry, R. S., and Sephton, M. A., 2006. Desert varnish - an environmental recorder on earth and a target material for Mars sample return. *Astronomy and Geophysics*, **47**(4), 34–35.
- Perry, R. S., Engel, M. H., Botta, O., and Staley, J. T., 2003. Amino acid analyses of desert varnish from the Sonoran and Mojave deserts. *Geomicrobiology Journal*, **20**, 427–438.
- Perry, R. S., Dodsworth, J., Staley, J. T., and Engel, M. H., 2004. Bacterial diversity in desert varnish. In *Third European Workshop on Exo/Astrobiology, Mars: The Search for Life ESA Publications Netherlands*. Noordwijk: ESA, SP-545, pp. 259–260.
- Perry, R. S., Kolb, V. M., Ajish, P. I., Lynne, B. Y., McLoughlin, N., Sephton, M. A., Wacey, D., and Green, O. R., 2005. Making silica rock coatings in the lab: synthetic Desert varnish. In Hoover, R. B., Levin, G. V., Rosanov, A. Y., and Gladstone, R. G. (eds.), *Astrobiology IX*. Bellingham: SPIE, pp. 265–275.
- Perry, R. S., Lynne, B. Y., Sephton, M., Kolb, V. M., Perry, C. C., and Staley, J. T., 2006. Baking black opal in the desert sun: the importance of silica in desert varnish. *Geology*, **34**, 537–540.
- Perry, R. S., McLoughlin, N., Lynne, B. Y., Sephton, M. A., Oliver, J., Perry, C. C., Campbell, K. A., Engel, M. H., Farmer, J. D., Brasier, M. D., and Staley, J. T., 2007. Defining biominerals and organominerals: direct and indirect indicators of life. *Sedimentary Geology*, **201**, 157–179.
- Phoenix, V. R. C. B. P., Engel, A. S., Tyler, S. W., and Ferris, F. G., 2006. Chilean high-altitude hot-spring sinters: a model system for UV screening mechanisms by early precambrian cyanobacteria. *Geobiology*, **4**, 15–28.
- Schelble, R. T., Westall, F., Allen, C. C., and Brearley, A. T., 2001. Hematite mineralized bacterial remains: Implications for Martian hematite deposits (Abstract). Abstracts of Papers, 32nd Lunar and Planetary Science Conference Abstracts (Online). Abstract #1438. (<http://www.lpi.usra.edu/publications/meetingpubs.html>)
- Smith, B. J., and Whalley, W. B., 1988. A note on the characteristics and possible origins of desert varnishes for southeast Morocco. *Earth Science Processes and Landforms*, **13**, 251–258.
- Staley, J. T., Adams, J. B., and Palmer, F. E., 1992. Desert varnish: a biological perspective. In Stotsky, G., and Bollag, J.-M. (eds.), *Soil Biochemistry*. New York: Marcel Dekker, pp. 173–195.
- Thiagarajan, N., and Lee, C.-T. A., 2004. Trace-element evidence for the origin of desert varnish by direct aqueous atmospheric deposition. *Earth and Planetary Science Letters*, **224**, 131–141.

Cross-references

Astrobiology
Endoliths
Extreme Environments
Microbial Silicification – Bacteria (or Passive)
Sinter

DETACHMENT

Definition

Detachment refers to the release and transport of microbial cells and their associated extracellular polymeric substances from an attached microbial biofilm to the fluid compartment bathing the film (Stewart, 1993).

Together with microbial attachment and growth, detachment is a primary process that balances biofilm accumulation and activity. Detachment processes control the (re-)dispersal of cells from biofilms into the planktonic community and hence are crucial for bacterial expansion and the exploration and colonization of new niches (Toutain et al., 2004). Bryers (1988) distinguished several categories of detachment processes: (i) *Erosion* is a more or less continuous removal of individual cells or small groups of cells resulting from the moving fluid in contact with the surface of the biofilm, whereas (ii) *abrasion* is caused by the collision of particles. (iii) *Sloughing* refers to the release of relatively large particles of biomass, whose size is comparable to or greater than the thickness of the biofilm itself. Furthermore, (iv) *predator grazing* may be considered as another form of detachment process.

Important factors controlling and/or inducing biofilm detachment include the activity of matrix-degrading enzymes, microbially generated gas bubbles, nutrient levels, microbial growth status, availability of multivalent cross-linking cations, fluid shear stress, and contact attrition (Hunt et al., 2004). In addition, there is increasing evidence that bacterial biofilms may actively regulate dispersion processes using cell-to-cell signaling (quorum-sensing). Dissolution of the biofilm matrix and release of bacteria are hypothesized to be triggered when a signal molecule, i.e., an excreted bacterial metabolite, accumulates to a threshold concentration (Hunt et al., 2004; Waters and Bassler, 2005).

Bibliography

- Bryers, J. D., 1988. Modeling biofilm accumulation. In Bazin, M. J., and Prosser, J. I. (eds.), *Physiological Models in Microbiology*. Boca Raton, FL: CRC Press, Vol. 2, pp. 109–144.
- Hunt, S. M., Werner, E. M., Huang, B., Hamilton, M. A., Stewart, P. S., 2004. Hypothesis for the role of nutrient starvation in biofilm detachment. *Applied and Environmental Microbiology*, **70**, 7418–7425.
- Stewart, P. S., 1993. A model of biofilm detachment. *Biotechnology and Bioengineering*, **41**, 111–117.
- Toutain, C. M., Caiazza, N. C., O'Toole, G. A., 2004. Molecular basis of biofilm development by *Pseudomonads*. In Ghannoum, M., and O'Toole, G. A. (eds.), *Microbial Biofilms*. Washington, DC: ASM, pp. 43–63.
- Waters, C. M., and Bassler, B. L., 2005. Quorum sensing: cell-to-cell communication in bacteria. *Annual Reviews of Cell and Developmental Biology*, **21**, 319–346.

Cross-references

Biofilms

Extracellular Polymeric Substances (EPS)

DIATOMS

Nicole Brinkmann¹, Thomas Friedl¹, Kathrin I. Mohr^{1,2}

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Infection Research, Braunschweig, Germany

Synonyms

Bacillariophyta

Definition

Diatoms (Chromalveolates supergroup, photosynthetic Stramenopiles, Bacillariophyta) are unicellular or colonial eukaryotic algae with unique cell walls composed of amorphous silica and consisting of two parts.

The diatoms (Bacillariophyta) represent an extremely diverse and successful lineage of photosynthetic Stramenopiles (Chromalveolates) with cell walls composed of amorphous silica and consisting of two parts,

termed frustules, as their most striking feature. The diatoms are of unicellular organization, but some form colonies. The diatom plastids are derived from red algal secondary symbiosis and are golden brown due to the high concentration of the carotenoid fucoxanthin. Diatoms are exceedingly abundant and thought to be the most important group of eukaryotic phytoplankton, responsible for approximately 40% of marine primary productivity (Falkowski et al., 1998). Their high abundance coupled with the resistance of diatom frustules to dissolution in normal waters has resulted in massive sedimentary accumulation and a significant fossil record.

Cell structure

Frustule: The diatom frustule is a highly patterned external wall composed of amorphous silica $[(\text{SiO}_2)_n(\text{H}_2\text{O})]$. Silica is often plentiful in natural waters (it is the second most abundant element in the Earth's crust) and is an energetically inexpensive source of wall material (Falkowski and Raven, 1997). The diatom frustule is constructed of two almost equal parts, termed valves, with the smaller valve (hypotheca) fitting into the larger (epitheca). Each theca is typically composed of two parts: the valve (which forms the larger outer surface) and a girdle (circular bands of silica attached to the edge of the valve). The frustule is present in all living diatoms, except following secondary loss in endosymbiotic diatoms living in some dinoflagellates (Tamura et al., 2005) and some foraminifera (Lee and Correia, 2005). Owing to their requirement for silica for cell wall biogenesis, the diatoms play a key role in the biogeochemical cycling of silica (Tréguer et al., 1995). Diatom frustule walls predominate in the sediments of the ocean floor, thus making the diatoms serious players in ocean biogeochemistry over geologically significant timescales (Kemp et al., 2000). Analysis of diatom frustule containing sediments can provide information on past environmental conditions (e.g., Reavie et al., 1998). Fossil diatom frustule deposits are even mined as diatomaceous earth and commercially used as filter material, mild abrasives, and absorbent or insulation materials (Lee, 2008). The precise method of frustule formation is still not fully understood but most models propose the formation of a matrix of organic fibrils and microtubules onto which the silica is deposited. More recently, Sumper and Kröger (2004) have suggested that silica formation takes place in intracellular compartments termed silica deposition vesicles (SDVs).

Two major categories of frustule shapes can be distinguished, that is, discoid or cylindrical cells with radial symmetry in valve view (centric diatoms) and elongated cells with a more or less bilateral symmetry (pennate diatoms). Some pennate diatoms have slits in their frustule that extends between both cell ends, the raphe system. Such diatoms are known as raphid diatoms and they possess the ability to accomplish rapid gliding motility. Other pennate diatoms lacking raphes are nonmotile and termed araphid diatoms (Round et al., 1990; Graham et al., 2009). The motive force for motility may come from

system of large fossils is excellently preserved (Franzen, 1994). Fundamental studies on biofilm soft-tissue relationship were carried out by Richter (1994) and Liebig (1997). Another intriguing example is the “soft-tissue” preservation in dinosaur bones (Kaye et al., 2008). Kaye and coworkers have found microbially altered soft tissue in a dinosaur bone and they give a critical explanation of this fact. Within their paper they discuss the processes of tissue preservation and also demonstrate the importance of recent microbial biofilm invasion and late diagenetic alteration of primarily formed pyrite framboids. The clou of this investigation is also that there is no real soft-tissue preservation – however, preserved biofilms cast the former tissue and give an idea about the original structures.

All “Hautschatten” soft-tissue preservations are probably preserved lithified biofilms and not real soft tissues.

Conclusion

Biofilms play a significant role in suboxic and anaerobic milieus preserving soft tissue remains. The body outline of dead organisms is exactly traced by the margins of the mineralised biofilms and give a perfect image of the entire fossil. These structures are often called after the German word “Hautschatten”-perservation and is commonly found under black shale condition.

Bibliography

- Briggs, D. E. G., 1999. Molecular taphonomy of animal and plant cuticles: selective preservation and diagenesis. *Philosophical Transactions of Royal Society Series B*, **354**, 7–16.
- Briggs, D. E. G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences*, **31**, 275–301.
- Briggs, D. E. G., and Kear, A. J., 1993. Fossilization of soft-tissue in the laboratory. *Science*, **259**, 1439–1442.
- Briggs, D. E. G., and Wilby, P. R., 1996. The role of calcium carbonate – calcium phosphate switch in the mineralization of soft bodied fossils. *Journal of the Geological Society, London*, **153**, 665–668.
- Franzen, J. L., 1994. Bacterial preservation of the digestive system in Messel Equoids. *Kaupia*, **4**, 3–11.
- Grimes, S. T., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Gupta, N. S., Michels, R., Briggs, D. E. G., Evershed, R. P., and Pancost, R. D., 2006. The organic preservation of fossil arthropods: an experimental study. *Proceedings of the Royal Society of London, Series B*, **273**, 2777–2783.
- Kaye, T. G., Gaugler, G., and Sawlowicz, Z., 2008. Dinosaur soft tissues interpreted as bacterial biofilms. *PLOS One*, **3**(7), e2808.
- Liebig, K., 1997. *Fossil Microorganisms from the Eocene Messel Oil Shale of Southern Hessen*. University Dissertation, Heidelberg, Germany.
- Richter, G., 1994. Bacteria and bacteria-like structures from the oil-shale of Messel. *Kaupia*, **4**, 21–28.
- Seilacher, A., 1970. Begriff und Bedeutung der Fossil-Lagerstätten. *Neues Jahrbuch Fur Geologie und Paläontologie, Monatshefte*, **1970**, 34–39.
- Seilacher, A., Reif, W. E., and Westphal, F., 1985. Sedimentological, ecological and temporal patterns of fossil lagerstätten.

Philosophical Transactions of the Royal Society of London, Series B, **311**, 5–23.

Toporski, J. K. W., Steele, A., Westall, F., Avet, R., Martill, D. M., and McKay, D. S., 2002. Morphologic and spectral investigation of exceptionally well-preserved bacterial biofilms from the Oligocene Enspel Formation, Germany. *Geochimica et Cosmochimica Acta*, **66**, 1773–1791.

Wuttke, M., 1983. “Weichteilerhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

[Bacteria](#)
[Beggiatoa](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Black Shales](#)
[Detachment](#)
[Ediacaran Biota](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Thiotrophic Bacteria](#)
[Whale and Wood Falls](#)

BIOGEOCHEMICAL CYCLES

A biogeochemical cycle defines the pathways by which chemical elements occurring in organisms are cycled between different living and nonliving compartments on the Earth (e.g., biosphere, atmosphere, hydrosphere, geosphere, etc.). Typical driving forces of a biogeochemical cycle are the metabolisms of living organisms, geological processes, or chemical reactions. For specific reading on major biogeochemical cycles, please refer to “[Carbon Cycle](#),” “[Nitrogen](#),” “[Sulfur Cycle](#),” “[Phosphorus](#),” “[Phosphorites](#).”

BIOLOGICAL CONTROL ON DIAGENESIS: INFLUENCE OF BACTERIA AND RELEVANCE TO OCEAN ACIDIFICATION

Fred T. Mackenzie¹, Andreas J. Andersson²

¹University of Hawaii, Honolulu, HI, USA

²Bermuda Institute of Ocean Sciences, Ferry Reach, St. George's, Bermuda

Introduction

Soon after carbonate mineral particles are deposited, a series of chemical, physical, and biological processes start that can significantly alter their mineralogy, isotopic composition and chemistry, and the fabric of the accumulating sediment. These processes include dissolution and precipitation reactions, abrasion, and transport, and

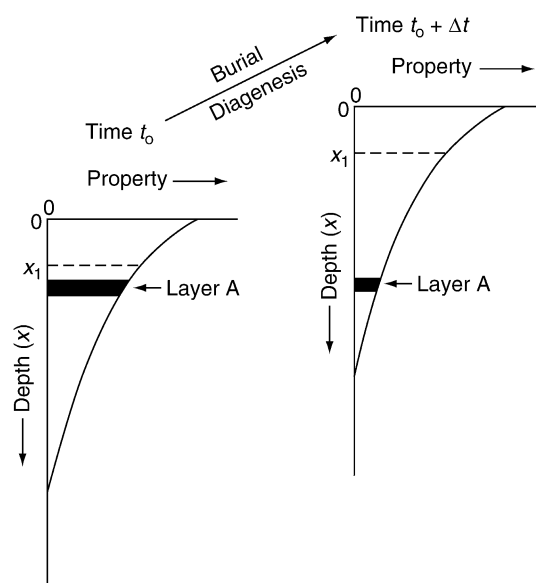
ingestion and boring by organisms. The processes occurring after deposition fall into the category of diagenesis. In particular, carbonate sediments consisting of the minerals low (<4 mol% MgCO_3) and high (>4 mol% MgCO_3) biogenic and inorganic magnesian calcite and aragonite and organic matter deposited in shallow waters are repeatedly deposited, resuspended, and then deposited again during periods of elevated turbulence, which are common especially during storms. Consequently, carbonate minerals may be subjected to several depositional environments before being permanently buried in a sediment. The study of carbonate diagenesis is further complicated by the fact that significant amounts of shallow-water carbonates are commonly associated with reefs, for which traditional models of sediment diagenesis are not always appropriate.

Soon after deposition, chemical and physical changes occur in carbonate sediments as a result of a myriad of diagenetic processes. Among the most important are dissolution-precipitation reactions and major alterations of pore water chemistry by bacterially mediated oxidation of organic matter. These changes generally occur most rapidly in the upper few to tens of centimeters of sediment. It is this topic that is most fully addressed in this article. In addition, transport within the sediment of both solid and dissolved components is also of fundamental importance in diagenesis. It can be caused by physical stirring resulting from the movement of infaunal organisms (e.g., worms, bivalves, shrimp), and is called bioturbation. Closely associated with bioturbation is bioirrigation, which is a result of organisms pumping water through their burrows. Deeper within the sediment and thus later in its history, advection of pore water and molecular diffusion in pore water are generally the dominant transport processes.

The diagenetic equation

Since the early 1960s, considerable effort has gone into modeling the diagenesis of sediments. A central theme of most models is that diagenetic change results from the sum of transport and chemical and biological reactions of various types. Diagenetic models can be divided into those assuming constant conditions (steady-state models) and those taking into account factors that may vary with time (e.g., temperature, salinity, material input rates, water depth). The primary idea in the steady-state models is that a property in the sediment, at a given depth below the sediment-water interface, is time-independent (Figure 1). Thus, if one were to measure the pore water acidity (pH) in a sediment at 20 cm depth and come back 10 years later and again measure the pH at the 20 cm depth, it would be the same. This statement would be true even though the sediment was not physically the same at the 20 cm depth because of deposition during the intervening 10 years.

The general diagenetic equation from which many important models have been derived (e.g., Berner, 1980) is:



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 1 Diagram illustrating under steady-state (constant) conditions how a property such as hydrogen ion concentration varies with depth as the sediment column accumulates. Note that a property at fixed depth x_1 does not change in magnitude as the sediment accumulates.

$$(\partial C_i / \partial t)_x = -\partial F_i / \partial x + \sum R_i \quad (1)$$

where C_i = concentration of solid or liquid component i in mass per unit volume of total sediments (solids plus pore water); F_i = flux of component i in terms of mass per unit area of total sediment per unit time; and R_i = rate of each diagenetic chemical, biochemical, and radiogenic reaction affecting i in terms of mass per unit volume of total sediment per unit time. An example of application of this equation would be the change in hydrogen ion concentration (pH) with time at a given depth in a sediment due to diffusive transport and the dissolution of calcium carbonate in the sediment. For a steady-state condition, Equation 1 is set equal to 0, because at a given depth x , the hydrogen ion concentration does not change with time. Steady-state models are generally more amenable to a mathematical solution than non-steady-state models. However, diagenesis in many shallow-water carbonate sediments is significantly influenced or even dominated by non-steady-state processes.

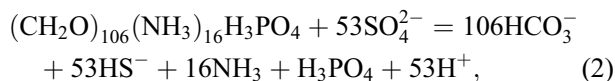
Dissolution of carbonates

Continental shelves and slopes comprise about 20% of the Earth's surface, and contain more than half of the sediments in the ocean. Recent estimates of marine carbonate burial rates indicate that between 35% and 70% of Holocene carbonate deposition has taken place on continental shelves and today's production of shallow-water biogenic carbonate ("calcareous") skeletons and tests represents about 28% of the total carbonate produced in the world's oceans.

A substantial fraction (>50%) of shallow-water produced CaCO_3 is at present time permanently buried in the sediments. In contrast, most of the CaCO_3 produced in the open ocean mainly by planktonic organisms such as coccolithophorids, forams, and pteropods dissolves before it reaches the ocean floor. As much as 50–90% of the material may dissolve in the upper 1,000 m owing to microbial decomposition of organic matter creating microenvironments corrosive to CaCO_3 particles. Typical examples of such environments include inside the guts of zooplankton or within aggregates of CaCO_3 and organic material referred to as “marine snow.” Carbonate material making it past the depth of the saturation horizon, the thermodynamic equilibrium boundary between the forward and backward reactions of precipitation and dissolution, will consequently become subject to dissolution regardless of the activity of microbes and the formation of microenvironments.

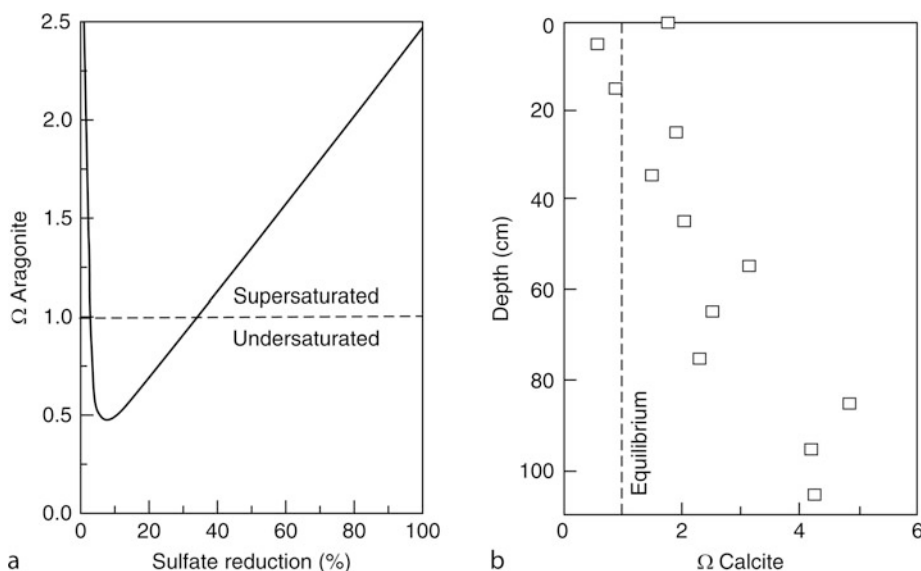
Since most shallow-water environments (coastal zone, continental shelf, banks, reefs, etc.) at present time are immersed in seawater supersaturated with respect to calcite, aragonite, and most Mg-calcite phases, dissolution of sedimentary CaCO_3 minerals in these environments is completely driven by microbially mediated processes associated with oxidation of organic material in the pore water-sediment system or boring autotrophic or heterotrophic microorganisms referred to as endoliths. In the presence of oxygen, the extent of CaCO_3 dissolution is almost completely controlled by the amount and the extent of organic matter decomposed or respired, but under anoxic conditions, oxidation of organic matter accompanied by reduction of sulfate can produce radically different

results. Most studies of the impact of chemical diagenesis on the carbonate chemistry of anoxic sediments have focused primarily on the fact that sulfate (SO_4^{2-}) reduction results in the production of alkalinity (mostly dissolved bicarbonate [HCO_3^-], carbonate [CO_3^{2-}] and carbonic acid [H_2CO_3] and CO_2 chemical species), which can cause precipitation of carbonate minerals (e.g., Berner, 1971). The reduction of the sulfate is promoted by bacteria, particularly *Desulfovibrio desulfuricans*, splitting the sulfur–oxygen bond and leading to the accumulation of dissolved chemical species of reduced sulfur (mainly H_2S and HS^- ; these sulfur species may contribute to the total alkalinity of the pore waters) in the pore waters and in sediments containing iron as ferrous sulfide minerals (mainly pyrite, “fool’s gold,” FeS_2) and free solid sulfur. Although the reaction is complex, this process can be schematically represented, using the average composition of organic matter of phytoplankton as substrate material, as:



which results in the commonly observed decrease in sulfate ion, and pH (measure of hydrogen ion concentration, H^+), and increase in total alkalinity, hydrogen sulfide (H_2S), phosphate (PO_4^{3-}), ammonia (NH_3), and total dissolved inorganic carbon (DIC) in sediment pore waters with increasing depth below the sediment–water interface.

During the early stages of sulfate reduction (~2 to 35%) (Figure 2), this reaction may not cause precipitation,

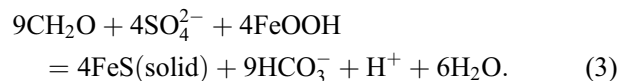


Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 2 The saturation state with respect to aragonite (Ω aragonite) of marine pore waters as a function of the amount of sulfate reduced to sulfide based on the model of Ben Yakoov (a). Saturation state with respect to calcite (Ω calcite) as a function of depth as observed in Mangrove Lake, Bermuda sediment pore waters. (From Morse and Mackenzie, 1990.)

but rather results in undersaturation conditions with respect to carbonate minerals because the impact of lowered pH (increasing H^+) has a larger negative effect on the total alkalinity than the positive effect on this chemical property arising from reduction of sulfate. Carbonate ion activity decreases rapidly as it is "titrated" by CO_2 from organic matter decomposition abetted by bacteria leading to a decrease in pore water saturation state. This process is evident in observational data for Fe-poor shallow water carbonate sediments from the Bahamas and has been confirmed in recent studies of sediments from Florida Bay, in the Checker Reef of Kaneohe Bay, Oahu, Hawaii, and in organic-rich sediments of Mangrove Lake, Bermuda (Morse and Mackenzie, 1990) and elsewhere. In Mangrove Lake, it was shown based on model calculations that the degree of undersaturation of anoxic pore waters with respect to carbonate mineral compositions can depend significantly on the carbon to nitrogen ratio (C/N) of the labile organic matter undergoing bacterial oxidation in the sediment pore water (Figure 3).

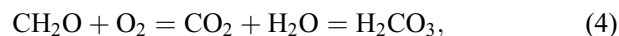
This sulfate reduction reaction in anoxic carbonate sediments has potential importance for carbonate dissolution in shallow-water, marine environments, but its global significance remains a question. An observation of interest is that even complete sulfate reduction can return the saturation state of the pore water only to about half of its original value. Thus, the sulfate reduction reaction by itself may not promote carbonate precipitation as total alkalinity increases and partial sulfate reduction may result in carbonate dissolution. The combination of the sulfate reduction reaction and reactions primarily with iron and

manganese oxide minerals in the sediments can lead to significant calcium carbonate precipitation. This type of net process can be represented schematically as (where CH_2O is a simplified formula for organic matter):

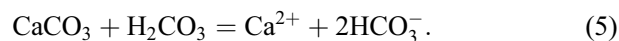


Reaction 3 results in the pH of the waters, in the absence of carbonate precipitation, being buffered at higher pH values. It is, therefore, reasonable to expect that the effectiveness of sulfate reduction in producing carbonate dissolution or precipitation may depend in part on the availability of reactive iron. This conclusion has been demonstrated in a study of the pore water geochemistry of aluminosilicate and carbonate-rich sediments from Kaneohe Bay, Oahu, Hawaii. The aluminosilicate sediments contain abundant pyrite whereas the pyrite content of the carbonate sediments in the bay is low. Pore waters collected from the aluminosilicate sediments have higher pH values than those collected from the carbonate-rich sediments. This observation is a result of the pH values in the pore waters of the aluminosilicate sediments being buffered at higher values than those for carbonate sediments because of Reaction 3 (Mackenzie et al., 1981).

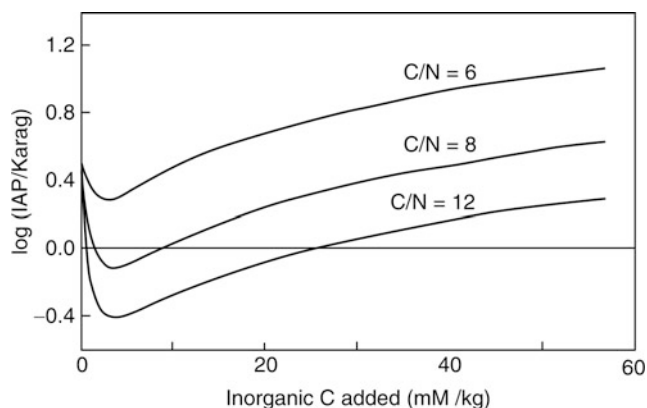
There are several processes that have been identified that can cause undersaturation of pore waters with respect to carbonate minerals, in addition to the previously discussed undersaturation conditions that may result during the early stages of sulfate reduction. These are early post-death microenvironments within organisms, oxidation of organic matter by processes preceding sulfate reduction, and oxidation of sulfides. In addition, both autotrophic and heterotrophic endoliths actively penetrate carbonate substrates by creating corrosive conditions with respect to these mineral phases. These processes commonly will be most important near the sediment–water interface. The most important process occurring prior to the onset of bacterial sulfate reduction is extensive organic matter degradation due to bacterially mediated oxygen reduction, a process important in both shallow-water and deep-sea carbonate sediments. The influence of benthic bacterial activity under aerobic conditions on carbonate mineral dissolution was nicely demonstrated by Moulin et al. (1985) for pore waters from sediments of the Gulf of Calvi, Corsica. Under aerobic conditions, the oxidation of organic matter may be written:



and if carbonate minerals were to dissolve because of the CO_2 produced by organic matter decomposition, then the reaction:

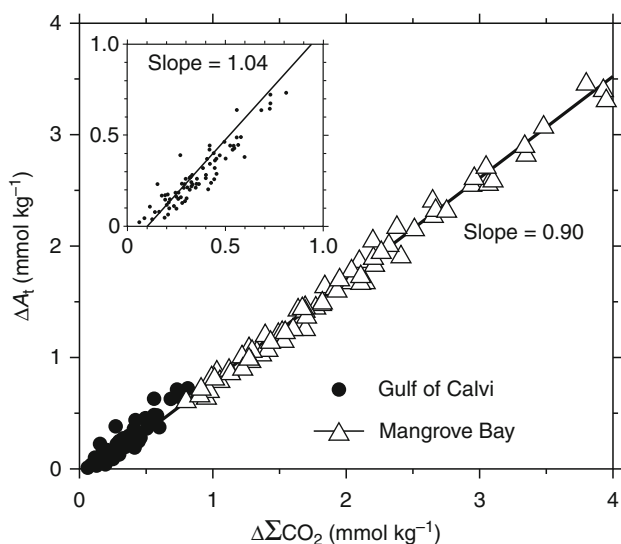


The overall net Reaction of 4 + 5 is:



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 3 Diagram illustrating how the C/N ratio of the organic matter deposited influences the saturation state with respect to aragonite ($\log(IAP/Karag)$; 0.0 = saturation) of the pore waters of Mangrove Lake, Bermuda organic-rich sediments. The saturation state is shown as a function of the amount of dissolved inorganic carbon (DIC) added to the pore waters from the oxidation of organic matter by aerobic oxygen consumption and anaerobic sulfate reduction processes. (From Morse and Mackenzie, 1990.)

Reaction 6 is possible only if the pore waters are undersaturated with a carbonate phase, and there are no other competing reactions, like significant ammonia release from decaying organic matter, to increase pore water saturation state. **Figure 4** shows the increase in total alkalinity versus increase in total CO_2 ($\Delta\Sigma\text{CO}_2$) as observed in shallowly buried pore waters in the Gulf of Calvi *Lithothamnion* sp. algal-rich sediments and in low and high magnesian calcite- and aragonite-rich sediments from Mangrove Bay, Bermuda. The slopes of the regression lines for the relationships are close to 1. The slope indicates that **Reaction 6** most likely gives rise to the increased total alkalinity observed in these pore waters; that is, the H_2CO_3 produced by organic matter oxidation is stoichiometrically utilized in dissolution of a carbonate mineral phase in the sediment. Close inspection of the regression line for the Gulf of Calvi sediments in **Figure 4** shows that it does not pass through the origin. This is because about 0.1 mmol kg^{-1} of H_2CO_3 has been added to the pore waters from aerobic bacterial respiration prior to carbonate dissolution. This initial input of H_2CO_3 lowers the saturation state of the shallowly buried pore waters to that required for carbonate mineral dissolution. These pore water observations have been confirmed in experiments using a biological reactor,



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 4 Increase in total alkalinity (ΔA_t , change in sediment pore water total alkalinity from that of overlying water column) and total DIC ($\Delta\Sigma\text{CO}_2$, change in sediment pore water total DIC from that of overlying water column) in pore waters of Gulf of Calvi, Corsica (insert, Moulin et al., 1985), and Mangrove Bay, Bermuda. Notice the slope of the lines of nearly 1 as predicted from **Reaction 6**. At zero total alkalinity the total DIC is not zero due to the addition of dissolved carbon species to the pore water column from the biological, mainly bacterial, remineralization of organic matter at very shallow depths (several centimeters) in the sediments above the depth, at which the waters become undersaturated with some magnesian calcite compositions and carbonate minerals dissolve with increasing depth.

and from solid phase mineralogical studies. High magnesian calcites are dissolved preferentially in these sediments, although the sediment contains a mixture of nearly pure calcite, aragonite, and magnesian calcites with up to 20 mol% MgCO_3 . In the Gulf sediments composed principally of the red alga *Lithothamnion* sp., this early diagenetic reaction has resulted in dissolution of 75% of the carbonate initially deposited. Furthermore, in the case of the study of Mangrove Bay, the pore waters were sampled every few hours during a complete, 24 h, diel cycle. The trend and relationship between changes in total alkalinity and total DIC are essentially the same as observed in the sediments of Gulf of Calvi. In addition, sea grasses, such as *Thalassia*, which covers vast areas of the bottom in Mangrove Bay probably enhances carbonate dissolution in these sediments by releasing oxygen through its roots and rhizomes into the sediments that consequently enhances decomposition of organic matter and production of H_2CO_3 . Evidence from the Bahamas bank has clearly demonstrated enhanced calcium carbonate dissolution owing to this process (Burdige and Zimmerman, 2002).

Other reactions of less importance than those above leading to undersaturated conditions with respect to calcium carbonate near the sediment–water interface include nitrate reduction and fermentation (e.g., Aller, 1980). Such reactions may also be important near the sediment–water interface of continental shelf and slope sediments, where bioturbation and bioirrigation can result in enhanced transport of reactants. Generally, as the water depth increases over continental slope sediments, the depths increase within the sediment at which significant sulfate reduction and reactions other than sulfate reduction influence carbonate chemistry.

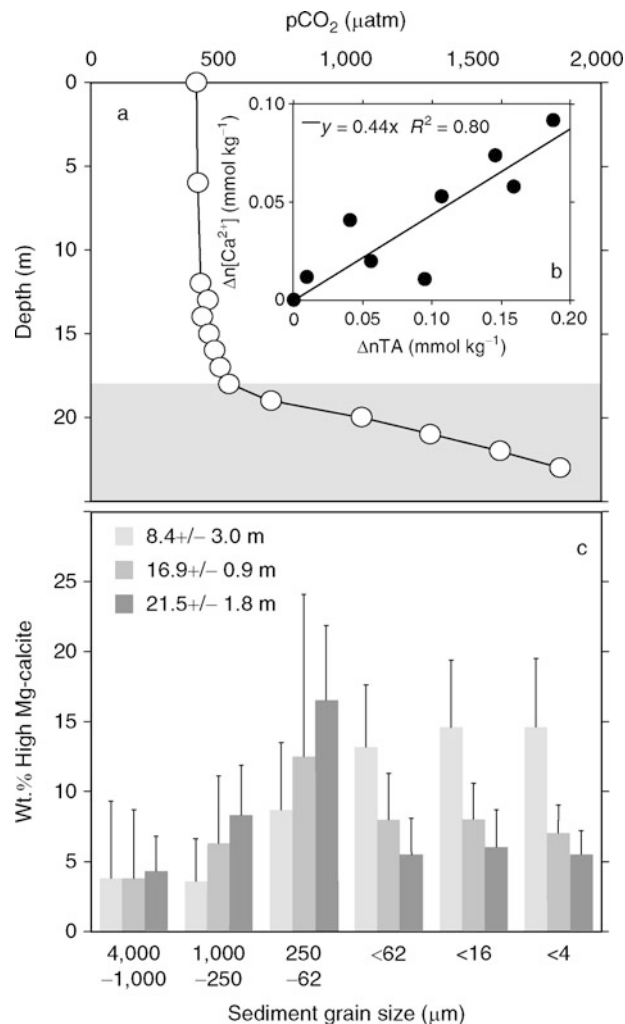
Carbonate dissolution and ocean acidification

Future increases in atmospheric CO_2 owing to human activities, such as burning of fossil fuels and land-use changes, result in increased uptake of part of this CO_2 by ocean surface seawater and formation of carbonic acid. As the term implies, dissociation of carbonic acid produces hydrogen ions, which results in increasing acidity and decreasing pH of seawater and therefore the name ocean acidification. Surface seawater pH has already decreased by approximately 0.1 pH units since the onset of the Industrial Revolution and is anticipated to decrease by another 0.2–0.3 pH units by the end of the twenty-first century (e.g., Caldeira and Wickett, 2003). Oceanic uptake of CO_2 also produces an increase in the seawater concentration of bicarbonate ions (HCO_3^-) and a decrease in the carbonate ion concentration (CO_3^{2-}). As a consequence, the saturation state with respect to calcium carbonate minerals (Ω), which is determined by dividing the in situ ion concentration product of calcium and carbonate ions by an experimentally determined solubility product ($\Omega = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K_{sp}^*$), will decrease. A lower carbonate saturation state of surface seawater implies that the initial composition of marine sediment pore waters will be closer to undersaturation with

respect to carbonate minerals, and thus, microbial decomposition of less organic material is required to produce undersaturated conditions and subsequent dissolution of carbonate mineral phases in the pore water–sediment system. Furthermore, while most shallow-water environments at present time are supersaturated with respect to calcite, aragonite, and most Mg-calcite phases (in general, Mg-calcite containing 8–12 mol% MgCO_3 or more is more soluble than both calcite and aragonite), surface seawater in high and intermediate latitude environments could become undersaturated with respect to most Mg-calcite phases and even aragonite during the present century. Consequently, as a result of human-induced ocean acidification, dissolution of carbonate minerals and sediments in shallow-water ocean environments is most likely to increase.

Increased dissolution of calcium carbonate minerals and sediments owing to human-induced ocean acidification will most likely occur selectively according to mineral stability. This could lead to a change in the average mineral composition of contemporary carbonate sediments in favor of stable mineral phases such as calcite and low Mg-calcite phases over metastable phases such as aragonite and high Mg-calcite. Although such a transition is slow relative to the rate at which seawater chemistry is changing owing to human activities, evidence for increasing carbonate dissolution and changing sediment composition exists based on numerical model simulations, saturation, and dissolution experiments, and mesocosm experiments, as well as from observations from natural environments. One such environment is Devil's Hole, located within Harrington Sound, Bermuda. During summertime, the water column of Devil's Hole becomes significantly stratified owing to the exponential decrease of solar irradiance as a function of depth. Organic matter produced in the surface mixed layer sinks and is decomposed by microbes in the subthermocline layer. As a consequence, the CO_2 , pH and the seawater saturation state with respect to carbonate minerals in this layer reach values typically not observed for most shallow-water environments today, but similar to values anticipated during the next several decades of the twenty-first century as a result of ocean acidification. In spite of significant production of fine grained Mg-calcite minerals throughout Harrington Sound, for example, by coralline algae, forams, and echinoderms, as well as the abundant occurrence of these mineral particles in sediments located at depths above the thermocline, little evidence exists for their presence in the sediments of Devil's Hole. These mineral phases are most likely selectively dissolved, a conclusion that is also supported by observed changes in seawater carbonate chemistry in the subthermocline layer in Devil's Hole (Figure 5; Neumann, 1965; Andersson et al., 2007).

In addition to the chemical changes imposed on surface seawater owing to rising atmospheric CO_2 , future dissolution of shallow-water carbonate minerals could also increase owing to increased deposition and subsequent increased microbial decomposition of organic matter in marine coastal sediments. Human activities on land have



Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification, Figure 5 (a) Partial pressure of CO_2 (pCO_2) in the water column of Devil's Hole, Harrington Sound, Bermuda. The pCO_2 of the subthermocline layer (gray) is significantly higher than anticipated from equilibrium with the atmosphere owing to microbial decomposition of organic matter. As a result, calcium carbonate minerals are selectively dissolved, which is demonstrated in (b) and (c). The former shows changes in salinity normalized ($S = 36$) calcium concentration ($\Delta n[\text{Ca}^{2+}]$) as a function of total alkalinity ($\Delta n\text{TA}$) in the subthermocline layer. The observed relationship clearly demonstrates that carbonate dissolution is taking place and the slope of a best fit line (0.44) suggests that the average composition of the dissolving phases has an Mg-content of 12 mol% MgCO_3 . (c) Relative weight percent high Mg-calcite as a function of sediment grain size and depth in Devil's Hole and Harrington Sound. For small grain sizes ($<62 \mu\text{m}$), the high Mg-calcite content decreases as a function of depth. The figure has been compiled based on data from Neumann (1965), Balzer and Wefer (1981), and Andersson et al. (2007).

significantly increased the amount of organic matter transported via rivers and runoff to the global coastal ocean relative to preindustrial conditions, and the total amount is expected to continue to increase with increased

deposition of this material to the sediments. Furthermore, input of nutrients to the global coastal ocean region have also significantly increased, which may stimulate increased production of organic matter and subsequent deposition of this material to the sediments within this region. As long as surface seawaters are supersaturated with respect to most carbonate phases, microbial decomposition of organic material is the main driver of carbonate dissolution. Consequently, even if the organic material deposited to the sediment–water remained constant, but the microbial activity increased, for example, owing to increasing temperature, carbonate dissolution would also increase. For the next several decades, human activities are likely to continue to lead to acidification of surface seawater and continuous increases in the amount of organic material being deposited to shallow-water sediments, and concurrently with increasing atmospheric concentrations of CO₂, surface and sea surface temperatures are likely to increase with potential increases in microbial and endolithic activity. As a result, dissolution of calcium carbonate minerals, particularly in coastal ocean environments, probably will increase due to all of these forcings. It is possible that under a business as usual scenario of fossil fuel CO₂ emissions, the production of carbonates in the global coastal ocean will be exceeded by their dissolution by year 2150 or earlier.

Summary

Diagenesis refers to those physical, chemical and biological processes that alter the original physical and chemical characteristics and properties of minerals and sediments. Calcium carbonate minerals constitute a substantial fraction of shallow-water sediments and may be subject to significant diagenetic alterations after deposition including dissolution and precipitation reactions. These reactions are largely mediated by microbial processes including oxidation of organic matter under both oxic and anoxic conditions as well as by endoliths, which actively penetrate carbonate substrates. The extent of calcium carbonate dissolution is to a large degree controlled by the amount of organic matter decomposed. Carbonate dissolution is likely to increase in the future in response to human-induced ocean acidification arising from the burning of fossil fuels, but also due to increased riverine transport of organic matter and nutrients to the coastal ocean, enhanced production in situ of organic matter in coastal waters, and subsequent deposition and microbial decomposition of the organic matter in marine sediments.

Acknowledgments

This work was supported by the US National Science Foundation grant OCE-0749401.

Bibliography

Aller, R. C., 1980. Diagenetic processes near the sediment–water interface of Long Island Sound. I. Decomposition and nutrient element geochemistry (S, N, P). *Advances in Geophysics*, **22**, 237–350.

- Andersson, A. J., Bates, N. R., and Mackenzie, F. T., 2007. Dissolution of carbonate sediments under rising pCO₂ and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, **13**, 237–264.
- Balzer, W., and Wefer, G., 1981. Dissolution of carbonate minerals in a subtropical shallow marine environment. *Marine Chemistry*, **10**, 545–558.
- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.
- Berner, R. A., 1980. *Early Diagenesis, A Theoretical Approach*. Princeton, NJ: Princeton University Press.
- Burdige, D. J., and Zimmerman, R. C., 2002. Impact of sea grass density on carbonate dissolution in Bahamian sediments. *Limnology and Oceanography*, **47**, 1751–1763.
- Caldeira, K., and Wickett, M. E., 2003. Anthropogenic carbon and ocean pH. *Nature*, **425**, 365.
- Mackenzie, F. T., Ristvet, B. L., Thorstenson, D. C., Lerman, A., and Leeper, R. H., 1981. Reverse weathering and chemical mass balance in a coastal environment. In Martin, J. M., Burton, J. D., and Eisma, D. (eds.), *River Inputs to Ocean Systems*. Switzerland: UNEP-UNESCO, pp. 152–187.
- Morse, J. W., and Mackenzie, F. T., 1990. *Geochemistry of Sedimentary Carbonates*. Amsterdam: Elsevier.
- Moulin, E., Jordens, A., and Wollast, R., 1985. Influence of the aerobic bacterial respiration on the early dissolution of carbonates in coastal sediments. In *Proceedings Progress in Belgium Oceanographic Research*, Brussels, Belgium, pp. 196–208.
- Neumann, A. C., 1965. Processes of recent carbonate sedimentation in Harrington Sound Bermuda. *Bulletin of Marine Science*, **15**, 987–1035.

Cross-references

[Alkalinity](#)
[Bioerosion](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbon \(Organic, Degradation\)](#)
[Carbonate Environments](#)
[Carbonates](#)
[Endoliths](#)
[Iron Sulfide Formation](#)
[Microbial Biomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)

BIOLOGICAL VOLCANIC ROCK WEATHERING

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The biologically induced breakdown of volcanic rocks, including crystalline rocks and glasses.

Volcanic rock weathering and global processes

The weathering of volcanic minerals is known to make a significant contribution to the global silicate weathering budget (Dessert et al., 2003), thus influencing carbon dioxide drawdown and climate control, since carbon

Important factors controlling and/or inducing biofilm detachment include the activity of matrix-degrading enzymes, microbially generated gas bubbles, nutrient levels, microbial growth status, availability of multivalent cross-linking cations, fluid shear stress, and contact attrition (Hunt et al., 2004). In addition, there is increasing evidence that bacterial biofilms may actively regulate dispersion processes using cell-to-cell signaling (quorum-sensing). Dissolution of the biofilm matrix and release of bacteria are hypothesized to be triggered when a signal molecule, i.e., an excreted bacterial metabolite, accumulates to a threshold concentration (Hunt et al., 2004; Waters and Bassler, 2005).

Bibliography

- Bryers, J. D., 1988. Modeling biofilm accumulation. In Bazin, M. J., and Prosser, J. I. (eds.), *Physiological Models in Microbiology*. Boca Raton, FL: CRC Press, Vol. 2, pp. 109–144.
- Hunt, S. M., Werner, E. M., Huang, B., Hamilton, M. A., Stewart, P. S., 2004. Hypothesis for the role of nutrient starvation in biofilm detachment. *Applied and Environmental Microbiology*, **70**, 7418–7425.
- Stewart, P. S., 1993. A model of biofilm detachment. *Biotechnology and Bioengineering*, **41**, 111–117.
- Toutain, C. M., Caiazza, N. C., O'Toole, G. A., 2004. Molecular basis of biofilm development by *Pseudomonads*. In Ghannoum, M., and O'Toole, G. A. (eds.), *Microbial Biofilms*. Washington, DC: ASM, pp. 43–63.
- Waters, C. M., and Bassler, B. L., 2005. Quorum sensing: cell-to-cell communication in bacteria. *Annual Reviews of Cell and Developmental Biology*, **21**, 319–346.

Cross-references

Biofilms

Extracellular Polymeric Substances (EPS)

DIATOMS

Nicole Brinkmann¹, Thomas Friedl¹, Kathrin I. Mohr^{1,2}

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Infection Research, Braunschweig, Germany

Synonyms

Bacillariophyta

Definition

Diatoms (Chromalveolates supergroup, photosynthetic Stramenopiles, Bacillariophyta) are unicellular or colonial eukaryotic algae with unique cell walls composed of amorphous silica and consisting of two parts.

The diatoms (Bacillariophyta) represent an extremely diverse and successful lineage of photosynthetic Stramenopiles (Chromalveolates) with cell walls composed of amorphous silica and consisting of two parts,

termed frustules, as their most striking feature. The diatoms are of unicellular organization, but some form colonies. The diatom plastids are derived from red algal secondary symbiosis and are golden brown due to the high concentration of the carotenoid fucoxanthin. Diatoms are exceedingly abundant and thought to be the most important group of eukaryotic phytoplankton, responsible for approximately 40% of marine primary productivity (Falkowski et al., 1998). Their high abundance coupled with the resistance of diatom frustules to dissolution in normal waters has resulted in massive sedimentary accumulation and a significant fossil record.

Cell structure

Frustule: The diatom frustule is a highly patterned external wall composed of amorphous silica $[(\text{SiO}_2)_n(\text{H}_2\text{O})]$. Silica is often plentiful in natural waters (it is the second most abundant element in the Earth's crust) and is an energetically inexpensive source of wall material (Falkowski and Raven, 1997). The diatom frustule is constructed of two almost equal parts, termed valves, with the smaller valve (hypotheca) fitting into the larger (epitheca). Each theca is typically composed of two parts: the valve (which forms the larger outer surface) and a girdle (circular bands of silica attached to the edge of the valve). The frustule is present in all living diatoms, except following secondary loss in endosymbiotic diatoms living in some dinoflagellates (Tamura et al., 2005) and some foraminifera (Lee and Correia, 2005). Owing to their requirement for silica for cell wall biogenesis, the diatoms play a key role in the biogeochemical cycling of silica (Tréguer et al., 1995). Diatom frustule walls predominate in the sediments of the ocean floor, thus making the diatoms serious players in ocean biogeochemistry over geologically significant timescales (Kemp et al., 2000). Analysis of diatom frustule containing sediments can provide information on past environmental conditions (e.g., Reavie et al., 1998). Fossil diatom frustule deposits are even mined as diatomaceous earth and commercially used as filter material, mild abrasives, and absorbent or insulation materials (Lee, 2008). The precise method of frustule formation is still not fully understood but most models propose the formation of a matrix of organic fibrils and microtubules onto which the silica is deposited. More recently, Sumper and Kröger (2004) have suggested that silica formation takes place in intracellular compartments termed silica deposition vesicles (SDVs).

Two major categories of frustule shapes can be distinguished, that is, discoid or cylindrical cells with radial symmetry in valve view (centric diatoms) and elongated cells with a more or less bilateral symmetry (pennate diatoms). Some pennate diatoms have slits in their frustule that extends between both cell ends, the raphe system. Such diatoms are known as raphid diatoms and they possess the ability to accomplish rapid gliding motility. Other pennate diatoms lacking raphes are nonmotile and termed araphid diatoms (Round et al., 1990; Graham et al., 2009). The motive force for motility may come from

raphe-associated microtubules and attached motor proteins which drag polysaccharide rods that are liberated from the cell by exocytosis at the raphe fissure (Schmidt, 1997). Mucilage that is secreted from the raphe of some pennate diatoms may also form a stalk used for stable attachment of the cell to substrates.

Cell division and sexual reproduction: The regular asexual method of reproduction is by cell division. The valves of the parent cell become the epithecas of the daughter cells with each daughter cell producing a new hypotheca (see Lee, 2008). As a result of cell division, one of the daughter cells is of the same size as the parent cell, and the other is smaller. Once a certain minimal size (about one-third of the maximum size) is reached after several generations, this triggers the start of sexual reproduction whereby gametes are formed. As a result of the fusion of two gametes, a large diploid auxospore (zygote) is formed reestablishing the maximum size of the diatom cell. Vegetative cells of diatoms are diploid, and gamete production involves meiosis. Centric diatoms are oogamous, producing one or two egg cells per parental cell and form 4–128 sperm per parent cell as the result of mitotic divisions following mitosis (Graham et al., 2009). Centric diatom sperms are flagellates, but there is only a single flagellum (insel flagellum, see entry *Algae (Eukaryotic)*). Pennate diatoms are usually isogamous, that is, both gametes are similar in size and neither is flagellate. In addition, also the asexual formation of resting spores may act as a second mechanism of size restoration (Lee, 2008).

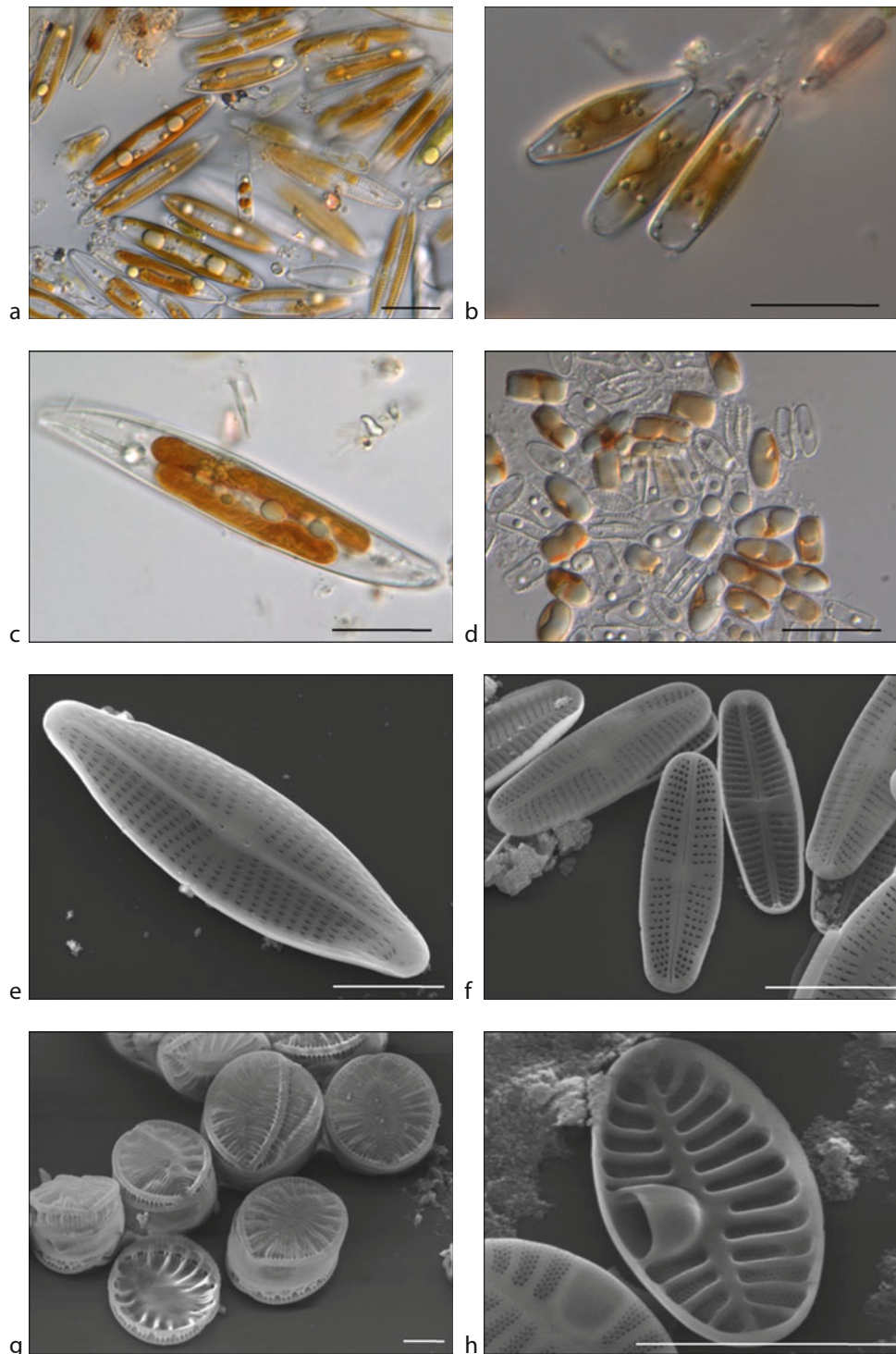
Plastids and storage products: Plastids are double membrane-bounded organelles usually containing the photosynthetic apparatus or some part of it. The diatom plastids are bounded by four membranes and a chloroplast endoplasmatic reticulum (see entry *Algae (Eukaryotic)*) is present. These are typical features of the plastids of most members of photosynthetic Stramenopiles which are thought to be derived from a single red algal secondary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003). The thylakoid membranes within the plastid have the typical structure of the photosynthetic Stramenopiles (heterokont algae), grouped into lamellae of three, all enclosed by a girdle lamella. Centric diatoms generally have large numbers of small discoid plastids, whereas pennate diatoms tend to have fewer plastids, sometimes only one. Due to the presence of the brown carotenoid fucoxanthin which is present in most photosynthetic Stramenopiles (see entry *Algae (Eukaryotic)*) the diatom plastids appear brown. Other characteristic accessory pigments are the chlorophylls c_1 and c_2 . Fucoxanthin and chlorophylls are bound within the light-harvesting antenna complexes by Fucoxanthin- and chlorophyll a/c -binding proteins (FCP). The FCP proteins are integral membrane proteins localized on the thylakoid membranes within the plastid, and their primary function is to target light energy to chlorophyll a within the photosynthetic reaction centers. Diatoms have chrysolaminarin as a carbohydrate storage product which

is dissolved within vacuoles in the cytoplasm, another feature shared with many photosynthetic Stramenopiles. In addition, cytoplasmatic lipid droplets occur. Diatoms are the source of unique highly branched isoprenoid alkanes and steroids which can be used as biomarkers in the water column and in sediments (see entry *Biomarkers (Molecular Fossils)*). An important survival strategy used by diatoms is to produce long-lived resting spores to buffer environmental deterioration (Round et al., 1990), although these only occur in small quantities in sediments.

Diversity and ecology

Diatoms are one of the most easily recognizable groups of major eukaryotic algae because of their unique architecturally complex siliceous cell walls. The siliceous material is laid down in certain regular patterns that leave the wall ornamented and these frustule structures are extensively used as taxonomic “fingerprints.” Diatoms are probably the most species-rich group of eukaryotic algae, with over 250 genera of extant diatoms and perhaps as many as 100,000 species occurring in both aquatic and terrestrial habitats (Norton et al., 1996; Sims et al., 2006). Many more diatom species still remain to be described and diatom species may actually number in the millions (Norton et al., 1996). Diatoms comprise the main component of the open-water marine flora and a significant part of the freshwater algal flora. While pennate diatoms are represented in about equal numbers in the freshwater and marine habitats, the centric diatoms are present predominantly in the marine environment (Lee, 2008). Diatoms are probably the most important group of eukaryotic phytoplankton. Their buoyant cells, often augmented by chitin threads or colonial adaptations, enable them to keep within the photic zone better than many other algae. Moreover, some species are able to withstand deep mixing and low light levels (Kilham et al., 1986). Water depth, photon penetration, vertical mixing, and micro-substrate conditions determine the relative importance of each habitat. Diatom productivity follows a seasonal pattern in most lakes, controlled by the variability of climate, nutrient supply, mixing regimes, and in northern latitudes the period of ice cover. Besides planktonic diatoms there are many benthic forms, growing on sediments or attached to rocks or macroalgae, and some species can also be found in soil (Lee, 2008). In freshwaters, the diatoms make up a large part of the periphyton (i.e., organisms attached to submerged vegetation or inorganic substrates), where they are attached to the substrate (e.g., forming biofilms together with cyanobacteria on tufa stromatolites in hardwater creeks, Arp et al., 2010; see Figure 1). Despite an increase in current retards the attachment of diatoms to the substrate, it causes faster growth leading to a higher standing crop in fast-flowing streams than those in slow-flowing streams (Lee, 2008).

Many diatoms exhibit growth habits and forms that trap sediments, and/or promote precipitation of carbonate



Diatoms, Figure 1 Raphid pennate diatoms recovered from tufa stromatolite biofilms of hardwater creeks: (a) diatom assemblage (biofilm sample, e.g., *Navicula* sp., *Gomphonema* sp., *Cymbella* sp.); (b) *Gomphonema* sp., (biofilm sample) with mucilaginous stalk; (c) *Gyrosigma* sp., (biofilms sample); (d) *Planothidium frequentissimum* (pure culture); (e) SEM of *Achnanthes saprophila* (pure culture; photograph taken by R. Jahn); (f) SEM of *Navicula veneta* (pure culture; photograph taken by R. Jahn); (g) SEM of *Surirella brebissonii* (pure culture; photograph taken by R. Jahn); (h) SEM of *Planothidium frequentissimum* (pure culture; photograph taken by R. Jahn), scale bar LM 20 μ m, SEM 5 μ m.

forming modern stromatolites (see entries “*Microbialites, Stromatolites, and Thombolites*,” “*Microbialites, Modern*”). Numerous authors have noted the presence of diatoms in living stromatolites in both marine environment (e.g., Golubic, 1976) and limnic and fluvial settings (e.g., Gomes, 1985). Several diatoms are conspicuous in building stromatolites, for example, *Mastogloia* sp., *Nitzschia* sp., *Amphora* sp., *Diploneis* sp., and function in a manner analogous to that of cyanobacteria in that they produce extracellular gel, are motile, phototropic, can trap and bind sediment, and produce surface irregularities of the mat (Awramik and Vanyo, 1986; Freytet and Verrecchia, 1998). Diatom mats are important sediment stabilizers in nonmarine environments (Winsborough, 1987) and in subtidal marine settings they appear to be capable of trapping and binding coarser sediment than do those dominated purely by cyanobacteria (Neumann et al., 1970). The presence of biofilms containing large amounts of solid CaCO_3 is often observed on structures submerged in hard freshwaters. The formation of such calcified biofilms may also involve trapping of calcium salts produced within the main body of water by the mucilage of diatom-rich films, and the mediation of CaCO_3 precipitation by biofilm algae, for example, via photosynthesis (Heath et al., 1995).

In warm oligotrophic seas, symbioses between nitrogen-fixing cyanobacteria and diatoms often play a significant role. *Richelia*, a heterocystous cyanobacterium, and the non-heterocystous *Trichodesmium* are believed to be the major N_2 fixers in tropical and subtropical oceans (Carpenter and Romans, 1991; Capone et al., 1997; Janson et al., 1999). In the diatom genera *Hemiaulus*, *Rhizosolenia*, and *Bacteriastrum*, *Richelia* lives as an endosymbiont between the cell wall and the frustule (Fogg, 1982; Villareal, 1994; Janson et al., 1995), with the cyanobacterial partner presumably supplying the eukaryotic partner with nitrogen and/or carbon (Rai et al., 2000). For an extensive bloom of the diatom *Hemiaulus hauckii* harboring *Richelia*, it was estimated that the N supply by N_2 fixation by the symbiosis exceeded that of nitrate flux from below the euphotic zone. High abundances of *Rhizosolenia-Richelia* and *Richelia-Chaetoceros* symbioses have been reported for the subtropical Pacific Ocean (Mague et al., 1974; Gómez et al., 2005); also free trichomes of *Richelia* have been observed there.

Evolutionary history, fossil record, and classification

Fossil diatoms are known from deposits extending from recent times back to about 180 million years ago (Kooistra et al., 2007). Molecular clock evidence suggests that diatoms appeared no earlier than 240 million years ago (Kooistra and Medlin, 1996). The oldest fossil diatoms frustules are from the Jurassic, and well-preserved, diverse floras are available from the Lower Cretaceous (Sims et al., 2006). The fossil evidence suggests that the first

diatoms lived in marine waters and had valves that were radially symmetrical. The main invasion of freshwaters appears to have been delayed until the Cenozoic. Pennate diatoms appear in the late Cretaceous and raphid diatoms in the Paleocene (Sims et al., 2006). Centric diatoms are oogamous and with numerous discoid plastids, pennate diatoms are isogamous with fewer plate-like plastids. These characters previously have led to treat the centric and pennate diatoms as two separate classes. Later, the raphid and araphid pennate diatoms were given equal taxonomic ranking so that three classes were recognized for the diatoms, Coscinodiscophyceae (centric diatoms), Fragilariophyceae (araphid pennate diatoms), and Bacillariophyceae (raphid pennate diatoms) (Round et al., 1990). In molecular phylogenies, however, it became evident that the centric diatoms are not monophyletic, but may represent two lineages to which class rank was given, Mediophyceae and Coscinodiscophyceae (Medlin and Kaczmarska, 2004; Sims et al., 2006). In turn, the molecular phylogenies show the raphid and araphid diatoms to form a monophyletic lineage, which has been recognized as the class Bacillariophyceae (Medlin and Kaczmarska, 2004; Sims et al., 2006).

Summary

Diatoms (Chromalveolates supergroup, photosynthetic Stramenopiles, Bacillariophyta) are unicellular or colonial eukaryotic algae with unique cell walls, termed frustule, composed of amorphous silica with regular patterns of ornamentation and consisting of two parts. They contain brown plastids derived from red algal secondary endosymbiosis. There are two major types: centric and pennate diatoms. Centric diatoms have a radial symmetry in valve view, containing several small plastids and are oogamous, that is, their gametes are an egg and motile spermatozooids with one flagellum. Centric diatoms have the oldest fossil record and make up two classes, Coscinodiscophyceae and Mediophyceae. Pennate diatoms have elongated cell walls with bilateral symmetry. Those pennate diatoms with a raphe exhibit gliding motility. They contain one or few plastids and are isogamous, that is, their gametes are of equal shape and without flagella. Raphid and araphid diatoms make up the class Bacillariophyceae. Diatoms are probably the most species-rich group of eukaryotic algae. They may be the most important group of eukaryotic phytoplankton in marine and freshwater environments, but are also abundant as benthic forms, growing on sediments or attached to submersed substrates, and can also be found in soils. They form important symbioses with nitrogen-fixing cyanobacteria in tropical seas. Diatom frustules predominate in the sediments of the ocean floor and the analysis of diatom frustule containing sediments can provide information on past environmental conditions. Diatoms produced significant fossil records and their fossil deposits are even mined and commercially

used. Based on fossil evidence, diatoms may be up to 240 million years old.

Bibliography

- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., De Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. *Tufa-forming biofilms of German karstwater streams: microorganisms, exopolymers, hydrochemistry and calcification*. London: Geological Society. Special publications, Vol. 336, pp. 83–118.
- Awramik, S. M., and Vanyo, J. P., 1986. Heliotropism in modern Stromatolites. *Science*, **231**, 1279–1281.
- Capone, D. G., Zehr, J. P., Paerl, H., Bergman, B., and Carpenter, E. J., 1997. *Trichodesmium*: a globally significant cyanobacterium. *Science*, **276**, 1221–1229.
- Carpenter, E. J., and Romans, K., 1991. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in North Atlantic Ocean. *Science*, **254**, 1356–1358.
- Falkowski, P. G., and Raven, J. A., 1997. *Aquatic photosynthesis*. Malden: Blackwell Science.
- Falkowski, P. G., Barber, R. T., and Smetacek, V., 1998. Biogeochemical controls and feedbacks on ocean primary production. *Science*, **281**, 200–206.
- Fogg, G. E., 1982. Marine plankton. In Carr, N. G., and Whitton, B. A. (eds.), *The Biology of Cyanobacteria*. Berkeley: University of California Press, pp. 491–513.
- Freytet, P., and Verrecchia, E. P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*, **45**, 535–563.
- Gomes, N. A., 1985. Modern stromatolites in a karst structure from the Malmani subgroup, Transvaal sequence, South Africa. *Transaction of the Geological Society of South Africa*, **88**, 1–9.
- Gómez, F., Furuya, K., and Takeda, S., 2005. Distribution of the cyanobacterium *Richelia intracellularis* as an epiphyte of the diatom *Chaetoceros compressus* in the western Pacific Ocean. *Journal of Plankton Research*, **27**, 323–330.
- Golubic, S., 1976. Organisms that build stromatolites. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 113–126.
- Graham, L. E., Graham, J. M., and Wilcox, L. W., 2009. *Algae*, 2nd edn. San Francisco: Pearson Benjamin Cummings.
- Heath, C. R., Leadbeater, B. C. S., and Callow, M. E., 1995. Effect of inhibitors on calcium carbonate deposition mediated by freshwater algae. *Journal of Applied Phycology*, **7**, 367–380.
- Janson, S., Rai, A. N., and Bergman, B., 1995. Intracellular cyanobiont *Richelia intracellularis*: ultrastructure and immunolocalisation of phycoerythrin, nitrogenase, *Rubisco* and glutamine synthetase. *Marine Biology*, **124**, 1–8.
- Janson, S., Wouters, J., Bergman, B., and Carpenter, E. J., 1999. Host specificity in the *Richelia*-diatom symbiosis revealed by *hetR* gene sequence analysis. *Environmental Microbiology*, **1**, 431–438.
- Keeling, P. J., 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany*, **91**, 1481–1493.
- Kemp, A. E. S., Pike, J., Pearce, R. B., and Lange, C. B., 2000. The “fall dump” – a new perspective on the role of a “shade flora” in the annual cycle of diatom production and export flux. *Deep-Sea Research*, **47**, 2129–2154.
- Kilham, P., Kilham, S. S., and Hecky, R. E., 1986. Hypothesized resource relationships among African planktonic diatoms. *Limnology and Oceanography*, **31**, 1169–1181.
- Kooistra, W. H. C. F., and Medlin, L. K., 1996. Evolution of the diatoms (Bacillariophyta). IV: a reconstruction of their age from small subunit rRNA coding regions and the fossil record. *Molecular Phylogenetics and Evolution*, **6**, 391–407.
- Kooistra, W. H. C. F., Gersonde, R., Medlin, L. K., and Mann, D. G., 2007. The origin and evolution of diatoms: their adaptation to a planktonic existence. In Falkowski, P. G., and Knoll, A. H., (eds.), *Evolution of Primary Producers in the Sea*. New York: Academic, pp. 210–249.
- Lee, J. J., and Correia, M., 2005. Endosymbiotic diatoms from previously unsampled habitats. *Symbiosis*, **38**, 251–260.
- Lee, R. E., 2008. *Phycology*, 4th edn. Cambridge: Cambridge University Press, ISBN 978-0521-68277-0.
- Mague, T. H., Weare, N. M., and Holm-Hansen, O., 1974. Nitrogen fixation in the North Pacific Ocean. *Marine Biology*, **24**, 109–119.
- McFadden, G. I., 2001. Primary and secondary endosymbiosis and the origin of plastids. *Journal of Phycology*, **37**, 951–959.
- Medlin, L. K., and Kaczmarska, I., 2004. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. *Phycologia*, **43**, 245–270.
- Neumann, A. C., Gebelein, C. D., and Scoffin, T. P., 1970. The composition, structure and erodability of subtidal mats. *Journal of Sedimentary Petrology*, **40**, 274–297.
- Norton, T. A., Melkonian, M., and Andersen, R. A., 1996. Algal biodiversity. *Phycologia*, **35**, 308–326.
- Palmer, J., 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology*, **39**, 4–12.
- Rai, A. N., Söderbäck, E., and Bergman, B., 2000. Cyanobacterium symbioses. *New Phytologist*, **147**, 449–481.
- Reavice, E. D., Smol, J. P., Carignan, R., and Lorrain, S., 1998. Diatom paleolimnology of two fluvial lakes in the St. Lawrence River. A reconstruction of environmental changes during the last century. *Journal of Phycology*, **34**, 446–456.
- Round, F. E., Crawford, R. M., and Mann, D. G., 1990. *The Diatoms. Biology and Morphology of the Genera*. Cambridge: Cambridge University Press, p. 747.
- Schmidt, A. M. M., 1997. Putative main function of actin bundles in raphid diatoms: necessity for a new locomotion model. *Phycologia*, **36**, 99 (abstract).
- Sims, P. A., Mann, D. G., and Medlin, L. K., 2006. Phycological reviews: evolution of the diatoms: insights from fossil, biological and molecular data. *Phycologia*, **45**, 361–402.
- Sumper, M., and Kröger, N., 2004. Silica formation in diatoms: the function of long-chain polyamines and silaffins. *Journal of Materials Chemistry*, **14**, 2059–2065.
- Tamura, M., Shimada, S., and Horiguchi, T., 2005. *Galeidinium rugatum* gen. et sp. nov. (Dinophyceae), a new coccoid dinoflagellate with a diatom endosymbiont. *Journal of Phycology*, **41**, 658–671.
- Tréguer, P., Nelson, D. M., van Bennekom, A. J., DeMaster, D. J., and Leynaert, A., 1995. The silica balance in the world ocean: a reestimate. *Science*, **268**, 375–379.
- Villareal, T. A., 1994. Widespread occurrence of the *Hemiaulus* – cyanobacterial symbiosis in the southwest North Atlantic Ocean. *Bulletin of Marine Science*, **54**, 1–7.
- Winsborough, B. M., and Golubic, S., 1987. The role of diatoms in stromatolite growth: two examples from modern freshwater settings. *Journal of Phycology*, **43**, 195–201.

Cross-references

Algae (Eukaryotic)
Carbon Cycle
Microbial Silicification – Bacteria (or Passive)
Microbialites, Modern
Microbialites, Stromatolites, and Thrombolites
Silica Biomineralization, Sponges
Stromatolites
Symbiosis
Tufa, Freshwater

DINOFLAGELLATES

Dinoflagellates are a highly diverse group of flagellated protists consisting of both photosynthetic and non-photosynthetic (heterotrophic) taxa in equal proportions (Taylor et al., 2008). Dinoflagellates are an important component of the modern plankton and have been common to abundant in marine and freshwater environments since the Mesozoic. More than 2,000 extant and 2,500 fossil species have been described. Being mostly biflagellated, swimming unicells of typically 10–100 μm in diameter, they can position themselves in the water column and take full advantage of the available light and nutrients. Many genera are indicative of (paleo-) environmental conditions such as water salinity, nutrient levels, and oceanic temperature zones. In modern and ancient sediments, dinoflagellate-derived organic matter contributions can be specified by distinctive steroid biomarkers (dinosterane and related compounds, see entry “*Biomarkers (Molecular Fossils)*”). Some dinoflagellates, called zooxanthellae, are capable of forming symbioses with a phylogenetically wide range of marine protists and invertebrate animals. For further information, see entries “*Algae (Eukaryotic)*” and “*Protozoa (Heterotroph, Eukaryotic)*.”

Bibliography

Taylor, F. J. R., Hoppenrath, M., and Saldarriaga, J. F., 2008. Dinoflagellate diversity and distribution. *Biodiversity and Conservation*, 17, 407–418.

DIVALENT EARTH ALKALINE CATIONS IN SEAWATER

Anton Eisenhauer
IFM-GEOMAR, Christian Albrechts Universität zu Kiel,
Kiel, Germany

Synonyms

Divalent cations in seawater

Definition

The most abundant divalent earth alkaline cations in seawater are Mg^{2+} , Ca^{2+} , and Sr^{2+} are simply termed the “divalent cations.” These ions and their dynamic change play a major role for the modern oceans. On long geological time scales dynamic changes of divalent cation concentrations in the oceans influenced the evolution of life and the climate evolution in the past.

Divalent cations in modern ocean water

Magnesium

Magnesium is a chemical element with the symbol *Mg*, the atomic number 12, and an atomic mass of 24.31.

Magnesium is the ninth most abundant element in the universe by mass. It constitutes about 2% of the Earth’s crust by mass, and it is the third most abundant element dissolved in seawater and it is the most abundant divalent cation followed by Ca and Sr in seawater (Brown et al., 1992). Mg ions are essential to all living cells, and is the 11th most abundant element by mass in the human body. In seawater ($S = 35$ psu), Mg is present at approximately 53 mM (mM = millimolar) (Brown et al., 1992). Only the monovalent sodium (Na^+ , 469 mM) is present at higher concentration. Magnesium is about five times more abundant than the third most abundant cation Ca (10 mM). Magnesium concentration is significantly lighter than calcium, so when compared on a weight basis, it is only about 3 times as concentrated (1285 vs. 420 ppm). Magnesium is present in seawater as a free divalent cation with water and other molecules attached to it (Brown et al., 1992; Kastner, 1999). It is estimated that each Mg^{2+} ion has approximately eight water molecules (ligands) tightly bound to it. A small portion (about 10%) of the magnesium is present as a soluble ion pair with sulfate (MgSO_4), and much smaller portions are paired with bicarbonate (MgHCO_3^+), carbonate (MgCO_3), fluoride (MgF^+), borate ($\text{MgB}(\text{OH})_4^+$), and hydroxide (MgOH^+).

While these ion pairs comprise only a small portion of the total magnesium concentration, they influence seawater chemistry to a large extent. In the case of carbonate (CO_3^{2-}), for example, the ion pairing to magnesium stabilizes the carbonate that it is present in far higher concentrations than it would be present in the absence of magnesium. This effect makes seawater a much better buffer in the pH range of 8.0 than it otherwise would be. Without this ion pairing, seawater pH might be significantly higher, and more susceptible to dynamic changes. The average residence time for a magnesium ion in seawater is on the order of several tens of millions of years. That time is substantially longer than that for Ca (~one million years) and aluminum (Al, 100 years), but less than Na (~250 million years). The long seawater residence time of Mg in seawater is a measure of its fairly unreactive chemical behavior indicating that it does get taken out of solution through various biological and chemical processes.

Sources and sinks for magnesium in the oceans

In the present-day ocean, the two most important calciumcarbonate (CaCO_3) polymorphs – low Mg-calcite and aragonite – are the most prevalent carbonates and major sinks for Mg replacing Ca to some extent in the crystal lattice. Mg also precipitates together with Ca directly from the water in some evaporitic settings, as well as by the dissolution and reprecipitation of calcite with magnesium replacing calcium in the crystal structure. Dolomite, a high magnesium type of calciumcarbonate usually does not form in the well-oxygenated modern oceans, although bacterial alteration of seawater is reported to induce dolomite precipitation (Kastner, 1999; McKenzie, 1981) in certain marine anoxic environments.

The amount of magnesium estimated to exist as dolomite deposits suggests the importance of dolomitization as a magnesium sink in the past, despite its low prevalence today (Wilkinson and Algeo, 1989). In general, the geological Mg record shows a general trend of increasing abundance of dolomite in sediments of increasing age (Wilkinson and Algeo, 1989). The relative lack of dolomite formation today might indicate differences in past seawater chemistry, climatic and environmental conditions that are more conducive to dolomite formation.

The interaction of hydrothermal waters with basalt at the mid-ocean ridge (MOR) effectively removes magnesium from the water to the basalt, and in exchange, calcium becomes enriched in the water (Mottl and Wheat, 1993). Estimates for the molar exchange rate of Mg to Ca at the MOR are close to unity. These models also predict that interaction at the MOR is a sufficient sink to explain the balance of magnesium in the ocean, as well as being a significant source for calcium.

Marine geological investigations at the mid-ocean ridge estimate a flux rate of the entire ocean volume through the accretion zone every 8 million years, or about 1.75×10^{17} g/year, a fraction of the flux rate for rivers of about 4.6×10^{19} g/year (Mottl and Holland, 1978). It has also been demonstrated through pore water profiles that magnesium and calcium chemically interact with the underlying crystalline basement resulting in a decrease of magnesium concentration and an increase in calcium concentration with depth below seafloor in the sediment column (Holland, 1984).

Calcium

Calcium (Ca) is the chemical element with the symbol *Ca* and atomic number 20. It has an atomic mass of 40.078. Calcium is a soft grey alkaline earth metal, and is the fifth most abundant element by mass in the Earth's crust. Calcium is also the fifth most abundant dissolved ion in seawater by both molarity and mass, after sodium, chloride, magnesium, and sulfate. Ca is essential for living organisms, particularly in cell physiology, where movement of the calcium ion Ca^{2+} into and out of the cytoplasm functions as a signal for many cellular processes. As a major material used in mineralization of bones and shells, calcium is the most abundant metal by mass in many animals.

Sources and sinks for calcium in seawater

Calcium is the second most abundant divalent cation of the major ions in seawater with a concentration of about 410 ppm (Brown et al., 1992). Ca variations in the oceans are often caused by changes in salinity, where the calcium goes up and down just as the salinity and the dynamic changes of the input of rivers, which are often greatly enriched in calcium relative to other ions such as sodium. Calcium is also frequently enriched in hydrothermal vent water released from the ocean bottom. The calcium is

dissolved from the hot basalt as the water passes through it, and is released to the ocean. Compared to other conservative cations Ca has a relative short mean residence time of about one million year or less which may reflect its important role in marine biomineralization and other chemical processes. One interesting aspect of calcium in seawater is that the calcium concentration can be higher in deep ocean water than in surface water due to the dissolution of CaCO_3 and the recycling of Ca in the deep ocean. Ca can also locally be depleted in places where precipitation of calcium carbonate is especially rapid. This includes the Bahamas Banks (where oolitic aragonite is precipitated), in parts of the Red Sea, and presumably in some lagoons where calcification is high and the water volume is small.

Chemical state of calcium in seawater

In fresh water below pH 11, Ca ions are essentially free. They are bound only to oriented water molecules and move about independently of all other ions in the solution. However, complexation may also occur with other chemical components such as dissolved phosphate, nitrate, or carbonate. This so-called hydration sphere is quite strongly attached to the ion in water, with about eight water molecules tightly attached in the first hydration shell. Beyond this first hydration shell other water molecules are arranged in a second shell in a looser way. All water molecules around the ion are very rapidly exchanging, but those closest to the ion exchange more slowly and move with it as it moves through the solution.

In seawater, the majority of calcium ions are still free. However, some (~10–15%) are present as an ion pair with sulfate, forming the neutral soluble ion pair CaSO_4 . Calcium similarly forms ion pairs with carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-). While these comprise a small fraction of the total calcium, the calcium carbonate ion pair comprises a fairly large portion of the total carbonate (together with Mg, about two third of the carbonate). These ion pairs consequently tend to lower the free concentration of carbonate, and thereby inhibit precipitation of CaCO_3 and increasing its solubility. In seawater, Ca also forms ion pairs with fluoride, hydroxide, borate, various forms of phosphate, and other less abundant ions. In almost all cases the effect of calcium is smaller than the effect of magnesium on these ions, both because the concentration of magnesium is higher.

Calcium carbonate in seawater

A very important aspect of Ca is its supersaturation in seawater. Supersaturation means that given the right circumstances, it will precipitate as solid calcium carbonate. Supersaturation can be described as:

$$K = [\text{Ca}^{++}][\text{CO}_3^{-}] \quad (1)$$

When $K = K_{sp}^*$ (the solubility product constant in seawater at any given temperature, pressure, and salinity), the solution is saturated. When K exceeds the K_{sp}^* , the solution is supersaturated and when the product of the concentration of calcium and carbonate is less than K_{sp}^* , the solution is undersaturated, and calcium carbonate can dissolve. In normal seawater, the product of calcium and carbonate is about 3 times the K_{sp}^* of aragonite and 5 times that of calcite.

The supersaturation (Ω) for calcium carbonate in seawater is given by:

$$\Omega = [Ca^{++}][CO_3^{--}]/K_{sp}^* \quad (2)$$

The higher Ω is, the more likely precipitation is to take place. At $S = 35$ psu and 1 atmosphere pressure, the K_{sp}^* decreases slightly as the temperature rises. Millero (1995) provides a series of long equations for calculating K_{sp}^* for both aragonite and calcite. For aragonite, the $\log K_{sp}^*$ drops from -6.19 at 25°C , to -6.23 at 40°C to -6.44 at 80°C . In relative terms, the K_{sp}^* has gone from 1 to 0.91 to 0.55 over this temperature range. Likewise for calcite, the relative K_{sp}^* has changed from 1 to 0.96 to 0.73 over this range.

Calcium (magnesium) carbonate precipitate from seawater

One situation in which calcium carbonate can precipitate involves adding calcium carbonate seed crystals to seawater. In many cases, this action will initiate precipitation of calcium carbonate (and magnesium as well). This precipitation typically does not proceed until supersaturation is gone, but may be stopped by some other processes. Furthermore, precipitation takes place if the supersaturation is pushed to unusually high levels. This can be caused by a rise in pH, a rise in temperature, or more obviously, by a rise in either calcium or carbonate.

However, chemical interaction of various cations, mainly Mg, permit the ocean to be supersaturated. Without these processes, it is unlikely that the ocean could remain supersaturated, and might even make it impossible for corals to maintain skeletons without expending considerable effort to prevent dissolution. Magnesium holds onto carbonate ions and reduces their free concentration, thereby reducing the likelihood of precipitation onto calcium carbonate surfaces. Furthermore, Mg gets onto the growing surface of the crystal, essentially poisoning further precipitation of calcium carbonate due to the higher activation hydration energy of Mg. Note that while both of these processes inhibit precipitation of calcium carbonate, the first actually increases the solubility, whereas the second does not. It is worth noting that the solubility of calcium carbonate in seawater is about 26 times higher than in freshwater at the same temperature. Other processes that inhibit crystal growth in seawater involve both

phosphate and organic compounds that get onto the growing crystal like Mg.

Bicarbonate acidity shift with temperature

As water is heated, the equilibrium between bicarbonate and carbonate (Equation 3) is shifted toward carbonate.



$$K_a^* = [CO_3^{--}][H^+]/[HCO_3^-] \quad (4)$$

$$[CO_3^{--}] = K_a^* [HCO_3^-]/[H^+] \quad (5)$$

$$pK_a^* = -\log K_a^* \quad (6)$$

This shift toward carbonate is evidenced by the shift in the seawater pK_a^* for bicarbonate from 9.00 at 25°C to 8.68 at 40°C and 8.16 at 80°C (calculated from equations provided by (Millero, 1995); the * simply indicates that it is in seawater at a given temperature, pressure, and salinity). From Equation 5 (and related derivations) it is evident that if K_a^* rises, $[CO_3^{2-}]$ and $[H^+]$ will rise, and $[HCO_3^-]$ will decline.

Strontium

Strontium in seawater

Strontium, with a molecular weight of ~ 87.5 g/mole is significantly heavier than calcium (40.07 g/mole) or magnesium (24.3 g/mole). In physical size, a strontium ion is about 13% larger than a calcium ion, and 70% larger than a magnesium ion. In seawater ($S = 35$ psu), Sr is present at approximately 90 μM . By concentration, it is the fifth most abundant cation but is the third most abundant divalent cation. In mass units, it is present in seawater at about 8 ppm. The strontium concentration is largely constant across the oceans, with local variations of only 2–3% relative to calcium (DeVilliers and Nelson, 1999; Bernat et al., 1972). As salinity varies in lagoons and estuaries strontium rises and falls accordingly. It was generally accepted that the strontium concentration was also unchanged with depth, but more recent measurements have shown that the strontium concentration rises with depth due to the presence of *acantharians* that deposit strontium sulfate skeletons, slightly depleting the surface of Sr. As these free floating organisms die and settle to the bottom, the deeper waters become enriched in strontium as the skeletons dissolve (DeVilliers and Nelson, 1999).

Most of the strontium is present as the free ion, with only water molecules attached to it (Millero, 1995). A small portion (about 10%) of the strontium is present as a soluble ion pair with sulfate ($SrSO_4$), and much smaller portions are paired with bicarbonate ($SrHCO^+$), carbonate ($SrCO_3$), fluoride (SrF^+), borate ($SrB(OH)^{4+}$), and hydroxide ($SrOH^+$). The average residence time for a strontium ion in the ocean is on the order of

10–20 million years. That time is similar to magnesium (20–50 million years) and is substantially longer than that for calcium (~1 million year) and aluminum (100 years). It is also substantially lesser than sodium (~250 million years). All three divalent cations are chemically very similar. Size is the primary difference which may cause some chemical differences, especially the solubility of certain strontium salts. Strontium carbonate (strontianite) is less soluble than calcium carbonate, which in turn is less soluble than magnesium carbonate. Interestingly, strontium sulfate (celestite) is much less soluble than calcium sulfate and magnesium sulfate. While none of these are actually saturated in seawater, strontium sulfate is close, at 30% of saturation (McManus et al., 2000). That difference is important, and in fact some organisms use strontium sulfate as a skeleton.

Organisms that use strontium for skeletal formation

Although shells of marine mineralizing species are usually made of calcium carbonate there are few species like *Cephalopods*, *Radiolaria*, *Gastropods* which enrich Sr in their calcium carbonate skeleton. However, there is one unique species *Acantharia* which is probably the main user of strontium in the ocean forming its skeleton completely out of SrSO_4 instead of CaCO_3 (c.f. Kinsman and Holland, 1969). These free floating unicellular microorganisms are related to *radiolaria*. They have radiating spines of strontium sulfate that are largely external to the central cytoplasm. Inside the cytosol the spines are connected. *Acantharia* live in the upper regions of the oceans where they deposit their strontium sulfate skeletons. Presumably, the skeletons of all Sr using species are protected from dissolution due to organic coatings. However, when, for example, *Acantharia* individuals die and sink, the protection of the strontium sulfate is lost, exposing the strontium sulfate to the open water, resulting in dissolution. They are populous enough that they are an important part of the strontium cycle in the oceans including river and hydrothermal vent. Certain species of *radiolaria* also use strontium sulfate, despite having silica skeletons. The radiolarian *Sphaerzoum punctatum*, for example, release flagellated swimmers during reproduction which contain celestite. These crystals are deposited inside a cytoplasmic vacuole, and have a 50–100 nm thick coating of organic material on it. Similar to *Acantharia* this coating may reduce the likelihood of dissolution of the celestite.

Reconstruction of past and present environmental information from divalent cation ratios (here Mg/Ca, Sr/Ca) and Ca isotope fractionation Mg/Ca-thermometer

The observation that magnesium was higher in marine carbonates precipitated in warmer water was confirmed for neritic foraminifer shells which are composed of high magnesium calcite (<5% MgCO_3). Another study suggested that pelagic foraminifera, which are composed

of low-magnesium calcite (<1% MgCO_3), might also follow this pattern (Savin and Douglas, 1973). Several studies also demonstrated that inorganic carbonates followed the same pattern (c.f. Katz, 1973). This latter point is important because it indicates that the temperature influence could not be entirely biological (Lea, 2003). The underlying basis for Mg/Ca paleothermometry is that the substitution of Mg in calcite is endothermic and therefore is favored at higher temperatures. The enthalpy change for the reaction based on the more recent thermodynamic is 21 kJ/mol which can be shown using the van't Hoff equation to equate an exponential increase in Mg/Ca of 3%/°C (Lea et al., 1999). Foraminifera shells differ from the thermodynamic prediction in two fundamental ways. First, foraminifera contain 5–10 times lower Mg than predicted from thermodynamic calculations (Lea, 2003). Second, the response of shell Mg to temperature is ~3 times larger than the thermodynamic prediction and inorganic observation, averaging $9 \pm 1\%/^\circ\text{C}$. Why the latter effect is so is not known, but it has several important implications for Mg/Ca paleothermometry. First, it increases the sensitivity of this approach. Second, it raises the question of why the response is so much greater in foraminifera and if the augmentation of the response depends on secondary factors that might change over geological time (Lea, 2003).

At present, several planctonic species (e.g., *G. sacculifer*, *G. bulloides*, *G. ruber* and *O. universa*) have been calibrated by culturing and fit with equations of the form:

$$\text{Mg/Ca} = (\text{mmol/mol}) = be^{mT} \quad (7)$$

Where b is the pre-exponential constant, m is the exponential constant, and T is the temperature (Lea et al., 1999). Fitting Mg/Ca-temperature data with an equation of this form has the dual advantage of allowing for an exponential response while also parametrizing, by the use of the natural logarithm. Calibration results for the three species mentioned above indicates exponential constants between 0.085 and 0.102, equivalent to 8.5%, 10.2% increase in Mg/Ca per°C (Nürnberg et al., 1996; Lea et al., 1999; Kisakürek et al., 2009).

Note that the Mg concentration of foraminifer shells, as well as other carbonates, is susceptible to change via dissolution. At present, scientists accept that dissolution alters the Mg/Ca content of foraminifera shells and instead are investigating the degree to which such changes occur and whether correction factors are possible, and the degree to which dissolution affects Mg/Ca ratios.

Results on quaternary timescale

Mg/Ca thermometry has led to a number of important findings for paleoceanographic and paleoclimatic research. These include documenting the history of subpolar Antarctic sea surface temperature (SST) variations (Mashiotto et al., 1999), tropical Atlantic and Pacific SST changes (Hastings et al., 1998; Nürnberg et al.,

2000), and changes in bottom water temperature in the Atlantic and Pacific.

Among these Mg/Ca results, the most important are those that are available for the tropics. Past SST changes in the tropics have been a contentious issue because the actual glacial–interglacial changes are relatively small ($<5^{\circ}\text{C}$) and therefore more difficult to detect. However, applying Mg/Ca-thermometry to a sediment core from the Ontong Java Plateau which lies on the equator in the center of the western Pacific warm pool and covering the last 500,000 years indicate that glacial SST cooling was systematically $\sim 3^{\circ}\text{C}$ cooler than modern conditions and that this cooling occurred during each of the last five major glacial episodes. Another important observation from Mg/Ca studies is the fact that glacial warming appears to lead ice sheet demise as seen from the oxygen isotope record ($\delta^{18}\text{O}$) by $\sim 3,000$ years (Lea et al., 2000). This observation suggests a prominent role for the tropics in pacing ice age cycles. However, local changes in salinity may also influence $\delta^{18}\text{O}$ -records resulting in apparent shifts between Mg/Ca and $\delta^{18}\text{O}$ -records.

Sr/Ca-thermometer

Early studies indicated that there was an inverse relationship between seawater temperature and strontium content of both inorganically precipitated calcium carbonate and coral aragonite of about $1\text{‰}/^{\circ}\text{C}$. Modern calibrations indicate a sensitivity of about $0.6\text{‰}/^{\circ}\text{C}$ decrease in Sr/Ca. The relationship between coral Sr/Ca and seawater temperature is given as a linear function of the form:

$$\text{Sr/Ca (mmol/mol)} = b + m(\text{SST}) \quad (8)$$

The thermodynamic prediction for strontium substitution in aragonite is actually an exponential response with an inverse temperature dependence, a consequence of the negative enthalpy (exothermic) nature of the reaction in which strontium substitutes for calcium in aragonite. The single inorganic aragonite precipitation study indicates a slope m of 0.039 and an intercept b of 10.66 mmol/mol (Kinsman and Holland, 1969). Calibrations are also available for a number of coral species, but mostly for species of *Porites* (c.f. Beck et al., 1992). Values of the intercept b , which determines the absolute Sr/Ca for a particular temperature, range from 10.3 to 11.3. Values of the slope m , determining the temperature sensitivity range from 0.036 to 0.086.

Results on historical timescale

Among other studies a few long time series of Sr/Ca have been published from Rorotonga and the Great Barrier Reef (GBR) in the South Pacific (Linsley et al., 2000). These SST records show clear interannual variability as well as distinct secular trends. For example, the GBR records which are an average of eight different coral cores, indicate a secular shift to warmer SST in the youngest part of the records (after ~ 1950). Results from the Rorotonga

site are somewhat different and suggest a prominent cooling ~ 1750 followed by a series of decadal oscillations that correlate in the twentieth century with the Pacific Decadal Oscillation (Mantua et al., 1997). The warm period recorded in the mid-eighteenth century at the Rorotonga site appears to be corroborated in the GBR sites, although with a reduced magnitude.

Ca isotope thermometry

The possibility of using calcium isotopes for paleothermometry is a very new idea that is based on the empirical observation of temperature-related fractionation between the isotopes ^{40}Ca and either ^{44}Ca or ^{42}Ca (expressed as $\delta^{44/40}\text{Ca}$ or $\delta^{44/42}\text{Ca}$). This potential paleothermometer has been calibrated in neritic benthic foraminifera (DeLaRocha and DePaolo, 2000), a spinose tropical planktonic foraminifera (Näglér et al., 2000), and a subtropical planktonic foraminifera (Gussone et al., 2003). Convincing evidence of the potential utility of this new paleothermometer comes from the study of (Näglér et al., 2000), which demonstrates an increase of 0.24‰ in shells of the planktic species *G. Sacculifer* $\delta^{44/40}\text{Ca}/^{\circ}\text{C}$. Down-core measurements from a core in the tropical Atlantic indicate that shells from glacial intervals are $\sim 0.5\text{--}1.0\text{‰}$ depleted in $\delta^{44/40}\text{Ca}$ consistent with colder glacial temperatures (Hippler et al., 2006). The $\delta^{44/40}\text{Ca}$ -temperature sensitivity is species dependent showing a much lower sensitivity for *O. universa* of only about $0.02\text{‰}/^{\circ}\text{C}$.

Summary

The divalent alkaline earth cations magnesium, calcium and strontium are key elements for the understanding of inorganic and biologically mediated mineralization and calcification. High resolution measurements of element partitioning and isotope fractionation will help to understand the processes controlling the trace element pathways from a bulk solution to the site of calcification. In particular, the recent findings of Fietzke and Eisenhauer (2006) of temperature dependent Sr isotope fractionation may further help to decipher biocalcification processes.

Bibliography

- Beck, J. W., Recy, J., Taylor, F., Edwards, R. L., and Cabioch, G., 1992. Abrupt changes in early Holocene tropical sea surface temperature derived from coral records. *Nature*, **385**, 705–707.
- Bernat, M., Church, T., and Allegre, C. J., 1972. Barium concentrations in Pacific and Mediterranean sea water profiles by direct isotope dilution mass spectrometry. *Earth and Planetary Science Letters*, **16**(1), 75–80.
- Brown, J., Colling, A., Park, D., Philips, J., Rotghery, D., and Wright, J., 1992. *Seawater: its Composition, Properties, and Behaviour*. Oxford: Pergamon Press.
- DeLaRocha, C. L., and DePaolo, D. J., 2000. Isotopic evidence for variations in the marine calcium cycle over the cenozoic. *Science*, **289**, 1176–1178.
- DeVilliers, S., and Nelson, B. K., 1999. Detection of low-temperature hydrothermal fluxes by seawater Mg and Ca anomalies. *Science*, **285**, 721–723.

- Fietzke, J., and Eisenhauer, A., 2006. Determination of temperature-dependent stable strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS. *Geochemistry, Geophysics, Geosystems*, **7**(8), doi:10.1029/2006GC001243.
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H., Lea, D. W., Bijma, J., and Nägler, T. F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta*, **67**(7), 1375–1382.
- Hastings, D. W., Russell, A. D., and Emerson, S. R., 1998. Foraminiferal magnesium in *G. sacculifer* as a paleotemperature proxy in the equatorial Atlantic and Caribbean surface oceans. *Paleoceanography*, **13**(2), 161–169.
- Hippler, D., Eisenhauer, A., et al. 2006. “Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140ka.” *Geochim Cosmochim Acta*, **70**, 90–100, doi:10.1016/j.gca.2005.07.022.
- Holland, H. D., 1984. *The Chemical Evolution of the Atmosphere and Oceans*. Princeton: Princeton University Press.
- Kastner, M., 1999. Oceanic minerals: their origin, nature of their environment, and significance. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 3380–3387.
- Katz, A., 1973. The interaction of magnesium with calcite during crystal growth at 25–90°C and one atmosphere. *Geochimica et Cosmochimica Acta*, **37**(6), 1563–1578, IN3, 1579–1586.
- Kinsman, D. J. J., and Holland, H. D., 1969. The co-precipitation of cations with CaCO_3 . The co-precipitation of Sr^{2+} with aragonite between 16° and 96°C. *Geochimica et Cosmochimica Acta*, **33**, 1–17.
- Kisakürek, B., Eisenhauer, A., et al. 2008. Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminifera, Globigerinoides ruber (white). *Earth and Planetary Science Letters*, **273**(3–4), 260–269.
- Lea, D. W., 2003. Elemental and isotopic proxies of past ocean temperatures. In Holland, H. D., and Turekian K. K. (eds.), *Treatise on Geochemistry*, Oxford: Elsevier, Vol. 6, pp. 365–390.
- Lea, D. W., Mashiotta, T. A., and Spero, H. J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochimica et Cosmochimica Acta*, **63**(16), 2369–2379.
- Lea, D. W., Pak, D. K., and Spero, H. J., 2000. Climate impact of late quaternary equatorial Pacific sea surface temperature variations. *Science*, **289**, 1719–1724.
- Linsley, B. K., Wellington, G. M., and Schrag, D. P., 2000. Decadal sea surface temperature variability in the subtropical South Pacific from 1726 to 1997 A.D. *Science*, **290**, 1145–1148.
- Mantua, J. N., Hare, S. R., Zhang, Y., Wallace, J. M., and Francis, R. C., 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. *American Meteorological Society*, **78**(6), 1069–1079.
- Mashiotta, T. M., Lea, D. W., and Spero, H. J., 1999. Glacial-interglacial changes in Subantarctic sea surface temperature and $\delta^{18}\text{O}$ -water using foraminiferal Mg. *Earth and Planetary Science Letters*, **170**, 417–432.
- McKenzie, J. A., 1981. Holocene dolomitization of calcium carbonate sediments from the coastal sabkhas of Abu Dhabi, UAE: a stable isotope study. *Journal of Geology*, **89**, 185–198.
- Millero, F. J., 1995. Thermodynamics of the carbon dioxide system in the oceans. *Geochimica et Cosmochimica Acta*, **59**(4), 661–677.
- Mottl, M. J., and Holland, H. D., 1978. Chemical exchange during hydrothermal alteration of basalt by seawater-I. Experimental results for major and minor components of seawater. *Geochimica et Cosmochimica Acta*, **42**(8), 1103–1115.
- Mottl, M. J., and Wheat, C. G., 1993. Hydrothermal circulation through mid-ocean ridge flanks: Fluxes of heat and magnesium. *Geochim Cosmochim Acta*, **58**, 2225–2237.
- Nägler, T., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -isotopes: new powerful tool for reconstruction of past sea surface temperatures. *Geochemistry, Geophysics, Geosystems*, **1**(2000GC000091).
- Nürnberg, D., Bijma, J., et al. 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochim Cosmochim Acta*, **60**, 803–814.
- Nürnberg, D., Müller, A., and Schneider, R. R., 2000. Paleo-sea surface temperature calculations in the equatorial east Atlantic from Mg/Ca ratios in planktic foraminifera: a comparison to sea surface temperature estimates from Uk^{37} , oxygen isotopes, and foraminiferal transfer function. *Paleoceanography*, **15**(1), 124–134.
- Savin, S. M., and Douglas, R. G., 1973. Stable isotope and magnesium geochemistry of recent planktonic foraminifera from South-Pacific. *Geological Society of America Bulletin*, **84**(7), 2327–2342.
- Wilkinson, B. H., and Algeo, T. J., 1989. Sedimentary carbonate record of calcium-magnesium cycling. *American Journal of Science*, **289**, 1158–1194.

Cross-references

Alkalinity
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Carbon Cycle
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Isotopes and Geobiology
 Salinity History of the Earth's Ocean
 Soda Ocean Hypothesis

DIVERSITY

The number of different species in a habitat; sometimes (falsely) used as a synonym for →community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

DOLOMITE, MICROBIAL

Jennifer A. Roberts, Paul A. Kenward
 University of Kansas, Lawrence, KS, USA

Synonyms

Bacterial dolomite; Organogenic dolomite

Definition

Dolomite. Widely distributed rock-forming mineral, $\text{CaMg}(\text{CO}_3)_2$. Usually, white or colorless, but can be yellowish and brown with rhombohedral crystals exhibiting curved, composite faces. It also occurs with massive and granular habit and has rhombohedral {1011} cleavage. **Dolomitization.** The process of converting calcite in limestones to dolomite by the action of magnesium-bearing solutions.

- Fietzke, J., and Eisenhauer, A., 2006. Determination of temperature-dependent stable strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS. *Geochemistry, Geophysics, Geosystems*, **7**(8), doi:10.1029/2006GC001243.
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H., Lea, D. W., Bijma, J., and Nägler, T. F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta*, **67**(7), 1375–1382.
- Hastings, D. W., Russell, A. D., and Emerson, S. R., 1998. Foraminiferal magnesium in *G. sacculifer* as a paleotemperature proxy in the equatorial Atlantic and Caribbean surface oceans. *Paleoceanography*, **13**(2), 161–169.
- Hippler, D., Eisenhauer, A., et al. 2006. “Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140ka.” *Geochim Cosmochim Acta*, **70**, 90–100, doi:10.1016/j.gca.2005.07.022.
- Holland, H. D., 1984. *The Chemical Evolution of the Atmosphere and Oceans*. Princeton: Princeton University Press.
- Kastner, M., 1999. Oceanic minerals: their origin, nature of their environment, and significance. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 3380–3387.
- Katz, A., 1973. The interaction of magnesium with calcite during crystal growth at 25–90°C and one atmosphere. *Geochimica et Cosmochimica Acta*, **37**(6), 1563–1578, IN3, 1579–1586.
- Kinsman, D. J. J., and Holland, H. D., 1969. The co-precipitation of cations with CaCO_3 . The co-precipitation of Sr^{2+} with aragonite between 16° and 96°C. *Geochimica et Cosmochimica Acta*, **33**, 1–17.
- Kisakürek, B., Eisenhauer, A., et al. 2008. Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminifera, Globigerinoides ruber (white). *Earth and Planetary Science Letters*, **273**(3–4), 260–269.
- Lea, D. W., 2003. Elemental and isotopic proxies of past ocean temperatures. In Holland, H. D., and Turekian K. K. (eds.), *Treatise on Geochemistry*, Oxford: Elsevier, Vol. 6, pp. 365–390.
- Lea, D. W., Mashiotta, T. A., and Spero, H. J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochimica et Cosmochimica Acta*, **63**(16), 2369–2379.
- Lea, D. W., Pak, D. K., and Spero, H. J., 2000. Climate impact of late quaternary equatorial Pacific sea surface temperature variations. *Science*, **289**, 1719–1724.
- Linsley, B. K., Wellington, G. M., and Schrag, D. P., 2000. Decadal sea surface temperature variability in the subtropical South Pacific from 1726 to 1997 A.D. *Science*, **290**, 1145–1148.
- Mantua, J. N., Hare, S. R., Zhang, Y., Wallace, J. M., and Francis, R. C., 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. *American Meteorological Society*, **78**(6), 1069–1079.
- Mashiotta, T. M., Lea, D. W., and Spero, H. J., 1999. Glacial-interglacial changes in Subantarctic sea surface temperature and $\delta^{18}\text{O}$ -water using foraminiferal Mg. *Earth and Planetary Science Letters*, **170**, 417–432.
- McKenzie, J. A., 1981. Holocene dolomitization of calcium carbonate sediments from the coastal sabkhas of Abu Dhabi, UAE: a stable isotope study. *Journal of Geology*, **89**, 185–198.
- Millero, F. J., 1995. Thermodynamics of the carbon dioxide system in the oceans. *Geochimica et Cosmochimica Acta*, **59**(4), 661–677.
- Mottl, M. J., and Holland, H. D., 1978. Chemical exchange during hydrothermal alteration of basalt by seawater-I. Experimental results for major and minor components of seawater. *Geochimica et Cosmochimica Acta*, **42**(8), 1103–1115.
- Mottl, M. J., and Wheat, C. G., 1993. Hydrothermal circulation through mid-ocean ridge flanks: Fluxes of heat and magnesium. *Geochim Cosmochim Acta*, **58**, 2225–2237.
- Nägler, T., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -isotopes: new powerful tool for reconstruction of past sea surface temperatures. *Geochemistry, Geophysics, Geosystems*, **1**(2000GC000091).
- Nürnberg, D., Bijma, J., et al. 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochim Cosmochim Acta*, **60**, 803–814.
- Nürnberg, D., Müller, A., and Schneider, R. R., 2000. Paleo-sea surface temperature calculations in the equatorial east Atlantic from Mg/Ca ratios in planktic foraminifera: a comparison to sea surface temperature estimates from Uk^{37} , oxygen isotopes, and foraminiferal transfer function. *Paleoceanography*, **15**(1), 124–134.
- Savin, S. M., and Douglas, R. G., 1973. Stable isotope and magnesium geochemistry of recent planktonic foraminifera from South-Pacific. *Geological Society of America Bulletin*, **84**(7), 2327–2342.
- Wilkinson, B. H., and Algeo, T. J., 1989. Sedimentary carbonate record of calcium-magnesium cycling. *American Journal of Science*, **289**, 1158–1194.

Cross-references

Alkalinity
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Carbon Cycle
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Isotopes and Geobiology
 Salinity History of the Earth's Ocean
 Soda Ocean Hypothesis

DIVERSITY

The number of different species in a habitat; sometimes (falsely) used as a synonym for →community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

DOLOMITE, MICROBIAL

Jennifer A. Roberts, Paul A. Kenward
 University of Kansas, Lawrence, KS, USA

Synonyms

Bacterial dolomite; Organogenic dolomite

Definition

Dolomite. Widely distributed rock-forming mineral, $\text{CaMg}(\text{CO}_3)_2$. Usually, white or colorless, but can be yellowish and brown with rhombohedral crystals exhibiting curved, composite faces. It also occurs with massive and granular habit and has rhombohedral {1011} cleavage.
Dolomitization. The process of converting calcite in limestones to dolomite by the action of magnesium-bearing solutions.

Introduction

The mineral dolomite is notoriously difficult to synthesize under laboratory conditions at low temperature (less than 50°C) and despite its abundance in the ancient rock record, scarcely forms in modern environments. This apparent departure from the geologists' guiding principle that the "present is the key to the past," established by Hutton's principle of uniformitarianism, is at the heart of the "Dolomite Problem," which is a twofold issue (e.g., McKenzie, 1991).

The dolomite problem

The first aspect of the problem is the abundance of massive dolomites, comprised of secondary or replacement dolomite, in ancient rock, but their striking absence in modern environments. Secondary dolomite is formed through the process of dolomitization, in which the mineral calcite is replaced by dolomite through interaction with Mg-rich fluids (Pursar et al., 1994; Burns et al., 2000, and references therein). The second aspect of the dolomite problem is the difficulty associated with the synthesis of primary dolomite (precipitation of the mineral directly from aqueous solution) at low temperature. Despite conditions favoring its formation and ample time for precipitation, researchers have been unsuccessful in synthesizing dolomite abiotically under laboratory conditions (e.g., Land, 1998), and attribute this lack of success to slow reaction kinetics (Hardie, 1987; Sibley et al., 1987). Recent research has confirmed long-held assertions (e.g., Nadson, 1928; Neher, 1959) that microorganisms play a fundamental role in the formation of dolomite at low-temperature.

Microorganisms as catalysts for dolomite formation

Vasconcelos et al. (1995) successfully synthesized ferroan dolomite in the presence of a native microbial consortium collected from a modern dolomite-precipitating environment, ushering in extensive scrutiny of microorganisms and microbial processes that facilitate dolomite formation. Modern dolomite-forming environments are isolated, and in those that have been extensively studied, dolomite formation is intimately linked to microbial populations and associated activity, for example, the Coorong region of Australia in coastal lakes (Wright, 1999; Wright and Wacey, 2005), Lagoa Vermelha, Brazil, a hypersaline coastal lagoon (Vasconcelos and McKenzie, 1997), and the Persian Gulf sabkhas, evaporitic salt pans (Barbieri et al., 2006). Evidence of primary dolomite precipitation from shallow and deep sea marine sediments, soils, and groundwater has been documented (e.g., Hardie, 1987; Lumsden, 1988; Whipkey et al., 2002; Roberts et al., 2004), but the volume of dolomite in these settings is typically minor, forming as thin beds or nodules. All of these environments and their native microbial communities provide an insight into the populations and the mechanisms by which microorganisms mediate dolomite formation.

Microorganisms implicated in dolomite formation

Whereas a wide variety of microorganisms has been implicated in dolomite formation, the best documented metabolic guild is those bacteria carrying out dissimilatory sulfate reduction under anaerobic conditions. These bacteria utilize organic carbon coupled to sulfate as a terminal electron acceptor for energy, thereby removing sulfate and generating alkalinity. Researchers have inferred their presence from geochemical indicators (e.g., Vasconcelos and McKenzie, 1997), culturing experiments (Warthmann et al., 2000; Wright and Wacey, 2005), and non-culture-based identification. Some of the organisms identified include *Desulfostipes sapovorans* and *Desulfosarcina* spp. (Wright and Wacey, 2005) and *Desulfovibrio brasiliensis* (Warthmann et al., 2000).

Two other types of bacterial metabolic pathways have also been shown to mediate low-temperature dolomite formation. Two moderately halophilic aerobic heterotrophs, *Halomonas meridiana* and *Virgibacillus marismortui*, produce dolomite under laboratory conditions (Sánchez-Román et al., 2008). These organisms utilize nitrogenated organic matter coupled to oxygen as a terminal electron acceptor, consuming acidity and producing alkalinity. The other bacterial metabolic guild implicated in dolomite precipitation is the sulfide oxidizers, which oxidize H₂S, producing sulfate and acidity (Moreira et al., 2004). This interaction has been inferred from geochemical and isotopic data from Lagoa Araruama, Lagoa Vermelha, and Brejo do Espinho, Brazil, and the production of H₂S in these environments is produced via bacterial sulfate reduction, also thought to produce microbial dolomites.

Additional metabolic pathways linked to dolomite precipitation are part of the Archaeal domain, and involve either the production or utilization of methane. Anaerobic methane oxidizers, which utilize methane and generate alkalinity, in concert with methanogens, were found to control low temperature dolomite formation in deep-sea sediments as inferred from geochemical and isotopic data (Moore et al., 2004). Isotopic signatures from a range of ancient sediments indicate that the activity of methanogens is linked to dolomite formation (e.g., Mazullo, 2000). Additionally, recent studies have linked methanogenesis to the precipitation of dolomite in shallow groundwater (Roberts et al., 2004). Laboratory experiments identified two methanogenic metabolic pathways associated with dolomite formation, acetoclastic and autotrophic. Acetoclastic methanogens cleave acetate forming methane and carbon dioxide, whereas autotrophic methanogens combine carbon dioxide and hydrogen to form methane. These pathways were associated with *Methanosaeta* spp. and *Methanobacterium* spp., respectively (Kenward et al., 2009).

Mechanisms for microbial mediation of dolomite

Although it is clear that the presence of microorganisms under some geochemical conditions promotes dolomite

precipitation, there is still debate as to *how* microorganisms are involved in facilitating precipitation.

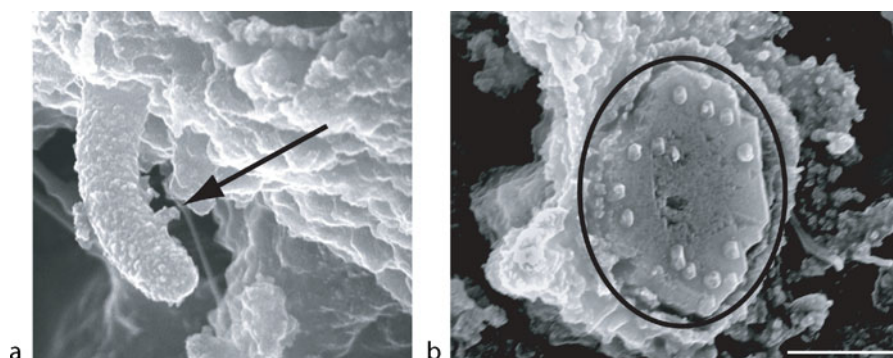
The most important criterion for the precipitation of dolomite is that supersaturated conditions exist. Many of the microbial guilds discussed previously are capable of driving supersaturation of dolomite as a result of their metabolic pathways. Sulfate reduction, aerobic heterotrophic respiration, anaerobic methane oxidation, and acetoclastic methanogenesis produce alkalinity as a by-product of metabolism, which increases supersaturation with respect to carbonate minerals. Sulfide oxidation produces acidity, which lowers the saturation state of competing carbonate minerals, thus, favoring the supersaturation of dolomite. Sulfate reduction, aerobic respiration of nitrogenated carbon compounds, autotrophic methanogenesis, and anaerobic methane oxidation increase pH in the system, which also favors supersaturation of the carbonate minerals (Madigan and Martinko, 2005).

Although microbial metabolism can generate solutions supersaturated with respect to dolomite, it has been demonstrated that supersaturation alone does not lead to the precipitation of dolomite and, therefore, microbes likely facilitate dolomite formation by removing additional kinetic barriers present at low temperatures. For example, the sulfate inhibition model asserts that sulfate is a kinetic inhibitor of dolomite precipitation because of the strong neutral complex it forms with magnesium (MgSO_4^0), essentially decreasing the Mg available to form dolomite (Baker and Kastner, 1981). This was demonstrated at low sulfate concentrations, hence, favoring those microbial interactions which either remove sulfate or occur under these conditions (i.e., sulfate reduction, methanogenesis, and anaerobic methane oxidation). Other researchers have disputed this model due to the fact that many modern dolomites are formed under conditions with sulfate concentrations equal to or above that of seawater (Hardie, 1987) suggesting that sulfate inhibition is not the sole kinetic barrier to dolomite formation. Laboratory studies have shown that at very low or very high sulfate concentrations,

dolomite formation is promoted (Brady et al., 1996; Siegel, 1961). Recently, Sánchez-Román et al. (2009) demonstrated that two moderately halophilic aerobic heterotrophic bacteria could promote dolomite precipitation at sulfate concentrations ranging from 0 to 56 mM. Taken together, these data suggest that sulfate may indeed be an inhibitor to dolomite formation under certain conditions, but is likely not the sole kinetic barrier to precipitation.

One common observation in modern microbial dolomites is the intimate association between the precipitated dolomite and the cell wall of the microorganism or extracellular polymeric substances (EPS) produced by the microorganisms. These observations, in combination with evidence of cell-associated mineral nucleation (e.g., Schultze-Lam et al., 1996; Fortin et al., 1997; Douglas and Beveridge, 1998), have lead to the hypothesis that these biological surfaces are involved in the nucleation of dolomite. Laboratory experiments analyzing the charge character of the cell wall or structure, have implicated nucleation directly onto the cell wall (Figure 1; van Lith et al., 2003a; Roberts et al., 2004; Kenward et al., 2009; Bosak and Newman, 2003). Other laboratory investigations mimicking EPS-like conditions or utilizing EPS directly, have shown that nucleation occurs on these substances (Figure 1; Sánchez-Román et al., 2008; Bontognali et al., 2008). Nucleation onto EPS, rather than the cell wall, may benefit the cell by avoiding entombment and is consistent with the fact that cellular material is rarely observed in microbial carbonates (Bontognali et al., 2008).

There are a wide range of potential mechanisms by which the microbial cell wall or EPS may influence mineral precipitation mechanisms and kinetics, including heterogeneous nucleation on the cell wall; enhanced rates of growth via the catalytic influence of organic molecules or surface functional groups; substrate-based coprecipitation; and oriented (epitaxial) aggregation growth (e.g., Fowle and Fein, 2001; Banfield et al., 2000; Chan et al., 2004; Rancourt et al., 2005;



Dolomite, Microbial, Figure 1 Scanning electron photomicrographs of dolomite formed in freshwater in the presence of methanogenic microorganisms. **Panel a** shows a cell embedded in EPS and encrusted with dolomite. Arrow indicates one well-formed crystal. **Panel b** shows a more developed platy crystal of dolomite. Oval denotes boundary between EPS and crystal edge. Note nanoglobules in EPS. Images courtesy of Paul A. Kenward with special thanks to the University of Kansas MAI.

Elhadj et al., 2006; see also Chapters *Microbialites, Modern and Extracellular Polymeric Substances (EPS)*). In particular, Elhadj et al. (2006) demonstrated that small organic molecules, such as those found in cellular material and EPS, influenced calcite growth rates via both electrostatic interactions and their ability to facilitate the release of water molecules around the calcium cation during precipitation. Desolvation of cations has been considered the rate limiting step in many precipitation reactions (Nielsen, 1984) and is consistent with the findings of Brady et al. (1996), who found that dehydration and carbonation of Mg^{2+} was the probable rate-limiting step for dolomite formation at room temperature.

Microbial signatures preserved in dolomite

Two primary signatures preserved in ancient rock are commonly used to classify dolomites as microbial in origin: morphology and textures of the rocks and carbon isotopic values. It has been recognized for some time that biogenic processes impact carbonate mineral morphology and, since the identification of aragonite needle dumbbells as biogenic features (Chafetz and Folk, 1984), these features and others have been considered proxies for bacterially mediated carbonate precipitation. Laboratory studies of microbial dolomite have produced dumbbells (Warthmann et al., 2000; van Lith et al., 2003b), spheroids (Sánchez-Román et al., 2008), nanoscale globules (Figure 1b; Sánchez-Román et al., 2008; Bontognali et al., 2008; Vasconcelos et al., 1995), nanoscale rhombs attached to cells (Figure 1a; Roberts et al., 2004), and platy crystal aggregates (Figure 1b; Kenward et al., 2009). Although many of these features are observed in ancient rocks, they are not limited to a biogenic origin and, therefore, not definitive evidence of microbial precipitation.

The composition and isotopic signature associated with carbonate precipitates has been linked with specific microbial communities, including iron reduction, sulfate reduction, and methanogenesis (e.g., Curtis et al., 1986). Those dolomites associated with sulfate reduction are typically low in Fe (due to pyrite precipitation) and ^{13}C -depleted, whereas methanogenic dolomites tend to have higher Fe abundance and are ^{13}C -enriched. Potentially, diagnostic $\delta^{18}O_{\text{dolomite}}$ signatures related to temperature (or potentially depth) of precipitation have been difficult to interpret due to the lack of experimental data for low temperature dolomite. Vasconcelos et al. (2005), however, proposed a paleothermometer for dolomite precipitation based on dolomite produced by sulfate reducing bacteria. Despite these efforts, extraordinary fractionation of $\delta^{18}O_{\text{dolomite}}$ has been observed via methanogenic activity that is inconsistent with the current paleothermometer (e.g., Roberts et al., 2004).

Summary

For more than a century, the Dolomite Problem has stumped scientists. In recent years, progress towards

a solution has been made by understanding the role of microorganisms in precipitating the mineral dolomite in low temperature (less than 50°C) environments. Many microorganisms, in both the Archaeal and Bacterial domains, are involved in these reactions, suggesting that microbial dolomite can form in a variety of biogeochemical environments. Changes in environmental conditions favoring one microorganism over another throughout time, is a potential explanation for the observed changes in abundance of dolomite from the past to the present. The mechanism by which microorganisms actually facilitate precipitation and increase reaction progress, is still debated. However, recent data point to a mechanism involving the microbial cell wall or cellular exudates overcoming kinetic barriers rather than a metabolic or geochemical control.

Bibliography

- Baker, P. A., and Kastner, M., 1981. Constraints on the formation of sedimentary dolomite. *Science*, **213**, 214–216.
- Banfield, J. F., Welch, S. A., Zhang, H., Thompson-Ebert, T., and Penn, R. L., 2000. Aggregation based crystal growth and microstructure development in natural iron oxyhydroxide biomineralization products. *Science*, **289**, 751–754.
- Barbieri, R., Stivaletta, N., Marinangeli, L., and Ori, G. G., 2006. Microbial signatures in sabkha evaporite deposits of Chott el Gharsa (Tunisia) and their astrobiological implications. *Planetary and Space Science*, **54**, 726–736.
- Bontognali, T. R. R., Vasconcelos, C., Warthmann, R. J., Dupraz, C., Bernasconi, S. M., and McKenzie, J. A., 2008. Microbes produce nanobacteria-like structures, avoiding cell entombment. *Geology*, **36**, 663–666.
- Bosak, T., and Newman, D. K., 2003. Microbial nucleation of calcium carbonate in the Precambrian. *Geology*, **31**, 577–580.
- Brady, P. V., Krumhans, J. L., and Papenguth, H. W., 1996. Surface complexation clues to dolomite growth. *Geochimica et Cosmochimica Acta*, **60**, 727–731.
- Burns, S. J., McKenzie, J. A., and Vasconcelos, C., 2000. Dolomite formation and biochemical cycles in the Phanerozoic. *Sedimentology*, **47**, 49–61.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituent. *Journal of Sedimentary Petrology*, **54**, 289–316.
- Chan, C. S., De Stasio, G., Welch, S. A., Girasole, M., Frazer, B. H., Nesterova, M. V., Fakra, S., and Banfield, J. F., 2004. Microbial polysaccharides template assembly of nanocrystal fibers. *Science*, **303**, 1656–1658.
- Curtis, C. D., Coleman, M. L., and Love, L. G., 1986. Pore water evolution during sediment burial from isotopic and mineral chemistry of calcite, dolomite and siderite concretions. *Geochimica et Cosmochimica Acta*, **50**, 2321–2334.
- Douglas, S., and Beveridge, T. J., 1998. Mineral formation by bacterial in natural microbial communities. *FEMS Microbial Ecology*, **26**, 79–88.
- Elhadj, S., DeYoreo, J. J., Hoyer, J. R., and Dove, P. M., 2006. Role of molecular charge and hydrophobicity in regulating the kinetics of crystal growth. *Proceedings of the National Academy of Science*, **103**, 19237–19242.
- Fortin, D., Ferris, F. G., and Beveridge, T. J., 1997. Surface-mediated mineral development by bacteria. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Mineral, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America.

- Fowle, D. A., and Fein, J. B., 2001. Quantifying the effects of *Bacillus subtilis* cell walls on the precipitation of copper hydroxide from aqueous solution. *Geomicrobiology Journal*, **18**, 77–91.
- Hardie, L. A., 1987. Dolomitization: a critical view of some current views. *Journal of Sedimentary Petrology*, **57**, 166–183.
- Kenward, P. A., Goldstein, R. G., Gonzalez, L. A., and Roberts, J. A., 2009. Precipitation of low-temperature dolomite from an anaerobic microbial consortium: the role of methanogenic Archaea. *Geobiology*, **7**, 556–565.
- Land, L. S., 1998. Failure to precipitate dolomite at 25 (C from dilute solution despite 1000-fold oversaturation after 32 yr. *Aquatic Geochemistry*, **4**, 361–368.
- Lumsden, D. N., 1988. Characteristics of deep marine dolomite. *Journal of Sedimentary Petrology*, **58**, 1023–1031.
- Madigan, M. T., and Martinko, J. M., 2005. *Brock Biology of Microorganisms*, 11th edn. Upper Saddle River, NJ: Pearson Prentice Hall.
- Mazullo, S. J., 2000. Organogenic dolomitization in peritidal to deep-sea sediments. *Journal of Sedimentary Research*, **70**, 10–23.
- McKenzie, J. A., 1991. The dolomite problem: an outstanding controversy. In Muller, D. W., Weisser, H., and McKenzie, J. A. (eds.), *Controversies in Modern Geology*. New York: Academic Press, 490 p.
- Moore, T. S., Murray, R. W., Kurtz, A. C., and Schrag, D. P., 2004. Anaerobic methane oxidation and the formation of dolomite. *Earth and Planetary Science Letters*, **229**, 141–154.
- Moreira, N. F., Walter, L. M., Vasconcelos, C., McKenzie, J. A., and McCall, P. J., 2004. Role of sulfide oxidation in dolomitization: sediment and pore-water geochemistry of a modern hypersaline lagoon system. *Geology*, **32**, 701–704.
- Nadson, G. A., 1928. Breitag zur kenntnis der bakteriogenen kalkablagerungen. *Archiv fuer Hydrobiologie*, **19**, 154–164.
- Neher, J., 1959. Bakterien in tieferliegenden gesteinslagen. *Eclogae Geologicae Helvetiae*, **52**, 619–625.
- Nielsen, A. E., 1984. Electrolyte crystal-growth mechanisms. *Journal of Crystal Growth*, **67**, 289–310.
- Pursar, B. H., Tucker, M. E., and Zenger, D. H., 1994. Problems, progress and future research concerning dolomites and dolomitization. In Pursar, B., Tucker, M., and Zenger, D. (eds.), *Dolomites: A Volume in Honour of Dolomieu*. Publication of the International Association of Sedimentology, Vol. 21, pp. 3–20.
- Rancourt, D. G., Thibault, P. J., Mavrocordatos, D., and Lamarche, G., 2005. Hydrous ferrous oxide precipitation in the presence of nonmetabolizing bacteria: constraints on the mechanism of a biotic effect. *Geochimica et Cosmochimica Acta*, **69**, 553–577.
- Roberts, J. A., Bennett, P. C., Gonzalez, L. A., Macpherson, G. L., and Milliken, K. L., 2004. Microbial precipitation of dolomite in methanogenic groundwater. *Geology*, **32**, 277–280.
- Sánchez-Román, M., Vasconcelos, C., Schmid, T., Dittrich, M., McKenzie, J. A., Zenobi, R., and Rivadeneyra, M. A., 2008. Aerobic microbial dolomite at the nanometer scale: implications for the geologic record. *Geology*, **36**, 879–882.
- Sánchez-Román, M., McKenzie, J. A., Wagener, A., Rivadeneyra, M. A., and Vasconcelos, C., 2009. Presence of sulfate does not inhibit low-temperature dolomite formation. *Earth and Planetary Science Letters*, **285**, 131–139.
- Schultze-Lam, S., Fortin, D., Davis, B. S., and Beveridge, T. J., 1996. Mineralization of bacterial surfaces. *Chemical Geology*, **132**, 171–181.
- Sibley, D. F., Dedoes, R. E., and Bartlett, T. R., 1987. The kinetics of dolomitization. *Geology*, **15**, 1112–1114.
- Siegel, F. R., 1961. Factors influencing the precipitation of dolomite. *Kansas Geological Survey Bulletin*, **152**, 127–158.
- van Lith, Y., Warthmann, R., Vasconcelos, C., and McKenzie, J. A., 2003a. Microbial fossilization in carbonate sediments: a result of the bacterial surface involvement in dolomite precipitation. *Sedimentology*, **50**, 237–245.
- van Lith, Y., Warthmann, R., Vasconcelos, C., and McKenzie, J. A., 2003b. Sulphate-reducing bacteria induce low-temperature Ca-dolomite and high Mg-calcite formation. *Geobiology*, **1**, 71–79.
- Vasconcelos, C., and McKenzie, J. A., 1997. Microbial mediation of modern dolomite precipitation and diagenesis under anoxic conditions, Lagoa Vermelha, Rio de Janeiro, Brazil. *Journal of Sedimentary Research*, **67**, 378–390.
- Vasconcelos, C., McKenzie, J. A., Bernasconi, S., Grujic, D., and Tien, A. J., 1995. Microbial mediation as a possible mechanism for natural dolomite formation at low temperatures. *Nature*, **377**, 220–222.
- Vasconcelos, C., McKenzie, J. A., Warthmann, R., and Bernasconi, S. M., 2005. Calibration of the $\delta^{18}\text{O}$ paleothermometer for dolomite precipitated in microbial cultures and natural environments. *Geology*, **33**, 317–320.
- Warthmann, R., van Lith, Y., Vasconcelos, C., McKenzie, J. A., and Karpoff, A. M., 2000. Bacterially induced dolomite precipitation in anoxic culture experiments. *Geology*, **28**, 1091–1094.
- Whipkey, C. E., Capo, R. C., Hsieh, J. C. C., and Chadwick, O. A., 2002. Development of magnesian carbonates in Quaternary soils on the Island of Hawaii. *Journal of Sedimentary Research*, **72**, 158–165.
- Wright, D., 1999. The role of sulfate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia. *Sedimentary Geology*, **126**, 147–157.
- Wright, D. T., and Wacey, D., 2005. Precipitation of dolomite using sulphate reducing bacteria from the Coorong Region, South Australia: significance and implications. *Sedimentology*, **52**, 987–1008.

Cross-references

Archaea

[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)
[Biosignatures in Rocks](#)
[Calcite Precipitation, Microbially Induced](#)
[Cap Carbonates](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)

E

EARLY EARTH

See entries “*Origin of Life*,” “*Salinity History of the Earth’s Ocean*,” and “*Critical Intervals in Earth History*.”

EARLY PRECAMBRIAN EUKARYOTES

Joachim Reitner
University of Göttingen, Göttingen, Germany

Definition

Eukaryotes are probably the result of endosymbiosis, and basically three types exist. The basic ones are the simplest eukaryotic cells with a nucleus only (*Giardia*) (Graczk, 2005). Secondly, protists have mitochondria, which have been proposed to have an alpha-proteobacterial origin (Sagan (Margulis), 1967). Finally, beside mitochondria, cells of eukaryotic algae contain chloroplasts, former single-celled cyanobacteria achieved via endosymbiosis (Mereschkowsky, 1905; Wallin, 1920; Sagan (Margulis), 1967). The geological record of protists begins long before the Ediacaran vendobionts. Possibly stem group protists were found in ca. 3 Ga-old rocks (Javaux et al., 2010). Within the Late Mesoproterozoic rocks, the divergence of major eukaryotic clades is observed. Beside the fossil record of early eukaryotes, related biomarkers (steranes) were found in up to 2.7 Ga-old rocks, which may support the early origin of eukaryotes before the Great Oxidation Event (Brocks et al., 1999; Brocks et al., 2003a, b). However, the syngeneity of the biomarkers used for the interpretation has recently been questioned (Rasmussen et al., 2008) and the first appearance of eukaryotic biomarkers is still disputed (Brocks

et al., 1999; George et al., 2008; Rasmussen et al., 2008; Waldbauer et al., 2009).

The oldest known single-celled eukaryotes are acritarchs, probably ancestors of dinoflagellates. Acritarchs played a significant role in Proterozoic oceans, and the first record comes from the 3.2 Ga-old Moodies Group of South Africa (Javaux et al., 2010). This finding is surprising, since eukaryotic metabolisms normally need oxygen, and the Great Oxidation Event took place about 700 My later, between 2.32 and 2.45 Ga. A similar discrepancy emerged from the first appearance of eukaryotic biomarkers in 2.7 Ga-old rocks (Brocks et al., 1999). Although often controversially debated, biomarker records may give excellent indications to the diversification of major phylogenetic clades. For instance, the distribution of 24-isopropylcholestane revealed a high abundance of (demo-)sponges in the Cryogenian (McCaffrey et al., 1994; Love et al., 2009), thus suggesting sufficient oxygen in shelf regions already 100 My prior to the Cambrian explosion. Furthermore, early Precambrian eukaryotic life is not only indicated by biomarker findings. In some diagenetically early silicified stromatolites, well-preserved eukaryotic cells have been described. The best material is known from the 1.88 Gy-old Gunflint Chert (Ontario, Canada) (Fralick et al., 2002; Awramik and Barghoorn, 1977). *Eosphaera tyleri* from these cherts is probably an eukaryotic cell (Kazmierczak, 1979). Beside the single-celled eukaryotes, few larger fossils which are most likely multicellular organisms are known, such as *Grypania spiralis* from the Mesoproterozoic Gaoyuzhuang Formation (China) and disk-shaped centimeter-sized organisms like *Chuaria* (Knoll et al., 2006).

In the Cryogenian and Ediacaran, the diversity of eukaryotes, including fungi and lichens, increased significantly. It is obvious that the increase of diversity and size

is related with the Snowball Earth scenarios and increase of oxygen in the atmosphere.

Bibliography

- Awramik, S. M., and Barghoorn, E. S., 1997. The Gunflint microbiota. *Precambrian Research*, **5**, 121–142.
- Brocks, J. J., Summons, R. E., Logan, G. A., and Buick, R., 2003a. Molecular fossils in Archean rocks II: composition, thermal maturity and integrity of hydrocarbons extracted from sedimentary rocks of the 2.78–2.45 billion years old Mount Bruce Supergroup, Pilbara Craton, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4289–4319.
- Brocks, J. J., Buick, R., Summons, R. E., and Logan, G. A., 2003b. A reconstruction of Archean biological diversity based on molecular fossils from the 2.78–2.45 billion year old Mount Bruce Supergroup, Hamersley Basin, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4321–4335.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of Eukaryotes. *Science*, **285**, 1033–1036.
- Fralick, P., Davis, D. W., and Kissin, S. A., 2002. The age of the Gunflint Formation, Ontario, Canada: single zircon U–Pb age determinations from reworked volcanic ash. *Canadian Journal of Earth Science*, **39**, 1085–1091.
- George, S. C., Volk, H., Dutkiewicz, A., Ridley, J., and Buick, R., 2008. Preservation of hydrocarbons and biomarkers in oil trapped inside fluid inclusions for >2 billion years. *Geochimica et Cosmochimica Acta*, **72**, 844–870.
- Graczyk, T. K., 2005. Is Giardia a living fossil? *Trends in Parasitology*, **21**, 104–107.
- Javaux, E., Marshall, C. P., and Bekker, A., 2010. Organic-walled microfossils in 3.2-billion-year-old shallow-marine siliciclastic deposits. *Nature*, **463**, 934–938.
- Kazmierczak, J., 1979. The eukaryotic nature of Eosphaera-like feriferous structures from the Precambrian Gunflint Iron Formation, Canada: a comparative study. *Precambrian Research*, **9**, 1–22.
- Knoll, A. H., Javaux, E. J., Hewitt, D. and Cohen, P., 2006. Eukaryotic organisms in Proterozoic oceans. Philosophical Transactions of the Royal Society of London B, **361**, 1023–1038.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–721.
- MaCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Palaeoenvironmental implications of novel C30 steranes in Precambrian to Cenozoic age petroleum and bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Mereschkowski, C., 1905. Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biologisches Centralblatt*, **25**, 593–604.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J., and Kilburn, M. R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, **455**, 1101–1104.
- Sagan, L. (Margulis, L.) 1967. On the origin of mitosing cells. *Journal of Theoretical Biology*, **14**(3), 255–274.
- Wallin, I. E., 1923. The Mitochondria Problem. *The American Naturalist*, **57**(650), 255–261.
- Waldbauer, J. R., Sherman, L. S., Sumner, D. Y., and Summons, R. E., 2009. Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis. *Precambrian Research*, **169**, 28–47.

Cross-references

Algae (Eukaryotic)
 Biomarkers (Molecular Fossils)
 Critical Intervals in Earth History
 Ediacara Biota
 Geomycology
 Lateral Gene Transfer
 Origin of Metazoa
 Protozoa (Heterotroph, Eukaryotic)
 Sponges (Porifera) and Sponge Microbes
 Symbiosis

ECOLOGICAL NICHE

Different from the habitat (see entry *Habitat*) (spatial), the niche defines the function of an organism. See entry “*Microbial Communities, Structure, and Function*” for further reading.

EDIACARAN BIOTA

Dmitriy Grazhdankin
 Russian Academy of Sciences, Novosibirsk, Russia

Definition

Ediacaran period: An interval in the history of earth after the Marinoan/Varanger glaciation of the Neoproterozoic Era (see Chapter *Snowball Earth*), but before the Cambrian radiations. This interval marks the introduction of complex macroscopic organisms, leading to a revolution in the structure and evolution of marine paleocommunities, including the establishment of multi-level trophic structures, coevolutionary predator–prey interactions and infauunal activity.

Ediacaran biota: A highly distinctive assemblage of Ediacaran-age macroscopic organisms preserved as casts and moulds in siliciclastic, volcanoclastic, and carbonate sediments.

Introduction

Ediacaran biota is a heterogeneous assemblage representing the earliest known communities of macroscopic organisms. Diverse Ediacaran fossil assemblages are coeval with the earliest phosphatized metazoan embryos (Xiao et al., 1998; but see Bailey et al., 2007; Xiao et al., 2007), the earliest undisputed traces of metazoan activities (Jensen, 2003), and the time of eumetazoan crown-group divergence estimated by some (but not all) molecular clocks (Peterson and Butterfield, 2005). Nonetheless, placing the Ediacaran morphologies into a metazoan phylogeny has been an enduring challenge to paleobiologists (Gehling, 1991; Fedonkin, 1992; Jenkins,

1992; Seilacher, 1992; Conway Morris, 1993; Runnegar, 1995; Budd and Jensen, 2000; Dzik, 2003; Narbonne, 2005; Fedonkin et al., 2007a). Earlier attempts to classify these organisms, based on superficial similarities with modern taxa, resulted in interpretations relating them with crown-group animals, specifically, colonial hydrozoans, scyphozoans, sessile colonial anthozoans, annelids, primitive arthropods, and echinoderms (Glaessner, 1984). Over time, various members of the Ediacaran biota have been regarded as representatives of a number of groups spanning several Kingdoms including prokaryotic colonies (Steiner and Reitner, 2001); eukaryotic microbial colonies (Grazhdankin and Gerdes, 2007); xenophyophoran protists (Zhuravlev, 1993; Seilacher et al., 2003); intermediates between plants and animals (Pflug, 1972); marine fungi (Peterson et al., 2003); lichens (Retallack, 2007); sponges (Gehling and Rigby, 1996); ctenophores (Dzik, 2002); and quasi-molluscs (Fedonkin and Waggoner, 1997; Fedonkin et al., 2007b). Seilacher famously proposed in 1989 and 1992 that Ediacaran biota represents an entirely extinct lineage of macroscopic organisms, the Vendobionta, which were based on a “quilted pneu” or air-mattress type modular construction and lacked any significant tissue or organ differentiation. To date, no agreement has been reached concerning either the biological affinities of the Ediacaran biota or the degree of its phylogenetic heterogeneity. It does appear, however, that most Ediacaran organisms lack any compelling similarities with metazoans (Seilacher, 1989, 1992; Retallack, 1994; Grazhdankin and Seilacher, 2002, 2005; Narbonne, 2004; Brasier and Antcliffe, 2004).

Morphological disparity

The overall taxonomic diversity of the Ediacaran biota is modest. A little over 250 species have been described, of which half represents valid taxa and the rest either pseudofossils or synonyms reflecting life stage and preservational variations. At least eight high-level taxonomic groups can be recognized demonstrating considerable disparity within the Ediacaran biota. (1) Rangeomorpha comprises of fusiform, foliate, and plumose organisms characterized by branched tubular structures (Narbonne, 2004). (2) Frondomorpha are composed of three distinct parts: a large, relatively flattened foliate section, a central stem, and a holdfast or rooting anchor (Laflamme and Narbonne, 2008); the fossil record of these organisms may be biased towards holdfasts that have a much higher preservation potential compared to foliate sections (Gehling et al., 2000; Hofmann et al., 2008). (3) Discoidal structures exhibit dense concentric rugae or broad concentric rings, occasionally with radial furrows (Grazhdankin and Gerdes, 2007). (4) Palaeopascichnida consist of series (sometimes multiple series and even foam-like aggregations) of sausage-shaped or globular chambers that are occasionally branching (Seilacher et al., 2003). (5) Tribrachiomorpha, a bizarre group of discoidal organisms with prominent three-fold symmetry

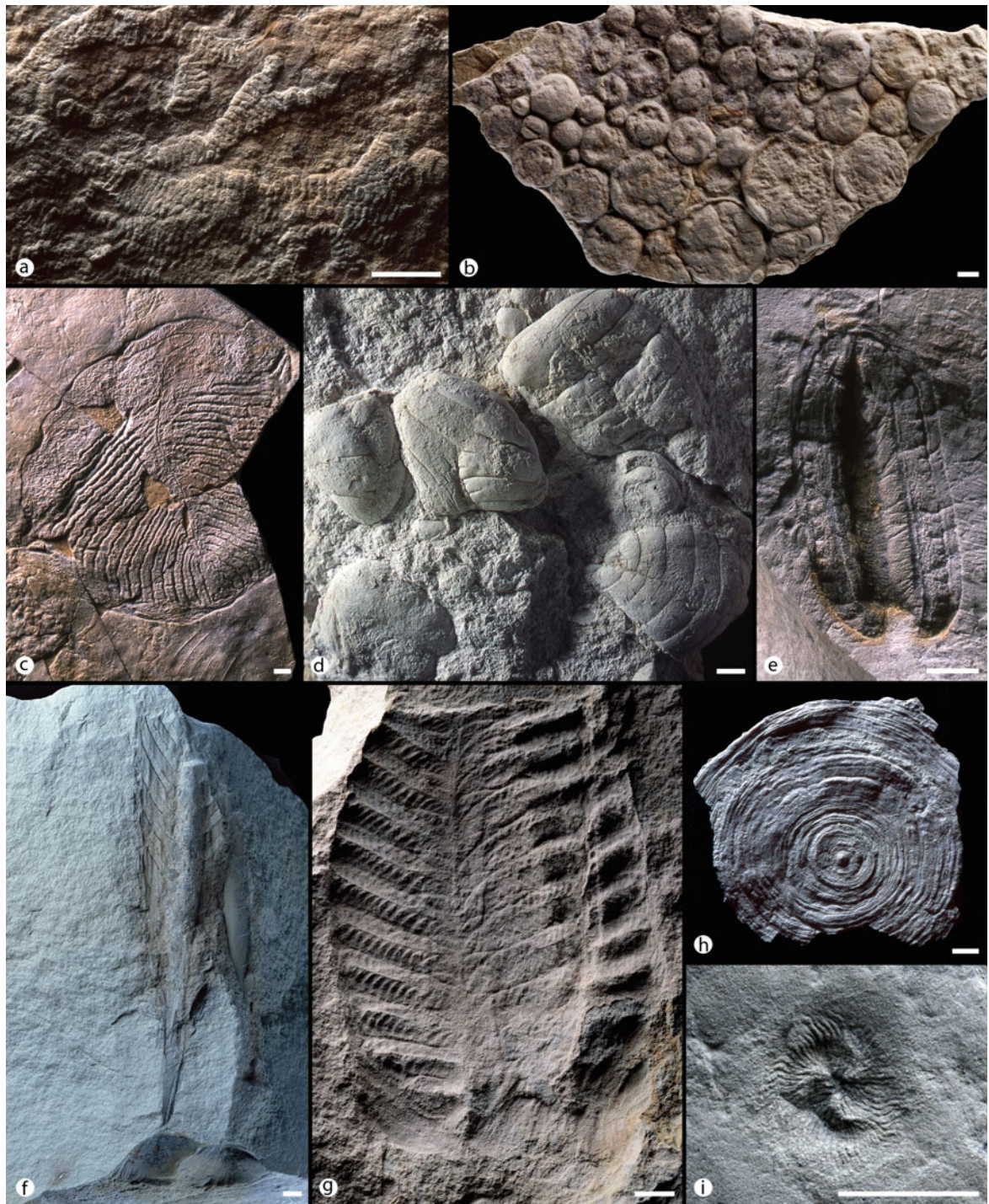
(Fedonkin, 1992). (6) Bilateromorpha, as the name says, comprise organisms with a bilaterally symmetric body (i.e., that consists of mirror image halves) (Fedonkin et al., 2007b). (7) Dickinsoniomorpha are flat and thin, at first glance bilaterally symmetric forms that consist of large number of segments, with one segment having a distinctive crescent shape and superficially resembling an arthropodal head-shield; the segments of the two sides do not line up perfectly along the midline, however, but are offset, are alternate, a kind of symmetry known as glide reflection (as opposed to mirror reflection symmetry) (Ivantsov, 1999, 2001, 2004, 2007). (8) Petalonamae, an imperfectly defined group dominated by serially quilted body plans, most exotic to mainstream biology; their mouldic preservation within sandstone is intriguing in that the specimens appear in variously curved and oriented shapes, approximately resembling internal moulds of pots and troughs (Pflug, 1972; Grazhdankin and Seilacher, 2002) (Figure 1).

Patterns of distribution

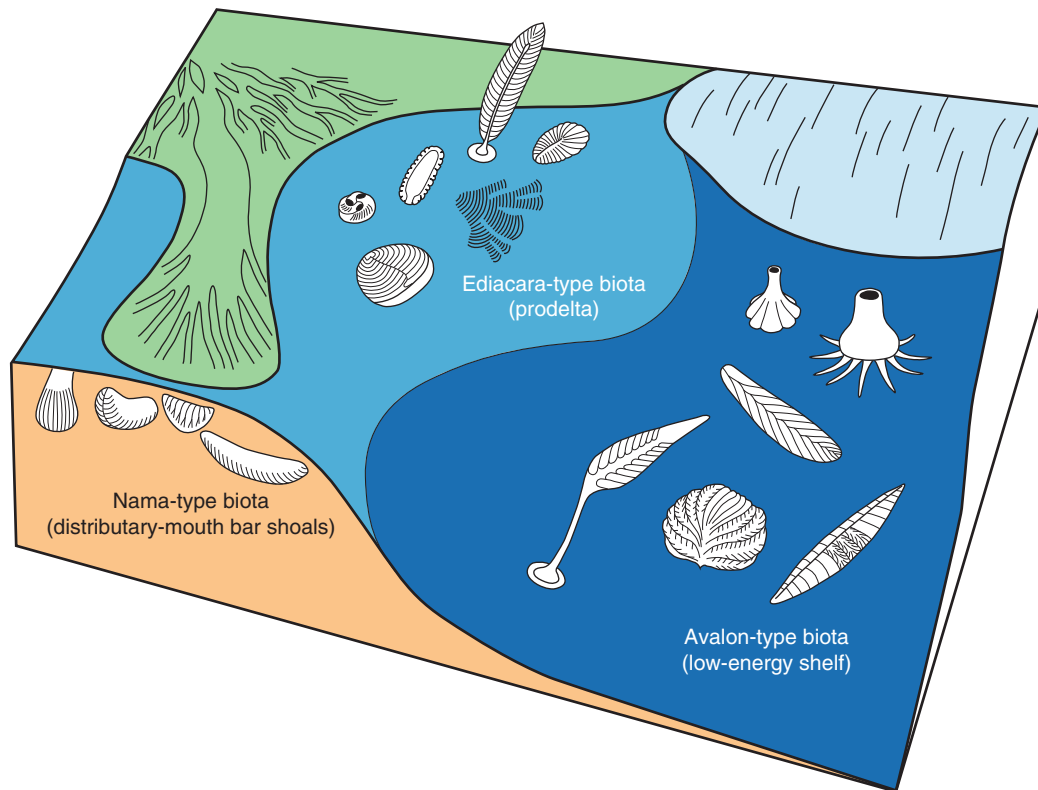
Ediacaran fossils have been documented globally within a discrete and well constrained interval of geological time between 575 and 544 Ma. Within this range, there is a clustering of three taxonomically distinct fossil assemblages: the Avalon-, Ediacara- and Nama-type biotas (Waggoner, 2003). The Avalon-type biota consists of rangeo- and frondomorphs. The Ediacara-type biota is the most diverse assemblage characterized by discoidal structures, frondo-, tribrachio-, bilatero-, and dickinsoniomorphs. The Nama-type biota is dominated by petalonamae. Palaeopascichnida are unique in that they occur in all three assemblages. The Avalon-, Ediacara-, and Nama-type biotas have been interpreted as evolutionary lineages, or biogeographic provinces, or preservational artefacts (Waggoner, 1999, 2003; Narbonne, 2005; Shen et al., 2008). It remains the case, however, that the Avalon-, Ediacara-, and Nama-type biotas were each confined to a certain habitat. These assemblages thus represent three principal ecotypes on a global scale, with Avalon-type biotas distributed in quiet waters on muddy substrates; Ediacara-type biotas inhabiting microbial substrates in shallow marine wave- and current-agitated settings; and Nama-type biotas found in extremely shallow-water river-mouth bar shoals (Grazhdankin, 2004) (Figure 2). Within each ecotype, species distribution and abundances exhibit extreme heterogeneity and patchiness on a population scale (Clapham and Narbonne, 2002; Clapham et al., 2003; Droser et al., 2006; Droser and Gehling, 2008). This may result from a variety of factors, including competitive interactions, initial distribution of colonists, history of disturbance, or underlying heterogeneity of the abiotic environment.

Preservation

Ediacaran organisms lack hard skeletons, yet the fossil preservation of their soft parts has almost no counterpart



Ediacaran Biota, Figure 1 Morphological disparity of the Ediacaran biota. (a) *Palaeopascichnus delicatus*, a member of Palaeopascichnida (hyporelief). (b) *Nemiana simplex*, a member of Psammocorallia, a group of radially symmetrical sand bodies, with smooth hemispherical lower surface (hyporelief). (c) *Yorgia waggoneri*, a member of Dickinsoniomorpha (hyporelief). (d) *Ventogyrus chistyakovi*, a member of Petalonamae (hyporelief). (e) *Kimberella quadrata*, a member of Bilateromorpha (hyporelief). (f) *Charniodiscus concentricus*, a member of Frondomorpha (vertical preservation in sandstone). (g) *Rangea schneiderhoejni*, a member of Rangeomorpha (hyporelief). (h) *Cyclomedusa davidi*, a discoidal structure (hyporelief). (i) *Tribrachidium heraldicum*, a member of Tribrachiomorpha (hyporelief). (a), (c–i) Southeast White Sea area, Russia. (b), Podolia, Ukraine. Scale bar, 10 mm.



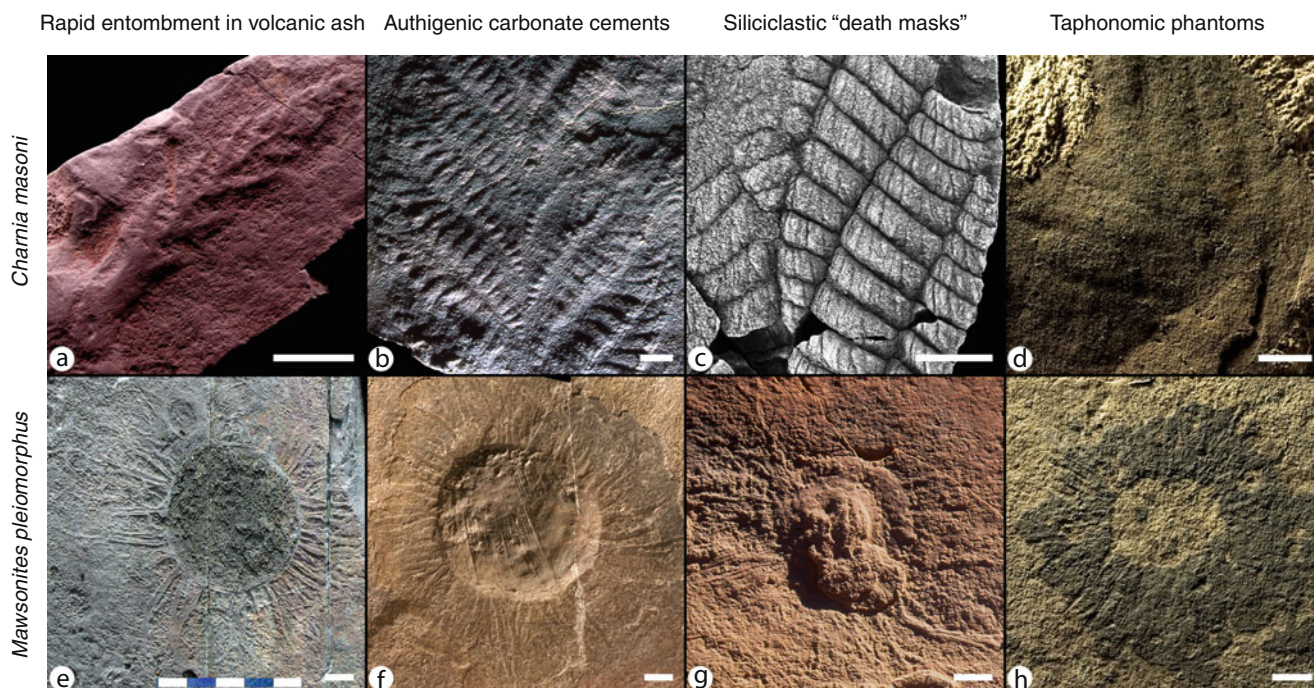
Ediacaran Biota, Figure 2 Patterns of distribution in the Ediacaran biotas. The Avalon-, Ediacara- and Nama-type biotas were each confined to a certain habitat.

to the classic examples in Phanerozoic (Conway Morris and Grazhdankin, 2005) (Figure 3). The preservation of Ediacaran fossils in coarse-grained sandstones might suggest that the organisms had an unusually tough integument (Seilacher, 1992), comparable with chitin of fungal cell walls (Retallack, 1994, 2007). A unique histology of Ediacaran bodies, such as presence of biomaterials indigestible for contemporary microorganisms or possible absence of degrading enzymes such as collagenase is sometimes invoked (Runnegar, 1991; Dzik, 1999). However, examples of Ediacaran fossils encrusted with aggregations of microcrystalline pyrite suggest that the Ediacaran tissues were not immune to microbial degradation after death, and that their decay fueled reduction of seawater sulfate. The most widely accepted view is that Ediacaran taphonomy represents a non-uniformitarian mode of preservation, associated with extensive pre-Cambrian microbial mat cover; certainly many of these fossils occur on microbially bound bedding surfaces characterized by finely-wrinkled, shagreen texture, and other microbially induced sedimentary structures (Gehling, 1991, 1999; Seilacher, 1999). Microbial mats and biofilms may have provided an adhesive surface to anchor benthic organisms during storms or inundation events, as well as protected the substrate from erosion. Storm deposits could

be subsequently recolonized by a new *microbial mat* that would in turn reduce particle and fluid mixing between oxic and anoxic layers in the sediment thereby facilitating early diagenesis. Sediments sealed by *microbial mats* would effectively act as closed dynamic systems of physical, chemical, and biochemical processes (see Chapter *Sediment Diagenesis – Biologically Controlled*). A smothered microbial mat itself could provide a template for precipitation of early diagenetic minerals and act as detailed “death-masks” of the associated Ediacaran corpses.

Biogeochemical perturbations

The sudden appearance and global dispersal of densely populated macroscopic communities of Ediacaran organisms would have had global ecological repercussions, and the Ediacaran period is noted for pronounced biogeochemical perturbations. Prior to appearance of the Ediacaran biota (~580 Myr ago), $\Delta\delta^{34}\text{S}$, the difference between coeval carbonate-associated sulfate and pyrite was conspicuously low while the ratios of “highly reactive iron” (i.e., the iron that is geochemically and biologically active during early sediment diagenesis) to total iron were high (Fike et al., 2006; Canfield et al., 2007), both indicating low ambient oxygen (at least locally).



Ediacaran Biota, Figure 3 *Charnia masoni* and *Mawsonites pleiomorphus* seen in four different taphonomic windows: rapid entombment in volcanic ash; authigenic carbonate cementation; siliciclastic microbial “death masks”; and taphonomic phantoms through deformation of associated carbonaceous films. Styles of mouldic preservation do not appear to be taxon selective, and therefore cannot alone be responsible for the morphological distinctiveness of the Ediacaran biota. (a), (c), Southeast White Sea area, Russia. (b), (d), (f), (h), Olenek Uplift, Russia. (e), Bonavista Peninsula, Newfoundland. (g), Chace Range, South Australia.

Following the appearance of the Ediacaran biota, highly reactive iron fell to essentially modern levels, $\Delta\delta^{34}\text{S}$ rose to $>46\text{‰}$ for the first time, and $\delta^{13}\text{C}_{\text{carb}}$ briefly dropped to an all time low of -15‰ PDB (though this was not tracked by $\delta^{13}\text{C}_{\text{org}}$). Current interpretation is that these shifts all mark a substantial rise of oxygen – from perhaps <10 to $>20\%$ PAL – and that the Ediacaran biota evolved immediately in response to the permissive ecology (Fike et al., 2006; Canfield et al., 2007). Alternatively, the rise of oxygen in Ediacaran time could be the consequence of a new-found sink for organic carbon, namely the production of abundant organic-carbon-adsorbing clay minerals – a consequence of newly evolved, soil-forming, terrestrial ecosystems (Kennedy et al., 2006). This, of course, echoes observations of pedogenic features (pertaining to processes of soil formation) in proximity to some Ediacaran fossils (Retallack, 1994, 2007). Thus, some biogeochemical perturbations during the Ediacaran period may have been the consequence of biotic evolution, rather than its cause (Butterfield, 2007).

Summary

Ediacaran biota is a geobiological phenomenon. From the evolutionary point of view it looks like an assortment of multiple experiments in multi-cellularity and/or

macroscopic size. In the ecological perspective, the Ediacaran biota witnessed what is arguably the earth’s most profound shift in ecosystem structure over the past 4 billion years, from an archaic world of limited morphological diversity and extreme evolutionary stasis to one of high diversity and rapid Phanerozoic-like turnover (Butterfield, 2007). The fossilized Ediacaran biota is seen through a unique taphonomic window, a product of intimate short-time relationships between macroscopic organisms and microbial substrates. Irrespective of whether the Ediacaran biota is related to Metazoa that define the Phanerozoic, it represents a key step in the origin of the modern biosphere.

Bibliography

- Bailey, J. V., Joye, S. B., Kalanetra, K. M., Flood, B. E., and Corsetti, F. A., 2007. Evidence of giant sulphur bacteria in Neoproterozoic phosphorites. *Nature*, **445**, 198–201.
- Brasier, M., and Antcliffe, J., 2004. Decoding the Ediacaran enigma. *Science*, **305**, 1115–1117.
- Budd, G. E., and Jensen, S., 2000. A critical reappraisal of the fossil record of the bilaterian phyla. *Biological Reviews*, **75**, 253–295.
- Butterfield, N. J., 2007. Macroevolution and macroecology through deep time. *Palaeontology*, **50**, 41–55.

- Canfield, D. E., Poulton, S. W., and Narbonne, G. M., 2007. Late-Neoproterozoic deep-ocean oxygenation and the rise of animal life. *Science*, **315**, 92–95.
- Clapham, M. E., and Narbonne, G. M., 2002. Ediacaran epifaunal tiering. *Geology*, **30**, 627–630.
- Clapham, M. E., Narbonne, G. M., and Gehling, J. G., 2003. Paleocology of the oldest known animal communities: Ediacaran assemblages at Mistaken Point, Newfoundland. *Paleobiology*, **29**, 527–544.
- Conway Morris, S., 1993. The fossil record and the early evolution of the Metazoa. *Nature*, **361**, 219–225.
- Conway Morris, S., and Grazhdankin, D., 2005. Enigmatic worm-like organisms from the Upper Devonian of New York: an apparent example of Ediacaran-like preservation. *Palaeontology*, **48**, 395–410.
- Droser, M. L., and Gehling, J. G., 2008. Synchronous aggregate growth in an abundant new Ediacaran tubular organism. *Science*, **319**, 1660–1662.
- Droser, M. L., Gehling, J. G., and Jensen, S., 2006. Assemblage palaeoecology of the Ediacara biota: the unabridged edition? *Palaeogeography, Palaeoclimatology, Palaeoecology*, **232**, 131–147.
- Dzik, J., 1999. Organic membranous skeleton of the Precambrian metazoans from Namibia. *Geology*, **27**, 519–522.
- Dzik, J., 2002. Possible ctenophoran affinities of the Precambrian 'sea-pen' *Rangia*. *Journal of Morphology*, **252**, 315–334.
- Dzik, J., 2003. Anatomical information content in the Ediacaran fossils and their possible zoological affinities. *Integrative and Comparative Biology*, **43**, 114–126.
- Fedonkin, M. A., 1992. Vendian faunas and the early evolution of Metazoa. In Lipps, J. H., and Signor, P. W. (eds.), *Origin and Early Evolution of the Metazoa*. New York: Plenum Press, pp. 87–129.
- Fedonkin, M. A., and Waggoner, B. M., 1997. The late Precambrian fossil *Kimberella* is a mollusc-like bilaterian organism. *Nature*, **388**, 868–871.
- Fedonkin, M. A., Gehling, J. G., Grey, K., Narbonne, G. M., and Vickers-Rich, P., 2007a. *The Rise of Animals: Evolution and Diversification of the Kingdom Animalia*. Baltimore: The Johns Hopkins University Press.
- Fedonkin, M. A., Simonetta, A., and Ivantsov, A. Y., 2007b. New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. In Vickers-Rich, P., and Komarow, P. (eds.), *The Rise and Fall of the Ediacaran Biota*. Bath, UK: The Geological Society, pp. 157–179. (Geological Society, London, Special Publications, 286).
- Fike, D. A., Grotzinger, J. P., Pratt, L. M., and Summons, R. E., 2006. Oxidation of the Ediacaran ocean. *Nature*, **444**, 744–747.
- Gehling, J. G., 1991. The case for Ediacaran fossil roots to the Metazoan tree. In Radhakrishna, B. P. (ed.), *The World of Martin F. Glaessner*. Bangalore: Geological Society of India, pp. 181–224. (Geological Society of India Memoir, 20).
- Gehling, J. G., 1999. Microbial mats in the terminal Proterozoic siliciclastics: Ediacaran death masks. *Palaaios*, **14**, 40–57.
- Gehling, J. G., and Rigby, J. K., 1996. Long expected sponges from the Neoproterozoic Ediacara fauna of South Australia. *Journal of Paleontology*, **70**, 185–195.
- Gehling, J. G., Narbonne, G. M., and Anderson, M. M., 2000. The first named Ediacaran body fossil, *Aspidella terranova*. *Palaeontology*, **43**, 427–456.
- Glaessner, M. F., 1984. *The Dawn of Animal Life: A Biohistorical Study*. Cambridge, UK: Cambridge University Press.
- Grazhdankin, D., 2004. Patterns of distribution in the Ediacaran biotas: facies versus biogeography and evolution. *Paleobiology*, **30**, 203–221.
- Grazhdankin, D., and Gerdes, G., 2007. Ediacaran microbial colonies. *Lethaia*, **40**, 201–210.
- Grazhdankin, D., and Seilacher, A., 2002. Underground Vendobionta from Namibia. *Palaeontology*, **45**, 57–78.
- Grazhdankin, D., and Seilacher, A., 2005. A re-examination of the Nama-type Vendian organism *Rangia schneiderhoehni*. *Geological Magazine*, **142**, 571–582.
- Hofmann, H. J., O'Brien, S. J., and King, A. F., 2008. Ediacaran biota on Bonavista Peninsula, Newfoundland, Canada. *Journal of Paleontology*, **82**, 1–36.
- Ivantsov, A. Y., 1999. A new dickinsonid from the Upper Vendian of the White Sea Winter Coast (Russia, Arkhangelsk Region). *Paleontological Journal*, **33**, 211–221.
- Ivantsov, A. Y., 2001. *Vendia* and other Precambrian 'arthropods.' *Paleontological Journal*, **35**, 335–343.
- Ivantsov, A. Y., 2004. New Proarticulata from the Vendian of the Arkhangelsk Region. *Paleontological Journal*, **38**, 247–253.
- Ivantsov, A. Y., 2007. Small Vendian transversely articulated fossils. *Paleontological Journal*, **41**, 113–122.
- Jenkins, R. J. F., 1992. Functional and ecological aspects of Ediacaran assemblages. In Lipps, J. H., and Signor, P. W. (eds.), *Origin and Early Evolution of the Metazoa*. New York: Plenum Press, pp. 131–176.
- Jensen, S., 2003. The Proterozoic and earliest Cambrian trace fossil record; patterns, problems and perspectives. *Integrative and Comparative Biology*, **43**, 219–228.
- Kennedy, M., Droser, M. L., Mayer, L. M., Pevear, D., and Mrofka, D., 2006. Late Precambrian oxygenation; inception of the clay mineral factory. *Science*, **311**, 1446–1449.
- Laflamme, M., and Narbonne, G. M., 2008. Ediacaran fronds. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **258**, 162–179.
- Narbonne, G. M., 2004. Modular construction of early Ediacaran complex life forms. *Science*, **305**, 1141–1144.
- Narbonne, G. M., 2005. The Ediacara biota: Neoproterozoic origin of animals and their ecosystems. *Annual Review of Earth and Planetary Sciences*, **33**, 421–442.
- Peterson, K. J., and Butterfield, N. J., 2005. Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 9547–9552.
- Peterson, K. J., Waggoner, B., and Hagadorn, J. W., 2003. A fungal analog for Newfoundland Ediacaran fossils? *Integrative and Comparative Biology*, **43**, 127–136.
- Pflug, H. D., 1972. Zur Fauna der Nama-Schichten in Südwest-Afrika. III. Erniettomorpha, Bau und Systematik. *Palaeontographica*, **A139**, 134–170.
- Retallack, G. J., 1994. Were the Ediacaran fossils lichens? *Paleobiology*, **20**, 523–544.
- Retallack, G. J., 2007. Growth, decay and burial compaction of *Dickinsonia*, an iconic Ediacaran fossil. *Alcheringa*, **31**, 215–240.
- Runnegar, B. N., 1991. Oxygen and the early evolution of the Metazoa. In Bryant, C. (ed.), *Metazoan Life Without Oxygen*. London: Chapman and Hall, pp. 65–87.
- Runnegar, B. N., 1995. Vendobionta or Metazoa? Developments in understanding the Ediacara 'fauna'. *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen*, **195**, 303–318.
- Seilacher, A., 1989. Vendozoa: organismic construction in the Proterozoic biosphere. *Lethaia*, **22**, 229–239.
- Seilacher, A., 1992. Vendobionta and Psammocorallia: lost constructions of Precambrian evolution. *Journal of the Geological Society, London*, **149**, 607–613.
- Seilacher, A., 1999. Biomat-related lifestyles in Precambrian. *Palaaios*, **14**, 86–93.

- Seilacher, A., Grazhdankin, D., and Legouta, A., 2003. Ediacaran biota: the dawn of animal life in the shadow of giant protists. *Palaeontological Research*, **7**, 43–54.
- Shen, B., Dong, L., Xiao, S., and Kowalewski, M., 2008. The Avalon explosion: evolution of Ediacaran morphospace. *Science*, **319**, 81–84.
- Steiner, M., and Reitner, J., 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. *Geology*, **29**, 1119–1122.
- Waggoner, B. M., 1999. Biogeographic analyses of the Ediacara biota: a conflict with paleotectonic reconstructions. *Paleobiology*, **25**, 440–458.
- Waggoner, B. M., 2003. The Ediacaran biotas in space and time. *Integrative and Comparative Biology*, **43**, 104–113.
- Xiao, S., Hagadorn, J. W., Zhou, C., and Yuan, X., 2007. Rare helical spheroidal fossils from the Doushantuo Lagerstätte: Ediacaran animal embryos come of age? *Geology*, **35**, 115–118.
- Xiao, S., Zhang, Y., and Knoll, A. H., 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature*, **391**, 553–558.
- Zhuravlev, A. Y., 1993. Were the Ediacaran Vendobionta multicellular? *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen*, **190**, 299–314.

Cross-references

Biofilms

[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)

[Critical Intervals in Earth History](#)

[Mat-Related Sedimentary Structures](#)

[Microbial Degradation](#)

[Sediment Diagenesis – Biologically Controlled](#)

[Snowball Earth](#)

ENDOLITHS

Bettina Weber, Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

Definition

Endoliths: Organisms, growing in the interior of rocks. They are cryptoendoliths when growing within structural cavities, chasmoendoliths when inhabiting fissures and cracks of the rock, and euendoliths when actively penetrating rocky substrates (Golubic et al., 1981).

Endolithically growing organisms

The endolithic growth form occurs in a variety of organism groups comprising cyanobacteria, red and green algae, fungi, lichens and nonphotoautotrophic bacteria. Most endolithic habitats (if not all) accommodate several of these organism groups and are accompanied by a large variety of nonphotoautotrophic bacteria (McNamara et al., 2006).

Biogeography

Polar climate

Probably the best known endolithic communities, first described by Friedmann and Ocampo in 1976, are those of the Beacon Sandstone in the dry valleys of southern

Victoria Land, Antarctica. In his studies, Friedmann (1977, 1978) specified cryptoendolithic lichens forming multicolored zones several millimeters wide under the surface of sandstone rocks as well as chasmoendolithic lichens mainly growing in granitic rock substrates. Fertile stages of both allowed them to be identified as *Buellia* sp. (Friedmann et al., 1980) as well as species of the genera *Lecidea* and *Acarospora* (Friedmann, 1982). At a later stage, two eukaryotic communities (one lichen and one green algae – *Hemichloris* – dominated) plus three cyanobacterial communities (a red *Gloeocapsa*, a *Hormathonema-Gloeocapsa* and a *Chroococcidiopsis* community) were distinguished at this site (Friedmann et al., 1988).

Within a coastal desert at Marble Point, near the frozen Ross Sea, Friedmann (1982) identified heavy chasmoendolithic growth within weathered marble, consisting of filamentous green algae and cyanobacteria, being accompanied by colonies of colorless bacteria.

After the first discovery of endoliths in Antarctica, chasmoendolithic algae were also detected in other parts of the continent, such as the Vestfold Hills, Princess Elisabeth Land and Mawson Rock, and Mac. Robertson Land, where Broady (1981) found a variety of green algae and cyanobacteria within quartz stones, charnockites, gneisses and other rocks. Small endolithic colonies with cyanobacterial, bacterial and fungal components growing within a gypsum crust on the surface of sandstone boulders were discovered by Hughes and Lawley (2003) on Alexander Island, Antarctic Peninsula.

The structure of endolithic communities collected from Granite Harbour at the Ross Sea coast was studied by De Los Rios et al. (2003, 2005) utilizing scanning electron microscopy with backscattered electron imaging (SEM-BSE). They describe an intimate contact between the organisms and the surrounding substrate and in a subsequent study, combined with molecular analyses (De Los Rios et al., 2007), they were able to distinguish two different types of endolithic biofilms.

An extensive distribution of a chasmo- and cryptoendolithic cyanobacterial community, dominated by the unicellular cyanobacterium *Chroococcidiopsis* sp., was reported from granite rocks of an area extending at 600 m elevation at Mt. Falconer, in the McMurdo Dry Valleys (Büdel et al., 2008; Figure 1a–c). The authors found that community to be highly active due to regular dewfall on the rock surface. Radiocarbon dating indicated that the community is by far younger than those reported from a 1,000 m higher elevation by Bonani et al. (1988).

Besides Antarctica, cryptoendolithic habitats were also discovered in the Canadian High Arctic (Omelson et al., 2006), hosting an assemblage of cyanobacteria, algae, fungi and heterotrophic bacteria within sandstone rocks.

Arid and semiarid climate

Endolithic cyanobacteria are known to occur in a variety of habitats within hot and tropical regions

- Seilacher, A., Grazhdankin, D., and Legouta, A., 2003. Ediacaran biota: the dawn of animal life in the shadow of giant protists. *Palaeontological Research*, **7**, 43–54.
- Shen, B., Dong, L., Xiao, S., and Kowalewski, M., 2008. The Avalon explosion: evolution of Ediacaran morphospace. *Science*, **319**, 81–84.
- Steiner, M., and Reitner, J., 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. *Geology*, **29**, 1119–1122.
- Waggoner, B. M., 1999. Biogeographic analyses of the Ediacara biota: a conflict with paleotectonic reconstructions. *Paleobiology*, **25**, 440–458.
- Waggoner, B. M., 2003. The Ediacaran biotas in space and time. *Integrative and Comparative Biology*, **43**, 104–113.
- Xiao, S., Hagadorn, J. W., Zhou, C., and Yuan, X., 2007. Rare helical spheroidal fossils from the Doushantuo Lagerstätte: Ediacaran animal embryos come of age? *Geology*, **35**, 115–118.
- Xiao, S., Zhang, Y., and Knoll, A. H., 1998. Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature*, **391**, 553–558.
- Zhuravlev, A. Y., 1993. Were the Ediacaran Vendobionta multicellular? *Neues Jahrbuch für Geologie und Paläontologie Abhandlungen*, **190**, 299–314.

Cross-references

Biofilms

[Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification](#)

[Critical Intervals in Earth History](#)

[Mat-Related Sedimentary Structures](#)

[Microbial Degradation](#)

[Sediment Diagenesis – Biologically Controlled](#)

[Snowball Earth](#)

ENDOLITHS

Bettina Weber, Burkhard Büdel

University of Kaiserslautern, Kaiserslautern, Germany

Definition

Endoliths: Organisms, growing in the interior of rocks. They are cryptoendoliths when growing within structural cavities, chasmoendoliths when inhabiting fissures and cracks of the rock, and euendoliths when actively penetrating rocky substrates (Golubic et al., 1981).

Endolithically growing organisms

The endolithic growth form occurs in a variety of organism groups comprising cyanobacteria, red and green algae, fungi, lichens and nonphotoautotrophic bacteria. Most endolithic habitats (if not all) accommodate several of these organism groups and are accompanied by a large variety of nonphotoautotrophic bacteria (McNamara et al., 2006).

Biogeography

Polar climate

Probably the best known endolithic communities, first described by Friedmann and Ocampo in 1976, are those of the Beacon Sandstone in the dry valleys of southern

Victoria Land, Antarctica. In his studies, Friedmann (1977, 1978) specified cryptoendolithic lichens forming multicolored zones several millimeters wide under the surface of sandstone rocks as well as chasmoendolithic lichens mainly growing in granitic rock substrates. Fertile stages of both allowed them to be identified as *Buellia* sp. (Friedmann et al., 1980) as well as species of the genera *Lecidea* and *Acarospora* (Friedmann, 1982). At a later stage, two euaryotic communities (one lichen and one green algae – *Hemichloris* – dominated) plus three cyanobacterial communities (a red *Gloeocapsa*, a *Hormathonema-Gloeocapsa* and a *Chroococcidiopsis* community) were distinguished at this site (Friedmann et al., 1988).

Within a coastal desert at Marble Point, near the frozen Ross Sea, Friedmann (1982) identified heavy chasmoendolithic growth within weathered marble, consisting of filamentous green algae and cyanobacteria, being accompanied by colonies of colorless bacteria.

After the first discovery of endoliths in Antarctica, chasmoendolithic algae were also detected in other parts of the continent, such as the Vestfold Hills, Princess Elisabeth Land and Mawson Rock, and Mac. Robertson Land, where Broady (1981) found a variety of green algae and cyanobacteria within quartz stones, charnockites, gneisses and other rocks. Small endolithic colonies with cyanobacterial, bacterial and fungal components growing within a gypsum crust on the surface of sandstone boulders were discovered by Hughes and Lawley (2003) on Alexander Island, Antarctic Peninsula.

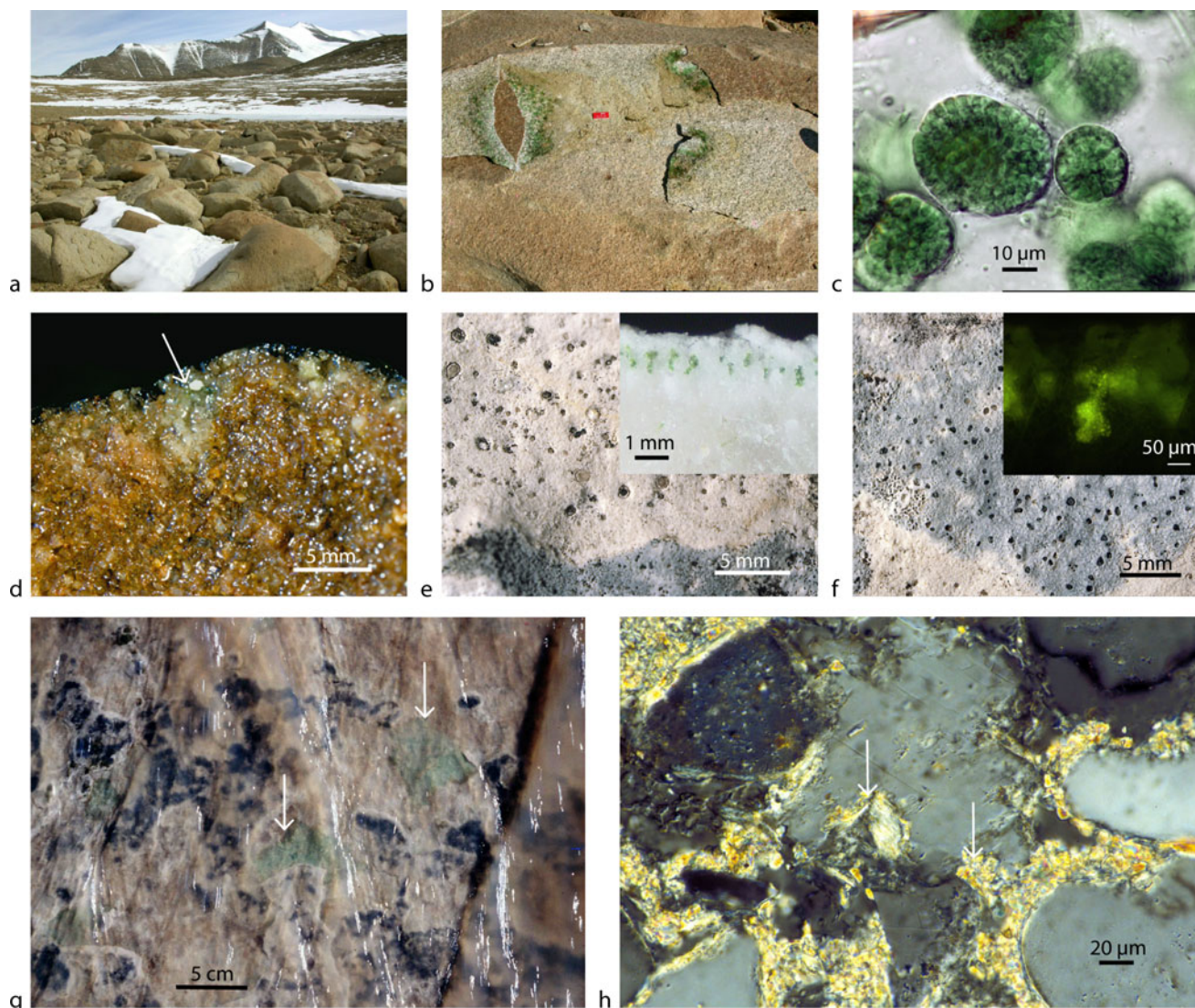
The structure of endolithic communities collected from Granite Harbour at the Ross Sea coast was studied by De Los Rios et al. (2003, 2005) utilizing scanning electron microscopy with backscattered electron imaging (SEM-BSE). They describe an intimate contact between the organisms and the surrounding substrate and in a subsequent study, combined with molecular analyses (De Los Rios et al., 2007), they were able to distinguish two different types of endolithic biofilms.

An extensive distribution of a chasmo- and cryptoendolithic cyanobacterial community, dominated by the unicellular cyanobacterium *Chroococcidiopsis* sp., was reported from granite rocks of an area extending at 600 m elevation at Mt. Falconer, in the McMurdo Dry Valleys (Büdel et al., 2008; Figure 1a–c). The authors found that community to be highly active due to regular dewfall on the rock surface. Radiocarbon dating indicated that the community is by far younger than those reported from a 1,000 m higher elevation by Bonani et al. (1988).

Besides Antarctica, cryptoendolithic habitats were also discovered in the Canadian High Arctic (Omelson et al., 2006), hosting an assemblage of cyanobacteria, algae, fungi and heterotrophic bacteria within sandstone rocks.

Arid and semiarid climate

Endolithic cyanobacteria are known to occur in a variety of habitats within hot and tropical regions



Endoliths, Figure 1 (a) Granite rock boulders as endolithic habitat, Mt. Falconer, McMurdo Dry Valleys, Antarctica. (b) Uncovered endolithic community, Mt. Falconer, Antarctica. Red scale = 4 cm. (c) Endolithic *Chroococcidiopsis* sp., Mt. Falconer, Antarctica. (d) *Synechococcus elongatus* endolithic in red sandstone (arrow), Spessart-Midlands, Germany. (e) *Hymenelia prevostii* has an oval, crustose, whitish-gray thallus. Brownish-pink apothecia are sunk into the thallus, separated from it by a narrow crack. The cross-fracture (inlet) is characterized by algal clusters of an elongated shape. (f) *H. coerulea* is characterized by its bluish to bluish-gray thallus that in some areas remains almost completely below the surface of the substrate. The mostly rimless apothecia are nonpruinose, small (0.2–0.4 mm) and are often situated on small elevations within the limestone. The cross-fracture (inlet) is characterized by smaller algal clusters (compared to *H. prevostii*) of a roundish shape; image taken by fluorescence microscopy, accomplished by mercury arc lamp activated in the blue region. (g) Exfoliation pattern of the South African Tshipise Sandstone along the growth zone of the organisms (green, arrows); the loosened exfoliative flakes are transported away by flowing water. (h) Geological thin section of the Tshipise Sandstone. Within the growth zone of the organisms, dissolution of the binding material, etch marks (arrow) on the quartz grains, and precipitation of calcium carbonate (arrow) are observed.

throughout the world. On the hillsides of the Negev Highlands, Friedmann et al. (1967) and later Danin and Garty (1983) observed both chasmoendolithic and coccoid euendolithic cyanobacteria within different rock types. A widespread and relatively uniform distribution of endolithic algae and cyanobacteria within sandstones was

discovered by Bell et al. (1986), along a transection covering both temperate and semidesert biomes of northern Arizona, southern Utah, and western New Mexico. Büdel and Wessels (1991) identified chasmoendolithic, cryptoendolithic, and hypolithic cyanobacteria within semiarid to arid regions of Africa, North America, Australia, and

Europe. The genus *Chroococcidiopsis* was found to occur almost worldwide within the cryptoendolithic habitat of hot deserts (Büdel, 1999; Weber et al., 1996, Figure 1c). Even within halite rocks in the hyperarid core of the Atacama Desert, this genus was observed to grow together with heterotrophic bacteria (Wierzchos et al., 2006).

In recent studies, endolithic cyanobacteria were also identified by means of molecular methods, like the endolithic communities within travertine deposits in Yellowstone National Park, which turned out to contain a variety of different cyanobacteria, including several new lineages (Norris and Castenholz, 2006). The endolithic communities in limestone from a Maya archaeological site only inhabited photosynthetically inactive bacteria, with cyanobacteria being restricted to the epilithic growth form (McNamara et al., 2006).

Descriptions of endolithic lichens within hot and tropical regions are much rarer. Wessels and Schoeman (1988) observed the endolithically growing *Lecidea* aff. *sarcogynoides* Koerb. on Clarens sandstone within the Golden Gate Highlands National Park, South Africa. The endolithic Sonoran Desert lichen *Verrucaria rubrocincta* Breuss was found to occur locally in abundance in caliche plates of open desert pavements (Bungartz et al., 2004).

Temperate climate

Endolithically growing organisms were first described by Bachmann (1890), who recognized the endolithic growth of *Verrucaria calciseda* DC. and also other species (e.g., *Sarcogyne pruinosa* Smft., *Aspicilia flavida* Hepp.) somewhat later (1892), by studying microscopical thin sections of local limestone samples. The chasmoendolithic growth of lichens, like *Aspicilia gibbosa* Ach., along mica minerals was first detected by Bachmann (1904), whereas Diels (1914) described a completely chasmoendolithic formation of algae inhabiting dolomite rocks in the Alps.

In a recent study, Hoppert et al. (2004) investigated the colonization of limestone in the Dachstein area of the Alps after continuous retreat of two glaciers, observing the endolithic hyphae of black fungi (Aureobasidiomycetes) as first colonizers 2 years after exposition of the rock, whereas the first green algae appeared between 5 and 12 years after exposure. Exposure times between 30 and 100 years resulted in macroscopically visible biofilms being dominated by endolithically growing lichens.

In contrast to that, endolithic growth below temperate-zone cliffs of the Niagara Escarpment, Canada, was only present under 6% of the surface area (Matthes-Sears et al., 1997), whereas the majority of the surface was covered by epilithic growth.

Aquatic habitats

Even in the coastal areas of marine environments, a variety of endolithically growing organisms are known to occur. At the limestone coasts of the northern Adriatic

near Rovinj, Schneider (1976) identified a variety of cyanobacteria and lichens. Le Campion-Alsumard (1979) described ten different species of cyanobacteria, which were often associated with green algae, fungi, and bacteria, revealing bathymetric distribution patterns up to 80 m below sea level in the region of Marseille.

In Shark Bay, western Australia, lithified stromatolites, which were formed by the coccoid cyanophyte *Entophysalis major* Ercegović, were described to be colonized by a thin bluish-black layer of predominantly epilithic thalli of the cyanobacterium *Hormathonema violaceo-nigrum* Ercegović, which sent short endolithic filaments about 50 μm deep into the substrate (Golubic, 1983). Papineau et al. (2005) utilized molecular techniques to characterize a diverse community consisting of bacteria and archaea with only a small fraction of cyanobacteria within this endolithic habitat.

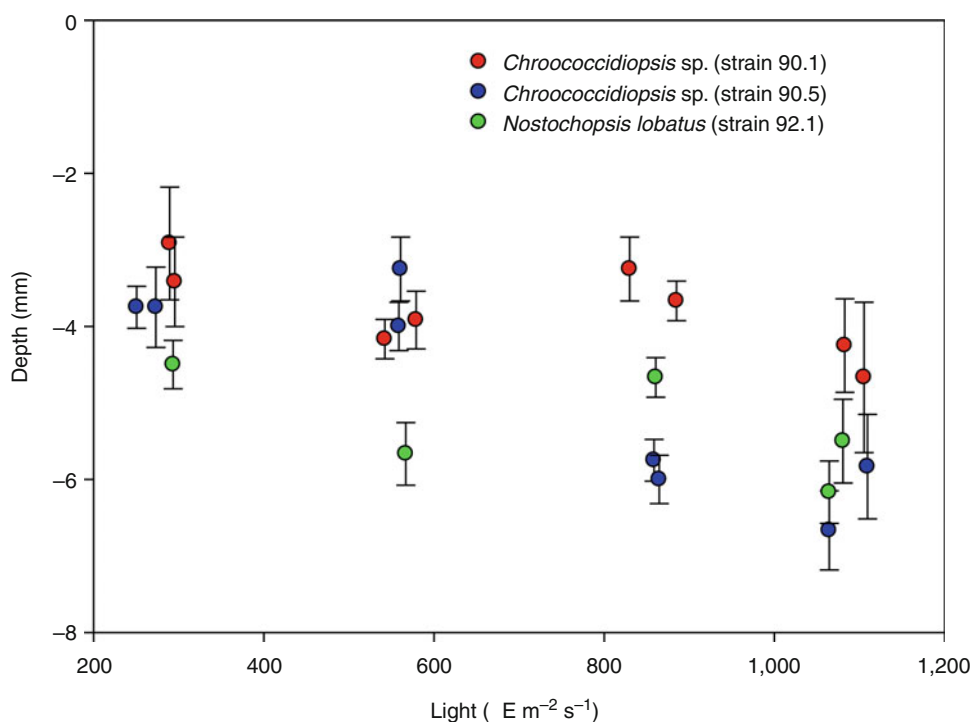
Recently, endolithic microbial communities have been discovered even in the extremely acidic (pH = 1) geothermal environment of Yellowstone National Park (Walker et al., 2005), where an acidophytic eucaryotic red alga (*Cyanidium* sp.) and actinobacteria (*Mycobacterium* spp.) made up the vast majority of the community. In contrast to that, geothermal rocks in the area of Rotorua, New Zealand, were found to be inhabited by a variety of endolithic cyanobacteria with the red algal genus *Cyanidium* occurring but playing only a minor role (Gaylarde et al., 2006).

In a pH-neutral and oxygen-rich creek in the midland area Spessart (Germany), the cyanobacterium *Synechococcus elongatus* Näg. was found to be growing cryptoendolithically within the bleached sandstone (Büdel et al., 1991, Figure 1d).

Advantages of endolithic growth

Light

It appears quite obvious that the overlying rock substrate effectively shields light from the endolithic organisms. Matthes et al. (2001) experienced an exponential decrease of light intensity in limestone with increasing depth and, using a normal photosensor, they measured 1% of the incoming light 1 mm within the dry substrate. Friedmann and Ocampo-Friedmann (1984) found that in a dry stage about 0.1% of the incoming light reached the upper level of the lichen zone, compared to about 1% in a wet stage. Weber et al. (1996) however explain that due to light dispersal and reflectance within the rock, scalar irradiance, measuring light intensity at an angle of nearly 360°, has to be investigated. By inoculating sterile quartz sand with cyanobacterial cultures, we were able to show that at higher light intensities, colonies of *Chroococcidiopsis* sp. (strain 90.1 and 90.5; culture collection of Department of Plant Ecology and Systematics, University of Kaiserslautern) and *Nostochopsis lobatus* (strain 92.1) settled significantly deeper within the substrate (Spearman's rho, strain 90.1: $n = 48$, $r_s = 0.442$, $p = 0.002$; strain 90.5: $n = 48$, $r_s = 0.752$, $p < 0.001$; strain 92.1: $n = 30$, $r_s = 0.494$,



Endoliths, Figure 2 Experimental setup with cyanobacterial cultures being inoculated on petri dishes with sterile quartz sand. After 5 months of growth under different light intensities, each sample was measured at six spots to obtain mean values and standard deviations. With increasing light intensity, each single strain as well as the organisms as one group settled significantly deeper within the substrate (Spearman's rho, strain 90.1: $n = 48$, $r_s = 0.442$, $p = 0.002$; strain 90.5: $n = 48$, $r_s = 0.752$, $p < 0.001$; strain 92.1: $n = 30$, $r_s = 0.494$, $p < 0.006$; all strains: $n = 126$, $r_s = 0.542$, $p < 0.001$).

$p < 0.006$; all strains: $n = 126$, $r_s = 0.542$, $p < 0.001$; Figure 2), strongly supporting the light shielding hypothesis. Measuring the light intensities needed by alpine endolithic lichens to reach the compensation and saturation point of photosynthesis, Weber et al. (2007) found relatively high cardinal points, also ascribing this to the light protecting properties of the limestone within this high light environment.

Temperature

In the cold deserts of Antarctica, Friedmann and Ocampo-Friedmann (1984) reported the endolithic environment to be much less affected by rapid temperature oscillations and coherent freezing and thawing cycles that occur at the rock surface as a result of the frequent gusts of katabatic winds. Friedmann (1980) measured temperature differences up to 7 K between the rock surface and 3 cm within the rock, while Friedmann et al. (1981) registered more balanced and up to 3 K higher temperatures within the lichen zone, as compared to the rock surface.

In the High Arctic, Omelon et al. (2006) measured only minor differences (0.1–0.3 K) between the mean temperature values and no major differences between the

minimum and maximum temperature values (0–1.1 K) at the rock surface and 5 mm below on a south-facing outcrop.

Similarly, Weber et al. (1996) measured the absolute temperature maxima to be only 1°C lower within the endolithic habitat of a South African sandstone plateau, as compared to the rock surface.

In summary, the temperature effect seems to be important in some Antarctic habitats where the endolithic growth prevents the organisms from frequent freezing and thawing. In warm climates, however, no major effect can be concluded from the minor temperature differences.

Humidity

Both in hot and cold deserts, porous rocks like sandstone are expected to function as a substantial water reservoir that allows the organisms to be active over a by far longer timespan than on the rock surface (Friedmann et al., 1981; Weber et al., 1996). The latter authors measured water content values between 4% and 79% in the upper 3 cm of the sandstone at the end of the dry season, and found these water contents to be positively correlated with the N- and C-content of the sandstone samples (Spearman's

rho; nitrogen: $n = 32$, $r_s = 0.585$, $p < 0.001$; carbon: $n = 32$, $r_s = 0.685$, $p < 0.001$). Similarly, Bell (1993) measured the internal relative humidity values within colorized sandstone of the Colorado Plateau to be usually above 80% and never below 60%, regardless of the season. In contrary to that, Friedmann (1980) described the water content of sandstone samples from Antarctica, from the American desert and from the Sinai Peninsula to be remarkably similar with only 0.1–0.2% of the weight of the rock. He expected this quantity of moisture to be sufficient for a nearly saturated atmosphere within cold desert rocks, whereas in hot deserts they would result in low humidity values. Büdel et al. (2008) also experienced the availability of water to be one of the most critical factors within Antarctic endolithic habitats. They observed that regular dewfall can occur by a temperature inversion between the rock surface and air, thus leading to dew precipitation on the rock surface. The penetration depth of the water was also found to be critical for the depth of growth of endolithic cyanobacteria.

In summary, elevated humidity values are widely accepted to favor the endolithic habitat, whereas the actual humidity values dependent on the climatic conditions have only rarely been measured within the different biomes.

Grazing organisms

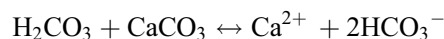
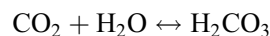
A less frequently named potential advantage is the endolithic shelter against grazing organisms. Schneider (1976) observed intense grazing of epilithic algae and cyanobacteria along the limestone coast of the Adria by gastropods, whereas endolithic organisms were shielded within the substrate. Tretiach (1995) described frequent grazing marks on epilithic lichens, whereas endolithic lichens that were particularly frequent in deeply shaded humid habitats revealed hardly any grazing marks by arthropods, gastropods or other small animals.

Therefore, in certain habitats, endolithic growth may be an effective shelter against grazing organisms, whereas it is not considered to be a major controlling factor.

Weathering mechanisms and biosignatures

Weathering features connected with the endolithic growth of organisms were observed as early as in 1880, when Sollas described the chemical weathering effect of the

endolithic lichen *Verrucaria rupestris* Schrad. (now *Verrucaria muralis* Ach.) growing on limestone. Bachmann (1892) observed that fungal hyphae and even fruiting bodies (e.g., apothecia) were able to grow within hard limestone rocks, and concluded that the hyphae secrete a substance dissolving the rock. In 1916, he was the first to propose the widely accepted weathering mechanism within carbonate rocks: the active organisms release CO_2 , which forms carbonic acid (H_2CO_3) in a humid environment that in turn, together with the calcium carbonate of the rock, forms water-soluble bicarbonate.



During the following decades there have been several descriptions of weathering patterns on limestone (e.g., Degelius, 1962; Le Campion-Alsumard, 1979; Danin et al., 1983; Gehrmann et al., 1992) and even stromatolites were observed to be weathered by the partly epi- and endolithically growing cyanobacterium *Hormathonema violaceo-nigrum* (Golubic, 1983). Weber et al. (Respiration-Induced Weathering Patterns of Two Endolithically Growing Lichens) studied the weathering patterns of two endolithic lichen species (*Hymenelia prevostii* (Duby) Krempelh. and *H. coerulea* (DC.) Massal., Figure 1e and f) in the Limestone Alps and observed the same general weathering pattern. However, *H. prevostii* revealed to develop more than twofold the biomass compared to *H. coerulea* and to modify a significantly wider zone within the substrate (Figure 1e and f; Table 1).

Pohl and Schneider (2002), however, described an overall protective effect of endoliths on carbonate rocks, since after primary colonization they did not observe a continuous loss of material but an internal recycling within the biofilm. Together with the coauthors, they even saw the upper substrate layer, which is usually impregnated by extracellular polymeric substances (EPS) of cyanobacteria, as a protective shield and recognized a stabilizing effect by the tightly woven internal cellular network of the organisms.

Weathering traces on silicate rocks (garnets) were also recognized already a long time ago (Bachmann, 1911), but weathering marks on quartz crystals were unknown

Endoliths, Table 1 Growth characteristics and weathering patterns of *Hymenelia prevostii* and *H. coerulea*. Differences of mean values were tested for statistical significance by *T*-test (chlorophyll content) and Mann-Whitney *U*-test (depth of beginning of algal layer and unaltered substrate)

Growth characteristics	<i>Hymenelia prevostii</i>	<i>Hymenelia coerulea</i>	Statistical significance
Chlorophyll content (mg chl $a + b \text{ m}^{-2}$)	322.4 ± 39.4 ($n = 3$)	141.7 ± 2.2 ($n = 2$)	$T = 6.469$, $p = 0.022$
Depth of beginning of algal layer (mm)	0.102 ± 0.034 ($n = 40$)	0.058 ± 0.019 ($n = 40$)	$U = 213.5$, $p < 0.001$
Depth of beginning of unaltered substrate (mm)	0.366 ± 0.091 ($n = 40$)	0.179 ± 0.036 ($n = 40$)	$U = 11.5$, $p < 0.001$

at that time. A distinctive weathering pattern of sandstone was discovered by Friedmann (1982), who described a dissolution of the cementing substance along the growth zone of endolithic lichens in the Antarctic cold desert. A very similar weathering pattern was observed by us (Weber et al., 1996) in the South African Tshipise Sandstone, where endolithic cyanobacteria caused the exfoliation of the substrate along the growth zone (Figure 1g). Such an exfoliation of sandstone was also described by Wessels and Schoeman (1988) for the endolithically growing lichen *Lecidea* aff. *sarcogynoides*. Since the silicate weathering could not be explained by an acidification as that of limestone, the driving mechanism was unknown until recently, when we could show that an alkalization process during photosynthesis was responsible (Büdel et al., 2004). This alkalization was shown to be responsible for a dissolution of the binding material, etch marks on the quartz grains, and a precipitation of calcium carbonate within the studied sandstone (Figure 1h), which resulted in the previously described exfoliation of the sandstone.

The formation of minerals, which could serve as biosignatures within rock deposits was already described in 1916 when Bachmann explained the deposition of calcium oxalate by endolithic cyanobacteria within limestone. Friedmann and Weed (1987) observed the formation of quartz rhinds on top of the endolithic organisms within the Beacon Sandstone, South Africa. This dense crystalline layer above the organisms was also recognized by us, but as stated earlier, we also observed the precipitation of calcium carbonate within the growth zone of the cyanobacteria (Figure 1h; Büdel et al., 2004).

The preservation of cell structures in the form of fossil endoliths is known from the silicified stromatolithic crusts of the 1,500–1,700 Ma old Dahong Yu Formation in China (Zhang and Golubic, 1987). But also ooid assemblages of several *Eohyella* species within 800 Ma old rocks were found to be comparable to modern ooids of the Bahama Bank and the Arabian Gulf (Knoll et al., 1986). Body fossils of endolithic bangiacean rhodophytes were found in sediments that are 420 Ma old (Kamierczak and Golubic, 1976), whereas Wierzchos et al. (2005) described the recent formation of cell-shaped structures within the sandstones of Antarctica. The latter authors propose these structures to be important markers in the search for former life on Mars. Golubic and Schneider (2003) as well as Banfield et al. (2001) point out that the activity of endolithic organisms left a rich fossil record of boring and mineralization patterns behind, which reveal the nature of palaeoenvironmental conditions under which they lived.

Conclusions

Endolithic growth is realized by a variety of organisms, such as cyanobacteria, algae, lichens, fungi, and bacteria, and occurs in various habitats all over the world. The advantages of endolithic growth are multifarious, like the

shield from high light intensities or grazing organisms or the occurrence of more favorable temperature and humidity conditions, with their relevance being variable within the different habitats. The endolithic organisms are known to have weathering effects on both calcareous and siliceous substrates with different underlying processes. The weathering marks and also newly formed minerals can serve as biosignatures, which allow to draw conclusions on endolithic growth in former times or on other planets.

Bibliography

- Bachmann, E., 1890. Die Beziehungen der Kalkflechten zu ihrem Substrat. *Berichte der Deutschen Botanischen Gesellschaft*, **8**, 141–145.
- Bachmann, E., 1892. Der Thallus der Kalkflechten. *Berichte der Deutschen Botanischen Gesellschaft*, **10**, 30–37.
- Bachmann, E., 1904. Die Beziehungen der Kieselflechten zu ihrem Substrat. *Berichte der Deutschen Botanischen Gesellschaft*, **22**, 101–104.
- Bachmann, E., 1911. Die Beziehungen der Kieselflechten zu ihrer Unterlage. II. Granat und Quarz. *Berichte der Deutschen Botanischen Gesellschaft*, **29**, 261–273.
- Bachmann, E., 1916. Kalklösende Algen. *Berichte der Deutschen Botanischen Gesellschaft*, **33**, 45–57.
- Banfield, J. F., Moreau, J. W., Chan, C. S., Welch, S. A., and Little, B., 2001. Mineralogical biosignatures and the search for life on Mars. *Astrobiology*, **1**(4), 447–465.
- Bell, R. A., 1993. Cryptoendolithic algae of hot semiarid lands and deserts. *Journal of Phycology*, **29**, 133–139.
- Bell, R. A., Athey, P. V., and Sommerfeld, M. R., 1986. Cryptoendolithic algal communities of the Colorado Plateau. *Journal of Phycology*, **22**, 429–435.
- Bonani, G., Friedmann, E. I., Ocampo-Friedmann, R., McKay, C., and Woelfli, W., 1988. Preliminary report on radiocarbon dating of cryptoendolithic microorganisms. *Berichte zur Polarforschung*, **58**, 2–3.
- Broady, P. A., 1981. The ecology of chasmolithic algae at coastal locations of Antarctica. *Phycologia*, **20**(3), 259–272.
- Büdel, B., 1999. Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. *European Journal of Phycology*, **34**, 361–370.
- Büdel, B., and Wessels, D. C. J., 1991. Rock inhabiting blue-green algae/cyanobacteria from hot arid regions. *Algological Studies*, **64**, 385–398.
- Büdel, B., Mollenhauer, D., and Mollenhauer, R., 1991. *Synechococcus elongatus* – cryptoendolithic growth within bleached sandstone from creeks in the midland area Spessart (Germany). *Algological Studies*, **64**, 357–360.
- Büdel, B., Weber, B., Kühl, M., Pfanz, H., Sültemeyer, D., and Wessels, D., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268.
- Büdel, B., Bendix, J., Bicker, F. T., and Green, T. G. A., 2008. Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor Valley, Antarctica. *Journal of Phycology*, **44**(6), 1415–1424.
- Bungartz, F., Grävie, L. A. J., and Nash, T. H. III, 2004. Anatomy of the endolithic sonoran desert lichen *Verrucaria rubrocincta* Breuss: implications for biodeterioration and biomineralization. *The Lichenologist*, **36**(1): 55–73.
- Danin, A., and Garty, J., 1983. Distribution of cyanobacteria and lichens on hillsides of the Negev highlands and their impact on

- biogenic weathering. *Zeitschrift fuer Geomorphologie, Neue Folge*, **27**(4), 423–444.
- Danin, A., Gerson, R., and Garty, J., 1983. Weathering patterns on hard limestone and dolomite by endolithic lichens and cyanobacteria: supporting evidence for Eolian contribution to Terra Rossa Soil. *Soil Science*, **136**(4), 213–217.
- Degelius, G., 1962. Über Verwitterung von Kalk- und Dolomitgestein durch Algen und Flechten. In Hedvall, J. A. (ed.), *Chemie im Dienst der Archäologie Bautechnik Denkmalpflege*. Lund: Hakam Ohlssons, pp. 156–164.
- De Los Rios, A., Wierzchos, J., Sancho, L. G., and Ascaso, C., 2003. Acid microenvironments in microbial biofilms of Antarctic endolithic microecosystems. *Environmental Microbiology*, **5**(4), 231–237.
- De Los Rios, A., Sancho, L. G., Grube, M., Wierzchos, J., and Ascaso, C., 2005. Endolithic growth of two *Lecidea* lichens in granite from continental Antarctica detected by molecular and microscopy techniques. *New Phytologist*, **165**, 181–190.
- De Los Rios, A., Grube, M., Sancho, L. G., and Ascaso, C., 2007. Ultrastructural and genetic characteristics of endolithic cyanobacterial biofilms colonizing Antarctic granite rocks. *FEMS Microbiology Ecology*, **59**, 386–395.
- Diels, L., 1914. Die Algenvegetation der Südtiroler Dolomitriffe. Ein Beitrag zur Ökologie der Lithophyten. *Berichte der Deutschen Botanischen Gesellschaft*, **32**, 502–526.
- Friedmann, E. I., 1977. Microorganisms in Antarctic desert rocks from dry valleys and Dufek Massif. *Antarctic Journal of the United States*, **12**(4), 26–30.
- Friedmann, E. I., 1978. Melting snow in the dry valleys is a source of water for endolithic microorganisms. *Antarctic Journal of the United States*, **13**(4), 162–163.
- Friedmann, E. I., 1980. Endolithic microbial life in hot and cold deserts. *Origins of Life*, **10**, 223–235.
- Friedmann, E. I., 1982. Endolithic microorganisms in the Antarctic cold desert. *Science*, **215**, 1045–1053.
- Friedmann, E. I., and Ocampo, R., 1976. Endolithic blue-green algae in the dry valleys: primary producers in the Antarctic desert ecosystem. *Science*, **193**, 1247–1249.
- Friedmann, E. I., and Ocampo-Friedmann, R., 1984. Endolithic microorganisms in extreme dry environments: analysis of a lithobiontic microbial habitat. In Klug, M. J., and Reddy, C. A. (eds.), *Current Perspectives in Microbial Ecology*. Washington, DC: ATM, pp. 177–185.
- Friedmann, E. I., and Weed, R., 1987. Microbial trace-fossil formation, biogeneous, and abiotic weathering in the Antarctic cold desert. *Science*, **236**, 703–705.
- Friedmann, E. I., Lipkin, Y., and Ocampo-Paus, R., 1967. Desert algae of the Negev (Israel). *Phycologia*, **6**, 185–200.
- Friedmann, E. I., Garty, J., and Kappen, L., 1980. Fertile stages of cryptoendolithic lichens in the dry valleys of Southern Victoria land. *Antarctic Journal of the United States*, **15**, 166–167.
- Friedmann, E. I., Friedmann, R. O., and McKay, C. P., 1981. Adaptations of cryptoendolithic lichens in the Antarctic desert. In Jouventin, P., Massé, L., and Tréhen, P. (eds.), *Colloque sur les Écosystèmes Subantarctiques*. Paimpont Paris No. 51: Comité National Français des Recherches Antarctiques, pp. 65–69.
- Friedmann, E. I., Hua, M., and Ocampo-Friedmann, R., 1988. Cryptoendolithic lichen and cyanobacterial communities of the Ross Desert, Antarctica. *Polarforschung*, **58**(2/3), 251–259.
- Gaylarde, P. M., Jungblut, A.-D., Gaylarde, C. C., and Neilan, B. A., 2006. Endolithic phototrophs from an active Geothermal region in New Zealand. *Geomicrobiology Journal*, **23**, 579–587.
- Gehrmann, C. K., Krumbein, W. E., and Petersen, K., 1992. Endolithic lichens and the corrosion of carbonate rocks – a study of biopitting. *International Journal of Mycology and Lichenology*, **5**(1–2), 37–48.
- Golubic, S., 1983. Stromatolites, fossil and recent: a case history. In Westbrook, P., and de Jong, E. W. (eds.), *Biomining and Biological Metal Accumulation*. Dordrecht, the Netherlands: D. Reidel, pp. 313–326.
- Golubic, S., and Schneider, J., 2003. Microbial endoliths as internal biofilms. In Krumbein, W. E., Dornieden, T., and Volkmann, M. (eds.), *Fossil and Recent Biofilms*. Dordrecht, the Netherlands: Kluwer, pp. 249–263.
- Golubic, S., Friedmann, I., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**(2), 475–478.
- Hoppert, M., Flies, C., Pohl, W., Günzl, B., and Schneider, J., 2004. Colonization strategies of lithobiontic microorganisms on carbonate rocks. *Environmental Geology*, **46**, 421–428.
- Hughes, K., and Lawley, B., 2003. A novel Antarctic microbial endolithic community within gypsum crusts. *Environmental Microbiology*, **5**(7), 555–565.
- Kamierczak, J., and Golubic, S., 1976. Oldest organic remains of boring algae from Polish Upper Silurian. *Nature*, **261**, 404–406.
- Knoll, A. H., Golubic, S., Green, J., and Swett, K., 1986. Organically preserved microbial endoliths from the late Proterozoic of East Greenland. *Nature*, **321**, 856–857.
- Le Campion-Alsumard, T., 1979. Les Cyanophycées endolithes marines. Systématique, ultrastructure, écologie et biodestruction. *Océanologica Acta*, **2**(2), 143–156.
- Matthes, U., Turner, S. J., and Larson, D. W., 2001. Light attenuation by limestone rock and its constraint on the depth distribution of endolithic algae and cyanobacteria. *International Journal of Plant Science*, **162**(2), 263–270.
- Matthes-Sears, U., Gerrath, J. A., and Larson, D. W., 1997. Abundance, biomass, and productivity of endolithic and epilithic lower plants on the temperate-zone cliffs of the Niagara Escarpment, Canada. *International Journal of Plant Science*, **158**(4), 451–460.
- McNamara, C. J., Perry, T. D., Bearce, K. A., Hernandez-Duque, G., and Mitchell, R., 2006. Epilithic and endolithic bacterial communities in limestone from a Maya archeological site. *Microbial Ecology*, **51**, 51–64.
- Norris, T. B., and Castenholz, R. W., 2006. Endolithic photosynthetic communities within ancient and recent travertine deposits in Yellowstone National Park. *FEMS Microbiology Ecology*, **57**(3), 470–483.
- Omelson, C. R., Pollard, W. H., and Ferris, F. G., 2006. Environmental controls on microbial colonization of high arctic cryptoendolithic habitats. *Polar Biology*, **30**, 19–29.
- Papineau, D., Walker, J. J., Mojzsis, S. J., and Pace, N. R., 2005. Composition and structure of microbial communities from stromatolites of Hamelin Pool in Shark Bay, Western Australia. *Applied and Environmental Microbiology*, **71**(8), 4822–4832.
- Pohl, W., and Schneider, J., 2002. Impact of endolithic biofilms on carbonate rock surfaces. In Siegesmund, S., Weiss, T., and Vollbrecht, A. (eds.), *Natural Stone, Weathering Phenomena, Conservation Strategies and Case Studies*. London: Geological Society, pp. 177–194.
- Schneider, J., 1976. Biological and inorganic factors in the destruction of limestone coasts. *Contributions to Sedimentology*, **6**, 1–112.
- Sollas, W. J., 1880. *On the activity of a lichen on limestone*. Report of the British Association for the Advancement of Science, p. 586.
- Tretiach, M., 1995. Ecophysiology of calcicolous endolithic lichens: progress and problems. *Giornale Botanico Italiano*, **129**(1), 159–184.

- Walker, J. J., Spear, J. R., and Pace, N. R., 2005. Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature*, **434**, 1011–1014.
- Weber, B., Wessels, D. C. J., and Büdel, B., 1996. Biology and ecology of cryptoendolithic cyanobacteria of a sandstone outcrop in the Northern Province, South Africa. *Algological Studies*, **83**, 565–579.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Wierzbos, J., Sancho, L. G., and Ascaso, C., 2005. Biomineralization of endolithic microbes in rocks from the McMurdo Dry Valleys of Antarctica: implications for microbial fossil formation and their detection. *Environmental Microbiology*, **7** (4), 566–575.
- Wierzbos, J., Ascaso, C., and McKay, C. P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology*, **6**(3), 1–8.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early proterozoic stromatolites, Hebei, China. *Acta Micropaleontologica Sinica*, **4**, 1–12.

Cross-references

[Algae \(Eukaryotic\)](#)
[Astrobiology](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococcidiopsis](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fungi and Lichens](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)

ENDOSYMBIOSIS

Endosymbiosis is a close, prolonged physical and/or metabolic association between distinct organisms, one of which lives inside the body of the other, either within its cells or in extracellular spaces.

Please refer to entry “[Symbiosis](#)” for further reading. See also entries “[Cyanobacteria](#),” “[Algae \(Eukaryotic\)](#),” “[Diatoms](#)” and “[Fungi and Lichens](#)” for particular forms of endosymbioses.

EVAPORITES

See entries “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Saline Lakes](#),” and “[Soda Lakes](#).”

EXOENZYMES

Kathrin Riedel, Alexander Grunau
 University of Zurich, Zurich, Switzerland

Synonyms

Excreted/secreted enzymes; Extracellular enzymes

Definition

Exoenzyme is an enzyme produced by a microorganism that secreted into the environment that is capable of degrading insoluble polymers.

Introduction

Osmotroph organisms, that is, archaea, bacteria, and fungi, are not able to ingest particulate matter directly but rather have to obtain nutrients through the active uptake of soluble compounds across their cell membrane. Organic polymers such as proteins, cellulose and hemicelluloses, lignin, chitin, starch, and lipids can only serve as sources for energy and anabolic processes if they are degraded into small, transportable molecules by secreted hydrolytic enzymes, collectively termed “exoenzymes.” The most relevant exoenzyme classes, their impact on biogeochemical cycles, symbiotic/pathogenic interactions and major biotechnological applications are summarized in the following paragraphs.

Exoenzymes – an overview

[Figure 1](#) illustrates the most relevant microbial exoenzymes and their substrates. Their catalytic mechanisms as well as selected review articles describing the respective enzymes in detail are shown in [Table 1](#). [Figure 2](#) summarizes the impact of microbial enzymes on environmental processes and their application in biotechnology; both aspects are discussed in detail in the following paragraphs.

Exoenzymes and their impact on environmental processes

Prokaryotic and eukaryotic microorganisms are one of the main driving forces of biogeochemical cycles as they can decompose virtually all natural compounds, thereby exerting a lasting effect on biosphere and climate (Madigan et al., 2008). Archaea, bacteria, and fungi are the only living agents that are capable of regenerating essential elements such as carbon, sulfur, nitrogen, and iron by decomposing dead organic matter. Moreover, some microorganisms are able to degrade plastics, particularly biopolymers, and highly complex xenobiotics such as crude oil, and thus contribute significantly to the cleanup or detoxification of environmental pollutants (bioremediation; Knackmuss, 1996).

- Walker, J. J., Spear, J. R., and Pace, N. R., 2005. Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature*, **434**, 1011–1014.
- Weber, B., Wessels, D. C. J., and Büdel, B., 1996. Biology and ecology of cryptoendolithic cyanobacteria of a sandstone outcrop in the Northern Province, South Africa. *Algological Studies*, **83**, 565–579.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Wierzbos, J., Sancho, L. G., and Ascaso, C., 2005. Biomineralization of endolithic microbes in rocks from the McMurdo Dry Valleys of Antarctica: implications for microbial fossil formation and their detection. *Environmental Microbiology*, **7**(4), 566–575.
- Wierzbos, J., Ascaso, C., and McKay, C. P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology*, **6**(3), 1–8.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early proterozoic stromatolites, Hebei, China. *Acta Micropaleontologica Sinica*, **4**, 1–12.

Cross-references

[Algae \(Eukaryotic\)](#)
[Astrobiology](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococcidiopsis](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fungi and Lichens](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)

ENDOSYMBIOSIS

Endosymbiosis is a close, prolonged physical and/or metabolic association between distinct organisms, one of which lives inside the body of the other, either within its cells or in extracellular spaces.

Please refer to entry “[Symbiosis](#)” for further reading. See also entries “[Cyanobacteria](#),” “[Algae \(Eukaryotic\)](#),” “[Diatoms](#)” and “[Fungi and Lichens](#)” for particular forms of endosymbioses.

EVAPORITES

See entries “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Saline Lakes](#),” and “[Soda Lakes](#).”

EXOENZYMES

Kathrin Riedel, Alexander Grunau
 University of Zurich, Zurich, Switzerland

Synonyms

Excreted/secreted enzymes; Extracellular enzymes

Definition

Exoenzyme is an enzyme produced by a microorganism that secreted into the environment that is capable of degrading insoluble polymers.

Introduction

Osmotroph organisms, that is, archaea, bacteria, and fungi, are not able to ingest particulate matter directly but rather have to obtain nutrients through the active uptake of soluble compounds across their cell membrane. Organic polymers such as proteins, cellulose and hemicelluloses, lignin, chitin, starch, and lipids can only serve as sources for energy and anabolic processes if they are degraded into small, transportable molecules by secreted hydrolytic enzymes, collectively termed “exoenzymes.” The most relevant exoenzyme classes, their impact on biogeochemical cycles, symbiotic/pathogenic interactions and major biotechnological applications are summarized in the following paragraphs.

Exoenzymes – an overview

[Figure 1](#) illustrates the most relevant microbial exoenzymes and their substrates. Their catalytic mechanisms as well as selected review articles describing the respective enzymes in detail are shown in [Table 1](#). [Figure 2](#) summarizes the impact of microbial enzymes on environmental processes and their application in biotechnology; both aspects are discussed in detail in the following paragraphs.

Exoenzymes and their impact on environmental processes

Prokaryotic and eukaryotic microorganisms are one of the main driving forces of biogeochemical cycles as they can decompose virtually all natural compounds, thereby exerting a lasting effect on biosphere and climate (Madigan et al., 2008). Archaea, bacteria, and fungi are the only living agents that are capable of regenerating essential elements such as carbon, sulfur, nitrogen, and iron by decomposing dead organic matter. Moreover, some microorganisms are able to degrade plastics, particularly biopolymers, and highly complex xenobiotics such as crude oil, and thus contribute significantly to the cleanup or detoxification of environmental pollutants (bioremediation; Knackmuss, 1996).

- Walker, J. J., Spear, J. R., and Pace, N. R., 2005. Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature*, **434**, 1011–1014.
- Weber, B., Wessels, D. C. J., and Büdel, B., 1996. Biology and ecology of cryptoendolithic cyanobacteria of a sandstone outcrop in the Northern Province, South Africa. *Algological Studies*, **83**, 565–579.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Wierzbos, J., Sancho, L. G., and Ascaso, C., 2005. Biomineralization of endolithic microbes in rocks from the McMurdo Dry Valleys of Antarctica: implications for microbial fossil formation and their detection. *Environmental Microbiology*, **7**(4), 566–575.
- Wierzbos, J., Ascaso, C., and McKay, C. P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology*, **6**(3), 1–8.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early proterozoic stromatolites, Hebei, China. *Acta Micropaleontologica Sinica*, **4**, 1–12.

Cross-references

[Algae \(Eukaryotic\)](#)
[Astrobiology](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococciopsia](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fungi and Lichens](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)

ENDOSYMBIOSIS

Endosymbiosis is a close, prolonged physical and/or metabolic association between distinct organisms, one of which lives inside the body of the other, either within its cells or in extracellular spaces.

Please refer to entry “[Symbiosis](#)” for further reading. See also entries “[Cyanobacteria](#),” “[Algae \(Eukaryotic\)](#),” “[Diatoms](#)” and “[Fungi and Lichens](#)” for particular forms of endosymbioses.

EVAPORITES

See entries “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Saline Lakes](#),” and “[Soda Lakes](#).”

EXOENZYMES

Kathrin Riedel, Alexander Grunau
 University of Zurich, Zurich, Switzerland

Synonyms

Excreted/secreted enzymes; Extracellular enzymes

Definition

Exoenzyme is an enzyme produced by a microorganism that secreted into the environment that is capable of degrading insoluble polymers.

Introduction

Osmotroph organisms, that is, archaea, bacteria, and fungi, are not able to ingest particulate matter directly but rather have to obtain nutrients through the active uptake of soluble compounds across their cell membrane. Organic polymers such as proteins, cellulose and hemicelluloses, lignin, chitin, starch, and lipids can only serve as sources for energy and anabolic processes if they are degraded into small, transportable molecules by secreted hydrolytic enzymes, collectively termed “exoenzymes.” The most relevant exoenzyme classes, their impact on biogeochemical cycles, symbiotic/pathogenic interactions and major biotechnological applications are summarized in the following paragraphs.

Exoenzymes – an overview

[Figure 1](#) illustrates the most relevant microbial exoenzymes and their substrates. Their catalytic mechanisms as well as selected review articles describing the respective enzymes in detail are shown in [Table 1](#). [Figure 2](#) summarizes the impact of microbial enzymes on environmental processes and their application in biotechnology; both aspects are discussed in detail in the following paragraphs.

Exoenzymes and their impact on environmental processes

Prokaryotic and eukaryotic microorganisms are one of the main driving forces of biogeochemical cycles as they can decompose virtually all natural compounds, thereby exerting a lasting effect on biosphere and climate (Madigan et al., 2008). Archaea, bacteria, and fungi are the only living agents that are capable of regenerating essential elements such as carbon, sulfur, nitrogen, and iron by decomposing dead organic matter. Moreover, some microorganisms are able to degrade plastics, particularly biopolymers, and highly complex xenobiotics such as crude oil, and thus contribute significantly to the cleanup or detoxification of environmental pollutants (bioremediation; Knackmuss, 1996).

- Walker, J. J., Spear, J. R., and Pace, N. R., 2005. Geobiology of a microbial endolithic community in the Yellowstone geothermal environment. *Nature*, **434**, 1011–1014.
- Weber, B., Wessels, D. C. J., and Büdel, B., 1996. Biology and ecology of cryptoendolithic cyanobacteria of a sandstone outcrop in the Northern Province, South Africa. *Algological Studies*, **83**, 565–579.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Wierzbos, J., Sancho, L. G., and Ascaso, C., 2005. Biomineralization of endolithic microbes in rocks from the McMurdo Dry Valleys of Antarctica: implications for microbial fossil formation and their detection. *Environmental Microbiology*, **7**(4), 566–575.
- Wierzbos, J., Ascaso, C., and McKay, C. P., 2006. Endolithic cyanobacteria in halite rocks from the hyperarid core of the Atacama Desert. *Astrobiology*, **6**(3), 1–8.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early proterozoic stromatolites, Hebei, China. *Acta Micropaleontologica Sinica*, **4**, 1–12.

Cross-references

[Algae \(Eukaryotic\)](#)
[Astrobiology](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococciopsia](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fungi and Lichens](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)

ENDOSYMBIOSIS

Endosymbiosis is a close, prolonged physical and/or metabolic association between distinct organisms, one of which lives inside the body of the other, either within its cells or in extracellular spaces.

Please refer to entry “[Symbiosis](#)” for further reading. See also entries “[Cyanobacteria](#),” “[Algae \(Eukaryotic\)](#),” “[Diatoms](#)” and “[Fungi and Lichens](#)” for particular forms of endosymbioses.

EVAPORITES

See entries “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Saline Lakes](#),” and “[Soda Lakes](#).”

EXOENZYMES

Kathrin Riedel, Alexander Grunau
 University of Zurich, Zurich, Switzerland

Synonyms

Excreted/secreted enzymes; Extracellular enzymes

Definition

Exoenzyme is an enzyme produced by a microorganism that secreted into the environment that is capable of degrading insoluble polymers.

Introduction

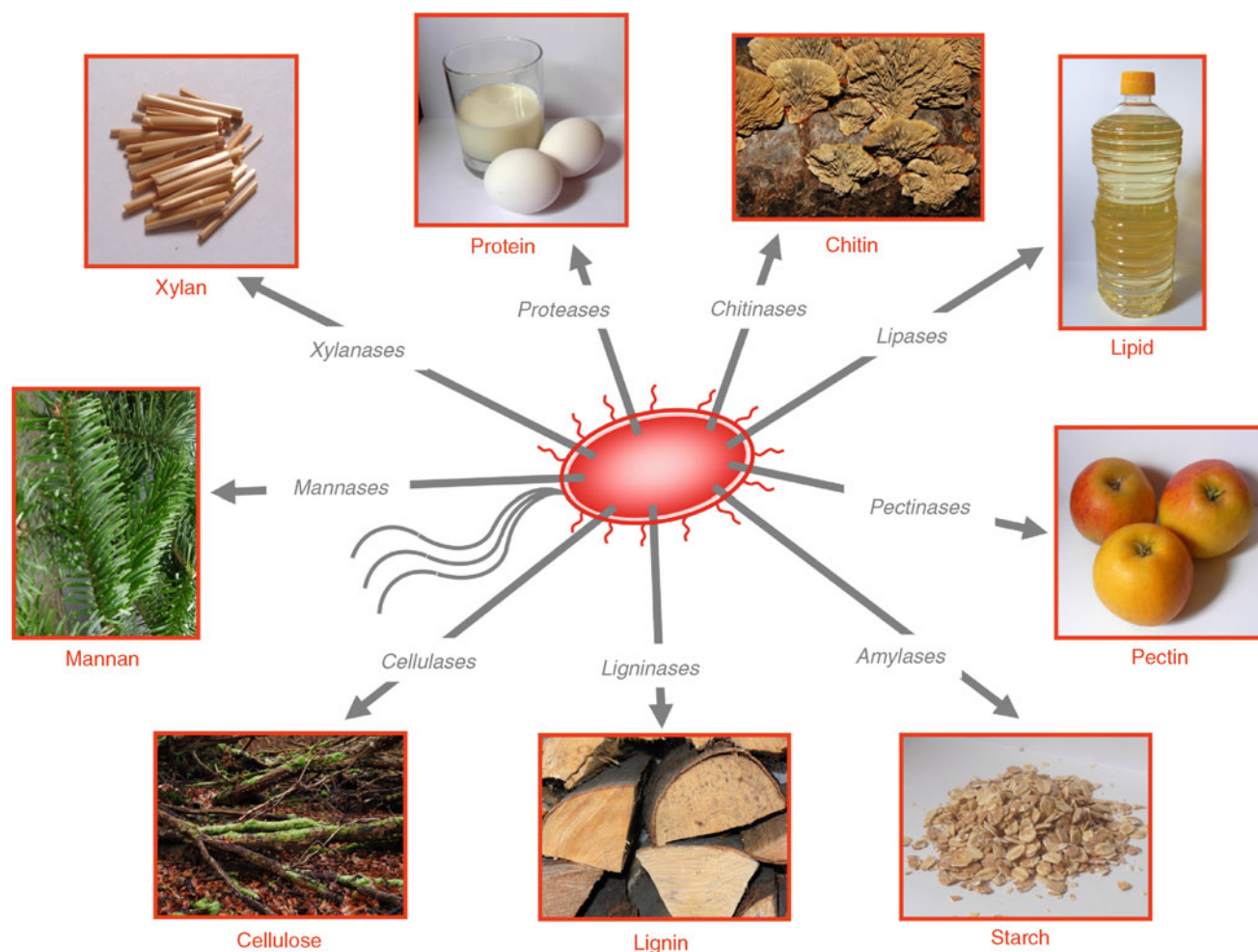
Osmotroph organisms, that is, archaea, bacteria, and fungi, are not able to ingest particulate matter directly but rather have to obtain nutrients through the active uptake of soluble compounds across their cell membrane. Organic polymers such as proteins, cellulose and hemicelluloses, lignin, chitin, starch, and lipids can only serve as sources for energy and anabolic processes if they are degraded into small, transportable molecules by secreted hydrolytic enzymes, collectively termed “exoenzymes.” The most relevant exoenzyme classes, their impact on biogeochemical cycles, symbiotic/pathogenic interactions and major biotechnological applications are summarized in the following paragraphs.

Exoenzymes – an overview

[Figure 1](#) illustrates the most relevant microbial exoenzymes and their substrates. Their catalytic mechanisms as well as selected review articles describing the respective enzymes in detail are shown in [Table 1](#). [Figure 2](#) summarizes the impact of microbial enzymes on environmental processes and their application in biotechnology; both aspects are discussed in detail in the following paragraphs.

Exoenzymes and their impact on environmental processes

Prokaryotic and eukaryotic microorganisms are one of the main driving forces of biogeochemical cycles as they can decompose virtually all natural compounds, thereby exerting a lasting effect on biosphere and climate (Madigan et al., 2008). Archaea, bacteria, and fungi are the only living agents that are capable of regenerating essential elements such as carbon, sulfur, nitrogen, and iron by decomposing dead organic matter. Moreover, some microorganisms are able to degrade plastics, particularly biopolymers, and highly complex xenobiotics such as crude oil, and thus contribute significantly to the cleanup or detoxification of environmental pollutants (bioremediation; Knackmuss, 1996).



Exoenzymes, Figure 1 Degradation of insoluble polymers by secreted microbial enzymes. Note that the bacterial cell represents microbes in general.

Symbiotic interactions between microorganisms and animals (e.g., termites and ruminants) owe a great debt to the beneficial activities of microbial exoenzymes (Madigan et al., 2008). For example, a varied intestinal flora of bacteria, fungi (and protozoa), enable herbivores to digest cellulose and other plant polysaccharides. A vital part of human nutrition thereby directly stems from this exoenzymatic activity in the intestinal tracts of livestock and other ruminants, converting low-energy plant foods into high-energy foods such as milk, dairy products, and meat. On the other hand, uncontrolled microbial growth together with the production of hydrolytic enzymes such as proteases, lipases, and cell-wall degrading enzymes also destroys vast amounts of food and can even lead to food-borne infections or poisoning.

In deleterious host-pathogen interactions, exoenzymes produced by the pathogens may act as virulence factors (Madigan et al., 2008). Prominent examples for such

pathogenic enzymes are (a) hyaluronidases and coagulases produced by human and animal pathogens that degrade the intercellular matrix component hyaluronic acid or dissolve fibrin clotting, respectively, enabling them to invade and colonize host tissues, or (b) pectinases and cellulases produced by plant pathogens that break up cell walls and facilitate invasion and degradation of living plants.

Exoenzymes – nature's biotechnology tools

Microbial enzymes have served humankind since pre-historic times in the traditional production and conservation of food such as wine and cheese. However, enzymes as molecular tools were not discovered until the second half of the nineteenth century and have since been extensively used in various industries (Damhus et al., 2008). Classical chemical transformation processes have various inherent drawbacks, for example, poor product yields, high energy costs, harsh and hazardous reaction conditions and unwanted, partly harmful by-products. In

Exoenzymes, Table 1 Exoenzymes, substrates, and reaction mechanisms

Enzyme (subclasses)	Substrate	Catalytic mechanism	Reviewed in
Amylases α -Amylases β -Amylases Pullulanases Isoamylases Glucoamylases	Starch: mixed storage polymer consisting of amylose and amylopectin	Hydrolysis of α -1,4- or α -1,6-glycosidic bonds	Vihinen and Mäntsälä (1989)
Cellulases Endocellulases/ carboxymethylcellulases Exocellulases/ cellobiohydrolases	Cellulose: main structural polymer of plants	Hydrolysis of β -1,4-glycosidic bonds	Doi (2008)
Chitinases Endochitinases Exochitinases	Chitin: <i>N</i> -acetyl D-glucosamine structural polymer of fungi, insects, crustaceans, and nematodes	Hydrolysis of β -1,4-glycosidic bonds	Dahiya et al. (2006)
Hemicellulases Xylanases Mannases (many more)	Various amorphous heteropolymers present in plant cell walls together with cellulose	Hydrolysis of hemicellulose in monomeric sugars and acetic acid	Shallom and Shoham (2003)
Hyaluronidases	Hyaluronic acid, a major constituent of the interstitial barrier	Hydrolysis of 1,4- β -D-glycosidic bonds	Stern and Jedrzejewski (2006)
Ligninases Various peroxidases Laccases Accessory enzymes	Lignin: second most abundant structural polymer in plants, highly recalcitrant to degradation	Oxidation of various phenolic and non-phenolic lignin compounds	Wong (2009)
Lipases	Lipids: long-chain acylglycerols	Hydrolysis or synthesis of ester bonds	Jaeger and Eggert (2002)
Pectinases Polygalacturonases Pectin esterases Pectin lyases Pectate lyases	Pectin: polygalacturonic acid polymer present in plant primary cell walls and middle lamellae	Hydrolysis of α -1,4-galactosiduronic bonds	Alkorta et al. (1998)
Proteases Endoproteases Exoproteases Coagulases	Proteins or peptides: amino acid polymers Fibrinogen	Hydrolysis of peptide bonds Conversion of fibrinogen to fibrin	Rao et al. (1998) Rivera et al. (2007)

contrast, enzymatic reactions proceed under mild conditions are highly specific and involve very fast reactions. Moreover, enzymes contribute to sustainable development as they can be isolated from microorganisms which are cultured using primarily renewable resources (Damhus et al., 2008).

Modern advancements in biotechnology, especially in the field of molecular genetics and protein engineering, have brought the application of a wide variety of biocatalysts to many different industries (Figure 2 and Table 2): (a) amylases, proteases, lipases, and pectinases are used in the food industry, ranging from traditional applications in wine and beer production, cheese ripening, baking, but also for fruit juice clarification and sweetener production; (a) xylanases, cellulases, and amylases are employed in the paper industry for bleaching, recycling, and coating; (c) lipases, proteases, amylases, and cellulases are added to laundry and automated dishwashing detergents to improve cleaning performance and reduce energy and water consumption; (d) in the textile industry cellulases, laccases, and peroxidases are used for

bio-bleaching of jeans fabrics and cellulases/proteases for bio-polishing of cotton fabrics, wool and silk products; (e) amylases and cellulases are employed for biofuel production from starch- and cellulose-containing raw materials.

Summary

Microorganisms are able to degrade all naturally occurring organic compounds and also certain xenobiotic substances that persist in oxic environments and thereby deserve to be considered omnipotent. To this end, archaea, bacteria, and fungi secrete specific enzymes, the so-called “exoenzymes” into the immediate surroundings that act to break down their substrates in part or even up to complete mineralization. Exoenzymes are thus of great importance for elemental recycling processes within biogeochemical cycles, yet they also contribute substantially to symbiotic or pathogenic interactions between microbes and higher organisms and are furthermore useful catalysts for various biotechnological and industrial applications.



Exoenzymes, Figure 2 Microbial enzymes: ecological impact (*left panel*) and biotechnological applications (*right panel*). The top panel shows a colony of *Streptomyces* sp. degrading carboxymethyl cellulose incorporated in the agar and stained with Congo-red.

Bibliography

- Alkorta, I., Garbisu, G., Llama, M. J., and Serra, J. L., 1998. Industrial application of pectic enzymes: a review. *Process Biochemistry*, **22**, 21–28.
- Dahiya, N., Tewari, R., and Hoondal, G. S., 2006. Biotechnological aspects of chitinolytic enzymes: a review. *Applied Microbiology and Biotechnology*, **71**, 773–782.
- Damhus et al., 2008. Brochure “Enzymes at Work”. 3rd Edition 2008. Editors: Ture Damhus, Svend Kaasgaard, Henrik Lundquist, and Hans Sejr Olsen, all of Novozymes A/S. <http://www.novozymes.com/NR/rdonlyres/DF82372E-521E-41A3-97CF-71B4A3B6907D/0/Enzymesatwork.pdf>.
- Doi, R. H., 2008. Cellulases of mesophilic microorganisms – cellulosome and noncellulosome producers. *Annals of the New York Academy of Sciences*, **1125**, 267–279.
- Jaeger, K.-E., and Eggert, T., 2002. Lipases for biotechnology. *Current Opinion in Biotechnology*, **13**, 390–397.
- Knackmuss, H.-J., 1996. Basic knowledge and perspectives of biodegradation of xenobiotic compounds. *Journal of Biotechnology*, **51**, 287–295.
- Madigan, M., Martinko, J., Dunlap, P., Clark, D., and Brock, T., 2008. *Brock Biology of Microorganisms*. Upper Saddle River, NJ: Pearson Education.
- Rao, M. B., Tanksale, A. M., Ghatge, M. S., and Deshpande, V. V., 1998. Molecular and biotechnological aspects of microbial proteases. *Microbiology and Molecular Biology Reviews*, **62**, 597–635.
- Rivera, J., Vannakambadi, G., Höök, M., and Speziale, P., 2007. Fibrinogen-binding proteins of Gram-positive bacteria. *Journal of Thrombosis and Haemostasis*, **98**, 503–511.
- Shallom, D., and Shoham, Y., 2003. Microbial hemicellulases. *Current Opinion in Microbiology*, **6**, 219–228.
- Stern, R., and Jedrzejewski, M. J., 2006. Hyaluronidases: their genomics, structures, and mechanisms of action. *Chemical Reviews*, **106**, 818–839.

Exoenzymes, Table 2 Exoenzymes, producing organisms, and their applications in biotechnology

Enzyme (class)	Source organism (examples)	Application/industry
Amylase (hydrolases)	<i>Rhizopus</i> sp. <i>Bacillus</i> sp.	Starch saccharification, washing powder, baking industry, biofuel production
Catalase (oxidoreductases)	<i>Micrococcus</i> sp. <i>Aspergillus</i> sp.	Analytical applications, textile industry, conservation of egg-products
Cellulase (hydrolases)	<i>Trichoderma</i> sp. <i>Clostridium</i> sp.	Textile industry, paper industry, biofuel production, washing powder
Chitinase (hydrolases)	<i>Enterobacter</i> sp. <i>Streptomyces</i> sp.	Mosquito control, control of plant pathogenic fungi
Glucose-isomerase (isomerases)	<i>Bacillus</i> sp. <i>Arthrobacter</i> sp.	Starch saccharification, soft drinks
Glucose-oxidase (oxidoreductases)	<i>Trichoderma</i> sp.	Baking industry, conservation of egg-products
Invertase (hydrolases)	<i>Saccharomyces</i> sp.	Sweets (prevention of sugar crystallization)
Laccase (oxidoreductases)	<i>Basidiomyces</i> sp.	Textile industry (jeans bleaching)
Lipase (hydrolases)	<i>Aspergillus</i> sp. <i>Burkholderia</i> sp.	Washing powder, cheese production
Pectate-lyase (lyases)	<i>Streptomyces</i> sp.	Fruit juice clarification, textile industry
Pectinase (hydrolases)	<i>Aspergillus</i> sp. <i>Clostridium</i> sp.	Fruit juice clarification, coffee- and cacao-production
Peroxidase (oxidoreductases)	<i>Bacillus</i> sp.	Textile industry (jeans bleaching)
Protease (hydrolases)	<i>Aspergillus</i> sp. <i>Bacillus</i> sp.	Baking industry, washing powder, meat tenderizer
Pullulanase (hydrolases)	<i>Bacillus</i> sp.	Starch saccharification
Xylanase (hydrolases)	<i>Klebsiella</i> sp. <i>Aspergillus</i> sp.	Baking industry, paper industry, textile industry, animal food additive

Vihinen, M., and Mäntsälä, P., 1989. Microbial amylolytic enzymes. *Critical Reviews in Biochemistry and Molecular Biology*, **24**, 329–418.

Wong, D. W. S., 2009. Structure and action mechanism of ligninolytic enzymes. *Applied Biochemistry and Biotechnology*, **157**, 174–209.

Cross-references

[Aerobic Metabolism](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Carbon Cycle](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fermentation](#)
[Fungi and Lichens](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Symbiosis](#)

EXTRACELLULAR POLYMERIC SUBSTANCES (EPS)

Alan W. Decho
University of South Carolina, Columbia, SC, USA

Synonyms

Capsule; Exopolymers; Exopolysaccharide; Microbial secretions; Slime

Definition

Extracellular polymeric substances (EPS) are molecules having a range of sizes, compositions, and chemical properties that are produced and secreted by bacteria and other microorganisms, and contribute to the cell adaptability, resiliency, and functional roles in environments.

Capsule refers an extracellular matrix that immediately surrounds a cell.

Biofilms refer to attached microbial cells surrounded within a matrix of EPS.

Introduction

It has been realized for a long time that microbial organisms may exist as free-living individuals as well as in groups, attached to surfaces or to each other, and that under many conditions cells often prefer to attach to surfaces. Under the fluctuating, often less-predictable conditions of natural systems, such attachment may confer advantages and a certain degree of stability to cells. It is now realized that most bacteria and other microorganisms (e.g., cyanobacteria, archaea, diatoms) exist as attached “biofilms.” The biofilm consists of microbial cells that have attached to a surface (or aggregated), and have surrounded themselves within a gelatinous matrix of *EPS*, and may exhibit varying degrees of community structure (Decho, 1990). Biofilm formation appears to be a common microbial process that occurs under a wide range of conditions and environments, and whose influences span aquatic, terrestrial, the epi- and

Exoenzymes, Table 2 Exoenzymes, producing organisms, and their applications in biotechnology

Enzyme (class)	Source organism (examples)	Application/industry
Amylase (hydrolases)	<i>Rhizopus</i> sp. <i>Bacillus</i> sp.	Starch saccharification, washing powder, baking industry, biofuel production
Catalase (oxidoreductases)	<i>Micrococcus</i> sp. <i>Aspergillus</i> sp.	Analytical applications, textile industry, conservation of egg-products
Cellulase (hydrolases)	<i>Trichoderma</i> sp. <i>Clostridium</i> sp.	Textile industry, paper industry, biofuel production, washing powder
Chitinase (hydrolases)	<i>Enterobacter</i> sp. <i>Streptomyces</i> sp.	Mosquito control, control of plant pathogenic fungi
Glucose-isomerase (isomerases)	<i>Bacillus</i> sp. <i>Arthrobacter</i> sp.	Starch saccharification, soft drinks
Glucose-oxidase (oxidoreductases)	<i>Trichoderma</i> sp.	Baking industry, conservation of egg-products
Invertase (hydrolases)	<i>Saccharomyces</i> sp.	Sweets (prevention of sugar crystallization)
Laccase (oxidoreductases)	<i>Basidiomycetes</i> sp.	Textile industry (jeans bleaching)
Lipase (hydrolases)	<i>Aspergillus</i> sp. <i>Burkholderia</i> sp.	Washing powder, cheese production
Pectate-lyase (lyases)	<i>Streptomyces</i> sp.	Fruit juice clarification, textile industry
Pectinase (hydrolases)	<i>Aspergillus</i> sp. <i>Clostridium</i> sp.	Fruit juice clarification, coffee- and cacao-production
Peroxidase (oxidoreductases)	<i>Bacillus</i> sp.	Textile industry (jeans bleaching)
Protease (hydrolases)	<i>Aspergillus</i> sp. <i>Bacillus</i> sp.	Baking industry, washing powder, meat tenderizer
Pullulanase (hydrolases)	<i>Bacillus</i> sp.	Starch saccharification
Xylanase (hydrolases)	<i>Klebsiella</i> sp. <i>Aspergillus</i> sp.	Baking industry, paper industry, textile industry, animal food additive

Vihinen, M., and Mäntsälä, P., 1989. Microbial amylolytic enzymes. *Critical Reviews in Biochemistry and Molecular Biology*, **24**, 329–418.

Wong, D. W. S., 2009. Structure and action mechanism of ligninolytic enzymes. *Applied Biochemistry and Biotechnology*, **157**, 174–209.

Cross-references

[Aerobic Metabolism](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Carbon Cycle](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fermentation](#)
[Fungi and Lichens](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Symbiosis](#)

EXTRACELLULAR POLYMERIC SUBSTANCES (EPS)

Alan W. Decho
University of South Carolina, Columbia, SC, USA

Synonyms

Capsule; Exopolymers; Exopolysaccharide; Microbial secretions; Slime

Definition

Extracellular polymeric substances (EPS) are molecules having a range of sizes, compositions, and chemical properties that are produced and secreted by bacteria and other microorganisms, and contribute to the cell adaptability, resiliency, and functional roles in environments.

Capsule refers an extracellular matrix that immediately surrounds a cell.

Biofilms refer to attached microbial cells surrounded within a matrix of EPS.

Introduction

It has been realized for a long time that microbial organisms may exist as free-living individuals as well as in groups, attached to surfaces or to each other, and that under many conditions cells often prefer to attach to surfaces. Under the fluctuating, often less-predictable conditions of natural systems, such attachment may confer advantages and a certain degree of stability to cells. It is now realized that most bacteria and other microorganisms (e.g., cyanobacteria, archaea, diatoms) exist as attached “biofilms.” The biofilm consists of microbial cells that have attached to a surface (or aggregated), and have surrounded themselves within a gelatinous matrix of *EPS*, and may exhibit varying degrees of community structure (Decho, 1990). Biofilm formation appears to be a common microbial process that occurs under a wide range of conditions and environments, and whose influences span aquatic, terrestrial, the epi- and

endo-biont communities of plants and animals, and as well as disease and health processes (Stoodley et al., 2002).

The EPS matrix

One major change that occurs upon attachment to a surface and the formation of a biofilm is the secretion of EPS. The EPS consist of a wide range of molecules that confer different physical/chemical properties and adaptations for cells. The EPS matrix consists of molecules having a range of molecular sizes and conformations. The physical structure of natural EPS is poorly understood but may contain waterchannels, physical forms ranging from tight, dense gels to looser, dispersed slimes, whose EPS diffusivities (i.e., the ability of molecules to diffuse through EPS) may vary considerably. This provides cells with the potential capability to bind and sequester important ions, nutrients, and other molecules, and create strong biogeochemical gradients over small (i.e., micrometer) spatial distances. Cells may additionally surround themselves with EPS *capsules*. Together, EPS form the three-dimensional architecture from which cells may structure interactions, and even construct organized macrostructures.

EPS is an operational designation that refers to the milieu of relatively “large” molecules contained in the extracellular matrix in proximity to cells. In the past, cold-ethanol precipitations were used to separate the large polysaccharide-rich fraction of EPS from other molecules in the extracellular matrix. It is now realized, however, that a host of other molecules (e.g., proteins, lipids, and even DNA, etc.) may be present in the extracellular matrix. It is now realized that in natural biofilms, the polysaccharide fraction represents only a (small) subset of a more diverse EPS matrix. It is now realized that in some natural systems, a large portion of the EPS cannot be defined as a protein, carbohydrate, or lipid, and hence does not conveniently fit into classical chemical categories.

Initially, EPS was largely considered to be a matrix of “*exopolysaccharides*” (i.e., extracellular polysaccharides); and implied that the terms *EPS* and *exopolysaccharides* were synonymous. However, this was, in part, based on the specific carbohydrate-focus of investigators at the time, and further due to an artifact of carbon-rich culture conditions that were needed to grow bacteria and produce abundant quantities of exopolysaccharide. Much has been learned about polysaccharide chemistry from these important seminal studies (for an early review see Sutherland, 1982).

Currently, it is assumed that EPS contains proteins that may act as both structural components for the EPS matrix and as extracellular enzymes. More recently, deoxyribonucleic acids (DNA) have been abundantly found in the EPS of certain bacteria grown in culture, and in EPS extracted from natural microbial communities. While it is likely that some of this DNA may originate from the lysis of bacteria cells, there is growing evidence that bacteria may actively secrete DNA as part of the EPS. This has important implications for understanding DNA as solely an information molecule. Also, recent

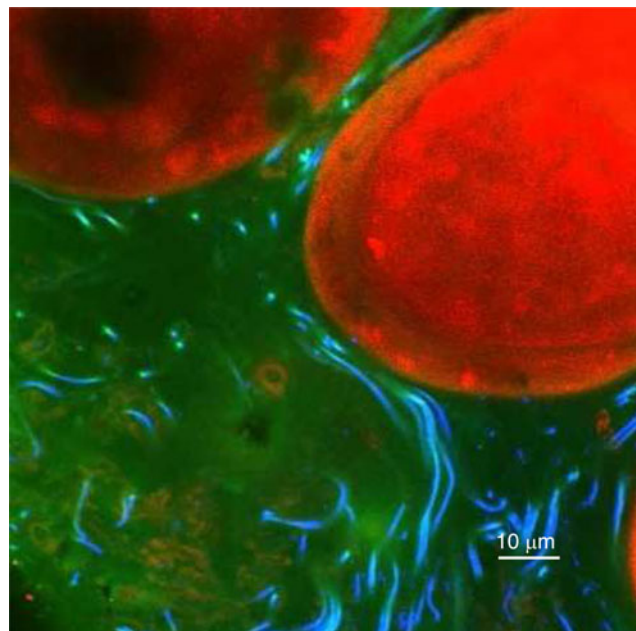
interest has centered on the occurrence of very refractory forms of proteins, called “amyloid proteins” in EPS. These heavily cross-linked proteins have been previously linked to disease processes in humans. In the EPS matrices of microbial communities, such amyloid proteins may provide a refractory “rebar” to resist degradation of the EPS.

Careful examinations of the EPS matrix are now being conducted using specialized equipment such as atomic force microscopy (AFM), nuclear magnetic resonance (NMR) spectroscopy, and confocal scanning laser microscopy (CSLM) in order to image and characterize the matrix under in situ conditions (Figure 1).

Roles of EPS in geobiology

A great deal of interest is developing in the role of EPS as it influences the broader area of geobiology. This interest centers on the role of biologically produced molecules in mineral formation. Certain molecules may directly or indirectly influence how minerals are formed through precipitation events.

The role of EPS in this process may be twofold (Dupraz et al., 2009). When EPS is newly secreted by microorganisms, certain functional groups (e.g., carboxyls) on the EPS may efficiently sequester Ca^{++} ions by forming bidentate bridges between adjacent EPS molecules, and thus inhibit complexation with available carbonate anions, and precipitation. However, partial degradation



Extracellular Polymeric Substances (EPS), Figure 1 Image showing the abundant extracellular polymeric substance (EPS) secretions (green) surrounding cyanobacteria and heterotrophic bacteria (blue) within a stromatolite microbial mat. The large round objects (red) are calcium carbonate sediments, called ooids. Image was collected using a confocal scanning laser microscopy (CSLM) (scale bar = 10 μm).

or steric-inhibition (i.e., blocking the binding) of these functional groups may allow Ca^{++} ions to accumulate locally reaching saturation levels in hydrated pockets within the EPS. Then, certain functional groups on EPS molecules may serve as a potential nucleation site to form a unidentate binding of a Ca^{++} cation, and allowing the cation to complex with a carbonate anion to initiate precipitation of aragonite (CaCO_3), a process which initiates the biogeomineral formation of calcium carbonate. This interest has been fueled by exciting studies of biologically mediated mineral formation that occurs within early oceans (Arp et al., 2001) and the important role of CaCO_3 in global carbon flux. The secretion of EPS by microbial flora likely developed early in the evolution of life, as evidenced by their roles in stromatolite formation, and broader microbial mat processes. Evidence supporting the presence of EPS in fossil microbial mats and other types of microbial-based fossils are now being found.

The presence of EPS in sediment systems strongly influences the physical stability of the sediments against resuspension by wave action and currents. Different physical forms of EPS, ranging from highly soluble (in water) to cohesive gels, have different (i.e., quantitative) stabilizing effects on sediments (Underwood and Paterson, 2003). Additionally, the presence of EPS contributes to the optical properties (i.e., reflectance, scattering, and absorbance) by sediments.

In open aquatic systems such as oceans, microorganisms often form aggregated communities, also called "marine snow," which occur suspended within the water column. Here, the EPS are especially important in the cohesion of cells and molecules into aggregates. Smaller non-cell containing forms of EPS, called "transparent exopolymer particles" (TEP) may aggregate into larger marine snow (Passow et al., 2001). These aggregation processes become especially pronounced during the later stage of phytoplankton blooms in oceans and lakes, and during extreme examples called "excessive mucilage events," such as those which occur periodically in the northern Adriatic Sea and other areas. Ultimately, the aggregation process eventually sends the microbial cells, EPS, and their sorbed ions and molecules to the sediments. This sedimentation process collectively carries with itself large quantities of carbon and important trace elements, and contributes to the global flux of these elements in oceans.

Conclusion

EPS are extracellular polymeric substances that are produced and secreted by microorganisms, and may vary in their physical state, chemical properties, and functional roles to cells. The EPS are often produced in association with a biofilm. The production of EPS influences many processes that have broader biological and geobiological importance. For example, EPS gels alter the physical stability and optical signatures of sediments and seawater,

and are involved in biogeomineral precipitation, and the global flux of carbon and other elements within oceans. Finally, biofilms and their EPS are prominent components of extreme environments ranging from cold Antarctic epipontic communities to anhydrophilic hypersaline ponds to the high temperatures/pressures of hydrothermal vent systems.

Summary

It is now realized that most bacteria and other microorganisms (e.g., cyanobacteria, Archea, diatoms) exist as attached "biofilms." The biofilm consists of microbial cells that have attached to a surface (or aggregated), and have surrounded themselves within a secreted gelatinous matrix of EPS. EPS is an operational designation that refers to the milieu of relatively "large" molecules contained in the extracellular matrix in proximity to cells. The EPS confer different physical/chemical properties and provides cells with the potential capability to bind and sequester important ions, nutrients, and other molecules, and create strong biogeochemical gradients over small (i.e., micrometer) spatial distances. Together, EPS form the three-dimensional architecture from which cells may structure interactions, and even construct organized macrostructures. A great deal of interest is developing in the role of EPS as it influences the broader area of geobiology. This interest centers on the role of biologically produced molecules in mineral formation.

Bibliography

- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in phanerozoic oceans. *Science*, **292**, 1701–1704.
- Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Reviews*, **28**, 73–154.
- Dupraz, C., Reid, R. P., Braissant, O., Decho, A. W., Norman, R. S., and Visscher, P. T., 2009. Processes of carbonate precipitation in modern microbial mats. *Earth Science Reviews*, **96**, 141–162.
- Passow, U., Shipe, R. F., Murray, A., Pak, D. K., Brzezinski, M. A., and Alldredge, A. L., 2001. Origin of transparent exopolymer particles (TEP) and their role in the sedimentation of particulate matter. *Continental Shelf Research*, **21**, 327–346.
- Stoodley, P., Sauer, K., Davies, D. G., and Costerton, J. W. 2002. Biofilms as complex differentiated communities. *Annual Review of Microbiology*, **56**, 187–209.
- Sutherland, I. W., 1982. Biosynthesis of microbial exopolysaccharides. *Advances in Microbial Physiology*, **23**, 79–150.
- Underwood, G. J. C., and Paterson, D. M., 2003. The importance of extracellular carbohydrate production by marine epipelagic diatoms. *Advances in Botanical Research*, **40**, 1–54.

Cross-references

Bacteria
 Calcite Precipitation, Microbially Induced
 Detachment
 Endoliths

Extreme Environments
 Geomycology
 Hydrothermal Environments, Terrestrial
 Mat-Related Sedimentary Structures
 Microbial Biomining
 Microbial Mats
 Stromatolites

EXTREME ENVIRONMENTS

Volker Thiel

University of Göttingen, Göttingen, Germany

Definition

The word “extreme” comes from the Latin word “*extremus*,” the superlative of “*exter*” (= on the outside) (Rothschild and Mancinelli, 2001). Whereas there is no general agreement on how to define an extreme environment, the term is commonly used for any setting that exhibits life conditions detrimental or fatal to higher organisms with respect to its physicochemical properties. Thus, extreme environments differ in one or more aspects from those which humans consider as “normal,” moderate conditions with circumneutral pH, temperatures between 20°C and 35°C, pressures around 0.1 MPA (1 atm), and adequate concentrations of nutrient and saline. It should be considered, however, that such definition represents an anthropocentric view and that what is extreme and what is normal from a microbial perspective remains questionable.

If not completely uninhabitable to life, extreme environments typically harbor specially adapted organisms, the so-called **extremophiles**. Most extremophiles are unicellular organisms, that is, protists, bacteria, and archaea. As a rule, extreme environments show a low diversity of multicellular organisms and only few animals are able to withstand the harsh conditions of particular extreme environments. Terms describing extremophiles usually combine an environment-specific prefix with the suffix “-phile” (Greek word for “-loving”). Replacing the suffix “-phile” by “-tolerant” implies that an organism *tolerates* rather than *requires* the respective conditions, and actually has its optimum at more moderate conditions. Prefixes may be combined for organisms that thrive in more than one extreme (e.g., “thermoacidophiles”). Such organisms are termed **polyextremophiles**.

Extreme environments can be categorized into several classes.

Acidic environments

Environments below pH 5, including, for example, sulfuric pools, geysers, and areas polluted by acid mine drainage are called acidic environments. Organisms dependent on acidic conditions below pH 5 (pH 7 = neutral) are called **acidophiles**. Examples are the bacterial genus *Acidithiobacillus* (used in biomining, see entry), *Ferroplasma acidophilum*, a sulfuric-acid producing

archaeon involved in acid mine drainage (see entry *Acid Rock Drainage*), the red alga *Cyanidium caldarium* (the most heat and acid tolerant alga known), the green alga *Dunaliella acidophila*, the fungus *Trichosporon cerebriae*, and the archaeum *Picrophilus* (Rothschild and Mancinelli, 2001). The major challenge for these organisms is to maintain their internal cellular pH at a constant, circumneutral level, that is, around pH 7. Individual strategies encompass, *inter alia*, (i) reinforcement of the cell membrane, (ii) secretion of extracellular polymeric substances (EPS) (see entry “*Extracellular Polymeric Substances (EPS)*”) to limit proton diffusion into the cell, (iii) secretion of buffer molecules capable of sequestering protons, including, for example, basic amino acids (lysine, histidine and arginine), and (iv) the ability to actively pump protons out of the cell (for reviews, see Baker-Austin and Dopson, 2007; Rothschild and Mancinelli, 2001).

Alkaline environments

Environments above pH 9 include, for example, hydrothermal springs (Figure 1a) and soda lakes (e.g., Lake Van, Mono Lake, Figure 1b) are called alkaline environments. Organisms dependent on alkaline (high) pH values > pH 9 are termed **alkaliphiles** (Horikoshi, 1998). Examples are the bacterium *Bacillus alcalophilus* (Horikoshi, 1998) and the archaeon *Natronococcus occultus* (Tindall et al., 1984). Like acidic habitats, alkaline environments pose particular requirements for the inhabiting organisms, specifically the ability to maintain internal pH at a level not higher than ~pH 8.5 (Horikoshi, 1998). Strategies to achieve this include, *inter alia*, (i) buffering cell cytoplasm using polyamines rich in amino acids with positively charged side groups (lysine, arginine, and histidine), (ii) introducing acidic compounds to protect the cell from the alkaline environment (e.g., teichoic acid, acidic amino acids, and uronic acids), (iii) Na⁺-ion channels actively driving the transfer of protons into the cell, (iv) synthesis of specific enzymes resistant to high pH. The enzymes of alkaliphiles found a variety of industrial applications. For instance, alkaline proteases, protein-hydrolyzing enzymes, are widely used in the detergent industry.

Hypersaline environments

Environments with salt concentrations greater than that of seawater (35‰; Figure 1, see also Chapters “*Saline Lakes*”, “*Deep Biosphere of Salt Deposits*”) are called hypersaline environments. Organisms dependent on salt concentrations greater than 35‰ (sea water) are called **halophiles**. Halophiles are known from the Archaea, Bacteria, and Eukarya (Grant et al., 1998; Gunde-Cimerron et al., 2005). Eukaryotes are represented, for instance by the green alga *Dunaliella*, and even animals occur in hypersaline environments, although in low species diversity (e.g., the brine shrimp *Artemia*, and the salt fly *Ephydra*). In the bacterial domain, halophiles represent many different taxonomic groups, whereas

Extreme Environments
 Geomycology
 Hydrothermal Environments, Terrestrial
 Mat-Related Sedimentary Structures
 Microbial Biomining
 Microbial Mats
 Stromatolites

EXTREME ENVIRONMENTS

Volker Thiel

University of Göttingen, Göttingen, Germany

Definition

The word “extreme” comes from the Latin word “*extremus*,” the superlative of “*exter*” (= on the outside) (Rothschild and Mancinelli, 2001). Whereas there is no general agreement on how to define an extreme environment, the term is commonly used for any setting that exhibits life conditions detrimental or fatal to higher organisms with respect to its physicochemical properties. Thus, extreme environments differ in one or more aspects from those which humans consider as “normal,” moderate conditions with circumneutral pH, temperatures between 20°C and 35°C, pressures around 0.1 MPA (1 atm), and adequate concentrations of nutrient and saline. It should be considered, however, that such definition represents an anthropocentric view and that what is extreme and what is normal from a microbial perspective remains questionable.

If not completely uninhabitable to life, extreme environments typically harbor specially adapted organisms, the so-called **extremophiles**. Most extremophiles are unicellular organisms, that is, protists, bacteria, and archaea. As a rule, extreme environments show a low diversity of multicellular organisms and only few animals are able to withstand the harsh conditions of particular extreme environments. Terms describing extremophiles usually combine an environment-specific prefix with the suffix “-phile” (Greek word for “-loving”). Replacing the suffix “-phile” by “-tolerant” implies that an organism *tolerates* rather than *requires* the respective conditions, and actually has its optimum at more moderate conditions. Prefixes may be combined for organisms that thrive in more than one extreme (e.g., “thermoacidophiles”). Such organisms are termed **polyextremophiles**.

Extreme environments can be categorized into several classes.

Acidic environments

Environments below pH 5, including, for example, sulfuric pools, geysers, and areas polluted by acid mine drainage are called acidic environments. Organisms dependent on acidic conditions below pH 5 (pH 7 = neutral) are called **acidophiles**. Examples are the bacterial genus *Acidithiobacillus* (used in biomining, see entry), *Ferroplasma acidophilum*, a sulfuric-acid producing

archaeon involved in acid mine drainage (see entry *Acid Rock Drainage*), the red alga *Cyanidium caldarium* (the most heat and acid tolerant alga known), the green alga *Dunaliella acidophila*, the fungus *Trichosporon cerebriae*, and the archaeum *Picrophilus* (Rothschild and Mancinelli, 2001). The major challenge for these organisms is to maintain their internal cellular pH at a constant, circumneutral level, that is, around pH 7. Individual strategies encompass, *inter alia*, (i) reinforcement of the cell membrane, (ii) secretion of extracellular polymeric substances (EPS) (see entry “*Extracellular Polymeric Substances (EPS)*”) to limit proton diffusion into the cell, (iii) secretion of buffer molecules capable of sequestering protons, including, for example, basic amino acids (lysine, histidine and arginine), and (iv) the ability to actively pump protons out of the cell (for reviews, see Baker-Austin and Dopson, 2007; Rothschild and Mancinelli, 2001).

Alkaline environments

Environments above pH 9 include, for example, hydrothermal springs (Figure 1a) and soda lakes (e.g., Lake Van, Mono Lake, Figure 1b) are called alkaline environments. Organisms dependent on alkaline (high) pH values > pH 9 are termed **alkaliphiles** (Horikoshi, 1998). Examples are the bacterium *Bacillus alcalophilus* (Horikoshi, 1998) and the archaeon *Natronococcus occultus* (Tindall et al., 1984). Like acidic habitats, alkaline environments pose particular requirements for the inhabiting organisms, specifically the ability to maintain internal pH at a level not higher than ~pH 8.5 (Horikoshi, 1998). Strategies to achieve this include, *inter alia*, (i) buffering cell cytoplasm using polyamines rich in amino acids with positively charged side groups (lysine, arginine, and histidine), (ii) introducing acidic compounds to protect the cell from the alkaline environment (e.g., teichoic acid, acidic amino acids, and uronic acids), (iii) Na⁺-ion channels actively driving the transfer of protons into the cell, (iv) synthesis of specific enzymes resistant to high pH. The enzymes of alkaliphiles found a variety of industrial applications. For instance, alkaline proteases, protein-hydrolyzing enzymes, are widely used in the detergent industry.

Hypersaline environments

Environments with salt concentrations greater than that of seawater (35‰; Figure 1, see also Chapters “*Saline Lakes*”, “*Deep Biosphere of Salt Deposits*”) are called hypersaline environments. Organisms dependent on salt concentrations greater than 35‰ (sea water) are called **halophiles**. Halophiles are known from the Archaea, Bacteria, and Eukarya (Grant et al., 1998; Gunde-Cimerron et al., 2005). Eukaryotes are represented, for instance by the green alga *Dunaliella*, and even animals occur in hypersaline environments, although in low species diversity (e.g., the brine shrimp *Artemia*, and the salt fly *Ephydra*). In the bacterial domain, halophiles represent many different taxonomic groups, whereas



Extreme Environments, Figure 1 Examples and features of extreme environments. (Images courtesy of J. Reitner and G. Arp) (a) A volcanic hot spring on Iceland, providing an environment for thermophilic bacteria and archaea. The spring bottom shows a temperature zonation of brown and black microbial mats. (b) Mono Lake, California, is an excellent example for both a hypersaline and an alkaline environment. Being a terminal lake, Mono Lake has no outlet to the ocean, and dissolved salts in the runoff have increased the water's alkalinity ($\text{pH} \sim 10$) and salt concentration (70‰). (c) Underwater view of the ~ 2 m deep hypersaline Lake 21 on Christmas Island (Republic of Kiribati). Kiritimati is a raised atoll, with a rather arid climate. Periodically flooding with sea water and subsequent evaporation cause the salinity of numerous inland lakes to increase to values about 120‰, four times that of sea water. The lake bottom is covered with microbial mats dominated by Cyanobacteria at the surface. (d) A calcifying microbial mat from the same setting as in (c), showing a complex layering of differently pigmented microbes. In the deeper parts of the mat, carbonate precipitation proceeds under anaerobic conditions, ultimately producing massive microbialites. (e) The Bonneville Salt Flats in northwestern Utah, USA, are a remnant of the ancient glacial Lake Bonneville. Up to 1.8 m, thick salt crusts provide an extremely hypertonic environment for halo- and xerophilic microorganisms. (f) Lake Clifton, 120 km south of Perth (Western Australia), provides a fragile ecosystem where the growth of a large thrombolite reef (see "Microbialites") is sustained by calcium carbonate rich aquifers water and strong seasonal trends in salinity (salinity 30–80 ‰). Notably, these modern thrombolitic microbialites coexist with a diverse invertebrate fauna.

those in the archaeal domain fell into a single order (*Halobacteriales*) and family (*Halobacteriaceae*; DasSarma and DasSarma, 2008). The most significant challenge for halophiles is to prevent the loss of water from the cell into the saline environment, and the accumulation of excess salt concentrations within the cell. The latter may give rise to the collapse of proteins and macromolecules, because the electric charges of the salt ions disturb the electrostatic interactions within biomacromolecules. Halophiles basically use two strategies to tackle these challenges. (i) Halophilic bacteria and algae accumulate organic compatible solutes, polar, highly soluble molecules uncharged at physiological pH, such as amino acids and their derivatives to counterbalance the osmotic pressure of the surrounding medium (Galinski, 1993; Oren, 1999). (ii) Halophilic archaea, thriving at NaCl concentrations higher than 1.5 M, use the so-called “salt-in strategy.” These most halophilic organisms concentrate K^+ ions within the cell in order to balance osmotic pressure. Their proteins are particularly rich in acidic amino acids, thus enhancing resistance against structural collapse. For more information, see entry “*Halobacteria – Halophiles*.” Several reports provided evidence that viable extremely halophilic microorganisms were enclosed in ancient salt sediments. Please refer to “*Deep Biosphere of Salt Deposits*” for details.

Extremely hot environments

Environments periodically or consistently above 40°C are called extremely hot environments (Stetter, 1998). Depending on the temperature, these settings harbor **thermophiles** (growth optimum 45–80°C, Martinko and Madigan, 2006) or **hyperthermophiles** (growth optimum above 80°C). (Organisms dependent on moderate temperatures between 10°C and 50°C (optimum 30–40°C) are called **mesophiles**.) Whereas thermophiles have a fairly broad ecological amplitude, including, for example, hot waters, sun-heated soils, and waste dumps, hyperthermophiles mostly occur at water-containing, geothermally heated terrestrial and marine springs and sediments (Figure 1a). As a rule, prokaryotes are able to grow at higher temperatures than eukaryotes (limit ~65°C). Accordingly, hyperthermophilic life is mainly represented by deeply branching bacteria, (e.g., *Aquifex*, *Thermotoga*) and archaea (e.g., *Sulfolobus*, *Methanothermus*; Stetter, 1998). The highest commonly accepted temperature for life has been reported for the hyperthermopiezophilic archaeon *Methanopyrus kandleri* (122°C; Takai et al., 2008). The challenge for organisms living in hot environments lies in avoiding thermal degradation of cellular biomacromolecules, which may, for example, result in unfolding of proteins. Individual strategies encompass stabilization of proteins by introducing additional disulfide bridges, ionic interactions, hydrogen bonds, and hydrophobic interactions. Another alternative is to introduce the proline residue at particular sites into proteins (the

proline rule; Imanaka, 2008). Because many scientists believe that prebiotic molecules and the first living organisms originated in hot environments, hyperthermophiles may provide insight into the early stages of life on Earth. Please refer to “*Hydrothermal Environments, Terrestrial*,” “*Hydrothermal Environments, Marine*,” “*Hot Springs and Geysers*,” and “*Origin of Life*” for further reading.

High-pressure environments

Environments under extreme hydrostatic or petrostatic (rock) pressure are high-pressure environments. Organisms thriving in these settings are called **piezophiles** (formerly barophiles). Broadly spoken, **piezophiles** organisms dependent on pressures greater than atmospheric pressure (0.1 MPa, Yayanos, 1998), although organisms living, for example, in shallow coastal waters are commonly neither considered as piezophiles nor as extremophiles. Based on their optimal growth pressure, piezophiles have further been classified as piezotolerant (0.1–10 MPa; 10 MPa $\approx$ 1,000 m water depth), piezophilic (10–50 MPa), and hyperpiezophilic (>50 MPa) bacteria and archaea (Fang and Bazylinski, 2008). Whereas, as a rule, the relative growth rate of these organisms decreases with pressure, the upper pressure limit of life has not yet been determined (Yayanos, 1998; Schrenk et al., 2010). High pressures affect biological systems by making the structures (e.g., membranes) more compact, as opposed to the effects of high temperature. Pressurization hinders any process resulting in a positive volume change, and vice versa. For example, if a reaction is accompanied by a volume decrease of 300 mL mol⁻¹, it is enhanced more than 200,000-fold by applying a pressure of 100 MPa ($\approx$ 10,000 m water depth; Abe et al., 1999). Likewise, hydrostatic pressure has been shown to exert a considerable influence on many protein–protein interactions, the efficacy of enzymatic catalysis, replication, and translation. Piezophiles have therefore evolved specific adaptations, for example, in terms of membrane lipid composition and cell division. Pressure has also a significant effect on microbial-mediated redox reactions, and metabolic versatility appears to be a specific adaptation to deep environments (Fang and Bazylinski, 2008; Lauro and Bartlett, 2008). For detailed reading, please refer to entry “*Piezophilic Bacteria*.”

Extremely cold environments

Environments with temperatures below 5°C for prolonged periods of time, such as cold polar regions, glaciers, sea-ice, deep-sea sediments, and permafrost soils are considered extremely cold environments. Organisms capable of growth and reproduction in these settings are called **psychrophiles** (cryophiles). They depend on low temperatures (< 0°C to 20°C) and have a growth optimum below 15°C (Morita, 1975). The lowest temperature limit for life seems to be around –20°C and has been reported for bacteria living in permafrost soil and in sea-ice (D’Amico

et al., 2006). Psychrophiles are found in all three domains of life. Eukaryotes such as *algae* (e.g., sea-ice diatoms, the “snow alga” *Chlamydomonas nivalis*), protozoans, fungi, and even small metazoans frequently occur, but psychrophiles are most abundant among diverse lineages within the bacteria (e.g., *Pseudomonas* spp., *Vibrio* spp., several cyanobacterial genera) and, less often cited, archaea (e.g., *Methanogenium* and *Methanococcus*) (Garrison, 1991; D’Amico et al., 2006). Main cold-induced challenges posed to psychrophiles within their habitats encompass (i) reduced enzyme activity, (ii) decreased membrane fluidity, (iii) altered transport of nutrients and waste products, (iv) decreased rates of transcription, translation, and cell division, (v) protein cold-denaturation and inappropriate protein folding, (viii) intracellular ice formation, and (ix) low water availability (see D’Amico et al., 2006, for a review). Psychrophilic organisms have successfully evolved strategies to overcome these negative effects of low temperatures. Such strategies involve modifications of the cell membrane composition toward a higher content of unsaturated, branched, or short-chain fatty acids, and large polar head groups (Chintalapati et al., 2004). Psychrophiles also synthesize specific antifreeze proteins, trehalose, and extracellular polymeric substances (EPS), which play an important role as cryoprotectants to keep the intercellular space liquid and protect the DNA at temperatures below water’s freezing point (see D’Amico et al., 2006, for a review). For further reading, please refer to “*Permafrost Microbiology*.”

Extremely dry environments

Environments without free water are considered extremely dry environments and they include hot and cold deserts, and some terrestrial endolithic habitats (see also Chapter “*Endoliths*”). Organisms dependent on very dry environments are termed **xerophiles**, whereas those tolerating only temporary desiccation are referred to as “xerotolerant.” Most organisms thriving in very dry environments are actually xerotolerants, which rely on at least periodically available free water. A strategy to cope with prolonged periods of dryness is to enter the state of anhydrobiosis, which is characterized by little intracellular water and no metabolic activity. Organisms that can become anhydrobiotic are found among bacteria, yeast, fungi, plants, and even animals such as nematodes and the brine shrimp *Artemia salina* (Rothschild and Mancinelli, 2001). The terms “xerophile” and “xerotolerant” are often used to include organisms thriving under conditions of low water activity $a_w < 0.80$. The a_w is a measure of the amount of water within a medium that an organism can use to support growth. It represents the ratio of the water vapor pressure of the substrate to that of pure water under the same conditions and is expressed as a fraction (pure water, $a_w = 1$; saturated NaCl, $a_w = 0.75$). The lowest a_w value recorded for growth to date was reported for the spoilage mould *Xeromyces bisporus* ($a_w = 0.61$; see Grant, 2004 for a review). According to this

definition, the most xerophilic organisms known, thrive in foods preserved by some form of dehydration or enhanced sugar levels, and in hypersaline environments where water availability is limited by a high concentration of salts (Grant, 2004). Whereas the former are dominated by xerophilic filamentous fungi and yeasts, high-salt environments are almost exclusively populated by prokaryotes. For the strategies employed by these organisms to cope with the osmotic stress exerted by these environments see above, and entry “*Halobacteria – Halophiles*.”

High-radiation environments

Environments exposed to high doses of ionizing radiation are high-radiation environments. Ionizing radiation is radiation with sufficient energy to ionize molecules, most commonly ultraviolet (UV) radiation and natural radioactivity. When such radiation passes through (living) matter, ions and free radicals are produced that react rapidly and modify molecules (Cox and Battista, 2005). Ionizing radiation is therefore potentially detrimental for life, mainly due to DNA damage resulting from the generation of reactive oxygen species and the hydrolytic cleavage of water. Organisms that are capable of withstanding high doses of ionizing radiation are called **radioresistant**. There is no clear pattern of evolution among ionizing-radiation-resistant species, and they occur scattered over the three domains of life. Well-studied microbial examples are the archaeon *Thermococcus gammatolerans* (Jolivet et al., 2003) and the bacterium *Deinococcus radiodurans* (Cox and Battista, 2005). Strategies to adapt to high doses of ionizing radiation involve (i) increasing the numbers of genome copies, (ii) tight spatial arrangement of nucleoids, (iii) accumulation of efficient radical scavengers, (iv) delaying DNA replication until damage repair has been completed, and (v) improved enzymatic genome-repair process. For further reading, please refer to entry “*Radioactivity*.”

Summary

“*Extreme environment*” refers to any setting that exhibits life conditions detrimental or fatal to higher organisms with respect to its physicochemical properties, in particular pH, temperature, pressure, saline concentrations, and radiation. Major classes of extreme environments encompass acidic ($\text{pH} < 5$), alkaline ($\text{pH} > 9$), hypersaline (salinity $> 35\%$), pressurized ($> 0.1 \text{ MPa}$), hot ($> 40^\circ\text{C}$), cold ($< 5^\circ\text{C}$), dry ($a_w < 0.80$), and high-radiation environments. These environments typically harbor specially adapted organisms, the so-called extremophiles. These can be classified into acidophiles, alkaliphiles, (hyper-) thermophiles, psychrophiles, xerophiles, and radioresistant organisms.

Bibliography

- Abe, F., Kato, C., and Horikoshi, K., 1999. Pressure-regulated metabolism in microorganisms. *Trends in Microbiology*, **7**, 447–453.
- Baker-Austin, C., and Dopson, M., 2007. Life in acid: pH homeostasis in acidophiles. *Trends in Microbiology*, **15**, 165–171.

- Chintalapati, S., Kiran, M. D., and Shivaji, S., 2004. Role of membrane lipid fatty acids in cold adaptation. *Cellular and Molecular Biology*, **50**, 631–642.
- Cox, M. M., and Battista, J. R., 2005. *Deinococcus radiodurans* — the consummate survivor” (PDF). *Nature reviews, Microbiology*, **3**, 882–892.
- D’Amico, S., Collins, T., Marx, J. C., Feller, G., and Gerday, C., 2006. Psychrophilic microorganisms: challenges for life. *EMBO Reports*, 385–389.
- DasSarma, P., and DasSarma, S., 2008. On the origin of prokaryotic “species”: the taxonomy of halophilic Archaea. *Saline Systems*, **4**, 5.
- Fang, J., and Bazylinski, D. A., 2008. Deep-sea geomicrobiology. In Michiels, C., and Bartlett, D. H. (eds.), *High-Pressure Microbiology*. Washington, DC: American Society for Microbiology, pp. 237–264.
- Galinski, E. A., 1993. Compatible solutes of halophilic eubacteria: molecular principles, water-solute interactions, stress protection. *Experientia*, **49**, 487–496.
- Garrison, D. L., 1991. Antarctic sea ice biota. *American Zoologist*, **31**, 17–33.
- Grant, W. D., 2004. Life at low water activity. *Philosophical Transactions Of the Royal Society of London Series B*, **359**, 1249–1267.
- Grant, W. D., Gemmel, R. T., and McGenity, T. J., 1998. Halophiles. In Horikoshi, K., and Grant, W. D. (eds.), *Extremophiles: Microbial Life in Extreme Environments*. New York: Wiley-Liss, pp. 93–132.
- Gunde-Cimeron, N., Oren, A., and Plemenita, A. (eds.) 2005. *Adaptation to Life in High Salt Concentrations in Archaea, Bacteria, and Eukarya*. The Netherlands: Springer, 577 p.
- Horikoshi, K., 1998. Alkaliphiles. In Horikoshi, K., and Grant, W. D. (eds.), *Extremophiles: Microbial Life in Extreme Environments*. New York: Wiley, pp. 155–179.
- Imanaka, T., 2008. Adaptation strategy of thermophiles toward hyperthermophily and their molecular bases. *Bulletin of the Chemical Society of Japan*, **81**, 171–182.
- Jolivet, E., L’Haridon, S., Corre, E., Forterre, P., and Prieur, D., 2003. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 847–851.
- Kamekura, M., 1998. Diversity of extremely halophilic bacteria. *Extremophiles*, **2**, 289–295.
- Lauro, F. M., and Bartlett, D. H., 2008. Prokaryotic lifestyles in deep sea habitats. *Extremophiles*, **12**, 15–25.
- Martinko, J. M., and Madigan, M. T., 2006. *Brock biology of microorganisms*, 11th edn. Englewood Cliffs, NJ: Prentice Hall.
- Morita, R. Y., 1975. Psychrophilic bacteria. *Bacteriological Reviews*, **39**, 144–167.
- Oren, A., 1999. Bioenergetic Aspects of Halophilism. *Microbiology and Molecular Biology Reviews*, **63**, 334–348.
- Rothschild, L. J., and Mancinelli, R. L., 2001. Life in extreme environments. *Nature*, **409**, 1092–1101.
- Round, F. E., 1981. *The Ecology of Algae*. Cambridge: Cambridge University press, 653 pp.
- Schrenk, M. O., Huber, J. A., and Edwards, K. J., 2010. Microbial Provinces in the Subseafloor. *Annual Review of Marine Science*, **2**, 279–304.
- Stetter, K. O., 1998. Hyperthermophiles: Isolation, classification and properties. In Horikoshi, K., and Grant, W. D., (eds.), *Extremophiles, Microbial Life in Extreme Environments*. New York: John Wiley & Sons, Inc., pp. 1–24.
- Takai, K., Nakamura, K., Toki, T., Tsunogai, U., Miyazaki, M., Hirayama, H., Nakagawa, S., Nunoura, T., and Horikoshi, K., 2008. Cell proliferation at 122°C and isotopically heavy CH₄ production by a hyperthermophilic methanogen under high-pressure cultivation. *Proceedings of the National Academy of Sciences of USA*, **105**, 10949–10954.
- Tindall, B. J., Ross, H. N. M., and Grant, W. D., 1984. *Natronobacterium* gen. nov. and *Natronococcus* gen. nov., two new genera of haloalkaliphilic archaeobacteria. *Systematic and Applied Microbiology*, **5**, 41–57.
- Yayanos, A. A., 1998. Empirical and theoretical aspects of life at high pressure in the deep sea. In Horikoshi, K., and Grant, W. D. (eds.), *Extremophiles, Microbial Life in Extreme Environments*. New York: Wiley, pp. 47–92.

Cross-references

[Acid Rock Drainage](#)
[Algae \(Eukaryotic\)](#)
[Alkalinity](#)
[Archaea](#)
[Bacteria](#)
[Chroococcidiopsis](#)
[Deep Biosphere of Salt Deposits](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Diatoms](#)
[Endoliths](#)
[Fungi and Lichens](#)
[Halobacteria – Halophiles](#)
[Hot Springs and Geysers](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Origin of Life](#)
[Permafrost Microbiology](#)
[Radioactivity \(Natural\)](#)
[Saline Lakes](#)
[Soda Lakes](#)
[Terrestrial Deep Biosphere](#)

F

FE(II)-OXIDIZING PROKARYOTES

Kristina L. Straub
University of Vienna, Vienna, Austria

Synonyms

Fe-oxidizers; Fe(II)-oxidizing prokaryotes/microorganisms; Ferrous iron-oxidizing prokaryotes/microorganisms; Iron-oxidizers; Iron-oxidizing prokaryotes/microorganisms.; Iron(II)-oxidizing prokaryotes/microorganisms

Definition

Fe(II)-oxidizing prokaryotes. Diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to oxidize Fe(II), ferrous iron, to Fe(III), ferric iron. The electrons obtained from the oxidation of Fe(II) are utilized for energy generation in aerobic or anaerobic respiration and/or for assimilative reduction reactions.

Introduction

Aerobic neutrophilic Fe(II)-oxidizing bacteria were among the first environmentally relevant prokaryotes that were discovered and studied in the nineteenth century. At that time, eye-catching ochre deposits in ponds and slowly running waters had attracted the attention of microbiologists. Microscopic analyses of such ochre deposits revealed the prevalence of twisted stalks and/or sheaths that were covered with iron minerals. Bacteria were suggested to be responsible for the production of these specific iron-covered structures, and subsequently the first aerobic Fe(II)-oxidizing prokaryotes were described: *Gallionella ferruginea* and *Leptothrix ochracea* (reviewed by Emerson, 2000; Canfield et al., 2005). In contrast, the anaerobic oxidation of Fe(II) by phototrophic bacteria (Widdel et al., 1993), nitrate-reducing bacteria

(Straub et al., 1996), and archaea (Hafenbradl et al., 1996) was not discovered until the end of the twentieth century. Aerobic and anaerobic Fe(II)-oxidizing prokaryotes play decisive roles in the biogeochemical cycle of iron. In comparison to other physiological groups, only few species of Fe(II)-oxidizing prokaryotes are available in pure culture. The capability to oxidize Fe(II) is apparently phylogenetically widespread because the known species belong to diverse phyla of the *Bacteria* and the *Archaea*. The mechanism(s) of prokaryotic Fe(II)-oxidation are largely unknown (Canfield et al., 2005).

Geochemical aspects of Fe(II)

Iron is the fourth most abundant element in the Earth's crust, and depending upon the geochemical conditions, it occurs either in soluble forms or in a variety of minerals (Cornell and Schwertmann, 2003). In natural habitats, Fe(III) minerals are reduced to Fe(II) mainly by the metabolic activities of prokaryotes. The concentration of dissolved Fe(II) is controlled by precipitation-dissolution reactions and by adsorption processes: Fe(II) precipitates with carbonates, phosphates, or sulfides and tends to adsorb to soil particles and mineral surfaces (Cornell and Schwertmann, 2003; Canfield et al., 2005). Chemical reoxidation reactions of Fe(II) depend on the pH and on the presence of appropriate oxidants. The reaction rate of Fe(II) with molecular oxygen is very low at acidic pH values, and hence Fe(II) is fairly stable in the presence of oxygen under acidic conditions. With increasing pH, the reaction rate of Fe(II) and molecular oxygen increases, and at fully aerated conditions Fe(II) is rapidly oxidized to ferric iron (Emerson, 2000; Canfield et al., 2005). In the absence of molecular oxygen, Fe(II) can be chemically oxidized at appreciable rates only by nitrite or Mn(IV). However, Mn(IV) minerals are insoluble, and the environmental significance of these nondiffusible oxidants is therefore limited (Canfield et al., 2005). It is thus

speculated that anaerobic Fe(II)-oxidizing prokaryotes are important catalysts for the reoxidation of Fe(II) in anoxic environments.

Fe(II) as electron donor in aerobic or anaerobic respiration

Dissimilatory Fe(II)-oxidizing prokaryotes utilize Fe(II) as electron donor in aerobic or anaerobic respiration, i.e., they gain energy by coupling the oxidation of Fe(II) to the reduction of molecular oxygen or to the reduction of nitrate. As in the oxidation of Fe(II) to Fe(III), only one electron can be obtained, dissimilatory Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to gain enough energy for maintenance and growth. Today, three physiological groups of prokaryotes are known to grow with Fe(II) as electron donor: acidophilic aerobic, neutrophilic aerobic, and neutrophilic nitrate-reducing prokaryotes. Due to energetic considerations, the oxidation of Fe(II) with nitrate at low pH values should also yield enough energy for prokaryotic growth. However, acidophilic Fe(II)-oxidation coupled to the reduction of nitrate has not been reported so far (Kappler and Straub, 2005; Appelo and Postma, 2007). The dissimilative oxidation of Fe(II) leads to the formation of Fe(III) minerals which are well soluble under acidic conditions but barely soluble at neutral pH. Hence, neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product.

Acidophilic aerobic Fe(II)-oxidizing prokaryotes

A variety of autotrophic and heterotrophic prokaryotes that thrive in environments of low pH (< 3) were described to grow by dissimilatory aerobic oxidation of ferrous iron (Johnson, 2007). At acidic pH values, Fe(II) is stable in the presence of molecular oxygen, and hence prokaryotes do not need to compete with the chemical oxidation. However, the energy gain of acidophilic aerobic Fe(II) oxidation is low because the redox potential of the redox pair $\text{Fe}^{3+}/\text{Fe}^{2+}$ is with +770 mV rather high. The difference to the redox potential of the redox pair $\text{O}_2/\text{H}_2\text{O}$ (> +1,000 mV below pH 3) allows therefore only little energy generation (Kappler and Straub, 2005; Appelo and Postma, 2007). Nevertheless, representatives of different phyla of the *Bacteria* (e.g., *Acidithiobacillus ferrooxidans*, *Sulfobacillus acidophilus*) and the *Archaea* (*Acidianus brierleyi*, *Sulfolobus metallicus*) exploit Fe(II) in aerobic respiration. Moreover, three species of the genus *Leptospirillum* are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Johnson, 2007). All other acidophilic Fe(II)-oxidizing prokaryotes make use of alternative electron donors (e.g., hydrogen, sulfur) and sulfur or Fe(III) as alternative electron acceptors. Species of acidophilic Fe(II)-oxidizing bacteria that also grow by dissimilatory reduction of Fe(III) were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993).

Acidithiobacillus ferrooxidans was the first acidophilic aerobic Fe(II)-oxidizing bacterium that was isolated (Temple and Colmer, 1951); it belongs to the *Gammaproteobacteria* and is so far the best studied of all acidophilic prokaryotes. However, details of the flow of electrons from Fe(II) to molecular oxygen are still debated. Apparently, Fe(II) is oxidized on the cell surface at the outer membrane. Subsequently, a variety of membrane-bound and periplasmic cytochromes plus the periplasmic copper protein rusticyanin transfer the electrons to a cytochrome *c* oxidase. There the electrons are used together with protons to reduce molecular oxygen to water (Johnson, 2007; Castelle et al., 2008).

Neutrophilic aerobic Fe(II)-oxidizing bacteria

So far, only few representatives of the *Alpha*-, *Beta*-, and *Gammaproteobacteria* are described to grow by dissimilatory aerobic oxidation of Fe(II) at neutral pH. This physiological group gains more energy than their acidophilic counterparts because the difference between the redox potentials of the relevant redox couples is greater at neutral than at acidic pH values ($\text{Fe(III)/Fe(II)} < +100$ mV; $\text{O}_2/\text{H}_2\text{O} +820$ mV; Kappler and Straub, 2005; Appelo and Postma, 2007). However, in oxic environments of neutral pH Fe(II) has a half-life in the order of few minutes and is chemically oxidized to ferric iron (Canfield et al., 2005). This competition with the chemical oxidation is likely the major reason why neutrophilic aerobic Fe(II)-oxidizing bacteria thrive preferentially in microoxic niches or oxic/anoxic boundaries. Laboratory studies showed that the activity of Fe(II)-oxidizing bacteria contributes up to 90% to the formation of Fe(III) under such conditions. Typical habitats for neutrophilic aerobic Fe(II)-oxidizing bacteria include groundwater springs, freshwater, and marine hydrothermal vents, wetlands, and plant rhizospheres (Emerson, 2000). Due to their production of conspicuous iron-encrusted twisted stalks or sheaths, *Gallionella ferruginea* and *Leptothrix ochracea* were the first Fe(II)-oxidizing bacteria that were already discovered in the nineteenth century. Only during the past few years, additional morphologically inconspicuous aerobic Fe(II)-oxidizing bacteria were isolated, e.g., *Ferritrophicum radicola* and *Sideroxydans paludicola* (Canfield et al., 2005; Weiss et al., 2007). According to physiological studies, several of the novel strains are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Weiss et al., 2007).

Neutrophilic anaerobic Fe(II)-oxidizing nitrate-reducing prokaryotes

Under anoxic conditions, bacteria (*Alpha*-, *Beta*-, *Gamma*- and *Deltaproteobacteria*) and archaea (*Archaeoglobales*) are capable of oxidizing Fe(II) with nitrate as the terminal electron acceptor. At pH 7, all redox pairs of the nitrate reduction pathway can accept electrons from Fe(II), because their redox potentials are more positive than that

of the iron redox couple (Straub et al., 1996). Nitrate-dependent Fe(II) oxidation was reported for diverse habitats such as rice paddy soil, activated sewage sludge, freshwater or marine sediments (Canfield et al., 2005). The first observation of this metabolism was made with a freshwater enrichment culture: Fe(II) was oxidized to Fe(III) and nitrate was concomitantly and stoichiometrically reduced to molecular nitrogen (Straub et al., 1996). As Fe(II) was the sole electron donor supplied, Fe(II) oxidation coupled to nitrate reduction definitely supported cell growth in this enrichment culture. The situation is not so clear in most pure cultures of Fe(II)-oxidizing nitrate-reducing bacteria (e.g., *Acidovorax* sp. strain BoFeN1 or *Thermomonas* sp. strain BrG3) because they need an organic co-substrate for growth. In such strains it is questioned whether Fe(II) oxidation is beneficial and supports cell growth or whether Fe(II) is just oxidized in a rather unspecific side reaction. All known species are able to utilize alternative electron donors (e.g., hydrogen, organic acids) and alternative electron acceptors (nitrite, oxygen, ferric iron). *Geobacter metallireducens*, isolated as Fe(III)-reducing bacterium, couples the oxidation of Fe(II) to the reduction of nitrate to ammonium; it is unclear if *G. metallireducens* obtains energy by this process (Lovley et al., 2004; Canfield et al., 2005). *Ferroglobus placidus* is so far the only representative of the domain *Archaea* that grows by coupling the dissimilatory oxidation of Fe(II) to the reduction of nitrate to nitric oxide and nitrogen dioxide. Alternatively, *F. placidus* utilizes hydrogen, sulfide, acetate, or monoaromatic compounds as electron donor and thiosulfate or Fe(III) as electron acceptor (Hafenbradl et al., 1996; Lovley et al., 2004).

Fe(II) as electron donor for assimilative reduction reactions

Various groups of prokaryotes are able to reduce oxidized inorganic compounds for the synthesis of biomass. Such assimilative reduction reactions require a source of electrons (Canfield et al., 2005). Phylogenetically and physiologically different groups of chemotrophic and phototrophic prokaryotes have the ability to exploit Fe(II) as source of electrons for assimilative reduction reactions such as the fixation of carbon dioxide. Chemotrophic acidophilic and neutrophilic, aerobic and anaerobic Fe(II)-oxidizing prokaryotes have been discussed before because they oxidize the major fraction of Fe(II) during respiratory energy generation. In contrast, phototrophic Fe(II)-oxidizing prokaryotes utilize light as energy source and oxidize Fe(II) exclusively for assimilative purposes. The assimilative oxidation of Fe(II) leads to the formation of Fe(III) which is barely soluble at neutral pH. Neutrophilic phototrophic Fe(II)-oxidizing bacteria therefore have to cope with a virtually insoluble metabolic end product.

Anaerobic phototrophic Fe(II) oxidation

Anoxygenic phototrophic bacteria were the first prokaryotes discovered that oxidize Fe(II) in the absence of

molecular oxygen (Widdel et al., 1993). So far, only few cultures of Fe(II)-oxidizing anoxygenic phototrophic bacteria were established either from freshwater or from marine sediments. They are affiliated with the purple sulfur (*Thiodictyon* sp. strain F4), the purple non-sulfur (e.g., *Rhodobacter* sp. strain SW2, *Rhodovulum robiginosum*), or the green sulfur bacteria (*Chlorobium ferrooxidans*). All strains have the ability to utilize alternative electron donors such as hydrogen, reduced sulfur compounds, or organic acids (Canfield et al., 2005). The discovery of anoxygenic phototrophic bacteria that directly catalyze the oxidation of Fe(II), provides an alternative or additional explanation for the origin of Precambrian banded iron formations (Widdel et al., 1993; Kappler and Straub, 2005).

Summary

Phylogenetically diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to oxidize Fe(II) during aerobic or anaerobic respiration and/or for assimilative reduction reactions. Only one electron can be obtained from the oxidation of Fe(II) to Fe(III) and Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(II)-oxidizing prokaryotes have to cope with different constraints: (1) Acidophilic Fe(II)-oxidizing prokaryotes gain less energy in respiration than their neutrophilic counterparts; (2) Neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product. It is discussed that anoxygenic phototrophic Fe(II)-oxidizing bacteria contributed to the generation of Precambrian banded iron formations.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Castelle, C., Guiral, M., Malarte, G., Ledgham, F., Leroy, G., Brugna, M., and Giudici-Ortoni, M. T., 2008. A new iron-oxidizing/O₂-reducing supercomplex spanning both inner and outer membranes, isolated from the extreme acidophile *Acidithiobacillus ferrooxidans*. *The Journal of Biological Chemistry*, **283**, 25803–25811.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Emerson, D., 2000. Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH. In Lovley, D. R. (ed.), *Environmental Microbe-Mineral Interactions*. Washington: ASM, pp. 31–52.
- Hafenbradl, D., Keller, M., Dirmeier, R., Rachel, R., Roánagel, P., Burggraf, S., Huber, H., and Stetter, K. O., 1996. *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe²⁺ at neutral pH under anoxic conditions. *Archives of Microbiology*, **166**, 308–314.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 257–270.

- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Straub, K. L., Benz, M., Schink, B., and Widdel, F., 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology*, **62**, 1458–1460.
- Temple, K. L., and Colmer, A. R., 1951. The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. *Journal of Bacteriology*, **62**, 605–611.
- Weiss, J. V., Rentz, J. A., Plaia, T., Neubauer, S. C., Merrill-Floyd, M., Lilburn, T., Bradburne, C., Megonigal, J. P., and Emerson, D., 2007. Characterization of neutrophilic Fe(II)-oxidizing bacteria isolated from the rhizosphere of wetland plants and description of *Ferritrophicum radicola* gen. nov. sp. nov., and *Sideroxydans paludicola* sp. nov. *Geomicrobiology Journal*, **24**, 559–570.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature*, **362**, 834–836.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Banded Iron Formations](#)
[Chemolithotrophy](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Leptothrix](#)
[Metals, Acquisition by Marine Bacteria](#)
[Nitrogen](#)
[Photosynthesis](#)
[Siderophores](#)

FE(III)-REDUCING PROKARYOTES

Kristina L. Straub
 University of Vienna, Vienna, Austria

Synonyms

Fe-reducers; Fe-reducing prokaryotes/microorganisms;
 Ferric iron-reducing prokaryotes/microorganisms; Iron-reducers; Iron-reducing prokaryotes/microorganisms;
 Iron(III)-reducing prokaryotes/microorganisms

Definition

Fe(III)-reducing prokaryotes. Various species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III), ferric iron, to Fe(II), ferrous iron, by the transfer of electrons. The electrons for Fe(III) reduction derive mainly from the metabolic oxidation of organic compounds or hydrogen.

Introduction

Reduction of Fe(III) by prokaryotes has been known since the beginning of the twentieth century but was not considered to be of importance. At that time, only few bacterial strains were known to reduce small amounts of Fe(III) during fermentation. In addition, it was misleadingly presumed that prokaryotes cause reduction of Fe(III) mainly indirectly by producing sulfide, releasing organic compounds, lowering the redox potential, or decreasing the pH. This perspective changed notably with the discovery of dissimilatory Fe(III)-reducing bacteria, i.e., bacteria that utilize Fe(III) as terminal electron acceptor in an anaerobic type of respiration (Balashova and Zavarzin, 1979, 1980; Lovley and Phillips, 1988; Myers and Nealson, 1988). In order to gain energy for maintenance and growth by this type of respiration, significant amounts of ferric iron have to be reduced. Additionally, it became clear that neither lowering the redox potential nor decreasing the pH is sufficient to cause Fe(III) reduction. Furthermore, it was demonstrated that only very few organic compounds (e.g., fructose, cysteine) were able to reduce Fe(III) chemically at significant rates. In the last 20 years, numerous dissimilatory Fe(III)-reducing prokaryotes were identified and it is now generally accepted that this physiological group of prokaryotes has a strong influence on the iron geochemistry of most environments. Because many Fe(III)-reducing prokaryotes also have the ability to catalyze the reduction of Mn(IV) or Mn(III) to Mn(II) they additionally influence the geochemistry of manganese (Lovley et al., 2004; Canfield et al., 2005; Kappler and Straub, 2005).

Evolutionary consideration

The ability to reduce Fe(III) occurs widely within the domains *Bacteria* and *Archaea*. Most known Fe(III)-reducing prokaryotes that grow by Fe(III) reduction belong to the phylum Proteobacteria with representatives in each of the five subdivisions *Alpha*- (e.g., *Acidiphilium rubrum*), *Beta*- (e.g., *Ferribacterium limneticum*), *Gamma*- (e.g., *Shewanella oneidensis*), *Delta*- (e.g., *Geobacter sulfurreducens*), and *Epsilonproteobacteria* (e.g., *Sulfurospirillum barnesii*). Several Gram-positive bacteria that belong to the phylum Firmicutes (e.g., *Thermoanaerobacter siderophilus*) and some separate bacterial lineages like *Geothrix fermentans* or *Geovibrio ferrireducens* also have the ability to reduce ferric iron (Lovley et al., 2004). Fe(III) reduction in the domain *Archaea* was discovered by testing pure cultures that had originally not been isolated with Fe(III): Representatives of the phyla Euryarchaeota (e.g., *Pyrococcus furiosus*) and Crenarchaeota (e.g., *Pyrobaculum islandicum*) showed reduction of ferric iron (Vargas et al., 1998). The great phylogenetic diversity of Fe(III)-reducing prokaryotes with representatives in all deeply branching phyla, along with iron isotope studies, supports the hypothesis that Fe(III) reduction was an early mode of energy metabolism. Further geochemical evidences

- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Straub, K. L., Benz, M., Schink, B., and Widdel, F., 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology*, **62**, 1458–1460.
- Temple, K. L., and Colmer, A. R., 1951. The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. *Journal of Bacteriology*, **62**, 605–611.
- Weiss, J. V., Rentz, J. A., Plaia, T., Neubauer, S. C., Merrill-Floyd, M., Lilburn, T., Bradburne, C., Megonigal, J. P., and Emerson, D., 2007. Characterization of neutrophilic Fe(II)-oxidizing bacteria isolated from the rhizosphere of wetland plants and description of *Ferritrophicum radicola* gen. nov. sp. nov., and *Sideroxydans paludicola* sp. nov. *Geomicrobiology Journal*, **24**, 559–570.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature*, **362**, 834–836.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Banded Iron Formations](#)
[Chemolithotrophy](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Leptothrix](#)
[Metals, Acquisition by Marine Bacteria](#)
[Nitrogen](#)
[Photosynthesis](#)
[Siderophores](#)

FE(III)-REDUCING PROKARYOTES

Kristina L. Straub
 University of Vienna, Vienna, Austria

Synonyms

Fe-reducers; Fe-reducing prokaryotes/microorganisms;
 Ferric iron-reducing prokaryotes/microorganisms; Iron-reducers; Iron-reducing prokaryotes/microorganisms;
 Iron(III)-reducing prokaryotes/microorganisms

Definition

Fe(III)-reducing prokaryotes. Various species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III), ferric iron, to Fe(II), ferrous iron, by the transfer of electrons. The electrons for Fe(III) reduction derive mainly from the metabolic oxidation of organic compounds or hydrogen.

Introduction

Reduction of Fe(III) by prokaryotes has been known since the beginning of the twentieth century but was not considered to be of importance. At that time, only few bacterial strains were known to reduce small amounts of Fe(III) during fermentation. In addition, it was misleadingly presumed that prokaryotes cause reduction of Fe(III) mainly indirectly by producing sulfide, releasing organic compounds, lowering the redox potential, or decreasing the pH. This perspective changed notably with the discovery of dissimilatory Fe(III)-reducing bacteria, i.e., bacteria that utilize Fe(III) as terminal electron acceptor in an anaerobic type of respiration (Balashova and Zavarzin, 1979, 1980; Lovley and Phillips, 1988; Myers and Nealson, 1988). In order to gain energy for maintenance and growth by this type of respiration, significant amounts of ferric iron have to be reduced. Additionally, it became clear that neither lowering the redox potential nor decreasing the pH is sufficient to cause Fe(III) reduction. Furthermore, it was demonstrated that only very few organic compounds (e.g., fructose, cysteine) were able to reduce Fe(III) chemically at significant rates. In the last 20 years, numerous dissimilatory Fe(III)-reducing prokaryotes were identified and it is now generally accepted that this physiological group of prokaryotes has a strong influence on the iron geochemistry of most environments. Because many Fe(III)-reducing prokaryotes also have the ability to catalyze the reduction of Mn(IV) or Mn(III) to Mn(II) they additionally influence the geochemistry of manganese (Lovley et al., 2004; Canfield et al., 2005; Kappler and Straub, 2005).

Evolutionary consideration

The ability to reduce Fe(III) occurs widely within the domains *Bacteria* and *Archaea*. Most known Fe(III)-reducing prokaryotes that grow by Fe(III) reduction belong to the phylum Proteobacteria with representatives in each of the five subdivisions *Alpha*- (e.g., *Acidiphilium rubrum*), *Beta*- (e.g., *Ferribacterium limneticum*), *Gamma*- (e.g., *Shewanella oneidensis*), *Delta*- (e.g., *Geobacter sulfurreducens*), and *Epsilonproteobacteria* (e.g., *Sulfurospirillum barnesii*). Several Gram-positive bacteria that belong to the phylum Firmicutes (e.g., *Thermoanaerobacter siderophilus*) and some separate bacterial lineages like *Geothrix fermentans* or *Geovibrio ferrireducens* also have the ability to reduce ferric iron (Lovley et al., 2004). Fe(III) reduction in the domain *Archaea* was discovered by testing pure cultures that had originally not been isolated with Fe(III): Representatives of the phyla Euryarchaeota (e.g., *Pyrococcus furiosus*) and Crenarchaeota (e.g., *Pyrobaculum islandicum*) showed reduction of ferric iron (Vargas et al., 1998). The great phylogenetic diversity of Fe(III)-reducing prokaryotes with representatives in all deeply branching phyla, along with iron isotope studies, supports the hypothesis that Fe(III) reduction was an early mode of energy metabolism. Further geochemical evidences

suggest that other major electron acceptors such as sulfate, nitrate, or molecular oxygen were not available on early Earth (Lovley et al., 2004; Canfield et al., 2005; Johnson et al., 2008).

Geochemical aspects of Fe(III)

Iron is the fourth most abundant element in the Earth's crust and Fe(III) minerals may account for a few percent dry weight of rocks, soils, and sediments. Hence, in most anoxic ecosystems Fe(III) minerals are the dominant electron acceptors for bacteria and archaea. Today, 16 different Fe(III) oxides, hydroxides, or oxide hydroxides are known and for simplicity they are often collectively termed Fe(III) oxides. They are all composed of Fe together with O and/or OH and differ in composition and crystal structure. Widespread Fe(III) oxides include ferrihydrite, goethite, hematite, lepidocrocite, and magnetite (Cornell and Schwertmann, 2003). In contrast to other electron acceptors such as oxygen, nitrate, or sulfate, Fe(III) oxides are poorly soluble at neutral pH. The solubility of Fe(III) oxides at neutral pH depends furthermore on the type of oxide, the degree of crystallinity and the crystal size, and may range from 10^{-9} M (e.g., ferrihydrite, low crystallinity, small crystals) to 10^{-18} M (e.g., goethite, high crystallinity, large crystals). At circumneutral pH, Fe(III)-reducing prokaryotes therefore have to cope with a virtually insoluble electron acceptor. With increasing acidity, the solubility of Fe(III) oxides increases and ferric iron is well soluble at pH values below 2.5 (Cornell and Schwertmann, 2003). Concomitant with the change in pH and Fe(III) oxide solubility, the redox potential of the transition between ferric and ferrous iron changes significantly. The redox potential of the redox pair $\text{Fe}^{3+}/\text{Fe}^{2+}$ is +770 mV only at acidic pH values. At neutral pH, with poorly crystalline Fe(III) oxides (ferrihydrite, lepidocrocite) as the oxidant, the redox potential ranges between +100 and -100 mV, while the redox potential of more crystalline Fe(III) oxides (goethite, hematite, magnetite) may be as low as -300 mV (Canfield et al., 2005; Straub et al., 2001). In respect of solubility and energetics, acidophilic and neutrophilic Fe(III)-reducing prokaryotes cope with entirely different substrates.

Reduction of Fe(III) by fermenting bacteria

The first Fe(III)-reducing prokaryotes that were studied in laboratory cultures belonged to the domain *Bacteria* and reduced only small amounts of Fe(III) during fermentative growth on organic substrates such as glucose or malate. This physiological group comprises of a variety of bacteria and includes anaerobes (e.g., *Clostridium pasteurianum*, *Vibrio* spp.), facultative anaerobes (e.g., *Escherichia coli*, *Paenibacillus polymyxa*), and aerotolerants (e.g., *Lactobacillus lactis*, *Lactococcus lactis*). Accordingly, Fe(III) reduction by fermenting bacteria was observed under both anoxic and oxic culture conditions. For fermenting bacteria, Fe(III) reduction is

only a minor pathway for electron disposal and research on this type of metabolism fell behind efforts to understand dissimilatory Fe(III) reduction. The mechanism(s) of electron transfer to Fe(III) during fermentation is unknown and it is also unclear whether this process yields energy for growth. Thermodynamic calculations suggest that Fe(III) reduction during fermentation results in a slightly greater energy yield than fermentation alone but experimental evidences are ambiguous (Lovley, 1991; Canfield et al., 2005). So far no representative of the domain *Archaea* was described to reduce Fe(III) during fermentative growth.

Dissimilatory Fe(III)-reducing prokaryotes

Dissimilatory Fe(III)-reducing prokaryotes utilize Fe(III) as terminal electron acceptor in anaerobic respiration, i.e., they gain energy by coupling the oxidation of an electron donor to the reduction of Fe(III). As in the reduction of Fe(III) to Fe(II) only one electron can be disposed, dissimilatory Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to gain enough energy for maintenance and growth. Numerous strains of phylogenetically dispersed, dissimilatory Fe(III)-reducing prokaryotes have been isolated into pure culture or have been detected by molecular techniques in a wide range of pristine or contaminated habitats, including freshwater and marine sediments, wetlands, soils, aquifers, the deep subsurface, deep subterranean thermal water, hydrothermal vents, and hot springs (Lovley et al., 2004). This widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Consistent with the great variety of ecosystems inhabited, pure cultures of Fe(III)-reducing prokaryotes show a wealth of physiological qualities in respect to oxygen concentration (strict or facultative anaerobes), temperature (growth reported between 4 and 121°C), pH (growth reported between pH 1 and 9), and salinity (freshwater and marine species). In addition, autotrophic growth with carbon dioxide as sole carbon source and fixation of molecular nitrogen was described for some species of Fe(III)-reducing prokaryotes. No prokaryote characterized so far, depends solely on Fe(III) as terminal electron acceptor for growth. In the absence of Fe(III), prokaryotes either grow by fermentation (e.g., *Thermotoga maritima*) or utilize alternative electron acceptors. Common alternative electron acceptors include oxygen (e.g., *Pantoea agglomerans*), nitrate (e.g., *Geobacter metallireducens*), manganese (e.g., *Deferribacter thermophilus*), reduced sulfur compounds (e.g., *Desulfuromusa kysingii*), and fumarate (e.g., *Desulfuromonas acetexigens*). Furthermore, some species transfer electrons to heavy metals (e.g., uranium, chromium), graphite electrodes, humic substances, or chlorinated compounds (e.g., tetrachloroethene, trichloroacetic acid). The vast majority of Fe(III)-reducing prokaryotes utilizes fermentation end products such as acetate, ethanol, or hydrogen as electrons donors. Only few prokaryotes are known to couple the dissimilatory reduction of Fe(III) to the oxidation of sugars (e.g., *Acidiphilium*

cryptum), amino acids (e.g., *Geothermobacter ehrlichii*), long-chain fatty acids (e.g., *Desulfuromonas palmitatis*), monoaromatic compounds (e.g., *Geobacter metallireducens*), or reduced inorganic sulfur compounds (e.g., *Acidithiobacillus ferrooxidans*).

Acidophilic Fe(III)-reducing bacteria

A variety of autotrophic and heterotrophic bacteria that thrive in environments of low pH (<3) can grow by dissimilatory Fe(III) reduction (Johnson, 2007). All known species are facultative anaerobes, and most of them synthesize cell compounds required for dissimilatory Fe(III) reduction only in the absence of oxygen. In contrast, some species of acidophilic Fe(III)-reducing bacteria (e.g., *Acidiphilium cryptum*) constitutively express all genes necessary for Fe(III) reduction (Johnson, 2007). This is surprising because the redox potential of the redox pair O_2/H_2O reaches values above +1,000 mV below a pH value of 3 (Appelo and Postma, 2007). Hence, cells gain more energy by aerobic respiration than by utilizing Fe(III) as terminal electron acceptor (redox potential of the redox pair Fe^{3+}/Fe^{2+} : +770 mV).

Several species of acidophilic Fe(III)-reducing bacteria (e.g., *Acidithiobacillus ferrooxidans*, *Sulfobacillus acidophilus*) also grow by oxidizing Fe(II) with molecular oxygen; such prokaryotes were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993). So far no representative of the domain *Archaea* was described to grow by dissimilatory Fe(III) reduction at acidic pH values.

Neutrophilic Fe(III)-reducing prokaryotes

The majority of dissimilatory Fe(III)-reducing prokaryotes that were isolated and characterized thrives at circumneutral pH and has to cope with a poorly soluble electron acceptor under natural conditions. In addition, the redox potential of the redox pair Fe(III)/Fe(II) is much lower (+100 to –300 mV, depending on the type of ferric iron oxide) than the redox potential of the redox pair O_2/H_2O (+820 mV) relevant for aerobic respiration. Therefore, at neutral pH, Fe(III) is a less valuable electron acceptor in terms of energy generation than molecular oxygen. Similarly, prokaryotes gain more energy from the respiration with nitrate or manganese as terminal electron acceptors. Hence, respiratory Fe(III) reduction at neutral pH occurs only under anoxic conditions in the absence of more favorable electron acceptors (Canfield et al., 2005). In some studies on neutrophilic Fe(III)-reducing prokaryotes, only chelated forms of ferric iron such as Fe(III) citrate or Fe(III) NTA (nitrilotriacetate) were applied. Caution is necessary in the interpretation of results from such studies because chelated Fe(III) forms are soluble at circumneutral pH, have redox potentials that are higher than those of non-chelated Fe(III) minerals, and may react rather unspecifically with electron-releasing cell compounds (Straub et al., 2001).

Most research on the biochemistry of microbial Fe(III) reduction focuses on members of the genera *Shewanella*

and *Geobacter*, two genera with significant differences in their basic physiology. *Shewanella* species are facultative anaerobes that belong to the *Gammaproteobacteria*. They grow very well in nutrient-rich medium and oxidize lactate or pyruvate incompletely to acetate. In contrast, species of the genus *Geobacter* belong to the *Deltaproteobacteria* and most of them are described as anaerobes. The most common electron donors utilized by *Geobacter* species include acetate, ethanol, and hydrogen. Despite differences in basic physiological features, various *c*-type cytochromes play a significant role in intracellular electron transfer processes in both *Shewanella* and *Geobacter* species.

Mechanisms of prokaryotic Fe(III) reduction

Considering the phylogenetically widespread ability of prokaryotes to reduce Fe(III) and the great prokaryotic physiological diversity, it is not surprising that Fe(III)-reducing prokaryotes developed different strategies to cope with Fe(III) as electron acceptor. According to experimental observations, prokaryotes developed the following strategies to transfer electrons from the cell to the surface of Fe(III) minerals: (a) Physical contact between cell and mineral surfaces allows for direct delivery of electrons; an attachment of cells to Fe(III) minerals has been repeatedly reported and seems to be the prerequisite for the delivery of electrons to Fe(III) in some Fe(III)-reducing prokaryotes such as *Geobacter metallireducens*. (b) Iron chelators increase the solubility of ferric iron; some evidence indicates that *Shewanella algae* and *Geothrix fermentans* produce and release Fe(III) chelators specifically during growth with Fe(III) minerals. (c) Electron-shuttling compounds accomplish the transfer of electrons from the cell to the mineral; diffusible electron-shuttling compounds help to bridge the spatial distance between cells and ferric iron (Lovley et al., 2004; Kappler and Straub, 2005). Reduction of Fe(III) by electron shuttling compounds is of particular importance in natural habitats where prokaryotic cells and Fe(III) minerals are not evenly distributed and Fe(III) might not be directly/physically accessible for prokaryotic cells. Naturally occurring electron-shuttling compounds that were shown to sustain prokaryotic growth include humic substances (Lovley et al., 1996) and sulfur compounds (Straub and Schink, 2004). Note that prokaryotes which reduce Fe(III) only via naturally occurring electron-shuttling compounds are no Fe(III)-reducing prokaryotes in a strict sense as their actual electron acceptor is the shuttling molecule and not Fe(III).

Summary

Phylogenetically diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III) during fermentative or respiratory growth by the transfer of electrons. Fe(III)-reducing prokaryotes have been recovered or detected in a great variety of pristine or contaminated habitats, and their widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Only one electron can be disposed

by the reduction of Fe(III) to Fe(II) and hence Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(III)-reducing prokaryotes have to cope with different iron species: Fe(III) minerals are soluble only below a pH value of 3. At circumneutral pH, Fe(III) is only poorly soluble and significantly less energy can be gained by Fe(III) respiration compared to aerobic respiration. However, Fe(III) is the dominant electron acceptor in many anoxic habitats and prokaryotes developed different strategies for its utilization.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Balashova, V. V., and Zavarzin, G. A., 1979. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiologia*, **48**, 773–778.
- Balashova, V. V., and Zavarzin, G. A., 1980. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiology*, **48**, 635–639.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington, DC: ASM, pp 257–270.
- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Johnson, C. M., Beard, B. L., and Roden, E. E., 2008. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient Earth. *Annual Review of Earth and Planetary Sciences*, **36**, 457–493.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, **55**, 259–287.
- Lovley, D. R., and Phillips, E. J. P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, **54**, 1472–1480.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445–448.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Myers, C. R., and Nealson, K. H., 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science*, **240**, 1319–1321.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* by electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744–5749.
- Straub, K. L., Benz, M., and Schink, B., 2001. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiology Ecology*, **34**, 181–186.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovley, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shewanella](#)
[Siderophores](#)

FERMENTATION

Fermentation is a route of obtaining energy from the oxidation of organic substrates using an endogenous electron acceptor (usually an organic compound). The energy yield in fermenting systems is limited when compared to respiration. Nevertheless, fermentation still allows growth under conditions when external, inorganic electron acceptors are lacking, or when iron as part of the electron carriers in the respiratory chain is not available and therefore an electron transport chain cannot be synthesized. Common fermentation substrates are sugars, which are present in large amounts in the environment, for example, as photosynthesis products and constituents of plant cell wall material. Microbial sugar fermentation pathways encompass lactic acid fermentation, alcoholic fermentation, and propionate fermentation, according to the major metabolic products generated. Another important fermentation pathway is acetoclastic methanogenesis, that is, fermentation of acetate to CO₂ and CH₄, which is performed by some methanogenic archaea (see entry “[Methane, Origin](#)”). [Hydrogen](#) is another common product of microbial fermentation processes.

For further details and references, please refer to entry “[Microbial Degradation](#).”

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Natuschka M. Lee¹, Daniela B. Meisinger¹,
 Michael Schmid², Michael Rothballer²
 Frank E. Löffler³

¹Technical University of Munich, Freising-Weihenstephan, Germany

²Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

³University of Tennessee, Knoxville, TN, USA

Definition

FISH (fluorescence in situ hybridization) is a hybridization technique employing reverse complementary

by the reduction of Fe(III) to Fe(II) and hence Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(III)-reducing prokaryotes have to cope with different iron species: Fe(III) minerals are soluble only below a pH value of 3. At circumneutral pH, Fe(III) is only poorly soluble and significantly less energy can be gained by Fe(III) respiration compared to aerobic respiration. However, Fe(III) is the dominant electron acceptor in many anoxic habitats and prokaryotes developed different strategies for its utilization.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Balashova, V. V., and Zavarzin, G. A., 1979. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiologia*, **48**, 773–778.
- Balashova, V. V., and Zavarzin, G. A., 1980. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiology*, **48**, 635–639.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington, DC: ASM, pp 257–270.
- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Johnson, C. M., Beard, B. L., and Roden, E. E., 2008. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient Earth. *Annual Review of Earth and Planetary Sciences*, **36**, 457–493.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, **55**, 259–287.
- Lovley, D. R., and Phillips, E. J. P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, **54**, 1472–1480.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445–448.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Myers, C. R., and Nealson, K. H., 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science*, **240**, 1319–1321.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* by electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744–5749.
- Straub, K. L., Benz, M., and Schink, B., 2001. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiology Ecology*, **34**, 181–186.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovley, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shewanella](#)
[Siderophores](#)

FERMENTATION

Fermentation is a route of obtaining energy from the oxidation of organic substrates using an endogenous electron acceptor (usually an organic compound). The energy yield in fermenting systems is limited when compared to respiration. Nevertheless, fermentation still allows growth under conditions when external, inorganic electron acceptors are lacking, or when iron as part of the electron carriers in the respiratory chain is not available and therefore an electron transport chain cannot be synthesized. Common fermentation substrates are sugars, which are present in large amounts in the environment, for example, as photosynthesis products and constituents of plant cell wall material. Microbial sugar fermentation pathways encompass lactic acid fermentation, alcoholic fermentation, and propionate fermentation, according to the major metabolic products generated. Another important fermentation pathway is acetoclastic methanogenesis, that is, fermentation of acetate to CO₂ and CH₄, which is performed by some methanogenic archaea (see entry “[Methane, Origin](#)”). [Hydrogen](#) is another common product of microbial fermentation processes.

For further details and references, please refer to entry “[Microbial Degradation](#).”

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Natuschka M. Lee¹, Daniela B. Meisinger¹,
 Michael Schmid², Michael Rothballer²
 Frank E. Löffler³

¹Technical University of Munich, Freising-Weihenstephan, Germany

²Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

³University of Tennessee, Knoxville, TN, USA

Definition

FISH (fluorescence in situ hybridization) is a hybridization technique employing reverse complementary

by the reduction of Fe(III) to Fe(II) and hence Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(III)-reducing prokaryotes have to cope with different iron species: Fe(III) minerals are soluble only below a pH value of 3. At circumneutral pH, Fe(III) is only poorly soluble and significantly less energy can be gained by Fe(III) respiration compared to aerobic respiration. However, Fe(III) is the dominant electron acceptor in many anoxic habitats and prokaryotes developed different strategies for its utilization.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Balashova, V. V., and Zavarzin, G. A., 1979. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiologia*, **48**, 773–778.
- Balashova, V. V., and Zavarzin, G. A., 1980. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiology*, **48**, 635–639.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington, DC: ASM, pp 257–270.
- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Johnson, C. M., Beard, B. L., and Roden, E. E., 2008. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient Earth. *Annual Review of Earth and Planetary Sciences*, **36**, 457–493.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, **55**, 259–287.
- Lovley, D. R., and Phillips, E. J. P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, **54**, 1472–1480.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445–448.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Myers, C. R., and Nealson, K. H., 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science*, **240**, 1319–1321.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* by electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744–5749.
- Straub, K. L., Benz, M., and Schink, B., 2001. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiology Ecology*, **34**, 181–186.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovley, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shewanella](#)
[Siderophores](#)

FERMENTATION

Fermentation is a route of obtaining energy from the oxidation of organic substrates using an endogenous electron acceptor (usually an organic compound). The energy yield in fermenting systems is limited when compared to respiration. Nevertheless, fermentation still allows growth under conditions when external, inorganic electron acceptors are lacking, or when iron as part of the electron carriers in the respiratory chain is not available and therefore an electron transport chain cannot be synthesized. Common fermentation substrates are sugars, which are present in large amounts in the environment, for example, as photosynthesis products and constituents of plant cell wall material. Microbial sugar fermentation pathways encompass lactic acid fermentation, alcoholic fermentation, and propionate fermentation, according to the major metabolic products generated. Another important fermentation pathway is acetoclastic methanogenesis, that is, fermentation of acetate to CO₂ and CH₄, which is performed by some methanogenic archaea (see entry “[Methane, Origin](#)”). [Hydrogen](#) is another common product of microbial fermentation processes.

For further details and references, please refer to entry “[Microbial Degradation](#).”

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Natuschka M. Lee¹, Daniela B. Meisinger¹,
 Michael Schmid², Michael Rothballer²
 Frank E. Löffler³

¹Technical University of Munich, Freising-Weihenstephan, Germany

²Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

³University of Tennessee, Knoxville, TN, USA

Definition

FISH (fluorescence in situ hybridization) is a hybridization technique employing reverse complementary

fluorescently labeled probes to detect and localize only those parts of the chromosome, a gene or its transcript with which they show a high degree of sequence similarity. Results are mostly evaluated by either fluorescence microscopy or cell sorting methods like flow cytometry. Depending on target gene, probe chemistry, labeling, and combination with other analytical tools, a wide range of different FISH applications can be employed for advanced in situ analysis of community structure, dynamics, localization, activity, function and interactions of different cellular species.

Introduction

Antonie van Leeuwenhoek's ingenious improvement of the microscope in the seventeenth century revolutionized biology as this initiated novel ways to explore our world and ultimately led to the discovery of a new dimension of life forms, the microorganisms. However, since traditional microscopy reveals mainly structural features, its usefulness to advanced biological research reached its limits with the beginning of the molecular era. Fortunately, new possibilities for advanced microscopy applications emerged with the parallel development of two techniques: (1) in situ hybridization techniques (ISH) employing labeled gene probes targeting specific regions of a particular gene, and, (2) fluorescence microscopy. Fluorescence in situ hybridization (FISH) makes use of reverse complementary gene probes labeled with fluorochromes that are hybridized to specific regions on a chromosome (e.g., a specific gene) of gene transcripts (e.g., rRNA), and are then viewed by fluorescence microscopy or evaluated by other cell sorting methods like flow cytometry. FISH revolutionized nearly all disciplines in microbiology, in particular environmental research fields like microbial ecology, environmental biotechnology and geomicrobiology, since this enabled a cultivation-independent exploration of whole cells of culturable as well as not yet cultured microorganisms in their natural, complex environment (Amann et al., 1995; Amann and Fuchs, 2008). However, along with the development of other novel molecular tools and the growing comprehension of the enormous complexity of the biology of microorganisms, the first generation of FISH tools reached its limits as well, especially for applications on oligotrophic ecosystems characterized by a low amount of cells with often low activities in a strongly confounding background, which is often the case in geomicrobiology. Different modifications of the first generation FISH protocol as well as different combinations with other analytical tools were undertaken in order to meet the increasing demands with respect to, e.g., improved targeting of cells with low ribosome content or probe-repelling cell wall chemistry, alternative strategies to increase probe specificities or signal intensities, advanced image analysis, elucidation of activity and function by targeting other genes than the 16S rRNA gene and combining with different analytical tools, and finally cell enrichment of probe-targeted cells for,

e.g., subsequent identification by sequencing (Wagner et al., 2003; Wagner et al., 2006; Amann and Fuchs, 2008; Orphan, 2009; Wagner, 2009). The development of novel FISH protocols in combination with other advanced analytical tools and microscopes is most likely to continue to play a crucial role in microbial ecology and geomicrobiology research. Only microscopy-based tools can provide us with the possibility to visualize the morphotype, growth characteristics, spatial distribution, and interactions of different taxa on a single cell level within complex communities. Furthermore, despite all progress in nucleic acid-based approaches like PCR and the "omics" approaches, only microscopy-based tools can link isolated extracted DNA/RNA markers, with intact, individual cells in their natural environment, and thus also exclude molecular biases often observed with nucleic acid extraction and PCR (Table 1). The greatest benefit will be reached when combining different FISH tools with various methods such as cultivation and molecular approaches. Ultimately, a systematic approach can be undertaken to reveal discrepancies between different tools caused by extant biases of each tool in terms of sensitivity and specificity, so that a validated, advanced insight into the true diversity, dynamics, function, activities, and relationships in the microbial world can be obtained.

Sample preparation, fixation and storage of samples

In contrast to nucleic acid-based tools, which simply require either addition of preservative agents or instant freezing of biomass and storage in sterile tubes for subsequent nucleic acid extraction, preparation of samples for FISH requires usually more consideration and effort, especially for long-term storage of samples of unknown microbial composition and/or employing certain types of FISH protocols that may disrupt the original structure of the sample.

Preparations for standard applications

1. *Sampling strategy*: While FISH may principally work equally well on nearly any kind of optimally fixed cells with high ribosome content, irrespective of cell titers or the nature of the environmental sample, our abilities to detect, visualize, and quantify the cells may be obstructed by tedious, time-consuming screening of samples with either too low or too high cell counts, confounding background caused by autofluorescence of the background or unspecific binding of probes or labels to nontarget cells/substances (Amann and Schleifer, 2001). Some of these problems can be minimized by considering how the sample should be optimally treated already during sampling, for example, by concentration, dilution, embedding, cell extraction, or particular consideration on chemical-physical conditions to avoid cells getting exposed to stress.
2. *Fixation time and protocols*: The purpose of the fixation is to maintain the original microbial composition

Fluorescence In Situ Hybridization (FISH), Table 1 Advantages and disadvantages of FISH versus disruptive tools like PCR with ribosomal biomarkers

Diagnostic possibilities	PCR/qPCR/RT PCR	FISH ^a
<i>Experimental conditions:</i>		
Preparation of biomass prior to analysis	Nucleic acid extraction	Fixation of cells ^b
Long-term storage of sample	−80°/−20°C	−20°/+4°C ^b
Labor time (after NA extraction or fixation)	>6 h	0.5 h to 96 h
<i>Relevant parameters for microbial ecology:</i>		
Quantify distribution and dynamics	Yes/partly	Partly
Link targeted gene directly to cells	No	Yes
Visualize morphology and growth characteristics	No	Yes
Visualize associations with other microorganisms	No	Yes
Three-dimensional reconstruction of complex aggregates	No	Yes
<i>Methodological strengths/weaknesses:</i>		
Simultaneous identification of several genes	Only with multiplex PCR	Yes ^c
Distinguish gene targets in live/inactive/dead cells	No (for DNA)	Partly ^d
Distinguish false-positive gene products (chimera)	No	Yes
Quick check of cultures for purity	No	Yes
Enrich for subsequent analysis (e.g., sequencing)	Yes	Partly
Amplification biases	Yes	No ^e
Quantification bias caused by multiple ribosomal gene copies	Yes (for qPCR)	No
Bias caused by unspecific target binding	Possible	Possible
Biases caused by matrix	Inhibition	Autofluorescence
Detection limit	>5 gene copies	10 ^{5–7} cells/ml ^f

^aIncluding different FISH protocols and advanced microscopy tools.

^bIn certain cases, samples may be stored at −80°C and the fixation omitted (Yilmaz et al., 2010b).

^cOnly with certain FISH protocols like mRNA-CARD FISH, RING-FISH, gene-FISH, and in situ RCA.

^dDepends on the FISH protocol and how this is combined with other analytical tools and appropriate references.

^eException, FISH protocols based on PCR.

^fDepends on concentration/dilution procedure.

of the samples as well as the morphological integrity of cells during the different hybridization steps (elevated temperature, exposure to enzymes, detergents and osmotic gradients) and prevent cell losses, while simultaneously making the cells permeable for the probe(s) (Amann, 1995). Unfortunately, there is not one plain fixation protocol for all cell types for all different FISH protocols. Different fixation protocols have been developed to cope with the complexity of archaea, bacteria, and Eukarya with regard to their cell envelope packages and the type of probes to be employed for a particular FISH protocol. A suboptimal fixation protocol may make cells impermeable for probes or even cause cell lysis, especially during longer storage times or when exposing the cells to certain procedures such as incubation with enzymes prior to the FISH procedure. Apart from the selection of an optimal fixative reagent, it is also relevant to pay attention to the concentration of the fixative reagent and the incubation time and conditions. Fixation times may range from a few min up to >24 h, depending on cell type and FISH protocol, and are generally followed by a washing step for removal of the fixative reagent. The two most common fixation procedures are based on either aldehydes (e.g., formalin, paraformaldehyde (PFA), glutaraldehyde) or on alcohols (e.g., ethanol, methanol). In the

majority of cases, Gram-negative cells within the domain Bacteria are usually fixed with 4% PFA, and Gram-positive cells within the domain Bacteria with ice cold 96% ethanol (Amann, 1995). To a certain extent, some Gram-negative species will also tolerate EtOH fixation, and some Gram-positive species (general exceptions are, e.g., *Actinomycetes* and *Streptococci*) may also tolerate PFA fixation (especially if the incubation time is short, see, e.g., de los Reyes et al., 1997). The previously mentioned fixation protocols may also work well for certain Archaea and Eukarya species, but in some cases, especially for certain Archaea species (Nakamura et al., 2006) or eukaryotes like fungi (Baschien et al., 2001; Tsuchiya and Taga, 2001; Weber et al., 2007) or protozoa, see, e.g., Fried et al. (2002); Petroni et al. (2003); Fried and Foissner (2007); Weber et al. (2007), other fixative reagents or post treatments with enzymes like chitinase or endo-isopeptidase are more suitable. When dealing with unknown samples, it is therefore recommended to explore the suitability of different fixation protocols. Irrespective of the fixation protocol used, the cells must be fixed as soon as possible with optimally prepared fixation solutions, preferably already during the sampling occasion to avoid population shifts and for optimal evaluation of true probe signal intensities as these

may in certain cases also serve as an indicator of activity (see, e.g., Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007).

3. *Storage of fixed cells:* Fixed samples (in wet or dry state, such as on a microscope slide or on a membrane filter) can be stored at room temperature or at +4°C for shorter periods. For optimal long-term storage at -20°C, fixed cells are generally dissolved in a 1:1 (v/v) solution with a 50% end concentration of ethanol in order to avoid freezing (Amann et al., 1994). Cells concentrated on a membrane filter are normally stored dry at -20°C (e.g., Teira et al., 2004). So far, no systematic studies on the maintenance of cell integrity or ribosome content in fixed cells after longer storage periods exist, but it is generally recommended that once the cells have been fixed, quantitative studies should be performed within days to avoid biases caused by unknown deterioration of cells or decrease of ribosome content.

Preparations for omics-based applications

For genomic or proteomic applications, unfixed biomass is desirable to minimize possible sequencing biases introduced by cross-linking and to avoid possible cell lysis difficulties, so that FISH targeted cells can be enriched by, e.g., flow cytometry and subjected to, e.g., whole genome application. Recently, Yilmaz et al. (2010b) demonstrated that the standard oligonucleotide FISH protocol can also be performed on at least two different types of frozen, non-fixed complex environmental samples using Archaea as well as Bacteria targeted probes. However, more research is needed to explore further possibilities as well as limits of this approach, such as if this is quantitative and if this applies to any kind of cells types or other environmental samples, irrespective of growth and storage conditions, as well as if this approach is also suitable for other types of FISH protocols.

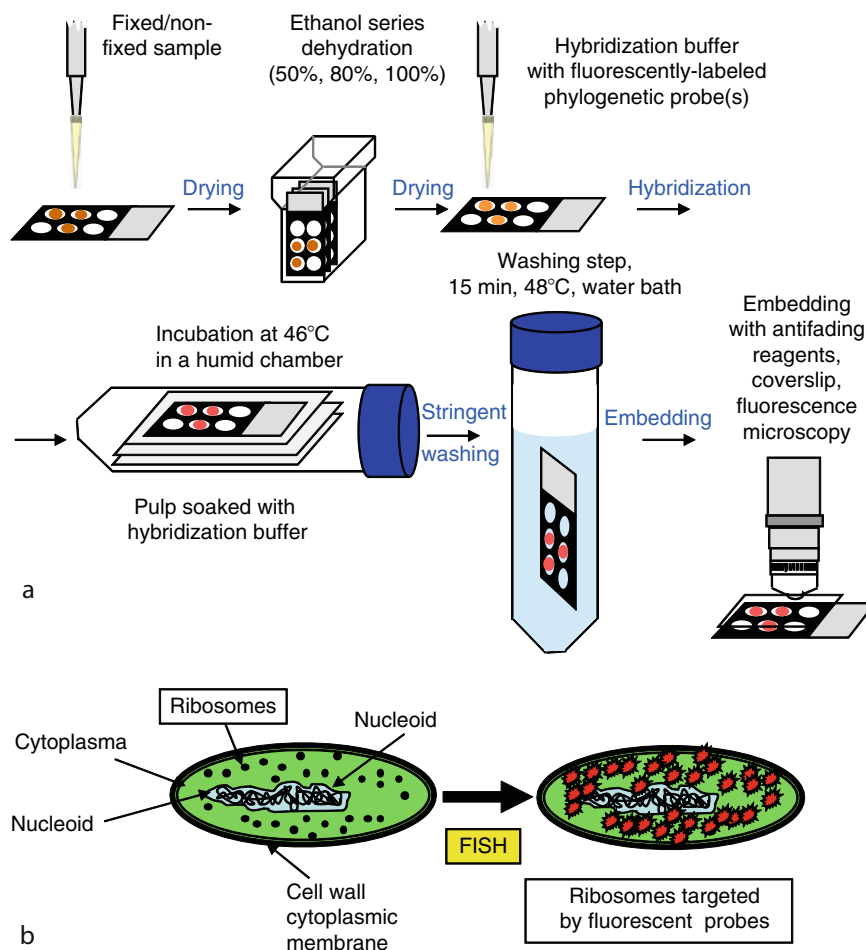
FISH for in situ detection of ribosomal genes

General background: The ribosomal gene was and will at least for the near future remain the standard gene for reconstruction of phylogeny, despite the growing amount of genome sequences which is presently revolutionizing our current concepts of taxonomy, phylogeny, and development of comprehensive biomarkers for improved identification (see, e.g., Konstantinidis et al., 2009). The main benefits of the ribosomal gene is that it is a functionally conserved molecule present in high numbers in all organisms from all domains and is composed of sequence regions of higher and lower evolutionary conservation. The variable conservation degree makes it particularly ideal for design of biomarkers for nucleic acid-based detection and quantification of different levels of microbial diversity (Ludwig et al., 1998; Priesse et al., 2007; Amaral-Zettler et al., 2008; Yarla et al., 2008). Although several different types of ribosomal subunits exist, only a few of these are currently used as targets for probes in

FISH studies. For most FISH studies, the main gene target is the 16S rRNA gene for prokaryotes, and the 18S-rRNA gene for eukaryotes (Ludwig et al., 1998; Metfies et al., 2008). However, other multicopy ribosomal genes or even stable multicopy RNA molecules have been (5S-rRNA, tmRNA), or may be occasionally used as alternative targets for FISH probes (23S-rRNA, 28S-rRNA, precursor rRNA, intergenic spacer region). These alternative gene targets may overcome some of the problems occasionally encountered when targeting the 16S rRNA gene, such as low information content and limited probe accessibility or information about activity status (Trebesius et al., 1994; Oerther et al., 2000; Schmid et al., 2001; Schonhuber et al., 2001; Zimmermann et al., 2001; Peplies et al., 2004). However, despite this, the 16S rRNA gene has remained the main gene target in FISH applications, especially for environmental systems with unknown diversity, since the 16S rRNA gene is nowadays routinely sequenced in most studies so that the amount of retrieved 16S rRNA gene sequences has thus come to outrange all other databases of other gene sequences.

Principle of FISH: The FISH procedure generally consists of the following steps (Adapted from Manz et al., 1992 and Amann, 1995):

1. Optional: For certain cases an additional post-treatment of the fixed/non-fixed biomass may be needed for increased permeabilization of probes through rigid cell envelope packages, or for removal of confounding background, by, e.g.:
 - Incubation with enzymes like lysozyme, protease, proteinase K, mutanolysine, endoisopeptidase, chitinase
 - Removal of wax or chemical precipitates by chemical solvents
 - Use of detergents (SDS) or other chemicals like hydrochloric acid
 - Physical treatment like exposure to microwave radiation
 - Addition of chemicals to avoid dissolution of essential compounds
2. Successive dehydration in an EtOH series (50%, 80%, 96%, each step between 1–3 min).
3. Hybridization of the fixed sample with the probe on a microscope slide or in solution under stringent conditions (Figure 1). Stringent conditions are achieved by keeping the hybridization temperature constant (+46°C) but varying the formamide concentration for different probes (Stahl and Amann, 2001).
4. Subsequent washing with preheated buffered washing solutions at a slightly higher temperature (+48°). After this, the hybridized samples can be evaluated or stored at dark prior to evaluation (for a shorter period at room temperature or at +4°, for longer periods, at -20°C).
5. Optional: Counter stain with, e.g., an appropriate nucleic acid stain like DAPI (4',6-diamidino-2-phenylindole) or SYBR Green (e.g., Wagner et al., 1994, or Engel et al., 2003).



Fluorescence In Situ Hybridization (FISH), Figure 1 (a) Schematic outline of the standard oligonucleotide FISH procedure on microscope slides. (b) Schematic outline of a cell before and after the standard oligonucleotide FISH procedure.

6. Evaluation is achieved by:

Visualization:

- Standard epifluorescence microscopy, or confocal laser scanning microscope for three-dimensional reconstruction (Wagner et al., 1998).
- Combination with other microscopes like electron microscopy (e.g., Gerard et al., 2005), RAMAN spectroscopy combined with a microscope (Wagner, 2009), or nanoSIMS (Behrens et al., 2008; Orphan, 2009).

For digital image analysis of FISH images, various software packages are available, such as CMEIAS, COMSTAT, and daime (Heydorn et al., 2000; Daims and Wagner, 2007; Zhou et al., 2007; Daims, 2009).

Other options:

- Membrane-based evaluation (e.g., dot blot, Wagner et al., 1994).
- Combination with other analytical tools (e.g., screening of uptake of radioactive tracers or stable isotopes

(Nielsen et al., 2005; Orphan, 2009) or other FISH protocols targeting non-ribosomal genes or their transcripts for exploring activities or functions.

- Reverse hybridization by microarray technology, where multiple probes are deposited on a support material to hybridize a labeled sample with the probes (DeSantis et al., 2005; Loy and Bodrossy, 2006; Taylor et al., 2010).
- Cell sorting or probe signal intensity evaluation by flow cytometry (Behrens, 2003; Amann and Fuchs, 2008), RING (Recognition of Individual Genes)-FISH (Zwirgmaier et al., 2004b), or magneto-FISH (Orphan, 2009).
- Whole genome amplification (Orphan, 2009; Yilmaz et al., 2010b).

Basic equipment and reagents for standard FISH applications:

- Hybridization oven (46°C)
- Water bath (48°C)
- Polypropylene screw top tubes, filter paper

- Reagents (for chemical composition, check, e.g., Amann, 1995):
 - NaCl
 - Tris/Phosphate buffer
 - EDTA
 - SDS
 - Formamide
 - 50%, 80%, 96% EtOH
 - Reagents/equipment for improved probe accessibility
- Labeled probes
- Counterstains like nucleic acid stains (e.g., DAPI (4',6-diamidino-2-phenylindole) or SYBR Green)
- Microscope slides (possibly coated with, e.g., poly-L-lysine (Sigma-Aldrich, www.sigmaaldrich.com/sigma-aldrich/home.html) or a gelatine-chromium sulphate solution (Amann, 1995))
- Mounting medium of appropriate pH (e.g., from Citifluor, www.citifluor.co.uk/; Vectashield, www.vectorlabs.com/; various mounting media from Invitrogen, www.invitrogen.com/site/us/en/home.html)
- Some form of fluorescence microscope.

FISH probes (probe design, probe evaluation, probe databases)

For a proper interpretation of FISH experiments, probes need to be optimally designed and handled. Several different types of softwares (e.g., the ARB software package, (www.arb-home.de/) and Primrose (www.bioinformatics-toolkit.org/index.html)) and detailed guidelines for probe design for particularly FISH experiments exist (Amann, 1995; Amann and Schleifer, 2001; Stahl and Amann, 2001), and (Hugenholtz et al., 2001). Below follows an updated summary of some of those guidelines, focusing mainly on applications with the ARB software package:

1. Construction of a molecular data base: Prior to all probe design, a comprehensive data base of all relevant complete gene sequences of the target group and relevant nontarget groups should be collected for a thorough evaluation of phylogenetic relationships. Partial sequences may also be included, but these should be handled more critically due to their limited value for a full evaluation of all potential probe targets. Sequences can be downloaded from different categories of databases: (1) all kinds of gene sequences: EMBL (www.embl.de/), NCBI (www.ncbi.nlm.nih.gov/), (2) regularly updated and curated ribosomal gene sequences: RDP (<http://rdp.cme.msu.edu/>), SILVA (www.arb-silva.de/), Green Genes (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>); the ribosomal internal spacer collection (<http://egg.umh.es/rissc/>); (3) functional genes: Fun Gene (<http://fungene.cme.msu.edu/index.spr>). Principally, most softwares that can align sequences, perform phylogenetic evaluations, and design biomarkers like PCR primers can be used for FISH probe design as well.

However, the bioinformatic software package ARB (www.arb-home.de/) using the curated SILVA database (www.arb-silva.de/) is highly recommended, as several useful and partly unique tools have been implemented into this package to facilitate a straightforward and advanced design, evaluation and visualization of probes and their target (Ludwig et al., 2004; Kumar et al., 2005; Kumar et al., 2006; Priesse et al., 2007).

2. Hierarchic probe design strategy: Where possible, it is recommended to design a hierarchic probe set consisting of several probes targeting different phylogenetic levels of the target organism/group (see, e.g., Amann and Schleifer, 2001; Amann and Fuchs, 2008). If the different probes are labeled with different fluorochromes and combined in one FISH experiment on a mixture of cells with different phylogenetic relationships, different overlaps of the probes will produce different color combinations depending on the taxonomical relationships. This facilitates a straightforward top to bottom identification of the taxonomical status (e.g., from phylum down to species level) of different cell species in a complex sample (Amann, 1996).
3. General rules for standard oligonucleotide probe design:
 - (a) Select the appropriate gene sequences of the desired target group. Depending on bioinformatic software, design the probe according to the recommended procedure. For example, if using the ARB software (Ludwig et al., 2004), select from the menu "probe design" an appropriate PT server, then define appropriate parameters such as length of probe (average length of probes for standard oligonucleotide probes is between 15–25 nucleotides), minimum group and maximum non-group hits, GC content, melting temperature range, range of nucleotide positions, and maximum hair-pin bonds.
 - (b) The selected probe should be completely specific (complementary) to a region of the selected target sequences and have at least one mismatch to the same target region in nontarget sequences. Optimally, the mismatch should be centralized in the nontarget sequences in order to maximize the destabilizing effect of the mismatch.
 - (c) Perform probe match to evaluate the specificity of the probe (e.g., in the bioinformatic software package ARB (www.arb-home.de/), the RDP Probe Match tool (<http://rdp.cme.msu.edu/probematch/search.jsp>), the probeBase probeCheck tool (<http://131.130.66.200/cgi-bin/probecheck/content.pl?id=home>) or general data bases like the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).
 - (d) Unfortunately, there is no such thing as a perfect probe that only targets the target group as the course of the evolution of gene sequences is a random process. During a recent review Amann and Fuchs (2008) showed that it is more advisable to

evaluate group coverages as the percentage of group members that can be identified relative to the total number of members in the target group. With this approach, three groups can be distinguished: true positives; false negatives; and false positives. Parallel to the constantly growing amount of gene sequences, the coverage of each probe target group is ultimately going to change and possibly demand more complex probe sets. For example, in the early days of the FISH technology, only one probe was needed to target all recognized species in the Bacteria domain (Amann et al., 1990). However, a decade later, Daims et al. (1999) showed that a mixture of three different Bacteria domain targeting probes were needed to cover all so far (up till 1999) recognized sublineages.

- (e) Evaluate the melting temperature of the probes (e.g., as described by (Hugenholtz et al., 2001) or the probeBase, www.microbial-ecology.net/probebase/). Optimally, the probe should have a melting temperature $> 57^{\circ}\text{C}$. If the melting temperature is $< 57^{\circ}\text{C}$, the temperature can be increased by shifting the position of the probe or expanding the length of the probe.
 - (f) Check probe accessibility. A reoccurring problem is the probe accessibility. The general explanation for is that certain regions of the secondary structure of the ribosomal gene are inaccessible for probes. Unfortunately, the accessible and inaccessible regions of the ribosomal molecules appear to vary from species to species, so that only precise estimations of probe accessibilities can be made for those species where a systematic probe accessibility mapping has been performed (see, e.g., Fuchs et al., 1998; Behrens, 2003a, 2006b). The general recommendation is therefore to design probes on different target positions and then to simply explore on a trial-and-error experimental basis which of the selected probes produce high probe signals. However, Yilmaz et al. (2006) showed that principally all positions on the ribosome molecule may yield satisfying probe signals if a sophisticated approach based on a thermodynamics-based probe design is employed. With this approach, FISH experiments of any given probe can be mathematically simulated. Based on this, theoretical predictions on, e.g., the probe accessibility, the formamide denaturation profile, and the potential of mismatch discriminations can be made (for further details see Yilmaz et al., 2010a). Based on this, further optimizations of the probe accessibility can be made such as modifying the length of the probe or the experimental hybridization conditions (e.g., increase of the hybridization time up to 96 h).
 - (g) Check for potential self-complementarity of the probe which may initiate duplex formations between the probe and the target (e.g., caused by hairpins or dimers).
 - (h) Naming of probe. There are three types of nomenclatures for probes: (1) the short name (e.g., EUB 338), which is often constructed by a shortage of the name of the target group (e.g., EUB for Eubacteria), followed by a number that denotes the first position of the probe on the target gene (e.g., nucleotide position 338 on the *E. coli* gene); (2) a name based on a comprehensive nomenclature to denote main features of the probe such as the target gene, target group, target group level, 3' end of the probe, and probe length (Alm et al., 1996); and (3) the probeBase accession number (e.g., pB-00159 for probe EUB 338) as assigned by the online probe database ProbeBase (www.microbial-ecology.net/probebase/).
4. Labeling of probes: The first rRNA-targeted oligonucleotide probes for FISH were radioactively labeled, developed by microautoradiography and evaluated by light microscopy (Giovannoni et al., 1988). Unfortunately, the work with radioactively labeled probes restricted the applicability of FISH. However, the applicability of FISH increased as soon as the first fluorescently labeled rRNA-targeted oligonucleotide probes were reported (DeLong et al., 1989). Probes are nowadays generally covalently labeled at the 5' end of the oligonucleotide, and the most commonly used fluorochromes for FISH are sulfoindocyanine fluorochromes like Cy3 and Cy5, fluorescein, ALEXA probes, rhodamine, and Texas red. The criteria for selecting appropriate fluorochromes are based on the capabilities of the available epifluorescence microscope and on the nature of the cells and their surrounding environment, where the aim is to avoid a confounding background and unspecific binding of fluorochromes to nontarget cells, and/or to minimize bleaching of probe signals. During the last decade, several different approaches have been used to increase the probe signal intensities in those cases where standard labeled probes produce too weak fluorescent signals. A simple strategy was to employ mixtures of mono-labeled probes targeting different sites of the ribosomal gene (e.g., Amann et al., 1990; Krumholz et al., 1990; Morris et al., 2002; Sunamura and Maruyama, 2006). Another strategy is to employ alternative labeling procedures of oligonucleotide probes, such as polynucleotide FISH (e.g., Trebesius et al., 1994), horseradish peroxidase (employed in catalyzed reporter deposition (CARD-FISH), e.g., Pernthaler et al., 2002a; Wendeberg, 2010) or various types of multiple labeling of probes (Amann and Schleifer, 2001) such as DOPE-FISH (Stoecker et al., 2010).
 5. Purchase and handling of probes: Probes are chemically synthesized and can be purchased with or without label. In earlier times, probes had to be manually labeled (Amann, 1995), but nowadays labeled probes can be easily purchased from most providers (e.g., Biomer, Invitrogen, MWG, SIGMA) of molecular reagents like PCR primers at a rather low cost (starting

from approximately 50 USD). Probes are normally shipped in lyophilized stage and must be dissolved in either sterile water or an appropriate buffer (depending fluorochrome chemistry) to a stock solution of normally 100 pmol/μl. It is recommended to store aliquots of working solutions in individual tubes in dark at -20°C, and to avoid excessive freeze-thawing of the aliquots. For long time storage or transport at room temperature it is recommended to lyophilize the probes.

6. Evaluation of probes:

(a) For any purchased probe, it is recommended to check for the probe's concentration, length, homogeneity, and the completeness of the labeling, to avoid unwanted competition between labeled and unlabeled probes for target sites as this may reduce the probe signal intensity. This can be done as described by Stahl and Amann (2001), or as recommended by the provider who performed the labeling.

(b) Newly designed probes are generally first tested under non-stringent conditions (i.e., by excluding formamide in the hybridization buffer). If positive probe signals are obtained in reference strains as expected, stringent conditions of the probe should be determined by an increasing formamide series, as described in Amann and Schleifer (2001). Probe signal intensities can be quantified by digital image analysis with, e.g., the daime software, as described by (Daims et al., 2009).

(c) Combination of probes. The great advantage of FISH over standard PCR applications is that several differently labeled gene probes can be simultaneously combined and thus enable a simultaneous identification of different probe-targeted species within a complex sample. The general guidelines for optimal combination of different probes are:

- Probes with identical formamide concentrations for optimal stringencies (at which the probe hybridizes to the specific target but not to nontarget groups) can be combined in the same hybridization experiment.
- Probes with different formamide concentrations for optimal stringencies cannot be combined in the same hybridization experiment. Instead, successive hybridizations must be performed for each probe, starting with the probe with the highest formamide concentration.

(d) Most standard epifluorescence microscopes are equipped with filter sets that allow separate recording of at least two to three differently labeled probes and one UV-based nucleic acid stain like DAPI (4',6-Diamidino-2-phenylindol). Such recorded images can then be merged into one image with image analysis software packages. However, recent developments based on, e.g., advanced confocal laser scanning microscopes with multichannel image analysis, special tools such as spectral imaging or FISH protocols employing quantum dot

labeled probes allow the simultaneous combination of a considerably higher amount of differently labeled probes (e.g., Ainsworth et al., 2006; Anderson, 2010; Bentolila et al., 2006).

7. Probe bases: Different attempts to construct databases with FISH probes have been made during the last two decades. However, the probeBase (www.microbial-ecology.net/probebase/) is currently the most regularly updated data base for ribosomal targeted probes, encompassing 2,568 different probes targeting species within Archaea, Bacteria, and, to some extent, Eukarya (further probes targeting protists and cyanobacteria can be retrieved from www.sb-roscoff.fr/Phyto/Data-bases/RNA_probes_introduction.php) for FISH as well as for ~16 different microarray applications (version September 2010). Here, many of the so far published probes can be easily searched by different criteria (target organism, target sites, probe name, probe sequence, probeBase accession number, references). Detailed information about the probe chemistry are listed and links are provided for online probe matches to other databases like the ProbeCheck (<http://131.130.66.200/cgi-bin/probecheck/content.pl?id=home>), Green Genes (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>), or RDP (<http://rdp.cme.msu.edu/>). Furthermore, up to 150 ribosomal gene sequences retrieved from own clone libraries can be uploaded to screen for probes targeting these sequences (Loy et al., 2007, 2008).
8. Controls: For a valid evaluation of probe signals, three different categories of controls are needed: (1) Positive controls where probes are employed that should or should not overlap with the intended target gene. (2) Negative controls, to explore the impact of confounding background caused by, e.g., autofluorescence, and of nonspecific binding of fluorochrome or probe to target cells as well as nontarget cells. To achieve this, two different negative controls are recommended: exclusion of probes during the hybridization experiment, and application of negative reference probes like the NON-SENSE probe (Loy et al., 2002) or the non-EUB probe (reverse complementary to the EUB probe, (Wallner et al., 1993)). (3) All experiments should be performed on appropriate optimally fixed strains carefully selected to serve as positive as well as negative controls.
9. Other probe types: Note that some of the rules applied for design and handling of standard oligonucleotide probes may not be applicable to other probe types, like polynucleotide probes, PNA FISH probes, or CARD FISH probes.

General guidelines for evaluation and presentation of FISH results

For optimal interpretation of FISH experiments, several parameters should be considered, as outlined below. Omitting any of these principles may lead to false interpretations of FISH results.

Checklist for optimal FISH experiments and evaluation of results:

1. Were optimal sampling strategies, fixation protocols, and storage conditions employed?
2. Were appropriate reference strains used simultaneously in parallel with the FISH experiments? If strains are lacking, clones based on CLONE-FISH (Schramm et al., 2002) may also be used to experimentally evaluate the FISH experiments.
3. Were the microscopical investigations performed with optimal microscope settings?
4. Were appropriate probes/probe combinations (positive as well as negative controls) used under stringent conditions?
 - (a) If the probe signals were positive, explore if nonspecific probe signals can be unequivocally excluded or if the identity of the probe signal can be confirmed with other measures.
 - (b) If the probe signals were negative on the sample subject to investigation but otherwise positive with positive controls, explore if this is caused either by cell losses during the hybridization procedure, low cell numbers in the original sample, or low activity (and thus low ribosome content and probe signal intensity).
5. If quantifications of FISH images were performed, explore if the amount of sample applied to the microscope slide was appropriate. A too high or too low cell concentration may result in an over- or underestimation of cell numbers. Evaluate also if the amount of microscope slide fields investigated was high enough to ensure statistical evaluations of error ranges. For further evaluation of quantification, spiking of sample may be recommendable (SPIKE-FISH, Daims et al., 2001).
6. When presenting FISH results, it is recommended to describe results as "cells targeted by probe X," instead of uncritically identifying the probe signals with the intended target group of the probe. This is especially important when applying probes to samples with unknown microbial composition and the applied probe is known to target false-positive as well as false-negative species and no further means have been undertaken to confirm the identity by, e.g., appropriate combinations of hierarchic FISH probe sets or subsequent sequencing (Amann and Fuchs, 2008).
2. Confounding background caused by auto-fluorescing compounds like photosynthetic pigments, proteins like cofactors (e.g., F420 for methanogens), other parameters in the surrounding environment (e.g., precipitates, fibers, minerals, humic substances); or by unspecific absorption of fluorochrome label.
3. Probe technical problems (e.g., probe permeabilization problems, probe accessibility in the ribosomal gene, probe specificity).
4. Limited information content of the 16S rRNA gene for taxonomical resolution of closely related species.
5. Limited possibilities to confirm true identity and explore phylogenetic relationships and identify novel gene groups or species.
6. Limited information on in situ physiology in terms of:
 - (a) Activity status (high ribosome content may reflect exponential growth stage, but this appears to be species dependent and needs to be explored for each particular species group (Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007);
 - (b) Function, since the ribosomal gene does not reveal information about function and since the genotype is not always linked to the phenotype (e.g., many functional microbial groups are polyphyletic, such as methanogens, sulphate reducers, denitrifiers, cyanobacteria, spirochaetes, reductive dechlorinators);
 - (c) Limited possibilities to monitor gene expressions (mRNA transcripts) or proteins.
7. Applications limited to prokaryotic and eukaryotic cells. However, the first notes on successful FISH applications on bacteriophages (personal communication, K. Zwirgmaier, D. Scanlan, A. Millard, University of Warwick, UK) was recently made with the Recognition of individual genes [RING]-FISH protocol (Zwirgmaier et al., 2004b).

To overcome these limitations, several different strategies have been employed, ranging from modifying different parts of the procedures of the first-generation FISH protocol, searching for alternative gene targets, probe chemistries and labeling procedures, to combining FISH with other markers (e.g., activity stains, enzymes, isotopes), analytical procedures, and advanced microscopes or other analytical tools for evaluation of several different parameters for a holistic interpretation of in situ microbial ecology. Today, at least four categories of advanced FISH strategies can be recognized, which can be combined in a multitude of ways for various novel applications.

Category I – increasing the probe signal intensity:

Drawbacks of oligonucleotide FISH and the benefits of novel FISH technologies

The first generation of FISH protocol based on oligonucleotide probes revolutionized nearly all disciplines in microbiology. However, along with applications of this protocol on different ecosystems and species, many of the limits of the first-generation FISH protocol became obvious:

1. Successful detection dependent on ribosome content (minimum 370 ± 16 rRNA molecules per *E. coli* cell (Hoshino et al., 2008)).
- Incubate sample prior to fixation with substrates or stress-promoting compounds like chloramphenicol (Kalmbach et al., 1997; Ouverney et al., 1997).
- Employ non-fluorescent labeling of probes for increased detection of cells in, e.g., a strongly autofluorescing background based on, e.g., radiolabels (Giovannoni

et al., 1988); or enzymes (Zarda et al., 1991; Amann et al., 1992; Lim et al., 1993).

- Employ alternative probe design based on helper probes for oligonucleotide probes to increase probe accessibility (Fuchs, 2000).
- Employ alternative probe chemistries for increased hybridization efficiencies and specificities based on peptide nucleic acid probes (PNA) (Perry-O'Keefe et al., 2001), or locked nucleic acid (LNA) probes (Kubota et al., 2006a).
- Explore probe accessibilities and thermodynamics-based probe design to predict probe accessibilities and optimal hybridization conditions (Yilmaz et al., 2006; Yilmaz et al., 2010a), and/or in nucleotide or probe quenching (Behrens, 2003; Behrens et al., 2004; Wagner et al., 2003).
- Employ self-ligating probes to overcome background autofluorescence and avoid washing steps (Sando and Kool, 2002).
- Employ PCR-generated probes to generate polynucleotide probes (Trebesius et al., 1994; Zimmermann et al., 2001; Meisinger et al., 2007; Pernthaler, 2002; Preston et al., 2002; Moraru et al., 2010).
- Employ modified fluorescent labeling technologies based on horse radish peroxidase labeled probes and catalyzed reporter deposition (CARD)-FISH (Pernthaler et al., 2002a, b; Hoshino et al., 2008; Pavlekovic et al., 2009); multiple labeling of probes (Amann and Schleifer, 2001; Stoecker et al., 2010) or quantum dot conjugated probes (Bentolila et al., 2006).

Category II – exploring activity or function:

- Combine FISH with rRNA dot slot to quantify cellular rRNA content (Ravenschlag et al., 2000).
- Employ FISH probes targeting the precursor 16S rRNA gene and intergenic spacer regions (Oerther et al., 2000; Schmid et al., 2001; Moter et al., 2010).
- Combine FISH with different activity indicating stains like redox dyes (Nielsen et al., 2003), halogenated thymidine analogue bromodeoxyuridine (BrdU) (Pernthaler et al., 2002), exo-enzyme labeled probes (Xia et al., 2007), or antibodies to target cell surface markers or proteins (Currin et al., 1990; Lubeck et al., 2000; Gmür and Lüthi-Schaller, 2007).
- Incubate sample with radioactively labeled substrates or stable isotopes to explore specific uptake of labeled substrates by micro-autoradiography (Nielsen et al., 2005), RAMAN spectroscopy (Wagner, 2009), or nano-SIMS (Behrens et al., 2008; Orphan, 2009).
- Target functional genes or even the mRNA gene by CARD-FISH (Wagner et al., 1998; Pernthaler and Amann, 2004; Orphan, 2009; Pilhofer et al., 2009), two-pass TSA-mRNA FISH (Kubota et al., 2006b), or by alternative labeling (Coleman et al., 2007).
- Target functional genes. For this, there are two categories: (1) PCR-generated probes like polynucleotide

FISH (Moraru et al., 2010) or (2) amplification of the target sequence like in situ PCR (Hodson et al., 1995; Rodríguez-Castaño, G., 2008; Tani et al., 1998), in situ loop-mediated isothermal amplification (Maruyama et al., 2003); recognition of individual genes [RING]-FISH (Zwirgmaier et al., 2004b; Pratscher et al., 2009; Hauer et al., in preparation), in situ rolling-circle amplification (Maruyama et al., 2005; Hoshino et al., 2010), cycling primed in situ amplification (CPRINS, Kenzaka, 2005; Tamaki et al., 2005), circularizable probes (Zhang et al., 2006), circularizable probes (Zhang et al., 2006; Hoshino et al., 2010), peptide nucleic acid assisted rolling circle amplification FISH (Smolina et al., 2007), and polynucleotide FISH (Moraru et al., 2010).

Category III – combining FISH with advanced cameras, microscopes, or other analytical tools:

- CCD camera (Amann and Schleifer, 2001); confocal laser scanning microscopy (Wagner et al., 1998), and advanced digital image processing (Daims, 2009; Daims and Wagner, 2007)
- Electron microscopy (Kenzaka et al., 2005; Ishidoshiro et al., 2005)
- Spectral imaging (Ainsworth et al., 2006)
- RAMAN spectroscopy (Wagner, 2009)
- nanoSIMS (Behrens et al., 2008; Orphan, 2009)
- Atomic force microscopy (Huang et al., 2009)

Category IV – enrichment of FISH targeted cells for subsequent analyses, and combination with high-throughput technologies:

- Automatic cell sorting and enrichment techniques based on flow cytometry (Amann and Fuchs, 2008)
- Magneto-FISH (Orphan, 2009; Stoffels et al., 1999)
- RING-FISH (Zwirgmaier et al., 2004a)
- Whole genome amplification (Yilmaz et al., 2010b)

Many of these improvements minimize at least some of the drawbacks observed with the first generation FISH protocol; however, these new approaches do also introduce other drawbacks. For example, CARD-FISH has replaced the standard FISH protocol in several cases because it produces stronger probe signals (Pernthaler et al., 2002a, b) and just recently it was shown that it can be combined with polynucleotide probes targeting functional genes in a subsequent step (Moraru et al., 2010). Nevertheless, compared to standard oligonucleotide FISH, CARD-FISH is a rather time-consuming FISH protocol and allows only the application of one probe per hybridization experiment. Furthermore, as peroxidase can be found in nearly all organisms, there may be a risk for unspecific probe reactions. Fortunately, the peroxidase can be denatured prior to the FISH procedure, however, optimal denaturation conditions appear to be species dependent and may thus require individual optimizations (Pavlekovic et al., 2009). Similarly, the recent developments of various protocols to target non-ribosomal genes

like various functional genes and mRNA transcripts, or combining with various analytical tools, present different advantages as well as disadvantages. For example, considerable higher costs, advanced equipment, tedious protocols, loss of probe specificity, less flexibility in terms of combination with other probes or protocols or on various organisms, problems with adequate preservation of gene target due to high decay rate (e.g., precursors or mRNA), or unpredictable cross-reactions (e.g., when incubating a complex community with radioactive substrates or stable isotopes). However, irrespective of these drawbacks that may eventually be overcome in future, it is crucial to keep in mind that our greatest hindrance for successful, unequivocal interpretations of FISH experiments in the environment is the unknown biodiversity and multitude facets of dynamics and interactions, which paradoxically seem to grow with each advancement in the science of microbiology. Thus, it appears likely that the development of novel FISH technologies will continue in parallel with the development of novel tools and our increased understanding of microbial diversity.

Different FISH applications in geomicrobiology

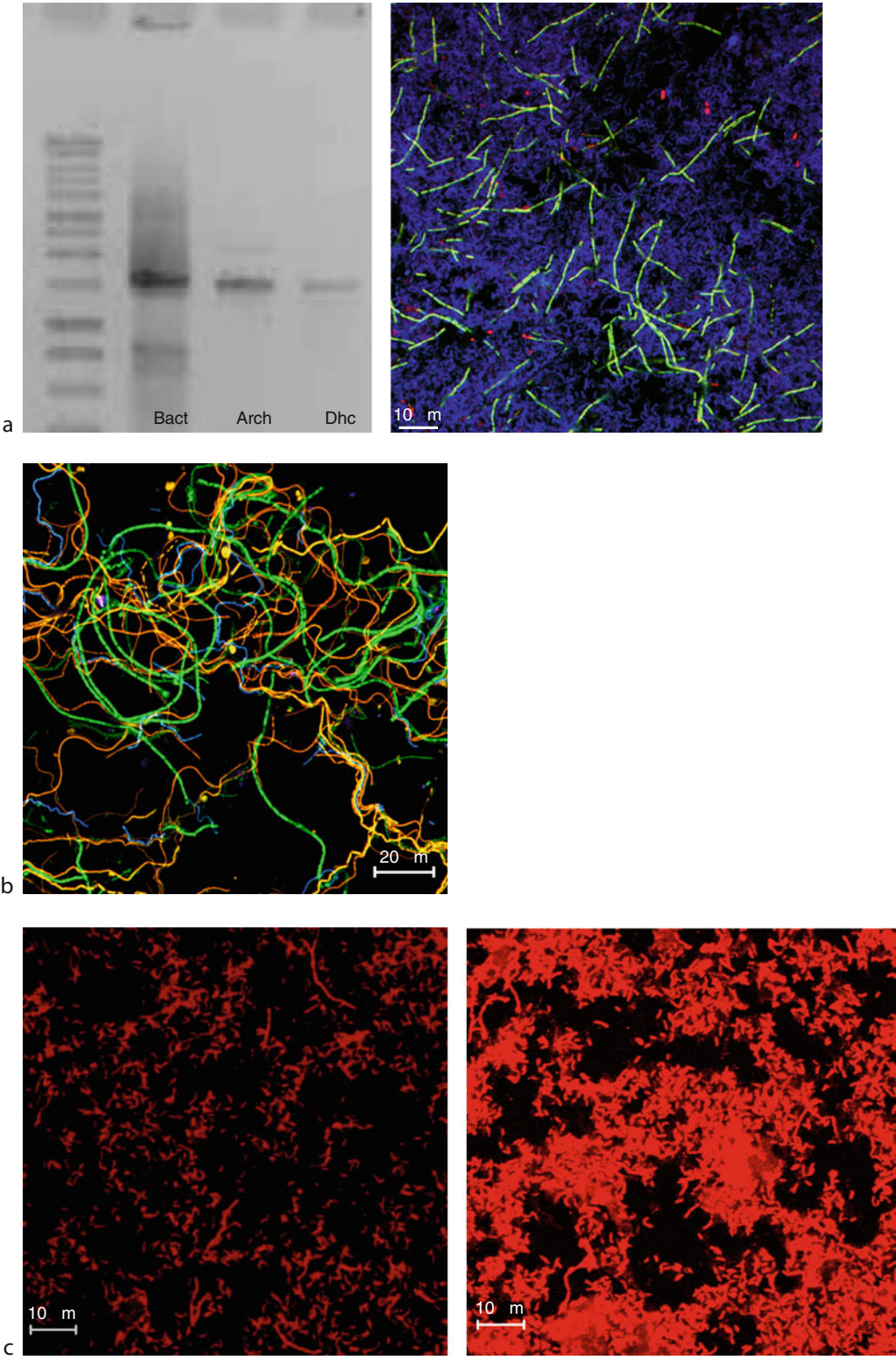
The fields explored in geomicrobiology are extremely diverse, ranging from the deep biosphere to the atmosphere surrounding our planet Earth, and from there to the outer space. The conditions for the different ecosystems will therefore vary tremendously so that different sampling technologies and modifications of FISH protocols must be employed in order to achieve successful FISH results. So far, FISH has not been employed in geomicrobiology as often and as successfully as in other microbiological fields like environmental biotechnology (e.g., wastewater treatment plants, Nielsen et al., 2009) or in medical sciences (e.g., Bridger et al., 2010). This is because many of the ecosystems explored in geomicrobiology are often exposed to extreme conditions and generally contain low amounts of usually novel microorganisms with slow or unknown growth rates. Furthermore, special considerations must be made in terms of interpreting results with regard to the risk of contamination of samples during sampling (e.g., during drilling experiments) and to the impact of geochemical conditions on so far published FISH protocols. Because of these obstacles, many of the recent advanced developments of FISH protocols have actually been driven by microbial ecologists and geomicrobiologists (Orphan, 2009). Below follow some examples of how FISH has been employed for some of the most widely explored ecosystems in geomicrobiology (see also images in Figure 2):

Aquatic systems: In general, successful FISH experiments can be performed on aquatic systems, since at least one of the main parameters for good microbial growth and dispersion of cells and nutrients, water, is abundant. Nevertheless, several parameters may introduce several biases, e.g., low nutrient status and/or naturally occurring

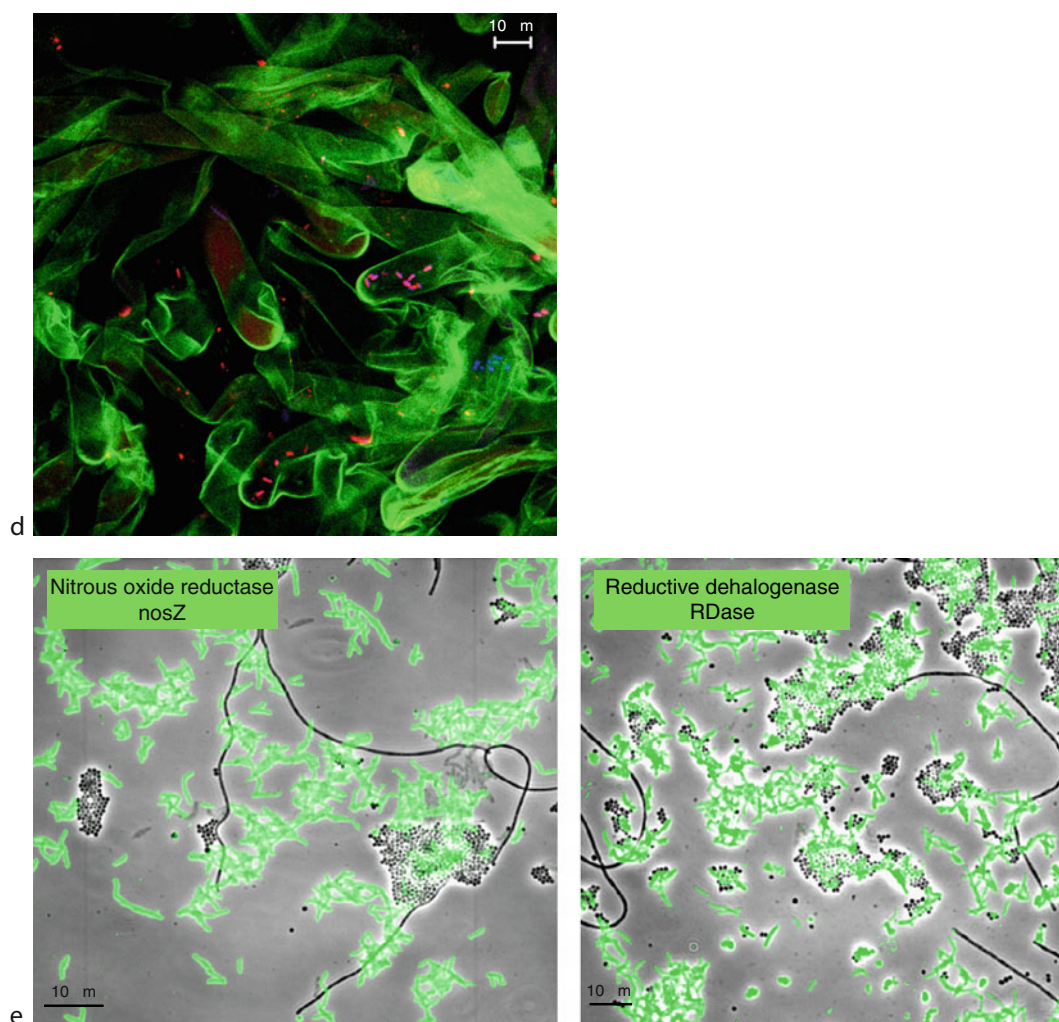
autofluorescing particles (Vesey et al., 1997). Aquatic systems with high nutrient content such as contaminated lakes or even anthropogenic systems like wastewater treatment plants will contain greater amounts of highly active bacteria than oligotrophic systems like aquifers and thus produce stronger FISH probe signals. For improved FISH on oligotrophic systems, different approaches have been undertaken:

Aquifers/ground water/drinking water: These ecosystems are generally dominated by planctonic cells in rather low cell numbers due to extreme conditions like oligotrophy, different redox states (e.g., anaerobic conditions), or toxic compounds (e.g., heavy metals, chlorinated drinking water or contaminated aquifers). Due to this, the general turn-over activities and thus also the ribosome content in the cells are generally extremely low. For adequate FISH evaluation, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris removed (e.g., by cell extraction, Caracciolo et al., 2005). Early studies reported that standard FISH protocols may work well on certain aquatic samples like lakes and bottled water (Pernthaler et al., 1998; Glöckner et al., 2000; Watanabe et al., 2000; Flies et al., 2005; Loy et al., 2005); however, more recent studies report that either rainbow FISH (Sunamura and Maruyama, 2006) or CARD-FISH are more recommendable on drinking water or groundwater (Sekar et al., 2003; Nielsen et al., 2006; Wilhartitz et al., 2007; Meisinger et al., 2010a).

Ocean: Similar to the freshwater systems mentioned above, oceanic ecosystems are generally dominated by planctonic cells in rather low cell numbers. However, in contrast to the conditions in the aquifers in the subsurface, the cells in oceans are often exposed to more dynamic conditions. Despite this, the ribosome content in the cells and the turn-over activities may be rather low at least for certain species – depending on location and other conditions such as oxygen concentration, light frequencies, redox conditions, nutrient conditions, flux rates. For adequate FISH evaluation of cells, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris must be removed (e.g., by cell extraction). Early studies demonstrated that standard FISH protocols may work well (Murray et al., 1998; Glöckner, 1999; Cottrell and Kirchman, 2000; Morris et al., 2004); however during the last decade, most studies have tried to increase probe signal intensities by modifying standard FISH protocols or applying other FISH tools like polynucleotide FISH (DeLong et al., 1999; Pernthaler et al., 2002; Preston et al., 2002) or CARD-FISH (Pernthaler et al., 2002a; b; Ishii et al., 2004; Teira et al., 2004; Herndl et al., 2005). In addition to this, various combinations with different activity targeted studies based on incubation with chloramphenicol (Ouverney and Fuhrman, 1997), radioactive substrates like thymidine (Pernthaler et al., 2002) or bromodeoxyuridine (BrdU, Pernthaler and Pernthaler, 2005), or other substrates such as amino acids



Fluorescence In Situ Hybridization (FISH), Figure 2 (Continued)



Fluorescence In Situ Hybridization (FISH), Figure 2 Demonstration of different images from different ecosystems produced by different FISH techniques.

(Ouverney and Fuhrman, 2000; Cottrell, 2000; Cottrell, 2003), have been undertaken.

Soil/sediments/subseafloor: Soil and sediments are rather difficult environments for successful FISH studies, as these habitats are extremely diverse, complex, and subject to harsh and fluctuating conditions in terms of, e.g., water content and redox conditions. Thus, more than most other ecosystems, the biosphere of microorganisms in soil is often characterized by micro-niches which may be exposed to dramatic changes with regard to space and time. In addition to this, many substances in soil or sediments produce strongly confounding background such as humic compounds, mineral precipitates or other microscopic particles of unknown character. For successful FISH experiments, two different main approaches have been made (for a review, see Schmid et al., 2006; Schmid et al., 2007): (1) Spatial separations of cells or micro-niches by approaches such as embedding and

sectioning of soil columns or by confocal laser scanning microscopy of soil aggregates, different cell extraction procedures such as density centrifugation or cation exchange membranes (McDonald, 1986; Smith and Stribley, 1994; Caracciolo et al., 2005; Kurola et al., 2005; Lunau et al., 2005; Bertaux et al., 2007), or flow cytometry (Kalyuzhnaya et al., 2006; Podar et al., 2007). (2) The second approach employed a variety of different FISH protocols. The early studies reported generally only partial success or even contradictory results between FISH and other analytical tools (Hahn et al., 1992; Ludwig et al., 1997; Zarda et al., 1997; Chatzinotas, 1998; Llobet-Brossa et al., 1998; Christensen, 1999; Hristova et al., 2000; Ravensschlag et al., 2000; Janvier et al., 2003; Kobabe et al., 2004; Rusch and Amend, 2004; Flies et al., 2005; Knittel et al., 2005). However, during the last years, most studies have now replaced the standard FISH tool with CARD-FISH and nanoSIMS (Ishii et al., 2004;

Mussmann et al., 2005; Schippers et al., 2005; Tal et al., 2005; Orphan 2009).

Karst regions/rock/deep biosphere: During the last decade, our understanding of the role of microorganisms and their interactions with rocks and minerals over geological timescales has expanded considerably. The interaction of minerals with water and microbes do not only influence soil fertility, transport compounds, and pollutants through the subsurface, but they also contribute to dissolution of rocks or precipitation of various mineral formations like speleothems in caves and may thus even play a key role in global climate change (Northup and Lavoie, 2001; Brown and Lee, 2007; Cockell and Herrera, 2008). Several attempts have been made to screen for microbial life in rocks and set up validated guidelines for this (Barton et al., 2001; Barton et al., 2006; Tobin et al., 1999). The reoccurring concern is to not only prove extant microbial processes (Orphan and House, 2009) but also current cellular-based in situ activities in often carbon-poor systems. Since it may be difficult to retrieve sufficient amounts of RNA or other activity biomarkers from these systems due to low cell counts and activities, FISH, in particular in combination with advanced tools like nanoSIMS, may play a crucial role for demonstrating whole cells with targetable genes (Orphan, 2009). Despite these obstacles, standard FISH as well as CARD-FISH protocols have been successfully applied on, e.g., microbial mats in association with water and energy-rich substances for life based on chemolithoautotrophy in rocky environments or caves (Engel et al., 2003; Macalady et al., 2006; Meisinger et al., 2007; Meisinger et al., 2010b). However, certain types of microbial mats may be strongly hampered by confounding background caused by various mineral precipitates (Engel et al., 2010; Koeberich, 2008). Rigid environments like calcified biofilms or speleothems thus demand sophisticated modifications of standard FISH protocols as well as CARD-FISH (e.g., Shiraishi et al., 2008).

Extremophiles in various geological environments: Extremophiles thrive in extreme ecosystems which are characterized by extreme physical or geochemical conditions. Such conditions may pose a particular challenge for FISH experiments. So far, FISH has only been practiced in certain types of extremophilic ecosystems with mixed success, e.g., in hot springs (Nubel et al., 2002; Simbahan et al., 2005; Nakagawa et al., 2006; Weidler et al., 2008), polar regions (Junge et al., 2004), halophilic systems (Rossello-Mora et al., 2003; Maturano et al., 2006), mines/bioleaching systems (Schrenk et al., 1998; Edwards et al., 1999; Bond et al., 2000; Takai, 2002; Baker et al., 2004), hydrothermal vents (Harmsen et al., 1997; Schrenk et al., 2003; Nakagawa et al., 2006), and methane hydrate seeps (Boetius and al, 2000).

Summary

FISH (fluorescence in situ hybridization) is a valuable tool for in situ identification of microbial community structure, cell morphology, dynamics and spatial distribution.

If combined with different analytical tools additional information of activity, function and interactions of different species on single cell level can be performed. However, for successful FISH experiments, it is crucial to optimize and validate all steps throughout the FISH protocol, ranging from proper sampling technique and fixation, selection of appropriate gene markers, hybridization conditions and relevant controls, to optimal detection and evaluation strategies.

Bibliography

- Ainsworth, T. D., Fine, M., Blackall, L. L., and Hoegh-Guldberg, O., 2006. Fluorescence *in situ* hybridization and spectral imaging of coral-associated bacterial communities. *Applied and Environmental Microbiology*, **72**, 3016–3020.
- Alm, E., Oerther, D., Larsen, N., Stahl, D., and Raskin, L., 1996. The oligonucleotide probe database. *Applied and Environmental Microbiology*, **62**, 3557–3559.
- Amann, R., and Fuchs, B. M., 2008. Single-cell identification in microbial communities by improved fluorescence *in situ* hybridization techniques. *Nature Reviews Microbiology*, **6**, 339–348.
- Amann, R., Ludwig, W., and Schleifer, K.-H., 1994. Identification of uncultured bacteria: a challenging task for molecular taxonomists. *ASM News*, **60**, 360–365.
- Amann, R., Ludwig, W., and Schleifer, K.-H., 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Amann, R., and Schleifer, K.-H., 2001. Nucleic acid probes and their application in environmental microbiology. In Garrity, G. M. (ed.), *Bergey's Manual of Systematic Bacteriology*. New York: Springer, 2nd edn. pp. 67–82.
- Amann, R., Snaird, J., Wagner, M., Ludwig, W., and Schleifer, K. H., 1996. *In situ* visualization of high genetic diversity in a natural microbial community. *Journal of Bacteriology*, **178**, 3496–3500.
- Amann, R. I., 1995. *In situ* identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In *Molecular Microbial Ecology Manual*. Vol. 3.3.6, pp. 1–15.
- Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., and Stahl, D., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology*, **56**, 1919–1925.
- Amann, R. I., Krumholz, L., and Stahl, D. A., 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *Journal of Bacteriology*, **172**, 762–770.
- Amann, R. I., Zarda, B., Stahl, D. A., and Schleifer, K. H., 1992. Identification of individual prokaryotic cells by using enzyme-labeled, rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology*, **58**, 3007–3011.
- Amaral-Zettler, L., Peplies, J., Ramette, A., Fuchs, B., Ludwig, W., and Glöckner, F. O., 2008. Proceedings of the international workshop on Ribosomal RNA technology, April 7–9, 2008, Bremen, Germany. *Systematic and Applied Microbiology*, **31**, 258–268.
- Anderson, R., 2010. Multiplex fluorescence in situ hybridization (M-FISH). *Methods Molecular Biology*, **659**, 83–97.
- Baker, B. J., Lutz, M. A., Dawson, S. C., Bond, P. L., and Banfield, J. F., 2004. Metabolically active eukaryotic communities in extremely acidic mine drainage. *Applied and Environmental Microbiology*, **70**, 6264–6271.

- Barton, H. A., Spear, J. R., and Pace, N. R., 2001. Microbial life in the underworld: biogenicity in secondary mineral formations. *Geomicrobiology Journal*, **18**, 359–368.
- Barton, H. A., Taylor, N. M., Lubbers, B. R., and Pemberton, A. C., 2006. DNA extraction from low-biomass carbonate rock: an improved method with reduced contamination and the low-biomass contaminant database. *Journal of Microbiological Methods*, **66**, 21–31.
- Baschien, C., Manz, W., Neu, T. R., and Szewzyk, U., 2001. I. Decomposition and microbial activities fluorescence *in situ* hybridization of freshwater fungi. *International Review of Hydrobiology*, **86**, 371–381.
- Behrens, S., Fuchs, B. M., and Amann, R., 2004. The effect of nucleobase-specific fluorescence quenching on *in situ* hybridization with rRNA-targeted oligonucleotide probes. *Systematic and Applied Microbiology*, **27**, 565–572.
- Behrens, S., Fuchs, B. M., Mueller, F., and Amann, R., 2003. Is the *in situ* accessibility of the 16S rRNA of *Escherichia coli* for Cy3-labeled oligonucleotide probes predicted by a three-dimensional structure model of the 30S ribosomal subunit? *Applied and Environmental Microbiology*, **69**, 4935–4941.
- Behrens, S., Losekann, T., Pett-Ridge, J., Weber, P. K., Ng, W.-O., Stevenson, B. S., Hutcheon, I. D., Relman, D. A., and Spormann, A. M., 2008. Linking microbial phylogeny to metabolic activity at the single-cell level by using enhanced element labeling-catalyzed reporter deposition fluorescence *in situ* hybridization (EL-FISH) and NanoSIMS. *Applied and Environmental Microbiology*, **74**, 3143–3150.
- Behrens, S., Rühland, C., Inácio, J., Huber, H., Fonseca, Á., Spencer-Martins, I., Fuchs, B. M., and Amann, R., 2003. *In situ* accessibility of small-subunit rRNA of members of the domains bacteria, archaea, and eucarya to Cy3-labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **69**, 1748–1758.
- Bentolila, I. A., and Weiss, S., 2006. Single-step multicolor fluorescence *in situ* hybridization using semiconductor quantum dot–DNA conjugates. *Cell Biochemistry and Biophysics*, **45**, 59–70.
- Bertaux, J., Gloger, U., Schmid, M., Hartmann, A., and Scheu, S., 2007. Routine fluorescence *in situ* hybridization in soil. *Journal of Microbiological Methods*, **69**, 451–460.
- Boetius, A., et al., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623.
- Bond, P. L., Druschel, G. K., and Banfield, J. F., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Bridger, J. M., and Volpi, E. V., 2010. *Fluorescence In Situ Hybridization. Methods in Molecular Biology*. Totowa: Humana Press, Vol. 659.
- Brown, D. J., and Lee, M., 2007. From microscopic minerals to global climate change? *Geology Today*, **23**, 172–177.
- Caracciolo, A. B., Grenni, P., Cupo, C., and Rossetti, S., 2005. *In situ* analysis of native microbial communities in complex samples with high particulate loads. *FEMS Microbiology Letters*, **253**, 55–58.
- Chatzinotas, A. S. R., Schönhuber, W., Amann, R., Daae, F. L., Torsvik, V., Zeyer, J., and Hahn, D., 1998. Analysis of broad-scale differences in microbial community composition of two pristine forest soils. *Systematic and Applied Microbiology*, **21**, 579–587.
- Christensen, H., Hansen, M., and Sørensen, J., 1999. Counting and size classification of active soil bacteria by fluorescence *in situ* hybridization with an rRNA oligonucleotide probe. *Applied and Environmental Microbiology*, **65**, 1753–1761.
- Cockell, C. S., and Herrera, A., 2008. Why are some microorganisms boring? *Trends in Microbiology*, **16**, 101–106.
- Coleman, J. R., Culley, D. E., Chrisler, W. B., and Brockman, F. J., 2007. mRNA-targeted fluorescent *in situ* hybridization (FISH) of gram-negative bacteria without template amplification or tyramide signal amplification. *Journal of Microbiological Methods*, **71**, 246–255.
- Cottrell, M. T., and Kirchman, D. L., 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnology and Oceanography*, **48**, 168–178.
- Cottrell, M. T., and Kirchman, D. L., 2000. Natural assemblages of marine proteobacteria and members of the cytophaga-flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology*, **66**, 1692–1697.
- Cottrell, M. T., and Kirchman, D. L., 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **66**, 5116–5122.
- Currin, C. A., Pearl, H. W., Suba, G. K., and Alberte, R. S., 1990. Immunofluorescence detection and characterization of N₂-fixing microorganisms from aquatic environments. *Limnology and Oceanography*, **35**, 59–71.
- Daims, H., 2009. Use of fluorescence *in situ* hybridization and the daim image analysis program for the cultivation-independent quantification of microorganisms in environmental and medical samples. *Cold Spring Harbor Protocols*, pdb.prot5253.
- Daims, H., and Wagner, M., 2007. Quantification of uncultured microorganisms by fluorescence microscopy and digital image analysis. *Applied Microbiology and Biotechnology*, **75**, 237–248.
- Daims, H., Bruhl, A., Amann, R., Schleifer, K. H., and Wagner, M., 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology*, **22**, 434–444.
- Daims, H., Ramsing, N. B., Schleifer, K.-H., and Wagner, M., 2001. Cultivation-independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **67**, 5810–5818.
- de los Reyes, F., Ritter, W., and Raskin, L., 1997. Group-specific small-subunit rRNA hybridization probes to characterize filamentous foaming in activated sludge systems. *Applied and Environmental Microbiology*, **63**, 1107–1117.
- DeLong, E. F., Taylor, L. T., Marsh, T. L., and Preston, C. M., 1999. Visualization and enumeration of marine planktonic archaea and bacteria by using polyribonucleotide probes and fluorescent *in situ* hybridization. *Applied and Environmental Microbiology*, **65**, 5554–5563.
- DeLong, E. F., Wickham, G. S., and Pace, N. R., 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. *Science*, **243**, 1360–1363.
- DeSantis, T. Z., Stone, C. E., Murray, S. R., Moberg, J. P., and Andersen, G. L., 2005. Rapid quantification and taxonomic classification of environmental DNA from both prokaryotic and eukaryotic origins using a microarray. *FEMS Microbiology Letters*, **245**, 271–278.
- Edwards, K. J., Gihring, T. M., and Banfield, J. F., 1999. Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Applied and Environmental Microbiology*, **65**, 3627–3632.
- Engel, A. S., Lee, N., Porter, M. L., Stern, L. A., Bennett, P. C., and Wagner, M., 2003. Filamentous “Epsilonproteobacteria” dominate microbial mats from sulfidic cave springs. *Applied and Environmental Microbiology*, **69**, 5503–5511.

- Engel, A. S., Meisinger, D. B., Porter, M. L., Payn, R. A., Schmid, M., Stern, L. A., Schleifer, K. H., and Lee, N. M., 2010. Linking phylogenetic and functional diversity to nutrient spiraling in microbial mats from Lower Kane Cave (USA). *The ISME Journal*, **4**, 98–110.
- Flies, C. B., Peplies, J., and Schuler, D., 2005. Combined approach for characterization of uncultivated magnetotactic bacteria from various aquatic environments. *Applied and Environmental Microbiology*, **71**, 2723–2731.
- Fried, J., and Foissner, W., 2007. Differentiation of two very similar glaucocid ciliate morphospecies (ciliophora, tetrahymenida) by fluorescence *in situ* hybridization with 18S rRNA targeted oligonucleotide probes. *Journal of Eukaryotic Microbiology*, **54**, 381–387.
- Fried, J., Ludwig, W., Psenner, R., and Schleifer, K. H., 2002. Improvement of ciliate identification and quantification: a new protocol for fluorescence *in situ* hybridization (FISH) in combination with silver stain techniques. *Systematic and Applied Microbiology*, **25**, 555–571.
- Fuchs, B., Wallner, G., Beisker, W., Schwiapl, I., Ludwig, W., and Amann, R., 1998. Flow cytometric analysis of the *in situ* accessibility of *Escherichia coli* 16S rRNA for fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **64**, 4973–4982.
- Fuchs, B. M., Glöckner, F. O., Wulf, J., and Amann, R., 2000. Unlabeled helper oligonucleotides increase the *in situ* accessibility to 16S rRNA of fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **66**, 3603–3607.
- Gerard, E., Guyot, F., Philippot, P., and Purificacion, L.-G., 2005. Fluorescence *in situ* hybridisation coupled to ultra small immunogold detection to identify prokaryotic cells using transmission and scanning electron microscopy. *Journal of Microbiological Methods*, **63**, 20–28.
- Giovannoni, S. J., DeLong, E. F., Olsen, G. J., and Pace, N. R., 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. *Journal of Bacteriology*, **170**, 720–726.
- Glöckner, F. O., Fuchs, B. M., and Amann, R., 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **65**, 3721–3726.
- Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A., and Amann, R., 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Applied and Environmental Microbiology*, **66**, 5053–5065.
- Gmür, R., and Lüthi-Schaller, H., 2007. A combined immunofluorescence and fluorescent *in situ* hybridization assay for single cell analyses of dental plaque microorganisms. *Journal of Microbiological Methods*, **69**, 402–405.
- Hahn, D., Amann, R. I., Ludwig, W., Akkermans, A. D. L., and Schleifer, K. H., 1992. Detection of micro-organisms in soil after *in situ* hybridization with rRNA-targeted, fluorescently labelled oligonucleotides. *Journal of General Microbiology*, **138**, 879–887.
- Harmsen, H., Prieur, D., and Jeanthon, C., 1997. Group-specific 16S rRNA-targeted oligonucleotide probes to identify thermophilic bacteria in marine hydrothermal vents. *Applied and Environmental Microbiology*, **63**, 4061–4068.
- Hauer, K., Pavlekovic, M., Chee-Sanford, J., Sanford, R. A., Löffler, F. E., and Lee, N. M., *In situ* detection of functional genes by RING-FISH in *Anaeromyxobacter* sp. (in press)
- Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J., 2005. Contribution of archaea to total prokaryotic production in the deep Atlantic ocean. *Applied and Environmental Microbiology*, **71**, 2303–2309.
- Heydorn, A., Ersboll, B. K., Hentzer, M., Parsek, M. R., Givskov, M., and Molin, S., 2000. Experimental reproducibility in flow-chamber biofilms. *Microbiology*, **146**, 2409–2415.
- Hodson, R. E., Dustman, W. A., Garg, R. P., and Moran, M. A., 1995. *In situ* PCR for visualization of microscale distribution of specific genes and gene products in prokaryotic communities. *Applied and Environmental Microbiology*, **61**, 4074–4082.
- Hoshino, T., Yilmaz, L. S., Noguera, D. R., Daims, H., and Wagner, M., 2008. Quantification of target molecules needed to detect microorganisms by fluorescence *in situ* hybridization (FISH) and catalyzed reporter deposition-FISH. *Applied and Environmental Microbiology*, **74**, 5068–5077.
- Hoshino, T., and Schramm, A., 2010. Detection of denitrification genes by *in situ* rolling circle amplification-fluorescence *in situ* hybridization to link metabolic potential with identity inside bacterial cells. *Environmental Microbiology*, **12**, 2508–2517.
- Hristova, K. R., Mau, M., Zheng, D., Aminov, R. I., Mackie, R. I., Gaskins, H. R., and Raskin, L., 2000. Desulfotomaculum genus- and subgenus-specific 16S rRNA hybridization probes for environmental studies. *Environmental Microbiology*, **2**, 143–159.
- Huang, J., Ma, L., Sundararajan, S., Fei, S.-z., and Li, L., 2009. Visualization by atomic force microscopy and FISH of the 45S rDNA gaps in mitotic chromosomes of *Lolium perenne*. *Protoplasma*, **236**, 59–65.
- Hugenholtz, P., Tyson, G. W., and Blackall, L. L., 2001. Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence *in situ* hybridization. In *Gene Probes: Principles and Protocols*. Vol. 176. London: Humana Press.
- Ishii, K., Mu[ss]mann, M., MacGregor, B. J., and Amann, R., 2004. An improved fluorescence *in situ* hybridization protocol for the identification of bacteria and archaea in marine sediments. *FEMS Microbiology Ecology*, **50**, 203–212.
- Janvier, M., Regnault, B., and Grimont, P., 2003. Development and use of fluorescent 16S rRNA-targeted probes for the specific detection of *Methylophaga* species by *in situ* hybridization in marine sediments. *Research in Microbiology*, **154**, 483–490.
- Junge, K., Eicken, H., and Deming, J. W., 2004. Bacterial activity at -2 to -20°C in Arctic wintertime sea ice. *Applied and Environmental Microbiology*, **70**, 550–557.
- Kalmbach, S., Manz, W., and Szwezyk, U., 1997. Dynamics of biofilm formation in drinking water: phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and *in situ* hybridization. *FEMS Microbiology Ecology*, **22**, 265–279.
- Kalyuzhnaya, M. G., Zabinsky, R., Bowerman, S., Baker, D. R., Lidstrom, M. E., and Chistoserdova, L., 2006. Fluorescence *in situ* hybridization-flow cytometry-cell sorting-based method for separation and enrichment of type I and type II methanotroph populations. *Applied and Environmental Microbiology*, **72**, 4293–4301.
- Kenzaka, T., Ishidoshiro, A., Yamaguchi, N., Tani, K., and Nasu, M., 2005. rRNA sequence-based scanning electron microscopic detection of bacteria. *Applied and Environmental Microbiology*, **71**, 5523–5531.
- Kenzaka, T., Tamaki, S., Yamaguchi, N., Tani, K., and Nasu, M., 2005. Recognition of individual genes in diverse microorganisms by cycling primed *in situ* amplification. *Applied and Environmental Microbiology*, **71**, 7236–7244.
- Knittel, K., Losekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Kobabe, S., Wagner, D., and Pfeiffer, E.-M., 2004. Characterisation of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridisation. *FEMS Microbiology Ecology*, **50**, 13–23.

- Koebberich, M., 2008. Methodenevaluierung zur fluoreszenz in situ hybridisierung des eisenoxidierenden bakteriums *Gallionella ferruginea*. Bachelor of science thesis of Georg August University of Göttingen, Germany. (Supervisors: J. Reitner and N. V. Queric, Geobiology Center).
- Konstantinidis, K. T., Serres, M. H., Romine, M. F., Rodrigues, J. L. M., Auchtung, J., McCue, L.-A., Lipton, M. S., Obraztsova, A., Giometti, C. S., Nealson, K. H., Fredrickson, J. K., and Tiedje, J. M., 2009. Comparative systems biology across an evolutionary gradient within the *Shewanella* genus. *Proceedings of the National Academy of Sciences*, **106**, 15909–15914.
- Kubota, K., Ohashi, A., Imachi, H., and Harada, H., 2006a. Improved *in situ* hybridization efficiency with locked-nucleic-acid-incorporated DNA probes. *Applied and Environmental Microbiology*, **72**, 5311–5317.
- Kubota, K., Ohashi, A., Imachi, H., and Harada, H., 2006b. Visualization of *mcr* mRNA in a methanogen by fluorescence *in situ* hybridization with an oligonucleotide probe and two-pass tyramide signal amplification (two-pass TSA-FISH). *Journal of Microbiological Methods*, **66**, 521–528.
- Kumar, Y., Westram, R., Behrens, S., Fuchs, B., Glockner, F., Amann, R., Meier, H., and Ludwig, W., 2005. Graphical representation of ribosomal RNA probe accessibility data using ARB software package. *BMC Bioinformatics*, **6**, 61.
- Kumar, Y., Westram, R., Kipfer, P., Meier, H., and Ludwig, W., 2006. Evaluation of sequence alignments and oligonucleotide probes with respect to three-dimensional structure of ribosomal RNA using ARB software package. *BMC Bioinformatics*, **7**, 240.
- Kurola, J., Wittmann, C., Salkinoja-Salonen, M., Aarnio, T., and Romantschuk, M., 2005. Application of cation-exchange membranes for characterisation and imaging ammonia-oxidising bacteria in soils. *FEMS Microbiology Ecology*, **53**, 463–472.
- Lim, E., Amaral, L., Caron, D., and DeLong, E., 1993. Application of rRNA-based probes for observing marine nanoplanktonic protists. *Applied and Environmental Microbiology*, **59**, 1647–1655.
- Llobet-Brossa, E., Rossello-Mora, R., and Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **64**, 2691–2696.
- Loy, A., Arnold, R., Tischler, P., Rattei, T., Wagner, M., and Horn, M., 2008. Probecheck – a central resource for evaluating oligonucleotide probe coverage and specificity. *Environmental Microbiology*, **10**, 2894–2898.
- Loy, A., Beisker, W., and Meier, H., 2005. Diversity of bacteria growing in natural mineral water after bottling. *Applied and Environmental Microbiology*, **71**, 3624–3632.
- Loy, A., and Bodrossy, L., 2006. Highly parallel microbial diagnostics using oligonucleotide microarrays. *Clinica Chimica Acta (From Real-Time PCR to Nanotechnology: Rapid and/or High-Throughput Diagnostic Methods for Nucleic Acids)*, **363**, 106–119.
- Loy, A., Lehner, A., Lee, N., Adamczyk, J., Meier, H., Ernst, J., Schleifer, K.-H., and Wagner, M., 2002. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. *Applied and Environmental Microbiology*, **68**, 5064–5081.
- Loy, A., Maixner, F., Wagner, M., and Horn, M., 2007. Probase – an online resource for rRNA-targeted oligonucleotide probes: new features 2007. *Nucleic Acids Research*, **35**, D800–04.
- Lubeck, P. S., Hansen, M., and Sorensen, J., 2000. Simultaneous detection of the establishment of seed-inoculated *Pseudomonas fluorescens* strain DR54 and native soil bacteria on sugar beet root surfaces using fluorescence antibody and *in situ* hybridization techniques. *FEMS Microbiology Ecology*, **33**, 11–19.
- Ludwig, W., Bauer, S. H., Bauer, M., Held, I., Kirchhof, G., Schulze, R., Huber, I., Spring, S., Hartmann, A., and Schleifer, K. H., 1997. Detection and *in situ* identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiology Letters*, **153**, 181–190.
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M., and Schleifer, K. H., 1998. Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis*, **19**, 554–568.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K.-H., 2004. ARB: a software environment for sequence data. *Nucleic Acids Research*, **32**, 1363–1371.
- Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., and Simon, M., 2005. An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environmental Microbiology*, **7**, 961–968.
- Macalady, J. L., Lyon, E. H., Koffman, B., Albertson, L. K., Meyer, K., Galdenzi, S., and Mariani, S., 2006. Dominant microbial populations in limestone-corroding stream biofilms, Frasassi Cave System, Italy. *Applied and Environmental Microbiology*, **72**, 5596–5609.
- Manz, W., Amann, R., Ludwig, W., Wagner, M., and Schleifer, K.-H., 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. *Systematic and Applied Microbiology*, **15**, 593–600.
- Maruyama, F., Kanzaka, T., Yamaguchi, N., Tani, K., and Nasu, M., 2003. Detection of bacteria carrying the *stx2* gene by *in situ* loop-mediated isothermal amplification. *Applied and Environmental Microbiology*, **69**, 5023–5028.
- Maruyama, F., Kanzaka, T., Yamaguchi, N., Tani, K., and Nasu, M., 2005. Visualization and enumeration of bacteria carrying a specific gene sequence by *in situ* rolling circle amplification. *Applied and Environmental Microbiology*, **71**, 7933–7940.
- Maturrano, L., Santos, F., Rossello-Mora, R., and Anton, J., 2006. Microbial diversity in Maras Salterns, a hypersaline environment in the Peruvian Andes. *Applied and Environmental Microbiology*, **72**, 3887–3895.
- McDonald, R. M., 1986. Sampling soil microfloras: dispersion of soil by ion exchange and extraction of specific micro organisms form suspension by elutriation. *Soil Biology. Biochemistry*, **18**, 399–406.
- Meisinger, D. B., Pavlekovic, M., Löffler, F. E., and Lee, N. M., 2010a. Comparison of FISH and PCR applications on contaminated groundwater. (in press).
- Meisinger, D. B., Spring, S., Schmid, M., Löffler, F. E., Porter, M. L., Engel, A. S., and Lee, N. M., 2010b. Novel *Chloroflexi* lineages of subclasses I and VI in chemolithoautotrophic microbial mats in the sulphidic Lower Kane Cave, USA. (in press).
- Meisinger, D. B., Zimmermann, J., Ludwig, W., Schleifer, K.-H., Wanner, G., Schmid, M., Stern, L. A., Bennett, P. C., Engel, A. S., and Lee, N., 2007. *In situ* detection of novel *Acidobacteria* in microbial mats from a chemolithoautotrophically-based cave ecosystem (Lower Kane Cave, WY, USA). *Environmental Microbiology*, **9**, 1523–1534.
- Metfies, K., and Medlin, L. K., 2008. Feasibility of transferring fluorescent *in situ* hybridization probes to an 18S rRNA gene phylochip and mapping of signal intensities. *Applied and Environmental Microbiology*, **74**, 2814–2821.
- Molin, S., and Givskov, M., 1999. Application of molecular tools for *in situ* monitoring of bacterial growth activity. *Environmental Microbiology*, **1**, 383–391.

- Moraru, C., Lam, P., Fuchs, B. H., Kuypers, M. M. M., and Amann, R., 2010. GeneFISH – an *in situ* technique for linking gene presence and cell identity in environmental microorganisms. *Environmental Microbiology*, doi:10.1111/j.1462-2920.2010.02281.x
- Morris, R. M., Rappe, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, **420**, 806–810.
- Morris, R. M., Rappe, M. S., Urbach, E., Connon, S. A., and Giovannoni, S. J., 2004. Prevalence of the chloroflexi-related SAR202 bacterioplankton cluster throughout the Mesopelagic zone and deep ocean. *Applied and Environmental Microbiology*, **70**, 2836–2842.
- Moter, A., Schmiedel, J., Petrich, A., Schmiedel, D., Mallmann, C., Musci, M., Hetzer, R., and Göbel, U., 2010. In situ measurement of biofilm activity: implications for diagnosis of infective endocarditis. In Poster presentation at *ISME 13 International Symposium on Microbial Ecology Microbes – Stewards of a Changing Planet*, Seattle, WA, USA, 22–27 August 2010. Abstract Category 31 Methodological Developments.
- Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F., 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. *Applied and Environmental Microbiology*, **64**, 2585–2595.
- Musmann, M., Ishii, K., Rabus, R., and Amann, R., 2005. Diversity and vertical distribution of cultured and uncultured Deltaproteobacteria in an intertidal mud flat of the Wadden Sea. *Environmental Microbiology*, **7**, 405–418.
- Nakagawa, T., Takai, K., Suzuki, Y., Hirayama, H., Konno, U., Tsunogai, U., and Horikoshi, K., 2006. Geomicrobiological exploration and characterization of a novel deep-sea hydrothermal system at the TOTO caldera in the Mariana Volcanic Arc. *Environmental Microbiology*, **8**, 37–49.
- Nakamura, K., Terada, T., Sekiguchi, Y., Shinzato, N., Meng, X.-Y., Enoki, M., and Kamagata, Y., 2006. Application of pseudomurein endoisopeptidase to fluorescence *in situ* hybridization of methanogens within the family methanobacteriaceae. *Applied and Environmental Microbiology*, **72**, 6907–6913.
- Nielsen, J. L., Aquino de Muro, M., and Nielsen, P. H., 2003. Evaluation of the redox dye 5-cyano-2,3-tolyl-tetrazolium chloride for activity studies by simultaneous use of microautoradiography and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **69**, 641–643.
- Nielsen, J. L., Klausen, C., Nielsen, P. H., Burford, M., and Jorgensen, N. O. G., 2006. Detection of activity among uncultured Actinobacteria in a drinking water reservoir. *FEMS Microbiology Ecology*, **55**, 432–438.
- Nielsen, J. L., and Nielsen, P. H., 2005. Advantages in microscopy: microautoradiography of single cells. In Leadbetter, J. R. (ed.), *Methods in Enzymology*. San Diego: Elsevier Academic Press, Vol. 397, pp. 237–56.
- Northup, D. E., and Lavoie, K. H., 2001. Geomicrobiology of caves: a review. *Geomicrobiology Journal*, **18**, 199–222.
- Nubel, U., Bateson, M. M., Vandieken, V., Wieland, A., Kuhl, M., and Ward, D. M., 2002. Microscopic examination of distribution and phenotypic properties of phylogenetically diverse chloroflexaceae-related bacteria in hot spring microbial mats. *Applied and Environmental Microbiology*, **68**, 4593–4603.
- Nielsen, P. H., Daims, H., and Lemmer, H., 2009. *FISH handbook for Biological Wastewater Treatment*. London: IWA Publishing.
- Oerther, D. B., Pernthaler, J., Schramm, A., Amann, R., and Raskin, L., 2000. Monitoring precursor 16S rRNAs of acinetobacter spp. in activated sludge wastewater treatment systems. *Applied and Environmental Microbiology*, **66**, 2154–2165.
- Orphan, V. J., 2009. Methods for unveiling cryptic microbial partnerships in nature. *Current Opinion in Microbiology*, **12**, 231–237.
- Orphan, V. J., and House, C. H., 2009. Geobiological investigations using secondary ion mass spectrometry: microanalysis of extant and paleo-microbial processes. *Geobiology*, **7**, 360–372.
- Ouverney, C., and Fuhrman, J., 1997. Increase in fluorescence intensity of 16S rRNA *in situ* hybridization in natural samples treated with chloramphenicol. *Applied and Environmental Microbiology*, **63**, 2735–2740.
- Ouverney, C. C., and Fuhrman, J. A., 2000. Marine planktonic archaea take up amino acids. *Applied and Environmental Microbiology*, **66**, 4829–4833.
- Rodriguez-castaño, G. P., 2008. *Sequence diversity and expression of novel bacterial nitrous oxide reductase (nosZ) genes in tropical environments*. Master of Science Thesis in Biology. Mayagüez Campus, USA, University of Puerto Rico.
- Pavlekovic, M., Schmid, M. C., Schmider-Poignee, N., Spring, S., Pilhofer, M., Gaul, T., Fiandaca, M., Löffler, F. E., Jetten, M., Schleifer, K.-H., and Lee, N. M., 2009. Optimization of three FISH procedures for *in situ* detection of anaerobic ammonium oxidizing bacteria in biological wastewater treatment. *Journal of Microbiological Methods*, **78**, 119–126.
- Peplies, J., Glockner, F. O., Amann, R., and Ludwig, W., 2004. Comparative sequence analysis and oligonucleotide probe design based on 23S rRNA genes of alphaproteobacteria from North Sea Bacterioplankton. *Systematic and Applied Microbiology*, **27**, 573–580.
- Pernthaler, A., and Amann, R., 2004. Simultaneous fluorescence *in situ* hybridization of mRNA and rRNA in environmental bacteria. *Applied and Environmental Microbiology*, **70**, 5426–5433.
- Pernthaler, A., and Pernthaler, J., 2005. Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. *Applied and Environmental Microbiology*, **71**, 4638–4644.
- Pernthaler, J., Glockner, F.-O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R., 1998. Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Applied and Environmental Microbiology*, **64**, 4299–4306.
- Pernthaler, A., Pernthaler, J., and Amann, R., 2002a. Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, **68**, 3094–3101.
- Pernthaler, A., Pernthaler, J., Schattenhofer, M., and Amann, R., 2002b. Identification of DNA-synthesizing bacterial cells in coastal North Sea Plankton. *Applied and Environmental Microbiology*, **68**, 5728–5736.
- Pernthaler, A., Preston, C. M., Pernthaler, J., DeLong, E. F., and Amann, R., 2002c. Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine bacteria and archaea. *Applied and Environmental Microbiology*, **68**, 661–667.
- Perry-O'Keefe, H., Stender, H., Broomer, A., Oliveira, K., Coull, J., and Hyldig-Nielsen, J. J., 2001. Filter-based PNA *in situ* hybridization for rapid detection, identification and enumeration of specific micro-organisms. *Journal of Applied Microbiology*, **90**, 180–189.
- Petroni, G., Rosati, G., Vannini, C., Modeo, L., Dini, F., and Verni, F., 2003. *In Situ* Identification by fluorescently labeled oligonucleotide probes of morphologically similar, closely related ciliate species. *Microbial Ecology*, **45**, 156–162.
- Pilhofer, M., Pavlekovic, M., Lee, N. M., Ludwig, W., and Schleifer, K.-H., 2009. Fluorescence *in situ* hybridization for intracellular localization of nifH mRNA. *Systematic and Applied Microbiology*, **32**, 186–192.
- Podar, M., Abulencia, C. B., Walcher, M., Hutchison, D., Zengler, K., Garcia, J. A., Holland, T., Cotton, D., Hauser, L.,

- and Keller, M., 2007. Targeted access to the genomes of low-abundance organisms in complex microbial communities. *Applied and Environmental Microbiology*, **73**, 3205–3214.
- Polerecky, L., Bissett, A., Al-Najjar, M., Faerber, P., Osmer, H., Suci, P. A., Stoodley, P., and de Beer, D., 2009. Modular spectral imaging system for discrimination of pigments in cells and microbial communities. *Applied and Environmental Microbiology*, **75**, 758–771.
- Pratscher, J., Stichternoth, C., Fichtl, K., Schleifer, K.-H., and Braker, G., 2009. Application of recognition of individual genes-fluorescence *in situ* hybridization (RING-FISH) to detect nitrite reductase genes (*nirK*) of denitrifiers in pure cultures and environmental samples. *Applied and Environmental Microbiology*, **75**, 802–810.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glockner, F. O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, **35**, 7188–7196.
- Ravenschlag, K., Sahm, K., Knoblauch, C., Jorgensen, B. B., and Amann, R., 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. *Applied and Environmental Microbiology*, **66**, 3592–3602.
- Rossello-Mora, R., Lee, N., Anton, J., and Wagner, M., 2003. Substrate uptake in extremely halophilic microbial communities revealed by microautoradiography and fluorescence *in situ* hybridization. *Extremophiles*, **7**, 409–413.
- Rossetti, S., Tomei, M. C., Blackall, L. L., and Tandoi, V., 2007. Bacterial growth kinetics estimation by fluorescence *in situ* hybridization and spectrofluorometric quantification. *Letters in Applied Microbiology*, **44**, 643–648.
- Rusch, A., and Amend, J. P., 2004. Order-specific 16S rRNA-targeted oligonucleotide probes for (hyper)thermophilic archaea and bacteria. *Extremophiles*, **8**, 357–366.
- Sando, S., and Kool, E. T., 2002. Imaging of RNA in bacteria with self-ligating quenched probes. *Journal of the American Chemical Society*, **124**, 9686–9687.
- Schatz, M. C., Phillippy, A. M., Gajer, P., DeSantis, T. Z., Andersen, G. L., Ravel, J., 2010. Integrated microbial survey analysis of prokaryotic communities for the PhyloChip microarray. *Applied and Environmental Microbiology*, **76**, 5636–5638.
- Schippers, A., Neretin, L. N., Kallmeyer, J., Ferdelman, T. G., Cragg, B. A., John Parkes, R., and Jorgensen, B. B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature*, **433**, 861–864.
- Schmid, M., Schmitz-Esser, S., Jetten, M., and Wagner, M., 2001. 16S-23S rDNA intergenic spacer and 23S rDNA of anaerobic ammonium-oxidizing bacteria: implications for phylogeny and *in situ* detection. *Environmental Microbiology*, **3**, 450–459.
- Schmid, M., Selesi, D., Rothballer, M., Schlöter, M., Hartmann, A., Lee, N., and Kandeler, E., 2006. Localization and visualization of microbial community structure and activity in soil microhabitats. In König, H., and Varma, A., (ed.), *Soil Biology*, Berlin Heidelberg: Springer, Vol. 6.
- Schmid, M., Rothballer, M., and Hartmann, A., 2007. Analysis of microbial communities in soil microhabitats using fluorescence *in situ* hybridization. In van Elsas, J. D., Jansson, J. K., and Trevors, J. (eds.), *Modern Soil Microbiology*, 2nd edn. Boca Raton: CRC-Press, Chap. 12, pp. 317–335.
- Schonhuber, W., Le Bourhis, G., Tremblay, J., Amann, R., and Kulakauskas, S., 2001. Utilization of tmRNA sequences for bacterial identification. *BMC Microbiology*, **1**, 20.
- Schramm, A., Fuchs, B. M., Nielsen, J. L., Tonolla, M., and Stahl, D. A., 2002. Fluorescence *in situ* hybridization of 16S rRNA gene clones (Clone-FISH) for probe validation and screening of clone libraries. *Environmental Microbiology*, **4**, 713–720.
- Schrenk, M. O., Edwards, K. J., Goodman, R. M., Hamers, R. J., and Banfield, J. F., 1998. Distribution of thiobacillus ferrooxidans and Leptospirillum ferrooxidans: implications for generation of acid mine drainage. *Science*, **279**, 1519–1522.
- Schrenk, M. O., Kelley, D. S., Delaney, J. R., and Baross, J. A., 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology*, **69**, 3580–3592.
- Sekar, R., Pernthaler, A., Pernthaler, J., Warnecke, F., Posch, T., and Amann, R., 2003. An improved protocol for quantification of freshwater actinobacteria by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **69**, 2928–2935.
- Shiraishi, F., Zippel, B., Neu, T. R., and Arp, G., 2008. *In situ* detection of bacteria in calcified biofilms using FISH and CARD-FISH. *Journal of Microbiological Methods*, **75**, 103–108.
- Simbahan, J., Kurth, E., Schelert, J., Dillman, A., Moriyama, E., Jovanovich, S., and Blum, P., 2005. Community analysis of a mercury hot spring supports occurrence of domain-specific forms of mercuric reductase. *Applied and Environmental Microbiology*, **71**, 8836–8845.
- Smith, N. C., and Stribley, D. P., 1994. A new approach to direct extraction of microorganisms from soil. In Dighton, K. R. J., and Giller, K. E. (ed.), *Beyond the Biomass*. Chichester: British Society of Soil Science (BSSS). A Wiley-Sayce Publication, Vol. 1194.
- Smolina, I., Lee, C., and Frank-Kamenetskii, M., 2007. Detection of low-copy-number genomic DNA sequences in individual bacterial cells by using peptide nucleic acid assisted rolling-circle amplification and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **73**, 2324–2328.
- Stahl, D. A., and Amann, R., 2001. Development and application of nucleic acid probes. In Stackebrandt, E., and Goodfellow, M. (ed.), *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley.
- Stoecker, K., Dorninger, C., Daims, H., and Michael, W., 2010. Double-labeling of oligonucleotide probes for fluorescence *in situ* hybridization (DOPE-FISH) improves signal intensity and increases rRNA accessibility. *Applied and Environmental Microbiology*, **76**(3), 922–926.
- Stoffels, M., Ludwig, W., and Schleifer, K. H., 1999. rRNA probe-based cell fishing of bacteria. *Environmental Microbiology*, **1**, 259–271.
- Sunamura, M., and Maruyama, A., 2006. A digital imaging procedure for seven-probe-labeling FISH (Rainbow-FISH) and its application to estuarine microbial communities. *FEMS Microbiology Ecology*, **55**, 159–166.
- Tal, Y., Watts, J. E. M., and Schreier, H. J., 2005. Anaerobic ammonia-oxidizing bacteria and related activity in Baltimore Inner Harbor Sediment. *Applied and Environmental Microbiology*, **71**, 1816–1821.
- Tani, K., Kurokawa, K., and Nasu, M., 1998. Development of a direct *in situ* PCR method for detection of specific bacteria in natural environments. *Applied and Environmental Microbiology*, **64**, 1536–1540.
- Taylor, M. W., Loy, A., and Wagner, M., 2010. Microarrays for studying the composition and function of microbial communities. In Seviour Robert, J., and Nielsen, P.H. (ed.), *The Microbiology of Activated Sludge*. London: IWA Publishing.
- Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J., 2004. Combining catalyzed reporter deposition-fluorescence *in situ* hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. *Applied and Environmental Microbiology*, **70**, 4411–4414.
- Tobin, K. J., Onstott, T. C., DeFlaun, M. F., Colwell, F. S., and Fredrickson, J., 1999. *In situ* imaging of microorganisms in geologic material. *Journal of Microbiological Methods*, **37**, 201–213.

- Trebesius, K., Amann, R., Ludwig, W., Muhlegger, K., and Schleifer, K., 1994. Identification of whole fixed bacterial cells with nonradioactive 23S rRNA-targeted polynucleotide probes. *Applied and Environmental Microbiology*, **60**, 3228–3235.
- Tsuchiya, D., and Taga, M., 2001., Application of fibre-FISH (fluorescence *in situ* hybridization) to filamentous fungi: visualization of the rRNA gene cluster of the ascomycete *Cochliobolus heterostrophus*. *Microbiology*, **147**, 1183–1187.
- Vesey, G., Deere, D., Gauci, M. R., Griffiths, K. R., Williams, K. L., and Veal, D. A., 1997. Evaluation of fluorochromes and excitation sources for immunofluorescence in water samples. *Cytometry*, **29**, 147–154.
- Wagner, M., 2009. Single-cell ecophysiology of microbes as revealed by Raman microspectroscopy or secondary ion mass spectrometry imaging. *Annual Review of Microbiology*, **63**, 411–429.
- Wagner, M., Erhardt, R., Manz, W., Amann, R., Lemmer, H., Wedi, D., and Schleifer, K.-H., 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus *acinetobacter* and its application for *in situ* monitoring in activated sludge. *Applied and Environmental Microbiology*, **60**, 792–800.
- Wagner, M., Horn, M., and Daims, H., 2003. Fluorescence *in situ* hybridisation for the identification and characterisation of prokaryotes. *Current Opinion in Microbiology*, **6**, 302–309.
- Wagner, M., Hutzler, P., and Amann, R., 1998. 3-D Analysis of complex microbial communities by combining confocal laser scanning microscopy and fluorescence *in situ* hybridization (FISH). In Wilkinson, M. H. F. and Schut, F. (ed.), *Digital Image Analysis of Microbes*. Chichester: Wiley, pp. 467–486.
- Wagner, M., Nielsen, P. H., Loy, A., Nielsen, J. L., and Daims, H., 2006. Linking microbial community structure with function: fluorescence *in situ* hybridization-microautoradiography and isotope arrays. In van der Meer, J. R., and Murrel, J. C. (eds.), *Current Opinion in Biotechnology, Analytical biotechnology*, **17**, 83–91.
- Wagner, M., Rath, G., Amann, R., Koops, H.-P., and Schleifer, K.-H., 1995. *In situ* identification of ammonia-oxidizing bacteria. *Systematic Applied Microbiology*, **18**, 251–264.
- Wagner, M., Schmid, M., Juretschko, S., Trebesius, K.-H., Bubert, A., Goebel, W., and Schleifer, K.-H., 1998. *In situ* detection of a virulence factor mRNA and 16S rRNA in *Listeria monocytogenes*. *FEMS Microbiology Letters*, **160**, 159–168.
- Wallner, G., Amann, R., and Beisker, W., 1993. Optimizing fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry*, **14**, 136–143.
- Watanabe, K., Watanabe, K., Kodama, Y., Sytsubo, K., and Harayama, S., 2000. Molecular characterization of bacterial populations in petroleum-contaminated groundwater discharged from underground crude oil storage cavities. *Applied and Environmental Microbiology*, **66**, 4803–4809.
- Weber, S. D., Ludwig, W., Schleifer, K.-H., and Fried, J., 2007. Microbial composition and structure of aerobic granular sewage biofilms. *Applied and Environmental Microbiology*, **73**, 6233–6240.
- Wendeberg, A., 2010. Fluorescence *in situ* hybridization for the identification of environmental microbes. *Cold Spring Harbor Protocols*, doi:10.1101/pdb.prot5366.
- Weidler, G. W., Gerbl, F. W., and Stan-Lotter, H., 2008. Crenarchaeota and their role in the nitrogen cycle in a subsurface radioactive thermal spring in the Austrian Central Alps. *Applied and Environmental Microbiology*, **74**: 5934–5942.
- Wilhartitz, I., Mach, R. L., Teira, E., Reinthaler, T., Herndl, G. J., and Farnleitner, A. H., 2007. Prokaryotic community analysis with CARD-FISH in comparison with FISH in ultra-oligotrophic ground- and drinking water. *Journal of Applied Microbiology*, **103**, 871–881.
- Xia, Y., Kong, Y., and Nielsen, P. H., 2007. *In situ* detection of protein-hydrolysing microorganisms in activated sludge. *FEMS Microbiology Ecology*, **60**, 156–165.
- Yarza, P., Richter, M., Peplies, J., Euzaby, J., Amann, R., Schleifer, K.-H., Ludwig, W., Glöckner, F. O., and Rosselló-Móra, R., 2008. The all-species living tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Systematic and Applied Microbiology*, **31**, 241–250.
- Yilmaz, L. S., Okten, H. E., and Noguera, D. R., 2006. Making all parts of the 16S rRNA of *Escherichia coli* accessible *in situ* to single DNA Oligonucleotides. *Applied and Environmental Microbiology*, **72**, 733–744.
- Yilmaz, L., Parnerkar, S., and Noguera, D., 2010a. Introducing mathfish: a website that runs mathematical models of fluorescent *in situ* hybridization for thermodynamics-based probe design. In Poster presentation at *ISME 13 International Symposium on Microbial Ecology Microbes - Stewards of a Changing Planet*, Seattle, WA, USA, 22–27 August 2010. Abstract Category 31 Methodological Developments.
- Yilmaz, S., Haroon, M., Rabkin, B., Tyson, G., and Hugenholtz, P., 2010b. Fixation-free fluorescence *in situ* hybridization for targeted enrichment of microbial populations. *The ISME Journal*, doi:10.1038/ismej.2010.73.
- Zarda, B., Amann, R., Wallner, G., and Schleifer, K.-H., 1991. Identification of single bacterial cells using digoxigenin-labelled, rRNA-targeted oligonucleotides. *Journal of General Microbiology*, **137**, 2823–2830.
- Zarda, B., Hahn, D., Chatzinotas, A., Schönhuber, W., Neef, A., Amann, R. I., and Zeyer, J., 1997. Analysis of bacterial community structure in bulk soil by *in situ* hybridization. *Archives of Microbiology*, **168**, 185–192.
- Zhang, D., Wu, J., Ye, F., Feng, T., Lee, I., and Yin, B., 2006. Amplification of circularizable probes for the detection of target nucleic acids and proteins. *Clinica Chimica Acta (From Real-Time PCR to Nanotechnology: Rapid and/or High-Throughput Diagnostic Methods for Nucleic Acids)*, **363**, 61–70.
- Zhou, Z., Pons, M. N., Raskin, L., and Zilles, J. L., 2007. Automated image analysis for quantitative fluorescence *in situ* hybridization with environmental samples. *Applied and Environmental Microbiology*, **73**, 2956–2962.
- Zimmermann, J., Ludwig, W., and Schleifer, K. H., 2001. DNA polynucleotide probes generated from representatives of the genus *acinetobacter* and their application in fluorescence *in situ* hybridization of environmental samples. *Systematic and Applied Microbiology*, **24**, 238–244.
- Zwirgmaier, K., Ludwig, W., and Schleifer, K.-H., 2004a. Improved method for polynucleotide probe-based cell sorting, using DNA-coated microplates. *Applied and Environmental Microbiology*, **70**, 494–497.
- Zwirgmaier, K., Ludwig, W., and Schleifer, K.-H., 2004b. Recognition of individual genes in a single bacterial cell by fluorescence *in situ* hybridization - RING-FISH. *Molecular Microbiology*, **51**, 89–96.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Basalt \(Glass, Endoliths\)](#)
[Biogeochemical Cycles](#)
[Biomarkers \(Molecular Fossils\)](#)
[Immunolocalization](#)
[Isotopes and Geobiology](#)
[Karst Ecosystems](#)
[Metagenomics](#)
[Microbial Communities, Structure, and Function](#)

Microbial Ecology of Submarine Caves
 Protozoa (Heterotroph, Eukaryotic)
 RNA World
 Scanning Probe Microscopy
 Speleothems
 Terrestrial Deep Biosphere

FORAMINIFERA

Alexander V. Altenbach
 Ludwig-Maximilians-University Munich, Munich,
 Germany

Synonyms

Forams (for short)

Definition

Foraminifera (from ancient Greek “hole bearers”, refers to the pores that early observers recognized in the calcitic tests) are amoeboid, eukaryotic protists with a large network of very thin cytoplasm extrusions (reticulopodia). Most species produce a genetically fixed shell (test), made of organic matter, agglutinated particles, calcite, aragonite, high-magnesium calcite, or opaline silica. The tests may be either simple bowls or tubes, or branched, coiled, substructured, and ornamented in diverse complexity. Several thousand species are found living in modern oceans, and this number still increases. Several times more extinct species are recovered from the fossil record. Foraminifera are considered an order (sometimes a class) in paleontology, subdivided by the structure and composition of modern and fossil tests. They are ranked among the “Granuloreticulosa” in biology, characterized by (1) their branching and anastomosing networks of reticulopodia, (2) a rapid bidirectional transport of intracellular granules and membrane domains driven by specific tubulin assemblies, and (3) a complex alternation of sexual and asexual reproductive cycles. Genetic investigations led to the definition of the first rank group “Foraminifera” within the Supergroup “Rhizaria”. Independent from such rankings, they range among the most common, diverse, and ubiquitous groups of single celled, test building eukaryotes on earth.

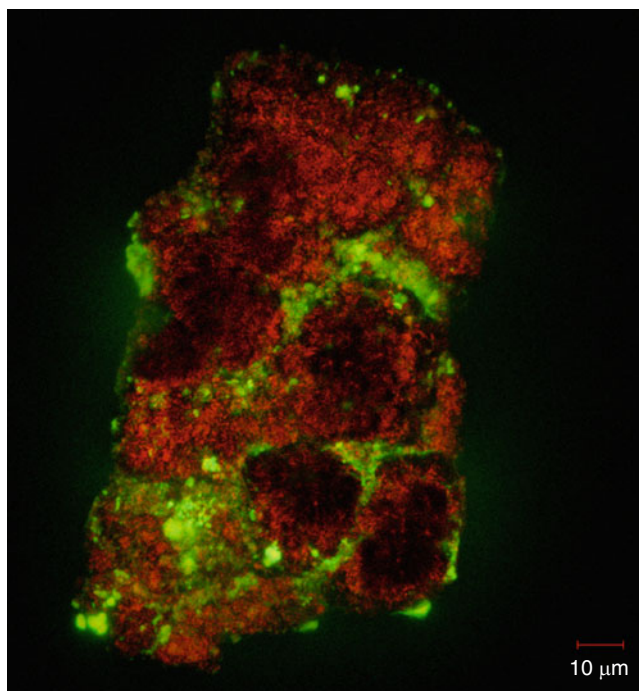
Introduction

Numerous foraminiferal species indicate specific environmental conditions, and the first and last appearance of extinct species exactly indicates time intervals in earth history. Investigations on the chemical and isotopical composition of their calcitic tests reveal environmental drifts in global ice volumes, palaeo-temperatures and -salinities of marine water masses, and marine palaeo-productivity (Hemleben et al., 1989; Murray, 2006). Despite the long term and extensive attention on foraminifera in

taxonomy (Loeblich and Tappan, 1988) and environmental sciences (Sen Gupta, 1999), our comprehensions on their evolution and ecophysiology are still in transition. Molecular ecology and metagenomics applied to the study of microbial biodiversity are changing our view of the biosphere. An impressive extension of foraminiferal clades has been uncovered by such molecular tools: naked freshwater amoebians, naked marine giant amoebians, and giant deep-sea protists formerly considered of unknown origin, where added to the first rank group Foraminifera (Adl et al., 2005; Pawlowski and Burki, 2009). Even amoeboid parasites of terrestrial plants are supposed to reach out from this lineage (Archibald and Keeling, 2004). Efforts to couple the growing phylogenetic diversity of single-celled organisms with ecological function lead to the discovery of novel metabolisms, processes, and symbiosis, and thus to a reevaluation of the geobiochemical impact of pro- and eukaryotes (Moya et al., 2008).

Earth history

The earliest fossils presumed for the foraminiferal lineage reach back into the Mesoproterozoic, despite severe problems in defining even Ediacaran remainders (Dong et al., 2008). The Cambrian provides already several foraminiferal families with organic-walled or agglutinated tests (Benton, 1993). A significant eukaryote radiation occurs in the Ordovician, coincident with increasing nutrient supply, enhanced primary production, and the propagation of planktonic metazoan stages. Such amplified planktonic food chains sponsored increased flux rates of organic matter at the sea floor, and thus the radiation of planktotrophy (Nützel et al., 2006). The calcitic fusulinids start in the Silurian (Loeblich and Tappan, 1988), and extend into the first rhizarian, rock forming mass deposition of biogenic shallow water carbonates, from the Pennsylvanian to the Permian. The fusulinid clade develops all principal morphological adaptations known from modern benthic foraminifera sheltering phototrophic symbionts, thus these mass deposits should result from host–symbiont interactions (Anderson and Lee, 1991). The structure of modern marine primary production was based on the evolution of diatoms, coccoliths, and modern dinoflagellates in the Triassic to Jurassic (Falkowski et al., 2004). Restructuring and enhancing marine primary productivity to an elevated level is recorded by the boost of rotaliid foraminifera, modern metazoans, and increasing bioturbation (Martin et al., 2008). One tribe of calcitic foraminifera, the globigerinids, completely adapted to this new planktonic food chain, and started to enter a purely pelagic live style in the Middle Jurassic. The boost of rock forming biogenic carbonate (chalk) and silica (diatomite) production from these new planktonic systems, however, was delayed to the Cretaceous in the marine realm, and to the Eocene for lacustrine diatomites. The first major radiation of planktonic foraminifera occurred during the mid-Cretaceous. The density structure of the upper water



Gold, Figure 2 Laser scanning micrograph (ex. 488 nm, em. 543 nm) of microorganisms (green) on a gold grain (red) from the Eder River, Germany. Eubacteria labeled by oligonucleotide probe EuB-CY3.

and not in the surrounding sediments. With a similarity of 99%, the sequence correlated to *Cupriavidus (Ralstonia) metallidurans*. Ninety percent of the bacteria in the laboratory experiment died because of the toxicity of AuCl_4^- . The surviving cells adapted themselves to the high concentration of Au(III) ions and precipitated gold. In addition to morphological studies (Bischoff, 1997), this provides evidence for bioaccumulation of secondary gold (e.g., nuggets, gold grains) by bacteria like *C. metallidurans* (Figure 2).

The basal biochemical microbial processes of the biomineralization of gold are not completely understood yet. Precipitation of gold provides the cellular defense of *C. metallidurans* and is controlled by a coupling of efflux, reduction and possibly methylation of gold complexes (Reith et al., 2009). Reith et al. (2007) have published a comprehensive review of the geomicrobiology of gold.

Summary

Different microorganisms are partially resistant to dissolved gold. Various microbial activities precipitate gold. Biofilms of *Cupriavidus metallidurans* accumulate gold naturally and contribute to the formation of secondary gold.

Bibliography

Benedetti, M., and Boulègue, J., 1991. Mechanism of gold transfer and deposition in a supergene environment. *Geochimica et Cosmochimica Acta*, **55**, 1539–1547.

- Bischoff, G. C. O., 1997. The biological origin of bacterioform gold from Australia. *Neues Jahrbuch Geologische Paläontologische Abhandlungen*, **H6**, 329–338.
- Karthikeyan, S., and Beveridge, T. J., 2002. *Pseudomonas aeruginosa* biofilms react with and precipitate toxic soluble gold. *Environmental Microbiology*, **4**(11), 667–675.
- Kashefi, K., Tor, J. M., Nevin, K. P., and Lovley, D. R., 2001. Reductive precipitation of gold by dissimilatory Fe(III) -reducing bacteria and archaea. *Applied and Environmental Microbiology*, **67**(7), 3275–3279.
- Lengke, M. F., and Southam, G., 2005. The effect of thiosulfate-oxidizing bacteria on the stability of the gold–thiosulfate complex. *Geochimica et Cosmochimica Acta*, **69**(15), 3759–3772.
- Lengke, M. F., Ravel, B., Fleet, M. E., Wanger, G., and Southam, G., 2006. Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)–chloride complex. *Environmental Science & Technology*, **40**(20): 6304–6309.
- Nakajima, A., 2003. Accumulation of gold by microorganisms. *World Journal of Microbiology*, **19**, 369–374.
- Nordstrom, D. K., and Southam G., 1997. Geomicrobiology of sulfide mineral oxidation. *Reviews in Mineralogy and Geochemistry*, **35**, 361–390.
- Reith, F., Rogers, S., McPhail, D. C., and Webb, D., 2006. Biomineralization of gold: biofilms on bacterioform gold. *Science*, **313**, 333–336.
- Reith, F., Lengke, M. F., Falconer, D., Craw, D., and Southam, G., 2007. The geomicrobiology of gold. *ISME Journal*, **1**, 567–584.
- Reith, F., Etschmann, B., Grosse, C., Moors, H., Benotmane, M. A., Monsieurs, P., Grass, G., Doonan, C., Vogt, S., Lai, B., Martinez-Criado, G., George, G. N., Nies, D. H., Mergeay, M., Pring, A., Southam, G., and Brugger, J., 2009. Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(1), 17757–17762.
- Sand, W., Gehrke, T., Jozsa, P.-G., and Schippers, A., 2001. (Bio) chemistry of bacterial leaching – direct vs. indirect bioleaching. *Hydrometallurgy*, **59**, 159–175.
- Southam, G., and Beveridge, T. J., 1996. The occurrence of sulfur and phosphorus within bacterially derived crystalline and pseudocrystalline octahedral gold formed in vitro. *Geochimica et Cosmochimica Acta*, **60**(22), 4369–4376.

Cross-references

[Algae \(Eukaryotic\)](#)
[Archaea](#)
[Bacteria](#)
[Biofilms](#)
[Fungi and Lichens](#)
[Microbial Biomineralization](#)
[Ores, Microbial Precipitation and Oxidation](#)

GONDWANALAND, FORMATION

Joseph G. Meert
 University of Florida, Gainesville, FL, USA

Definition

Gondwanaland or “Gondwana” is the name for the southern half of the Pangaeen supercontinent that existed some 300 million years ago. Gondwanaland is composed of the major continental blocks of South America, Africa,

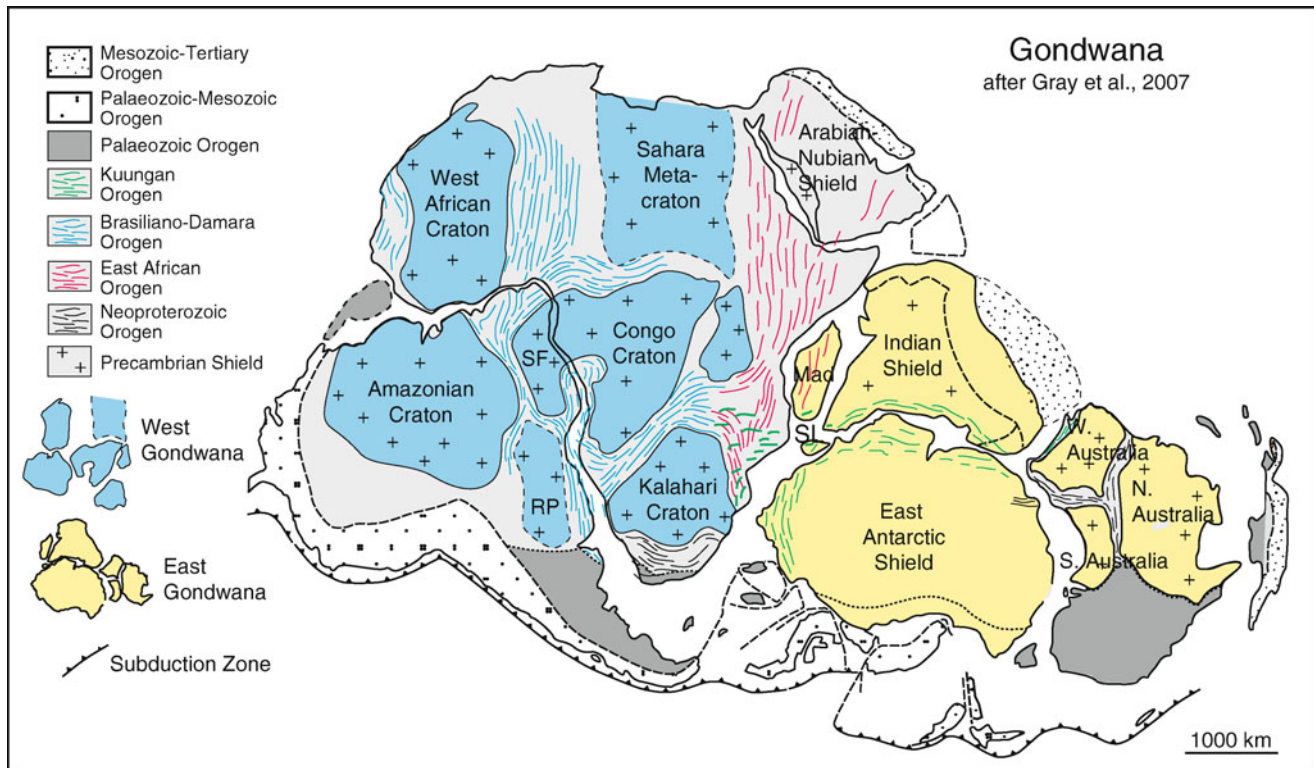
Arabia, Madagascar, Sri Lanka, India, Antarctica, and Australia (Figure 1). The name “Gondwana” is derived from a tribe in India (Gonds) and “wana” meaning “land of.” Gondwanaland is superficially divided into a western half (Africa and South America) and an eastern half (India, Sri Lanka, Madagascar, Antarctica, and Australia).

The archetypal view of Gondwanaland assembly was an Ediacaran–Cambrian-age coalescence of East Gondwana (India, Sri Lanka, Madagascar, Australia, and Antarctica) with West Gondwana (South America and Africa) along the Mozambique Belt (labeled East African Orogen in Figure 1). “Pan-African” tectonothermal belts outside of the Mozambique Belt (~500–600 Ma) were well-known at the time, but they were often treated as zones of ensialic activity rather than sites of continental collision. Although this rather simplistic view of Gondwanaland assembly is now strongly debated, the polyphase assembly model outlined below is not completely accepted (see Yoshida, 2007).

A new view of Gondwanaland assembly began with the work by Stern (1994) who demonstrated clear evidence of juvenile arc development and accretion in the Arabian–Nubian shield and continental collision to the south in Kenya and Tanzania. In the early 1990s, work by Dalziel (1991), Hoffman (1991), and Moores (1991) hinted that

the assembly of Gondwanaland followed the breakup of an earlier supercontinent called Rodinia (see Chapter *Breakup of Rodinia*). Although the exact continental configuration of Rodinia is still debated (for example, Meert and Torsvik, 2003), the assembly of West Gondwana is more or less viewed as a polyphase accretion of formerly disparate blocks while East Gondwana is generally treated as a coherent unit from ~1,100 Ma until its breakup in the Mesozoic (Meert, 2003). More recently, Fitzsimons (2000), Meert (2003), Collins and Pisarevsky (2005), and Kelsey et al. (2008) noted that at least two “Pan-African” age mountain belts cut across the East Antarctic shield and juxtapose distinct crustal fragments thought to comprise the East Antarctic “Grenvillian craton.” The existence of these belts preclude a united East Gondwana and favor a polyphase accretion of major blocks to form Greater Gondwanaland in the Cambrian. In this scenario, the assembly of Gondwanaland was accomplished along three major orogenic belts known as the Brasiliano, East African, and Kuungan orogenies (Figure 1). Additional data supporting a polyphase assembly of East Gondwana are derived from paleomagnetic and geochronologic studies.

No matter what the exact model of Gondwanaland assembly is, the formation of this supercontinent followed



Gondwanaland, Formation, Figure 1 The Gondwanaland supercontinent. The cratons comprising West Gondwana are shaded in light blue and those comprising East Gondwana are shaded in yellow. Neoproterozoic orogenic belts crisscross the supercontinent. Those associated with the final amalgamation of the supercontinent are the East African Orogen (750–620 Ma; red), the Brasiliano-Damara Orogen (630–520 Ma; blue), and the Kuungan Orogen (570–530 Ma; green).

shortly after the extreme glaciations during the late Neoproterozoic (Hoffman et al., 1998) and was nearly synchronous with the rapid evolutionary pulse that coincides with the Ediacaran and Cambrian radiations. Final assembly of Gondwanaland and a circum-Gondwanaland mountain chain is thought to have provided nutrients to the oceanic realm and also resulted in a large $^{87}\text{Sr}/^{86}\text{Sr}$ spike due to the erosion of the mountains (Squire et al., 2006). Therefore, links between the assembly of the Gondwanaland supercontinent and biological evolution are forwarded by numerous studies (Meert and Lieberman, 2008).

Bibliography

- Collins, A. S., and Pisarevsky, S. A., 2005. Amalgamating eastern Gondwana: the evolution of the circum-Indian orogens. *Earth Science Reviews*, **71**, 229–270.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Fitzsimons, I. C. W., 2000. A review of tectonic events in the East Antarctic Shield, and their implications for three separate collisional orogens. *Journal of African Earth Sciences*, **31**, 3–23.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside out? *Science*, **252**, 1409–1412.
- Hoffman, P. F., Kaufman, A. J., Halverson, G. P., and Schrag, D. P., 1998. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Kelsey, D. E., Wade, D. P., Collins, A. S., Hand, M., Sealing, C. R., and Netting, A., 2008. Discovery of a Neoproterozoic basin in the Prydz Belt in East Antarctica and its implications for Gondwana assembly and ultrahigh temperature metamorphism. *Precambrian Research*, **161**, 355–388.
- Meert, J. G., 2003. A synopsis of events related to the assembly of eastern Gondwana. *Tectonophysics*, **362**, 1–40.
- Meert, J. G., and Lieberman, B. S., 2008. The Neoproterozoic assembly of Gondwana and its relationship to the Ediacaran–Cambrian radiation. *Gondwana Research*, in press.
- Meert, J. G., and Torsvik, T. H., 2003. The making and unmaking of a supercontinent: Rodinia revisited. *Tectonophysics*, **375**, 261–288.
- Meert, J. G., Van der Voo, R., and Ayub, S., 1995. Paleomagnetic investigation of the neoproterozoic Gagwe lavas and Mbozi Complex, Tanzania and the assembly of Gondwana. *Precambrian Research*, **74**, 225–244.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Squire, R. J., Campbell, I. H., Allen, C. M., and Wilson, C. J. L., 2006. Did the Transgondwanan supermountain trigger the explosive radiation of animals on Earth? *Earth and Planetary Science Letters*, **250**, 116–133.
- Stern, R. J., 1994. Arc assembly and continental collision in the Neoproterozoic East Africa Orogen: implications for the consolidation of Gondwanaland. *Annual Reviews of Earth and Planetary Science*, **22**, 319–351.
- Yoshida, M., 2007. Geochronological data evaluation: implications for the Proterozoic tectonics of East Gondwana. *Gondwana Research*, **12**, 228–241.

Cross-references

[Breakup of Rodinia](#)
[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Origins of the Metazoa](#)
[Snowball Earth](#)
[Trace Fossils: Neoproterozoic](#)

GREAT OXYGENATION EVENT (GOE)

The term “Great Oxygenation Event” (GOE, also known as “Great Oxidation Event,” “oxygen catastrophe,” or “oxygen crisis”) describes a critical environmental change in Earth history that resulted from the appearance of diatomic oxygen (O_2), a waste product of oxygenic photosynthesis, in the atmosphere. The GOE occurred around 2.4 billion years (Ga) before present, close to the boundary between the Archean and the Proterozoic (2.5 Ga). For details, please refer to “[Critical Intervals in Earth History](#).”

GREEN ALGAE

Green algae are photosynthetic eukaryotes with simple plastids (derived from primary endosymbiosis) that are mostly microscopic and rarely more than a meter in greatest dimension. The approximate 6,000 extant species show an enormous diversity of growth habit and fine details of their cellular architecture. Green algae occur as unicellular or colonial, microscopic or macroscopic, motile planktonic, as well as benthic attached forms. Some (e.g., Dasycladales) are important carbonate producers in aquatic environments, others live in symbiotic associations with fungi to form lichens in terrestrial settings. Green algae living as endosymbionts inside heterotrophic organisms are a common phenomenon. The order Charales, in which full tissue differentiation occurs, is considered to include the closest relative of higher plants. For details, please refer to entry “[Algae \(Eukaryotic\)](#).”

GUILD

Metabolically related populations, e.g., sulfate-reducing microorganisms. See entry “[Microbial Communities, Structure, and Function](#)” for further reading.

F

FE(II)-OXIDIZING PROKARYOTES

Kristina L. Straub
University of Vienna, Vienna, Austria

Synonyms

Fe-oxidizers; Fe(II)-oxidizing prokaryotes/microorganisms; Ferrous iron-oxidizing prokaryotes/microorganisms; Iron-oxidizers; Iron-oxidizing prokaryotes/microorganisms.; Iron(II)-oxidizing prokaryotes/microorganisms

Definition

Fe(II)-oxidizing prokaryotes. Diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to oxidize Fe(II), ferrous iron, to Fe(III), ferric iron. The electrons obtained from the oxidation of Fe(II) are utilized for energy generation in aerobic or anaerobic respiration and/or for assimilative reduction reactions.

Introduction

Aerobic neutrophilic Fe(II)-oxidizing bacteria were among the first environmentally relevant prokaryotes that were discovered and studied in the nineteenth century. At that time, eye-catching ochre deposits in ponds and slowly running waters had attracted the attention of microbiologists. Microscopic analyses of such ochre deposits revealed the prevalence of twisted stalks and/or sheaths that were covered with iron minerals. Bacteria were suggested to be responsible for the production of these specific iron-covered structures, and subsequently the first aerobic Fe(II)-oxidizing prokaryotes were described: *Gallionella ferruginea* and *Leptothrix ochracea* (reviewed by Emerson, 2000; Canfield et al., 2005). In contrast, the anaerobic oxidation of Fe(II) by phototrophic bacteria (Widdel et al., 1993), nitrate-reducing bacteria

(Straub et al., 1996), and archaea (Hafenbradl et al., 1996) was not discovered until the end of the twentieth century. Aerobic and anaerobic Fe(II)-oxidizing prokaryotes play decisive roles in the biogeochemical cycle of iron. In comparison to other physiological groups, only few species of Fe(II)-oxidizing prokaryotes are available in pure culture. The capability to oxidize Fe(II) is apparently phylogenetically widespread because the known species belong to diverse phyla of the *Bacteria* and the *Archaea*. The mechanism(s) of prokaryotic Fe(II)-oxidation are largely unknown (Canfield et al., 2005).

Geochemical aspects of Fe(II)

Iron is the fourth most abundant element in the Earth's crust, and depending upon the geochemical conditions, it occurs either in soluble forms or in a variety of minerals (Cornell and Schwertmann, 2003). In natural habitats, Fe(III) minerals are reduced to Fe(II) mainly by the metabolic activities of prokaryotes. The concentration of dissolved Fe(II) is controlled by precipitation-dissolution reactions and by adsorption processes: Fe(II) precipitates with carbonates, phosphates, or sulfides and tends to adsorb to soil particles and mineral surfaces (Cornell and Schwertmann, 2003; Canfield et al., 2005). Chemical reoxidation reactions of Fe(II) depend on the pH and on the presence of appropriate oxidants. The reaction rate of Fe(II) with molecular oxygen is very low at acidic pH values, and hence Fe(II) is fairly stable in the presence of oxygen under acidic conditions. With increasing pH, the reaction rate of Fe(II) and molecular oxygen increases, and at fully aerated conditions Fe(II) is rapidly oxidized to ferric iron (Emerson, 2000; Canfield et al., 2005). In the absence of molecular oxygen, Fe(II) can be chemically oxidized at appreciable rates only by nitrite or Mn(IV). However, Mn(IV) minerals are insoluble, and the environmental significance of these nondiffusible oxidants is therefore limited (Canfield et al., 2005). It is thus

speculated that anaerobic Fe(II)-oxidizing prokaryotes are important catalysts for the reoxidation of Fe(II) in anoxic environments.

Fe(II) as electron donor in aerobic or anaerobic respiration

Dissimilatory Fe(II)-oxidizing prokaryotes utilize Fe(II) as electron donor in aerobic or anaerobic respiration, i.e., they gain energy by coupling the oxidation of Fe(II) to the reduction of molecular oxygen or to the reduction of nitrate. As in the oxidation of Fe(II) to Fe(III), only one electron can be obtained, dissimilatory Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to gain enough energy for maintenance and growth. Today, three physiological groups of prokaryotes are known to grow with Fe(II) as electron donor: acidophilic aerobic, neutrophilic aerobic, and neutrophilic nitrate-reducing prokaryotes. Due to energetic considerations, the oxidation of Fe(II) with nitrate at low pH values should also yield enough energy for prokaryotic growth. However, acidophilic Fe(II)-oxidation coupled to the reduction of nitrate has not been reported so far (Kappler and Straub, 2005; Appelo and Postma, 2007). The dissimilative oxidation of Fe(II) leads to the formation of Fe(III) minerals which are well soluble under acidic conditions but barely soluble at neutral pH. Hence, neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product.

Acidophilic aerobic Fe(II)-oxidizing prokaryotes

A variety of autotrophic and heterotrophic prokaryotes that thrive in environments of low pH (< 3) were described to grow by dissimilatory aerobic oxidation of ferrous iron (Johnson, 2007). At acidic pH values, Fe(II) is stable in the presence of molecular oxygen, and hence prokaryotes do not need to compete with the chemical oxidation. However, the energy gain of acidophilic aerobic Fe(II) oxidation is low because the redox potential of the redox pair $\text{Fe}^{3+}/\text{Fe}^{2+}$ is with +770 mV rather high. The difference to the redox potential of the redox pair $\text{O}_2/\text{H}_2\text{O}$ (> +1,000 mV below pH 3) allows therefore only little energy generation (Kappler and Straub, 2005; Appelo and Postma, 2007). Nevertheless, representatives of different phyla of the *Bacteria* (e.g., *Acidithiobacillus ferrooxidans*, *Sulfobacillus acidophilus*) and the *Archaea* (*Acidianus brierleyi*, *Sulfolobus metallicus*) exploit Fe(II) in aerobic respiration. Moreover, three species of the genus *Leptospirillum* are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Johnson, 2007). All other acidophilic Fe(II)-oxidizing prokaryotes make use of alternative electron donors (e.g., hydrogen, sulfur) and sulfur or Fe(III) as alternative electron acceptors. Species of acidophilic Fe(II)-oxidizing bacteria that also grow by dissimilatory reduction of Fe(III) were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993).

Acidithiobacillus ferrooxidans was the first acidophilic aerobic Fe(II)-oxidizing bacterium that was isolated (Temple and Colmer, 1951); it belongs to the *Gammaproteobacteria* and is so far the best studied of all acidophilic prokaryotes. However, details of the flow of electrons from Fe(II) to molecular oxygen are still debated. Apparently, Fe(II) is oxidized on the cell surface at the outer membrane. Subsequently, a variety of membrane-bound and periplasmic cytochromes plus the periplasmic copper protein rusticyanin transfer the electrons to a cytochrome *c* oxidase. There the electrons are used together with protons to reduce molecular oxygen to water (Johnson, 2007; Castelle et al., 2008).

Neutrophilic aerobic Fe(II)-oxidizing bacteria

So far, only few representatives of the *Alpha*-, *Beta*-, and *Gammaproteobacteria* are described to grow by dissimilatory aerobic oxidation of Fe(II) at neutral pH. This physiological group gains more energy than their acidophilic counterparts because the difference between the redox potentials of the relevant redox couples is greater at neutral than at acidic pH values ($\text{Fe(III)/Fe(II)} < +100$ mV; $\text{O}_2/\text{H}_2\text{O} +820$ mV; Kappler and Straub, 2005; Appelo and Postma, 2007). However, in oxic environments of neutral pH Fe(II) has a half-life in the order of few minutes and is chemically oxidized to ferric iron (Canfield et al., 2005). This competition with the chemical oxidation is likely the major reason why neutrophilic aerobic Fe(II)-oxidizing bacteria thrive preferentially in microoxic niches or oxic/anoxic boundaries. Laboratory studies showed that the activity of Fe(II)-oxidizing bacteria contributes up to 90% to the formation of Fe(III) under such conditions. Typical habitats for neutrophilic aerobic Fe(II)-oxidizing bacteria include groundwater springs, freshwater, and marine hydrothermal vents, wetlands, and plant rhizospheres (Emerson, 2000). Due to their production of conspicuous iron-encrusted twisted stalks or sheaths, *Gallionella ferruginea* and *Leptothrix ochracea* were the first Fe(II)-oxidizing bacteria that were already discovered in the nineteenth century. Only during the past few years, additional morphologically inconspicuous aerobic Fe(II)-oxidizing bacteria were isolated, e.g., *Ferritrophicum radicola* and *Sideroxydans paludicola* (Canfield et al., 2005; Weiss et al., 2007). According to physiological studies, several of the novel strains are obligate aerobic Fe(II)-oxidizing bacteria, i.e., Fe(II) is the only electron donor and molecular oxygen the only electron acceptor utilized (Weiss et al., 2007).

Neutrophilic anaerobic Fe(II)-oxidizing nitrate-reducing prokaryotes

Under anoxic conditions, bacteria (*Alpha*-, *Beta*-, *Gamma*- and *Deltaproteobacteria*) and archaea (*Archaeoglobales*) are capable of oxidizing Fe(II) with nitrate as the terminal electron acceptor. At pH 7, all redox pairs of the nitrate reduction pathway can accept electrons from Fe(II), because their redox potentials are more positive than that

of the iron redox couple (Straub et al., 1996). Nitrate-dependent Fe(II) oxidation was reported for diverse habitats such as rice paddy soil, activated sewage sludge, freshwater or marine sediments (Canfield et al., 2005). The first observation of this metabolism was made with a freshwater enrichment culture: Fe(II) was oxidized to Fe(III) and nitrate was concomitantly and stoichiometrically reduced to molecular nitrogen (Straub et al., 1996). As Fe(II) was the sole electron donor supplied, Fe(II) oxidation coupled to nitrate reduction definitely supported cell growth in this enrichment culture. The situation is not so clear in most pure cultures of Fe(II)-oxidizing nitrate-reducing bacteria (e.g., *Acidovorax* sp. strain BoFeN1 or *Thermomonas* sp. strain BrG3) because they need an organic co-substrate for growth. In such strains it is questioned whether Fe(II) oxidation is beneficial and supports cell growth or whether Fe(II) is just oxidized in a rather unspecific side reaction. All known species are able to utilize alternative electron donors (e.g., hydrogen, organic acids) and alternative electron acceptors (nitrite, oxygen, ferric iron). *Geobacter metallireducens*, isolated as Fe(III)-reducing bacterium, couples the oxidation of Fe(II) to the reduction of nitrate to ammonium; it is unclear if *G. metallireducens* obtains energy by this process (Lovley et al., 2004; Canfield et al., 2005). *Ferroglobus placidus* is so far the only representative of the domain *Archaea* that grows by coupling the dissimilatory oxidation of Fe(II) to the reduction of nitrate to nitric oxide and nitrogen dioxide. Alternatively, *F. placidus* utilizes hydrogen, sulfide, acetate, or monoaromatic compounds as electron donor and thiosulfate or Fe(III) as electron acceptor (Hafenbradl et al., 1996; Lovley et al., 2004).

Fe(II) as electron donor for assimilative reduction reactions

Various groups of prokaryotes are able to reduce oxidized inorganic compounds for the synthesis of biomass. Such assimilative reduction reactions require a source of electrons (Canfield et al., 2005). Phylogenetically and physiologically different groups of chemotrophic and phototrophic prokaryotes have the ability to exploit Fe(II) as source of electrons for assimilative reduction reactions such as the fixation of carbon dioxide. Chemotrophic acidophilic and neutrophilic, aerobic and anaerobic Fe(II)-oxidizing prokaryotes have been discussed before because they oxidize the major fraction of Fe(II) during respiratory energy generation. In contrast, phototrophic Fe(II)-oxidizing prokaryotes utilize light as energy source and oxidize Fe(II) exclusively for assimilative purposes. The assimilative oxidation of Fe(II) leads to the formation of Fe(III) which is barely soluble at neutral pH. Neutrophilic phototrophic Fe(II)-oxidizing bacteria therefore have to cope with a virtually insoluble metabolic end product.

Anaerobic phototrophic Fe(II) oxidation

Anoxygenic phototrophic bacteria were the first prokaryotes discovered that oxidize Fe(II) in the absence of

molecular oxygen (Widdel et al., 1993). So far, only few cultures of Fe(II)-oxidizing anoxygenic phototrophic bacteria were established either from freshwater or from marine sediments. They are affiliated with the purple sulfur (*Thiodictyon* sp. strain F4), the purple non-sulfur (e.g., *Rhodobacter* sp. strain SW2, *Rhodovulum robiginosum*), or the green sulfur bacteria (*Chlorobium ferrooxidans*). All strains have the ability to utilize alternative electron donors such as hydrogen, reduced sulfur compounds, or organic acids (Canfield et al., 2005). The discovery of anoxygenic phototrophic bacteria that directly catalyze the oxidation of Fe(II), provides an alternative or additional explanation for the origin of Precambrian banded iron formations (Widdel et al., 1993; Kappler and Straub, 2005).

Summary

Phylogenetically diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to oxidize Fe(II) during aerobic or anaerobic respiration and/or for assimilative reduction reactions. Only one electron can be obtained from the oxidation of Fe(II) to Fe(III) and Fe(II)-oxidizing prokaryotes have to oxidize large quantities of Fe(II) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(II)-oxidizing prokaryotes have to cope with different constraints: (1) Acidophilic Fe(II)-oxidizing prokaryotes gain less energy in respiration than their neutrophilic counterparts; (2) Neutrophilic Fe(II)-oxidizing prokaryotes have to cope with a virtually insoluble metabolic end product. It is discussed that anoxygenic phototrophic Fe(II)-oxidizing bacteria contributed to the generation of Precambrian banded iron formations.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Castelle, C., Guiral, M., Malarte, G., Ledgham, F., Leroy, G., Brugna, M., and Giudici-Ortoni, M. T., 2008. A new iron-oxidizing/O₂-reducing supercomplex spanning both inner and outer membranes, isolated from the extreme acidophile *Acidithiobacillus ferrooxidans*. *The Journal of Biological Chemistry*, **283**, 25803–25811.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Emerson, D., 2000. Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH. In Lovley, D. R. (ed.), *Environmental Microbe-Mineral Interactions*. Washington: ASM, pp. 31–52.
- Hafenbradl, D., Keller, M., Dirmeier, R., Rachel, R., Roánagel, P., Burggraf, S., Huber, H., and Stetter, K. O., 1996. *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe²⁺ at neutral pH under anoxic conditions. *Archives of Microbiology*, **166**, 308–314.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 257–270.

- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Straub, K. L., Benz, M., Schink, B., and Widdel, F., 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. *Applied and Environmental Microbiology*, **62**, 1458–1460.
- Temple, K. L., and Colmer, A. R., 1951. The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. *Journal of Bacteriology*, **62**, 605–611.
- Weiss, J. V., Rentz, J. A., Plaia, T., Neubauer, S. C., Merrill-Floyd, M., Lilburn, T., Bradburne, C., Megonigal, J. P., and Emerson, D., 2007. Characterization of neutrophilic Fe(II)-oxidizing bacteria isolated from the rhizosphere of wetland plants and description of *Ferritrophicum radicola* gen. nov. sp. nov., and *Sideroxydans paludicola* sp. nov. *Geomicrobiology Journal*, **24**, 559–570.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Assmus, B., and Schink, B., 1993. Ferrous iron oxidation by anoxygenic phototrophic bacteria. *Nature*, **362**, 834–836.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Banded Iron Formations](#)
[Chemolithotrophy](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Leptothrix](#)
[Metals, Acquisition by Marine Bacteria](#)
[Nitrogen](#)
[Photosynthesis](#)
[Siderophores](#)

FE(III)-REDUCING PROKARYOTES

Kristina L. Straub
 University of Vienna, Vienna, Austria

Synonyms

Fe-reducers; Fe-reducing prokaryotes/microorganisms;
 Ferric iron-reducing prokaryotes/microorganisms; Iron-reducers; Iron-reducing prokaryotes/microorganisms;
 Iron(III)-reducing prokaryotes/microorganisms

Definition

Fe(III)-reducing prokaryotes. Various species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III), ferric iron, to Fe(II), ferrous iron, by the transfer of electrons. The electrons for Fe(III) reduction derive mainly from the metabolic oxidation of organic compounds or hydrogen.

Introduction

Reduction of Fe(III) by prokaryotes has been known since the beginning of the twentieth century but was not considered to be of importance. At that time, only few bacterial strains were known to reduce small amounts of Fe(III) during fermentation. In addition, it was misleadingly presumed that prokaryotes cause reduction of Fe(III) mainly indirectly by producing sulfide, releasing organic compounds, lowering the redox potential, or decreasing the pH. This perspective changed notably with the discovery of dissimilatory Fe(III)-reducing bacteria, i.e., bacteria that utilize Fe(III) as terminal electron acceptor in an anaerobic type of respiration (Balashova and Zavarzin, 1979, 1980; Lovley and Phillips, 1988; Myers and Nealson, 1988). In order to gain energy for maintenance and growth by this type of respiration, significant amounts of ferric iron have to be reduced. Additionally, it became clear that neither lowering the redox potential nor decreasing the pH is sufficient to cause Fe(III) reduction. Furthermore, it was demonstrated that only very few organic compounds (e.g., fructose, cysteine) were able to reduce Fe(III) chemically at significant rates. In the last 20 years, numerous dissimilatory Fe(III)-reducing prokaryotes were identified and it is now generally accepted that this physiological group of prokaryotes has a strong influence on the iron geochemistry of most environments. Because many Fe(III)-reducing prokaryotes also have the ability to catalyze the reduction of Mn(IV) or Mn(III) to Mn(II) they additionally influence the geochemistry of manganese (Lovley et al., 2004; Canfield et al., 2005; Kappler and Straub, 2005).

Evolutionary consideration

The ability to reduce Fe(III) occurs widely within the domains *Bacteria* and *Archaea*. Most known Fe(III)-reducing prokaryotes that grow by Fe(III) reduction belong to the phylum Proteobacteria with representatives in each of the five subdivisions *Alpha*- (e.g., *Acidiphilium rubrum*), *Beta*- (e.g., *Ferribacterium limneticum*), *Gamma*- (e.g., *Shewanella oneidensis*), *Delta*- (e.g., *Geobacter sulfurreducens*), and *Epsilonproteobacteria* (e.g., *Sulfurospirillum barnesii*). Several Gram-positive bacteria that belong to the phylum Firmicutes (e.g., *Thermoanaerobacter siderophilus*) and some separate bacterial lineages like *Geothrix fermentans* or *Geovibrio ferrireducens* also have the ability to reduce ferric iron (Lovley et al., 2004). Fe(III) reduction in the domain *Archaea* was discovered by testing pure cultures that had originally not been isolated with Fe(III): Representatives of the phyla Euryarchaeota (e.g., *Pyrococcus furiosus*) and Crenarchaeota (e.g., *Pyrobaculum islandicum*) showed reduction of ferric iron (Vargas et al., 1998). The great phylogenetic diversity of Fe(III)-reducing prokaryotes with representatives in all deeply branching phyla, along with iron isotope studies, supports the hypothesis that Fe(III) reduction was an early mode of energy metabolism. Further geochemical evidences

suggest that other major electron acceptors such as sulfate, nitrate, or molecular oxygen were not available on early Earth (Lovley et al., 2004; Canfield et al., 2005; Johnson et al., 2008).

Geochemical aspects of Fe(III)

Iron is the fourth most abundant element in the Earth's crust and Fe(III) minerals may account for a few percent dry weight of rocks, soils, and sediments. Hence, in most anoxic ecosystems Fe(III) minerals are the dominant electron acceptors for bacteria and archaea. Today, 16 different Fe(III) oxides, hydroxides, or oxide hydroxides are known and for simplicity they are often collectively termed Fe(III) oxides. They are all composed of Fe together with O and/or OH and differ in composition and crystal structure. Widespread Fe(III) oxides include ferrihydrite, goethite, hematite, lepidocrocite, and magnetite (Cornell and Schwertmann, 2003). In contrast to other electron acceptors such as oxygen, nitrate, or sulfate, Fe(III) oxides are poorly soluble at neutral pH. The solubility of Fe(III) oxides at neutral pH depends furthermore on the type of oxide, the degree of crystallinity and the crystal size, and may range from 10^{-9} M (e.g., ferrihydrite, low crystallinity, small crystals) to 10^{-18} M (e.g., goethite, high crystallinity, large crystals). At circumneutral pH, Fe(III)-reducing prokaryotes therefore have to cope with a virtually insoluble electron acceptor. With increasing acidity, the solubility of Fe(III) oxides increases and ferric iron is well soluble at pH values below 2.5 (Cornell and Schwertmann, 2003). Concomitant with the change in pH and Fe(III) oxide solubility, the redox potential of the transition between ferric and ferrous iron changes significantly. The redox potential of the redox pair $\text{Fe}^{3+}/\text{Fe}^{2+}$ is +770 mV only at acidic pH values. At neutral pH, with poorly crystalline Fe(III) oxides (ferrihydrite, lepidocrocite) as the oxidant, the redox potential ranges between +100 and -100 mV, while the redox potential of more crystalline Fe(III) oxides (goethite, hematite, magnetite) may be as low as -300 mV (Canfield et al., 2005; Straub et al., 2001). In respect of solubility and energetics, acidophilic and neutrophilic Fe(III)-reducing prokaryotes cope with entirely different substrates.

Reduction of Fe(III) by fermenting bacteria

The first Fe(III)-reducing prokaryotes that were studied in laboratory cultures belonged to the domain *Bacteria* and reduced only small amounts of Fe(III) during fermentative growth on organic substrates such as glucose or malate. This physiological group comprises of a variety of bacteria and includes anaerobes (e.g., *Clostridium pasteurianum*, *Vibrio* spp.), facultative anaerobes (e.g., *Escherichia coli*, *Paenibacillus polymyxa*), and aerotolerants (e.g., *Lactobacillus lactis*, *Lactococcus lactis*). Accordingly, Fe(III) reduction by fermenting bacteria was observed under both anoxic and oxic culture conditions. For fermenting bacteria, Fe(III) reduction is

only a minor pathway for electron disposal and research on this type of metabolism fell behind efforts to understand dissimilatory Fe(III) reduction. The mechanism(s) of electron transfer to Fe(III) during fermentation is unknown and it is also unclear whether this process yields energy for growth. Thermodynamic calculations suggest that Fe(III) reduction during fermentation results in a slightly greater energy yield than fermentation alone but experimental evidences are ambiguous (Lovley, 1991; Canfield et al., 2005). So far no representative of the domain *Archaea* was described to reduce Fe(III) during fermentative growth.

Dissimilatory Fe(III)-reducing prokaryotes

Dissimilatory Fe(III)-reducing prokaryotes utilize Fe(III) as terminal electron acceptor in anaerobic respiration, i.e., they gain energy by coupling the oxidation of an electron donor to the reduction of Fe(III). As in the reduction of Fe(III) to Fe(II) only one electron can be disposed, dissimilatory Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to gain enough energy for maintenance and growth. Numerous strains of phylogenetically dispersed, dissimilatory Fe(III)-reducing prokaryotes have been isolated into pure culture or have been detected by molecular techniques in a wide range of pristine or contaminated habitats, including freshwater and marine sediments, wetlands, soils, aquifers, the deep subsurface, deep subterranean thermal water, hydrothermal vents, and hot springs (Lovley et al., 2004). This widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Consistent with the great variety of ecosystems inhabited, pure cultures of Fe(III)-reducing prokaryotes show a wealth of physiological qualities in respect to oxygen concentration (strict or facultative anaerobes), temperature (growth reported between 4 and 121°C), pH (growth reported between pH 1 and 9), and salinity (freshwater and marine species). In addition, autotrophic growth with carbon dioxide as sole carbon source and fixation of molecular nitrogen was described for some species of Fe(III)-reducing prokaryotes. No prokaryote characterized so far, depends solely on Fe(III) as terminal electron acceptor for growth. In the absence of Fe(III), prokaryotes either grow by fermentation (e.g., *Thermotoga maritima*) or utilize alternative electron acceptors. Common alternative electron acceptors include oxygen (e.g., *Pantoea agglomerans*), nitrate (e.g., *Geobacter metallireducens*), manganese (e.g., *Deferribacter thermophilus*), reduced sulfur compounds (e.g., *Desulfuromusa kysingii*), and fumarate (e.g., *Desulfuromonas acetexigens*). Furthermore, some species transfer electrons to heavy metals (e.g., uranium, chromium), graphite electrodes, humic substances, or chlorinated compounds (e.g., tetrachloroethene, trichloroacetic acid). The vast majority of Fe(III)-reducing prokaryotes utilizes fermentation end products such as acetate, ethanol, or hydrogen as electrons donors. Only few prokaryotes are known to couple the dissimilatory reduction of Fe(III) to the oxidation of sugars (e.g., *Acidiphilium*

cryptum), amino acids (e.g., *Geothermobacter ehrlichii*), long-chain fatty acids (e.g., *Desulfuromonas palmitatis*), monoaromatic compounds (e.g., *Geobacter metallireducens*), or reduced inorganic sulfur compounds (e.g., *Acidithiobacillus ferrooxidans*).

Acidophilic Fe(III)-reducing bacteria

A variety of autotrophic and heterotrophic bacteria that thrive in environments of low pH (<3) can grow by dissimilatory Fe(III) reduction (Johnson, 2007). All known species are facultative anaerobes, and most of them synthesize cell compounds required for dissimilatory Fe(III) reduction only in the absence of oxygen. In contrast, some species of acidophilic Fe(III)-reducing bacteria (e.g., *Acidiphilium cryptum*) constitutively express all genes necessary for Fe(III) reduction (Johnson, 2007). This is surprising because the redox potential of the redox pair O_2/H_2O reaches values above +1,000 mV below a pH value of 3 (Appelo and Postma, 2007). Hence, cells gain more energy by aerobic respiration than by utilizing Fe(III) as terminal electron acceptor (redox potential of the redox pair Fe^{3+}/Fe^{2+} : +770 mV).

Several species of acidophilic Fe(III)-reducing bacteria (e.g., *Acidithiobacillus ferrooxidans*, *Sulfobacillus acidophilus*) also grow by oxidizing Fe(II) with molecular oxygen; such prokaryotes were shown to catalyze the cycling of iron in laboratory experiments (Johnson et al., 1993). So far no representative of the domain *Archaea* was described to grow by dissimilatory Fe(III) reduction at acidic pH values.

Neutrophilic Fe(III)-reducing prokaryotes

The majority of dissimilatory Fe(III)-reducing prokaryotes that were isolated and characterized thrives at circumneutral pH and has to cope with a poorly soluble electron acceptor under natural conditions. In addition, the redox potential of the redox pair Fe(III)/Fe(II) is much lower (+100 to –300 mV, depending on the type of ferric iron oxide) than the redox potential of the redox pair O_2/H_2O (+820 mV) relevant for aerobic respiration. Therefore, at neutral pH, Fe(III) is a less valuable electron acceptor in terms of energy generation than molecular oxygen. Similarly, prokaryotes gain more energy from the respiration with nitrate or manganese as terminal electron acceptors. Hence, respiratory Fe(III) reduction at neutral pH occurs only under anoxic conditions in the absence of more favorable electron acceptors (Canfield et al., 2005). In some studies on neutrophilic Fe(III)-reducing prokaryotes, only chelated forms of ferric iron such as Fe(III) citrate or Fe(III) NTA (nitrilotriacetate) were applied. Caution is necessary in the interpretation of results from such studies because chelated Fe(III) forms are soluble at circumneutral pH, have redox potentials that are higher than those of non-chelated Fe(III) minerals, and may react rather unspecifically with electron-releasing cell compounds (Straub et al., 2001).

Most research on the biochemistry of microbial Fe(III) reduction focuses on members of the genera *Shewanella*

and *Geobacter*, two genera with significant differences in their basic physiology. *Shewanella* species are facultative anaerobes that belong to the *Gammaproteobacteria*. They grow very well in nutrient-rich medium and oxidize lactate or pyruvate incompletely to acetate. In contrast, species of the genus *Geobacter* belong to the *Deltaproteobacteria* and most of them are described as anaerobes. The most common electron donors utilized by *Geobacter* species include acetate, ethanol, and hydrogen. Despite differences in basic physiological features, various *c*-type cytochromes play a significant role in intracellular electron transfer processes in both *Shewanella* and *Geobacter* species.

Mechanisms of prokaryotic Fe(III) reduction

Considering the phylogenetically widespread ability of prokaryotes to reduce Fe(III) and the great prokaryotic physiological diversity, it is not surprising that Fe(III)-reducing prokaryotes developed different strategies to cope with Fe(III) as electron acceptor. According to experimental observations, prokaryotes developed the following strategies to transfer electrons from the cell to the surface of Fe(III) minerals: (a) Physical contact between cell and mineral surfaces allows for direct delivery of electrons; an attachment of cells to Fe(III) minerals has been repeatedly reported and seems to be the prerequisite for the delivery of electrons to Fe(III) in some Fe(III)-reducing prokaryotes such as *Geobacter metallireducens*. (b) Iron chelators increase the solubility of ferric iron; some evidence indicates that *Shewanella algae* and *Geothrix fermentans* produce and release Fe(III) chelators specifically during growth with Fe(III) minerals. (c) Electron-shuttling compounds accomplish the transfer of electrons from the cell to the mineral; diffusible electron-shuttling compounds help to bridge the spatial distance between cells and ferric iron (Lovley et al., 2004; Kappler and Straub, 2005). Reduction of Fe(III) by electron shuttling compounds is of particular importance in natural habitats where prokaryotic cells and Fe(III) minerals are not evenly distributed and Fe(III) might not be directly/physically accessible for prokaryotic cells. Naturally occurring electron-shuttling compounds that were shown to sustain prokaryotic growth include humic substances (Lovley et al., 1996) and sulfur compounds (Straub and Schink, 2004). Note that prokaryotes which reduce Fe(III) only via naturally occurring electron-shuttling compounds are no Fe(III)-reducing prokaryotes in a strict sense as their actual electron acceptor is the shuttling molecule and not Fe(III).

Summary

Phylogenetically diverse species of the prokaryotic domains *Bacteria* and *Archaea* have the ability to reduce Fe(III) during fermentative or respiratory growth by the transfer of electrons. Fe(III)-reducing prokaryotes have been recovered or detected in a great variety of pristine or contaminated habitats, and their widespread occurrence correlates well with the ubiquitous presence of Fe(III) minerals. Only one electron can be disposed

by the reduction of Fe(III) to Fe(II) and hence Fe(III)-reducing prokaryotes have to reduce large quantities of Fe(III) to sustain cell metabolism. Due to the complex geochemistry of iron, acidophilic and neutrophilic Fe(III)-reducing prokaryotes have to cope with different iron species: Fe(III) minerals are soluble only below a pH value of 3. At circumneutral pH, Fe(III) is only poorly soluble and significantly less energy can be gained by Fe(III) respiration compared to aerobic respiration. However, Fe(III) is the dominant electron acceptor in many anoxic habitats and prokaryotes developed different strategies for its utilization.

Bibliography

- Appelo, C. A. J., and Postma, D., 2007. *Geochemistry, Groundwater and Pollution*. Leiden: Balkema.
- Balashova, V. V., and Zavarzin, G. A., 1979. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiologia*, **48**, 773–778.
- Balashova, V. V., and Zavarzin, G. A., 1980. Anaerobic reduction of ferric iron by hydrogen bacteria. *Microbiology*, **48**, 635–639.
- Canfield, D. E., Thamdrup, B., and Kirstensen, E., 2005. *Aquatic Geomicrobiology*. San Diego: Elsevier.
- Cornell, R. M., and Schwertmann, U., 2003. *The Iron Oxides - Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: Wiley-VCH.
- Johnson, D. B., 2007. Physiology and ecology of acidophilic microorganisms. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington, DC: ASM, pp 257–270.
- Johnson, D. B., Ghauri, M. A., and McGinness, S., 1993. Biogeochemical cycling of iron and sulphur in leaching environments. *FEMS Microbiology Reviews*, **11**, 63–70.
- Johnson, C. M., Beard, B. L., and Roden, E. E., 2008. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient Earth. *Annual Review of Earth and Planetary Sciences*, **36**, 457–493.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. *Reviews in Mineralogy and Geochemistry*, **59**, 85–108.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, **55**, 259–287.
- Lovley, D. R., and Phillips, E. J. P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, **54**, 1472–1480.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445–448.
- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Myers, C. R., and Nealson, K. H., 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Science*, **240**, 1319–1321.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* by electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744–5749.
- Straub, K. L., Benz, M., and Schink, B., 2001. Iron metabolism in anoxic environments at near neutral pH. *FEMS Microbiology Ecology*, **34**, 181–186.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovley, D. R., 1998. Microbiological evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shewanella](#)
[Siderophores](#)

FERMENTATION

Fermentation is a route of obtaining energy from the oxidation of organic substrates using an endogenous electron acceptor (usually an organic compound). The energy yield in fermenting systems is limited when compared to respiration. Nevertheless, fermentation still allows growth under conditions when external, inorganic electron acceptors are lacking, or when iron as part of the electron carriers in the respiratory chain is not available and therefore an electron transport chain cannot be synthesized. Common fermentation substrates are sugars, which are present in large amounts in the environment, for example, as photosynthesis products and constituents of plant cell wall material. Microbial sugar fermentation pathways encompass lactic acid fermentation, alcoholic fermentation, and propionate fermentation, according to the major metabolic products generated. Another important fermentation pathway is acetoclastic methanogenesis, that is, fermentation of acetate to CO₂ and CH₄, which is performed by some methanogenic archaea (see entry “[Methane, Origin](#)”). [Hydrogen](#) is another common product of microbial fermentation processes.

For further details and references, please refer to entry “[Microbial Degradation](#).”

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Natuschka M. Lee¹, Daniela B. Meisinger¹,
 Michael Schmid², Michael Rothballer²
 Frank E. Löffler³

¹Technical University of Munich, Freising-Weihenstephan, Germany

²Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Neuherberg, Germany

³University of Tennessee, Knoxville, TN, USA

Definition

FISH (fluorescence in situ hybridization) is a hybridization technique employing reverse complementary

fluorescently labeled probes to detect and localize only those parts of the chromosome, a gene or its transcript with which they show a high degree of sequence similarity. Results are mostly evaluated by either fluorescence microscopy or cell sorting methods like flow cytometry. Depending on target gene, probe chemistry, labeling, and combination with other analytical tools, a wide range of different FISH applications can be employed for advanced in situ analysis of community structure, dynamics, localization, activity, function and interactions of different cellular species.

Introduction

Antonie van Leeuwenhoek's ingenious improvement of the microscope in the seventeenth century revolutionized biology as this initiated novel ways to explore our world and ultimately led to the discovery of a new dimension of life forms, the microorganisms. However, since traditional microscopy reveals mainly structural features, its usefulness to advanced biological research reached its limits with the beginning of the molecular era. Fortunately, new possibilities for advanced microscopy applications emerged with the parallel development of two techniques: (1) in situ hybridization techniques (ISH) employing labeled gene probes targeting specific regions of a particular gene, and, (2) fluorescence microscopy. Fluorescence in situ hybridization (FISH) makes use of reverse complementary gene probes labeled with fluorochromes that are hybridized to specific regions on a chromosome (e.g., a specific gene) of gene transcripts (e.g., rRNA), and are then viewed by fluorescence microscopy or evaluated by other cell sorting methods like flow cytometry. FISH revolutionized nearly all disciplines in microbiology, in particular environmental research fields like microbial ecology, environmental biotechnology and geomicrobiology, since this enabled a cultivation-independent exploration of whole cells of culturable as well as not yet cultured microorganisms in their natural, complex environment (Amann et al., 1995; Amann and Fuchs, 2008). However, along with the development of other novel molecular tools and the growing comprehension of the enormous complexity of the biology of microorganisms, the first generation of FISH tools reached its limits as well, especially for applications on oligotrophic ecosystems characterized by a low amount of cells with often low activities in a strongly confounding background, which is often the case in geomicrobiology. Different modifications of the first generation FISH protocol as well as different combinations with other analytical tools were undertaken in order to meet the increasing demands with respect to, e.g., improved targeting of cells with low ribosome content or probe-repelling cell wall chemistry, alternative strategies to increase probe specificities or signal intensities, advanced image analysis, elucidation of activity and function by targeting other genes than the 16S rRNA gene and combining with different analytical tools, and finally cell enrichment of probe-targeted cells for,

e.g., subsequent identification by sequencing (Wagner et al., 2003; Wagner et al., 2006; Amann and Fuchs, 2008; Orphan, 2009; Wagner, 2009). The development of novel FISH protocols in combination with other advanced analytical tools and microscopes is most likely to continue to play a crucial role in microbial ecology and geomicrobiology research. Only microscopy-based tools can provide us with the possibility to visualize the morphotype, growth characteristics, spatial distribution, and interactions of different taxa on a single cell level within complex communities. Furthermore, despite all progress in nucleic acid-based approaches like PCR and the "omics" approaches, only microscopy-based tools can link isolated extracted DNA/RNA markers, with intact, individual cells in their natural environment, and thus also exclude molecular biases often observed with nucleic acid extraction and PCR (Table 1). The greatest benefit will be reached when combining different FISH tools with various methods such as cultivation and molecular approaches. Ultimately, a systematic approach can be undertaken to reveal discrepancies between different tools caused by extant biases of each tool in terms of sensitivity and specificity, so that a validated, advanced insight into the true diversity, dynamics, function, activities, and relationships in the microbial world can be obtained.

Sample preparation, fixation and storage of samples

In contrast to nucleic acid-based tools, which simply require either addition of preservative agents or instant freezing of biomass and storage in sterile tubes for subsequent nucleic acid extraction, preparation of samples for FISH requires usually more consideration and effort, especially for long-term storage of samples of unknown microbial composition and/or employing certain types of FISH protocols that may disrupt the original structure of the sample.

Preparations for standard applications

1. *Sampling strategy*: While FISH may principally work equally well on nearly any kind of optimally fixed cells with high ribosome content, irrespective of cell titers or the nature of the environmental sample, our abilities to detect, visualize, and quantify the cells may be obstructed by tedious, time-consuming screening of samples with either too low or too high cell counts, confounding background caused by autofluorescence of the background or unspecific binding of probes or labels to nontarget cells/substances (Amann and Schleifer, 2001). Some of these problems can be minimized by considering how the sample should be optimally treated already during sampling, for example, by concentration, dilution, embedding, cell extraction, or particular consideration on chemical-physical conditions to avoid cells getting exposed to stress.
2. *Fixation time and protocols*: The purpose of the fixation is to maintain the original microbial composition

Fluorescence In Situ Hybridization (FISH), Table 1 Advantages and disadvantages of FISH versus disruptive tools like PCR with ribosomal biomarkers

Diagnostic possibilities	PCR/qPCR/RT PCR	FISH ^a
<i>Experimental conditions:</i>		
Preparation of biomass prior to analysis	Nucleic acid extraction	Fixation of cells ^b
Long-term storage of sample	−80°/−20°C	−20°/+4°C ^b
Labor time (after NA extraction or fixation)	>6 h	0.5 h to 96 h
<i>Relevant parameters for microbial ecology:</i>		
Quantify distribution and dynamics	Yes/partly	Partly
Link targeted gene directly to cells	No	Yes
Visualize morphology and growth characteristics	No	Yes
Visualize associations with other microorganisms	No	Yes
Three-dimensional reconstruction of complex aggregates	No	Yes
<i>Methodological strengths/weaknesses:</i>		
Simultaneous identification of several genes	Only with multiplex PCR	Yes ^c
Distinguish gene targets in live/inactive/dead cells	No (for DNA)	Partly ^d
Distinguish false-positive gene products (chimera)	No	Yes
Quick check of cultures for purity	No	Yes
Enrich for subsequent analysis (e.g., sequencing)	Yes	Partly
Amplification biases	Yes	No ^e
Quantification bias caused by multiple ribosomal gene copies	Yes (for qPCR)	No
Bias caused by unspecific target binding	Possible	Possible
Biases caused by matrix	Inhibition	Autofluorescence
Detection limit	>5 gene copies	10 ^{5–7} cells/ml ^f

^aIncluding different FISH protocols and advanced microscopy tools.

^bIn certain cases, samples may be stored at −80°C and the fixation omitted (Yilmaz et al., 2010b).

^cOnly with certain FISH protocols like mRNA-CARD FISH, RING-FISH, gene-FISH, and in situ RCA.

^dDepends on the FISH protocol and how this is combined with other analytical tools and appropriate references.

^eException, FISH protocols based on PCR.

^fDepends on concentration/dilution procedure.

of the samples as well as the morphological integrity of cells during the different hybridization steps (elevated temperature, exposure to enzymes, detergents and osmotic gradients) and prevent cell losses, while simultaneously making the cells permeable for the probe(s) (Amann, 1995). Unfortunately, there is not one plain fixation protocol for all cell types for all different FISH protocols. Different fixation protocols have been developed to cope with the complexity of archaea, bacteria, and Eukarya with regard to their cell envelope packages and the type of probes to be employed for a particular FISH protocol. A suboptimal fixation protocol may make cells impermeable for probes or even cause cell lysis, especially during longer storage times or when exposing the cells to certain procedures such as incubation with enzymes prior to the FISH procedure. Apart from the selection of an optimal fixative reagent, it is also relevant to pay attention to the concentration of the fixative reagent and the incubation time and conditions. Fixation times may range from a few min up to >24 h, depending on cell type and FISH protocol, and are generally followed by a washing step for removal of the fixative reagent. The two most common fixation procedures are based on either aldehydes (e.g., formalin, paraformaldehyde (PFA), glutaraldehyde) or on alcohols (e.g., ethanol, methanol). In the

majority of cases, Gram-negative cells within the domain Bacteria are usually fixed with 4% PFA, and Gram-positive cells within the domain Bacteria with ice cold 96% ethanol (Amann, 1995). To a certain extent, some Gram-negative species will also tolerate EtOH fixation, and some Gram-positive species (general exceptions are, e.g., *Actinomycetes* and *Streptococci*) may also tolerate PFA fixation (especially if the incubation time is short, see, e.g., de los Reyes et al., 1997). The previously mentioned fixation protocols may also work well for certain Archaea and Eukarya species, but in some cases, especially for certain Archaea species (Nakamura et al., 2006) or eukaryotes like fungi (Baschien et al., 2001; Tsuchiya and Taga, 2001; Weber et al., 2007) or protozoa, see, e.g., Fried et al. (2002); Petroni et al. (2003); Fried and Foissner (2007); Weber et al. (2007), other fixative reagents or post treatments with enzymes like chitinase or endo-isopeptidase are more suitable. When dealing with unknown samples, it is therefore recommended to explore the suitability of different fixation protocols. Irrespective of the fixation protocol used, the cells must be fixed as soon as possible with optimally prepared fixation solutions, preferably already during the sampling occasion to avoid population shifts and for optimal evaluation of true probe signal intensities as these

may in certain cases also serve as an indicator of activity (see, e.g., Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007).

3. *Storage of fixed cells:* Fixed samples (in wet or dry state, such as on a microscope slide or on a membrane filter) can be stored at room temperature or at +4°C for shorter periods. For optimal long-term storage at -20°C, fixed cells are generally dissolved in a 1:1 (v/v) solution with a 50% end concentration of ethanol in order to avoid freezing (Amann et al., 1994). Cells concentrated on a membrane filter are normally stored dry at -20°C (e.g., Teira et al., 2004). So far, no systematic studies on the maintenance of cell integrity or ribosome content in fixed cells after longer storage periods exist, but it is generally recommended that once the cells have been fixed, quantitative studies should be performed within days to avoid biases caused by unknown deterioration of cells or decrease of ribosome content.

Preparations for omics-based applications

For genomic or proteomic applications, unfixed biomass is desirable to minimize possible sequencing biases introduced by cross-linking and to avoid possible cell lysis difficulties, so that FISH targeted cells can be enriched by, e.g., flow cytometry and subjected to, e.g., whole genome application. Recently, Yilmaz et al. (2010b) demonstrated that the standard oligonucleotide FISH protocol can also be performed on at least two different types of frozen, non-fixed complex environmental samples using Archaea as well as Bacteria targeted probes. However, more research is needed to explore further possibilities as well as limits of this approach, such as if this is quantitative and if this applies to any kind of cells types or other environmental samples, irrespective of growth and storage conditions, as well as if this approach is also suitable for other types of FISH protocols.

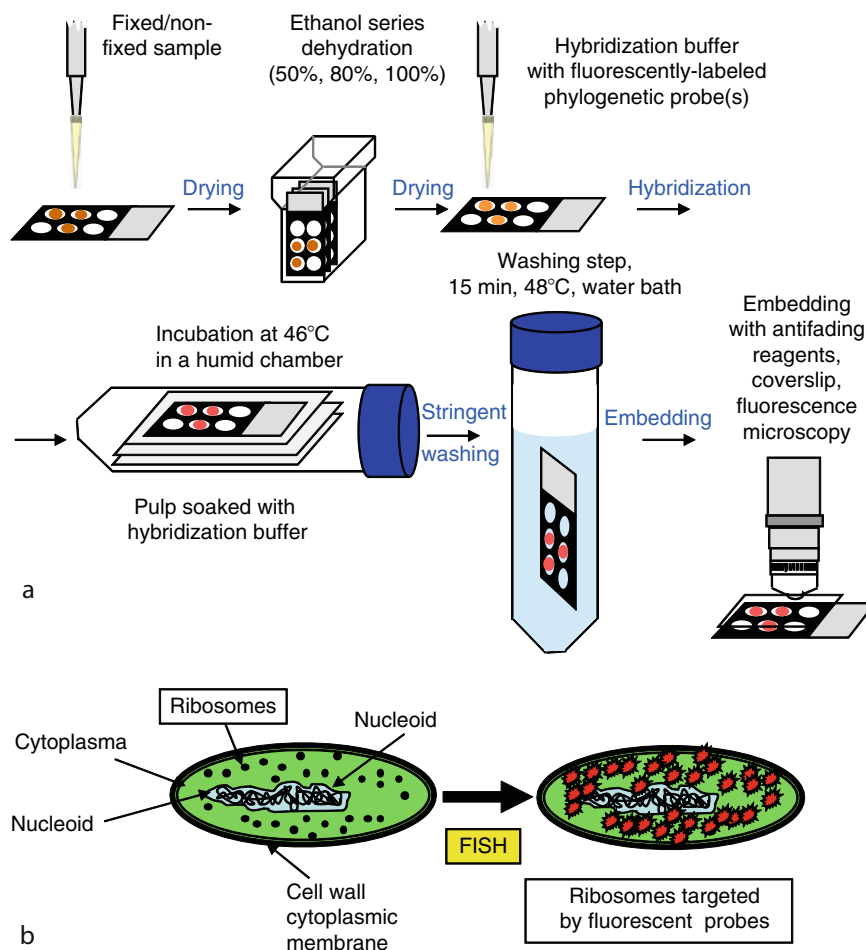
FISH for in situ detection of ribosomal genes

General background: The ribosomal gene was and will at least for the near future remain the standard gene for reconstruction of phylogeny, despite the growing amount of genome sequences which is presently revolutionizing our current concepts of taxonomy, phylogeny, and development of comprehensive biomarkers for improved identification (see, e.g., Konstantinidis et al., 2009). The main benefits of the ribosomal gene is that it is a functionally conserved molecule present in high numbers in all organisms from all domains and is composed of sequence regions of higher and lower evolutionary conservation. The variable conservation degree makes it particularly ideal for design of biomarkers for nucleic acid-based detection and quantification of different levels of microbial diversity (Ludwig et al., 1998; Priesse et al., 2007; Amaral-Zettler et al., 2008; Yarla et al., 2008). Although several different types of ribosomal subunits exist, only a few of these are currently used as targets for probes in

FISH studies. For most FISH studies, the main gene target is the 16S rRNA gene for prokaryotes, and the 18S-rRNA gene for eukaryotes (Ludwig et al., 1998; Metfies et al., 2008). However, other multicopy ribosomal genes or even stable multicopy RNA molecules have been (5S-rRNA, tmRNA), or may be occasionally used as alternative targets for FISH probes (23S-rRNA, 28S-rRNA, precursor rRNA, intergenic spacer region). These alternative gene targets may overcome some of the problems occasionally encountered when targeting the 16S rRNA gene, such as low information content and limited probe accessibility or information about activity status (Trebesius et al., 1994; Oerther et al., 2000; Schmid et al., 2001; Schonhuber et al., 2001; Zimmermann et al., 2001; Peplies et al., 2004). However, despite this, the 16S rRNA gene has remained the main gene target in FISH applications, especially for environmental systems with unknown diversity, since the 16S rRNA gene is nowadays routinely sequenced in most studies so that the amount of retrieved 16S rRNA gene sequences has thus come to outrange all other databases of other gene sequences.

Principle of FISH: The FISH procedure generally consists of the following steps (Adapted from Manz et al., 1992 and Amann, 1995):

1. Optional: For certain cases an additional post-treatment of the fixed/non-fixed biomass may be needed for increased permeabilization of probes through rigid cell envelope packages, or for removal of confounding background, by, e.g.:
 - Incubation with enzymes like lysozyme, protease, proteinase K, mutanolysine, endoisopeptidase, chitinase
 - Removal of wax or chemical precipitates by chemical solvents
 - Use of detergents (SDS) or other chemicals like hydrochloric acid
 - Physical treatment like exposure to microwave radiation
 - Addition of chemicals to avoid dissolution of essential compounds
2. Successive dehydration in an EtOH series (50%, 80%, 96%, each step between 1–3 min).
3. Hybridization of the fixed sample with the probe on a microscope slide or in solution under stringent conditions (Figure 1). Stringent conditions are achieved by keeping the hybridization temperature constant (+46°C) but varying the formamide concentration for different probes (Stahl and Amann, 2001).
4. Subsequent washing with preheated buffered washing solutions at a slightly higher temperature (+48°). After this, the hybridized samples can be evaluated or stored at dark prior to evaluation (for a shorter period at room temperature or at +4°, for longer periods, at -20°C).
5. Optional: Counter stain with, e.g., an appropriate nucleic acid stain like DAPI (4',6-diamidino-2-phenylindole) or SYBR Green (e.g., Wagner et al., 1994, or Engel et al., 2003).



Fluorescence In Situ Hybridization (FISH), Figure 1 (a) Schematic outline of the standard oligonucleotide FISH procedure on microscope slides. (b) Schematic outline of a cell before and after the standard oligonucleotide FISH procedure.

6. Evaluation is achieved by:

Visualization:

- Standard epifluorescence microscopy, or confocal laser scanning microscope for three-dimensional reconstruction (Wagner et al., 1998).
- Combination with other microscopes like electron microscopy (e.g., Gerard et al., 2005), RAMAN spectroscopy combined with a microscope (Wagner, 2009), or nanoSIMS (Behrens et al., 2008; Orphan, 2009).

For digital image analysis of FISH images, various software packages are available, such as CMEIAS, COMSTAT, and daime (Heydorn et al., 2000; Daims and Wagner, 2007; Zhou et al., 2007; Daims, 2009).

Other options:

- Membrane-based evaluation (e.g., dot blot, Wagner et al., 1994).
- Combination with other analytical tools (e.g., screening of uptake of radioactive tracers or stable isotopes

(Nielsen et al., 2005; Orphan, 2009) or other FISH protocols targeting non-ribosomal genes or their transcripts for exploring activities or functions.

- Reverse hybridization by microarray technology, where multiple probes are deposited on a support material to hybridize a labeled sample with the probes (DeSantis et al., 2005; Loy and Bodrossy, 2006; Taylor et al., 2010).
- Cell sorting or probe signal intensity evaluation by flow cytometry (Behrens, 2003; Amann and Fuchs, 2008), RING (Recognition of Individual Genes)-FISH (Zwirgmaier et al., 2004b), or magneto-FISH (Orphan, 2009).
- Whole genome amplification (Orphan, 2009; Yilmaz et al., 2010b).

Basic equipment and reagents for standard FISH applications:

- Hybridization oven (46°C)
- Water bath (48°C)
- Polypropylene screw top tubes, filter paper

- Reagents (for chemical composition, check, e.g., Amann, 1995):
 - NaCl
 - Tris/Phosphate buffer
 - EDTA
 - SDS
 - Formamide
 - 50%, 80%, 96% EtOH
 - Reagents/equipment for improved probe accessibility
- Labeled probes
- Counterstains like nucleic acid stains (e.g., DAPI (4',6-diamidino-2-phenylindole) or SYBR Green)
- Microscope slides (possibly coated with, e.g., poly-L-lysine (Sigma-Aldrich, www.sigmaaldrich.com/sigmaaldrich/home.html) or a gelatine-chromium sulphate solution (Amann, 1995))
- Mounting medium of appropriate pH (e.g., from Citifluor, www.citifluor.co.uk/; Vectashield, www.vectorlabs.com/; various mounting media from Invitrogen, www.invitrogen.com/site/us/en/home.html)
- Some form of fluorescence microscope.

FISH probes (probe design, probe evaluation, probe databases)

For a proper interpretation of FISH experiments, probes need to be optimally designed and handled. Several different types of softwares (e.g., the ARB software package, (www.arb-home.de/) and Primrose (www.bioinformatics-toolkit.org/index.html)) and detailed guidelines for probe design for particularly FISH experiments exist (Amann, 1995; Amann and Schleifer, 2001; Stahl and Amann, 2001), and (Hugenholtz et al., 2001). Below follows an updated summary of some of those guidelines, focusing mainly on applications with the ARB software package:

1. Construction of a molecular data base: Prior to all probe design, a comprehensive data base of all relevant complete gene sequences of the target group and relevant nontarget groups should be collected for a thorough evaluation of phylogenetic relationships. Partial sequences may also be included, but these should be handled more critically due to their limited value for a full evaluation of all potential probe targets. Sequences can be downloaded from different categories of databases: (1) all kinds of gene sequences: EMBL (www.embl.de/), NCBI (www.ncbi.nlm.nih.gov/), (2) regularly updated and curated ribosomal gene sequences: RDP (<http://rdp.cme.msu.edu/>), SILVA (www.arb-silva.de/), Green Genes (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>); the ribosomal internal spacer collection (<http://egg.umh.es/rissc/>); (3) functional genes: Fun Gene (<http://fungene.cme.msu.edu/index.spr>). Principally, most softwares that can align sequences, perform phylogenetic evaluations, and design biomarkers like PCR primers can be used for FISH probe design as well.

However, the bioinformatic software package ARB (www.arb-home.de/) using the curated SILVA database (www.arb-silva.de/) is highly recommended, as several useful and partly unique tools have been implemented into this package to facilitate a straightforward and advanced design, evaluation and visualization of probes and their target (Ludwig et al., 2004; Kumar et al., 2005; Kumar et al., 2006; Priesse et al., 2007).

2. Hierarchic probe design strategy: Where possible, it is recommended to design a hierarchic probe set consisting of several probes targeting different phylogenetic levels of the target organism/group (see, e.g., Amann and Schleifer, 2001; Amann and Fuchs, 2008). If the different probes are labeled with different fluorochromes and combined in one FISH experiment on a mixture of cells with different phylogenetic relationships, different overlaps of the probes will produce different color combinations depending on the taxonomical relationships. This facilitates a straightforward top to bottom identification of the taxonomical status (e.g., from phylum down to species level) of different cell species in a complex sample (Amann, 1996).
3. General rules for standard oligonucleotide probe design:
 - (a) Select the appropriate gene sequences of the desired target group. Depending on bioinformatic software, design the probe according to the recommended procedure. For example, if using the ARB software (Ludwig et al., 2004), select from the menu "probe design" an appropriate PT server, then define appropriate parameters such as length of probe (average length of probes for standard oligonucleotide probes is between 15–25 nucleotides), minimum group and maximum non-group hits, GC content, melting temperature range, range of nucleotide positions, and maximum hair-pin bonds.
 - (b) The selected probe should be completely specific (complementary) to a region of the selected target sequences and have at least one mismatch to the same target region in nontarget sequences. Optimally, the mismatch should be centralized in the nontarget sequences in order to maximize the destabilizing effect of the mismatch.
 - (c) Perform probe match to evaluate the specificity of the probe (e.g., in the bioinformatic software package ARB (www.arb-home.de/), the RDP Probe Match tool (<http://rdp.cme.msu.edu/probematch/search.jsp>), the probeBase probeCheck tool (<http://131.130.66.200/cgi-bin/probecheck/content.pl?id=home>) or general data bases like the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).
 - (d) Unfortunately, there is no such thing as a perfect probe that only targets the target group as the course of the evolution of gene sequences is a random process. During a recent review Amann and Fuchs (2008) showed that it is more advisable to

evaluate group coverages as the percentage of group members that can be identified relative to the total number of members in the target group. With this approach, three groups can be distinguished: true positives; false negatives; and false positives. Parallel to the constantly growing amount of gene sequences, the coverage of each probe target group is ultimately going to change and possibly demand more complex probe sets. For example, in the early days of the FISH technology, only one probe was needed to target all recognized species in the Bacteria domain (Amann et al., 1990). However, a decade later, Daims et al. (1999) showed that a mixture of three different Bacteria domain targeting probes were needed to cover all so far (up till 1999) recognized sublineages.

- (e) Evaluate the melting temperature of the probes (e.g., as described by (Hugenholtz et al., 2001) or the probeBase, www.microbial-ecology.net/probebase/). Optimally, the probe should have a melting temperature $> 57^{\circ}\text{C}$. If the melting temperature is $< 57^{\circ}\text{C}$, the temperature can be increased by shifting the position of the probe or expanding the length of the probe.
 - (f) Check probe accessibility. A reoccurring problem is the probe accessibility. The general explanation for is that certain regions of the secondary structure of the ribosomal gene are inaccessible for probes. Unfortunately, the accessible and inaccessible regions of the ribosomal molecules appear to vary from species to species, so that only precise estimations of probe accessibilities can be made for those species where a systematic probe accessibility mapping has been performed (see, e.g., Fuchs et al., 1998; Behrens, 2003a, 2006b). The general recommendation is therefore to design probes on different target positions and then to simply explore on a trial-and-error experimental basis which of the selected probes produce high probe signals. However, Yilmaz et al. (2006) showed that principally all positions on the ribosome molecule may yield satisfying probe signals if a sophisticated approach based on a thermodynamics-based probe design is employed. With this approach, FISH experiments of any given probe can be mathematically simulated. Based on this, theoretical predictions on, e.g., the probe accessibility, the formamide denaturation profile, and the potential of mismatch discriminations can be made (for further details see Yilmaz et al., 2010a). Based on this, further optimizations of the probe accessibility can be made such as modifying the length of the probe or the experimental hybridization conditions (e.g., increase of the hybridization time up to 96 h).
 - (g) Check for potential self-complementarity of the probe which may initiate duplex formations between the probe and the target (e.g., caused by hairpins or dimers).
 - (h) Naming of probe. There are three types of nomenclatures for probes: (1) the short name (e.g., EUB 338), which is often constructed by a shortage of the name of the target group (e.g., EUB for Eubacteria), followed by a number that denotes the first position of the probe on the target gene (e.g., nucleotide position 338 on the *E. coli* gene); (2) a name based on a comprehensive nomenclature to denote main features of the probe such as the target gene, target group, target group level, 3' end of the probe, and probe length (Alm et al., 1996); and (3) the probeBase accession number (e.g., pB-00159 for probe EUB 338) as assigned by the online probe database ProbeBase (www.microbial-ecology.net/probebase/).
4. Labeling of probes: The first rRNA-targeted oligonucleotide probes for FISH were radioactively labeled, developed by microautoradiography and evaluated by light microscopy (Giovannoni et al., 1988). Unfortunately, the work with radioactively labeled probes restricted the applicability of FISH. However, the applicability of FISH increased as soon as the first fluorescently labeled rRNA-targeted oligonucleotide probes were reported (DeLong et al., 1989). Probes are nowadays generally covalently labeled at the 5' end of the oligonucleotide, and the most commonly used fluorochromes for FISH are sulfoindocyanine fluorochromes like Cy3 and Cy5, fluorescein, ALEXA probes, rhodamine, and Texas red. The criteria for selecting appropriate fluorochromes are based on the capabilities of the available epifluorescence microscope and on the nature of the cells and their surrounding environment, where the aim is to avoid a confounding background and unspecific binding of fluorochromes to nontarget cells, and/or to minimize bleaching of probe signals. During the last decade, several different approaches have been used to increase the probe signal intensities in those cases where standard labeled probes produce too weak fluorescent signals. A simple strategy was to employ mixtures of mono-labeled probes targeting different sites of the ribosomal gene (e.g., Amann et al., 1990; Krumholz et al., 1990; Morris et al., 2002; Sunamura and Maruyama, 2006). Another strategy is to employ alternative labeling procedures of oligonucleotide probes, such as polynucleotide FISH (e.g., Trebesius et al., 1994), horseradish peroxidase (employed in catalyzed reporter deposition (CARD-FISH), e.g., Pernthaler et al., 2002a; Wendeberg, 2010) or various types of multiple labeling of probes (Amann and Schleifer, 2001) such as DOPE-FISH (Stoecker et al., 2010).
 5. Purchase and handling of probes: Probes are chemically synthesized and can be purchased with or without label. In earlier times, probes had to be manually labeled (Amann, 1995), but nowadays labeled probes can be easily purchased from most providers (e.g., Biomer, Invitrogen, MWG, SIGMA) of molecular reagents like PCR primers at a rather low cost (starting

from approximately 50 USD). Probes are normally shipped in lyophilized stage and must be dissolved in either sterile water or an appropriate buffer (depending fluorochrome chemistry) to a stock solution of normally 100 pmol/μl. It is recommended to store aliquots of working solutions in individual tubes in dark at -20°C, and to avoid excessive freeze-thawing of the aliquots. For long time storage or transport at room temperature it is recommended to lyophilize the probes.

6. Evaluation of probes:

(a) For any purchased probe, it is recommended to check for the probe's concentration, length, homogeneity, and the completeness of the labeling, to avoid unwanted competition between labeled and unlabeled probes for target sites as this may reduce the probe signal intensity. This can be done as described by Stahl and Amann (2001), or as recommended by the provider who performed the labeling.

(b) Newly designed probes are generally first tested under non-stringent conditions (i.e., by excluding formamide in the hybridization buffer). If positive probe signals are obtained in reference strains as expected, stringent conditions of the probe should be determined by an increasing formamide series, as described in Amann and Schleifer (2001). Probe signal intensities can be quantified by digital image analysis with, e.g., the daime software, as described by (Daims et al., 2009).

(c) Combination of probes. The great advantage of FISH over standard PCR applications is that several differently labeled gene probes can be simultaneously combined and thus enable a simultaneous identification of different probe-targeted species within a complex sample. The general guidelines for optimal combination of different probes are:

- Probes with identical formamide concentrations for optimal stringencies (at which the probe hybridizes to the specific target but not to nontarget groups) can be combined in the same hybridization experiment.
- Probes with different formamide concentrations for optimal stringencies cannot be combined in the same hybridization experiment. Instead, successive hybridizations must be performed for each probe, starting with the probe with the highest formamide concentration.

(d) Most standard epifluorescence microscopes are equipped with filter sets that allow separate recording of at least two to three differently labeled probes and one UV-based nucleic acid stain like DAPI (4',6-Diamidino-2-phenylindol). Such recorded images can then be merged into one image with image analysis software packages. However, recent developments based on, e.g., advanced confocal laser scanning microscopes with multichannel image analysis, special tools such as spectral imaging or FISH protocols employing quantum dot

labeled probes allow the simultaneous combination of a considerably higher amount of differently labeled probes (e.g., Ainsworth et al., 2006; Anderson, 2010; Bentolila et al., 2006).

7. Probe bases: Different attempts to construct databases with FISH probes have been made during the last two decades. However, the probeBase (www.microbial-ecology.net/probebase/) is currently the most regularly updated data base for ribosomal targeted probes, encompassing 2,568 different probes targeting species within Archaea, Bacteria, and, to some extent, Eukarya (further probes targeting protists and cyanobacteria can be retrieved from www.sb-roscoff.fr/Phyto/Data-bases/RNA_probes_introduction.php) for FISH as well as for ~16 different microarray applications (version September 2010). Here, many of the so far published probes can be easily searched by different criteria (target organism, target sites, probe name, probe sequence, probeBase accession number, references). Detailed information about the probe chemistry are listed and links are provided for online probe matches to other databases like the ProbeCheck (<http://131.130.66.200/cgi-bin/probecheck/content.pl?id=home>), Green Genes (<http://greengenes.lbl.gov/cgi-bin/nph-index.cgi>), or RDP (<http://rdp.cme.msu.edu/>). Furthermore, up to 150 ribosomal gene sequences retrieved from own clone libraries can be uploaded to screen for probes targeting these sequences (Loy et al., 2007, 2008).

8. Controls: For a valid evaluation of probe signals, three different categories of controls are needed: (1) Positive controls where probes are employed that should or should not overlap with the intended target gene. (2) Negative controls, to explore the impact of confounding background caused by, e.g., autofluorescence, and of nonspecific binding of fluorochrome or probe to target cells as well as nontarget cells. To achieve this, two different negative controls are recommended: exclusion of probes during the hybridization experiment, and application of negative reference probes like the NON-SENSE probe (Loy et al., 2002) or the non-EUB probe (reverse complementary to the EUB probe, (Wallner et al., 1993)). (3) All experiments should be performed on appropriate optimally fixed strains carefully selected to serve as positive as well as negative controls.

9. Other probe types: Note that some of the rules applied for design and handling of standard oligonucleotide probes may not be applicable to other probe types, like polynucleotide probes, PNA FISH probes, or CARD FISH probes.

General guidelines for evaluation and presentation of FISH results

For optimal interpretation of FISH experiments, several parameters should be considered, as outlined below. Omitting any of these principles may lead to false interpretations of FISH results.

Checklist for optimal FISH experiments and evaluation of results:

1. Were optimal sampling strategies, fixation protocols, and storage conditions employed?
2. Were appropriate reference strains used simultaneously in parallel with the FISH experiments? If strains are lacking, clones based on CLONE-FISH (Schramm et al., 2002) may also be used to experimentally evaluate the FISH experiments.
3. Were the microscopical investigations performed with optimal microscope settings?
4. Were appropriate probes/probe combinations (positive as well as negative controls) used under stringent conditions?
 - (a) If the probe signals were positive, explore if nonspecific probe signals can be unequivocally excluded or if the identity of the probe signal can be confirmed with other measures.
 - (b) If the probe signals were negative on the sample subject to investigation but otherwise positive with positive controls, explore if this is caused either by cell losses during the hybridization procedure, low cell numbers in the original sample, or low activity (and thus low ribosome content and probe signal intensity).
5. If quantifications of FISH images were performed, explore if the amount of sample applied to the microscope slide was appropriate. A too high or too low cell concentration may result in an over- or underestimation of cell numbers. Evaluate also if the amount of microscope slide fields investigated was high enough to ensure statistical evaluations of error ranges. For further evaluation of quantification, spiking of sample may be recommendable (SPIKE-FISH, Daims et al., 2001).
6. When presenting FISH results, it is recommended to describe results as "cells targeted by probe X," instead of uncritically identifying the probe signals with the intended target group of the probe. This is especially important when applying probes to samples with unknown microbial composition and the applied probe is known to target false-positive as well as false-negative species and no further means have been undertaken to confirm the identity by, e.g., appropriate combinations of hierarchic FISH probe sets or subsequent sequencing (Amann and Fuchs, 2008).
2. Confounding background caused by auto-fluorescing compounds like photosynthetic pigments, proteins like cofactors (e.g., F420 for methanogens), other parameters in the surrounding environment (e.g., precipitates, fibers, minerals, humic substances); or by unspecific absorption of fluorochrome label.
3. Probe technical problems (e.g., probe permeabilization problems, probe accessibility in the ribosomal gene, probe specificity).
4. Limited information content of the 16S rRNA gene for taxonomical resolution of closely related species.
5. Limited possibilities to confirm true identity and explore phylogenetic relationships and identify novel gene groups or species.
6. Limited information on in situ physiology in terms of:
 - (a) Activity status (high ribosome content may reflect exponential growth stage, but this appears to be species dependent and needs to be explored for each particular species group (Wagner et al., 1995; Molin and Givskov, 1999; Rossetti et al., 2007);
 - (b) Function, since the ribosomal gene does not reveal information about function and since the genotype is not always linked to the phenotype (e.g., many functional microbial groups are polyphyletic, such as methanogens, sulphate reducers, denitrifiers, cyanobacteria, spirochaetes, reductive dechlorinators);
 - (c) Limited possibilities to monitor gene expressions (mRNA transcripts) or proteins.
7. Applications limited to prokaryotic and eukaryotic cells. However, the first notes on successful FISH applications on bacteriophages (personal communication, K. Zwirgmaier, D. Scanlan, A. Millard, University of Warwick, UK) was recently made with the Recognition of individual genes [RING]-FISH protocol (Zwirgmaier et al., 2004b).

To overcome these limitations, several different strategies have been employed, ranging from modifying different parts of the procedures of the first-generation FISH protocol, searching for alternative gene targets, probe chemistries and labeling procedures, to combining FISH with other markers (e.g., activity stains, enzymes, isotopes), analytical procedures, and advanced microscopes or other analytical tools for evaluation of several different parameters for a holistic interpretation of in situ microbial ecology. Today, at least four categories of advanced FISH strategies can be recognized, which can be combined in a multitude of ways for various novel applications.

Category I – increasing the probe signal intensity:

Drawbacks of oligonucleotide FISH and the benefits of novel FISH technologies

The first generation of FISH protocol based on oligonucleotide probes revolutionized nearly all disciplines in microbiology. However, along with applications of this protocol on different ecosystems and species, many of the limits of the first-generation FISH protocol became obvious:

1. Successful detection dependent on ribosome content (minimum 370 ± 16 rRNA molecules per *E. coli* cell (Hoshino et al., 2008)).

- Incubate sample prior to fixation with substrates or stress-promoting compounds like chloramphenicol (Kalmbach et al., 1997; Ouverney et al., 1997).
- Employ non-fluorescent labeling of probes for increased detection of cells in, e.g., a strongly autofluorescing background based on, e.g., radiolabels (Giovannoni

et al., 1988); or enzymes (Zarda et al., 1991; Amann et al., 1992; Lim et al., 1993).

- Employ alternative probe design based on helper probes for oligonucleotide probes to increase probe accessibility (Fuchs, 2000).
- Employ alternative probe chemistries for increased hybridization efficiencies and specificities based on peptide nucleic acid probes (PNA) (Perry-O'Keefe et al., 2001), or locked nucleic acid (LNA) probes (Kubota et al., 2006a).
- Explore probe accessibilities and thermodynamics-based probe design to predict probe accessibilities and optimal hybridization conditions (Yilmaz et al., 2006; Yilmaz et al., 2010a), and/or in nucleotide or probe quenching (Behrens, 2003; Behrens et al., 2004; Wagner et al., 2003).
- Employ self-ligating probes to overcome background autofluorescence and avoid washing steps (Sando and Kool, 2002).
- Employ PCR-generated probes to generate polynucleotide probes (Trebesius et al., 1994; Zimmermann et al., 2001; Meisinger et al., 2007; Pernthaler, 2002; Preston et al., 2002; Moraru et al., 2010).
- Employ modified fluorescent labeling technologies based on horse radish peroxidase labeled probes and catalyzed reporter deposition (CARD)-FISH (Pernthaler et al., 2002a, b; Hoshino et al., 2008; Pavlekovic et al., 2009); multiple labeling of probes (Amann and Schleifer, 2001; Stoecker et al., 2010) or quantum dot conjugated probes (Bentolila et al., 2006).

Category II – exploring activity or function:

- Combine FISH with rRNA dot slot to quantify cellular rRNA content (Ravenschlag et al., 2000).
- Employ FISH probes targeting the precursor 16S rRNA gene and intergenic spacer regions (Oerther et al., 2000; Schmid et al., 2001; Moter et al., 2010).
- Combine FISH with different activity indicating stains like redox dyes (Nielsen et al., 2003), halogenated thymidine analogue bromodeoxyuridine (BrdU) (Pernthaler et al., 2002), exo-enzyme labeled probes (Xia et al., 2007), or antibodies to target cell surface markers or proteins (Currin et al., 1990; Lubeck et al., 2000; Gmür and Lüthi-Schaller, 2007).
- Incubate sample with radioactively labeled substrates or stable isotopes to explore specific uptake of labeled substrates by micro-autoradiography (Nielsen et al., 2005), RAMAN spectroscopy (Wagner, 2009), or nano-SIMS (Behrens et al., 2008; Orphan, 2009).
- Target functional genes or even the mRNA gene by CARD-FISH (Wagner et al., 1998; Pernthaler and Amann, 2004; Orphan, 2009; Pilhofer et al., 2009), two-pass TSA-mRNA FISH (Kubota et al., 2006b), or by alternative labeling (Coleman et al., 2007).
- Target functional genes. For this, there are two categories: (1) PCR-generated probes like polynucleotide

FISH (Moraru et al., 2010) or (2) amplification of the target sequence like in situ PCR (Hodson et al., 1995; Rodríguez-Castaño, G., 2008; Tani et al., 1998), in situ loop-mediated isothermal amplification (Maruyama et al., 2003); recognition of individual genes [RING]-FISH (Zwirgmaier et al., 2004b; Pratscher et al., 2009; Hauer et al., in preparation), in situ rolling-circle amplification (Maruyama et al., 2005; Hoshino et al., 2010), cycling primed in situ amplification (CPRINS, Kenzaka, 2005; Tamaki et al., 2005), circularizable probes (Zhang et al., 2006), circularizable probes (Zhang et al., 2006; Hoshino et al., 2010), peptide nucleic acid assisted rolling circle amplification FISH (Smolina et al., 2007), and polynucleotide FISH (Moraru et al., 2010).

Category III – combining FISH with advanced cameras, microscopes, or other analytical tools:

- CCD camera (Amann and Schleifer, 2001); confocal laser scanning microscopy (Wagner et al., 1998), and advanced digital image processing (Daims, 2009; Daims and Wagner, 2007)
- Electron microscopy (Kenzaka et al., 2005; Ishidoshiro et al., 2005)
- Spectral imaging (Ainsworth et al., 2006)
- RAMAN spectroscopy (Wagner, 2009)
- nanoSIMS (Behrens et al., 2008; Orphan, 2009)
- Atomic force microscopy (Huang et al., 2009)

Category IV – enrichment of FISH targeted cells for subsequent analyses, and combination with high-throughput technologies:

- Automatic cell sorting and enrichment techniques based on flow cytometry (Amann and Fuchs, 2008)
- Magneto-FISH (Orphan, 2009; Stoffels et al., 1999)
- RING-FISH (Zwirgmaier et al., 2004a)
- Whole genome amplification (Yilmaz et al., 2010b)

Many of these improvements minimize at least some of the drawbacks observed with the first generation FISH protocol; however, these new approaches do also introduce other drawbacks. For example, CARD-FISH has replaced the standard FISH protocol in several cases because it produces stronger probe signals (Pernthaler et al., 2002a, b) and just recently it was shown that it can be combined with polynucleotide probes targeting functional genes in a subsequent step (Moraru et al., 2010). Nevertheless, compared to standard oligonucleotide FISH, CARD-FISH is a rather time-consuming FISH protocol and allows only the application of one probe per hybridization experiment. Furthermore, as peroxidase can be found in nearly all organisms, there may be a risk for unspecific probe reactions. Fortunately, the peroxidase can be denatured prior to the FISH procedure, however, optimal denaturation conditions appear to be species dependent and may thus require individual optimizations (Pavlekovic et al., 2009). Similarly, the recent developments of various protocols to target non-ribosomal genes

like various functional genes and mRNA transcripts, or combining with various analytical tools, present different advantages as well as disadvantages. For example, considerable higher costs, advanced equipment, tedious protocols, loss of probe specificity, less flexibility in terms of combination with other probes or protocols or on various organisms, problems with adequate preservation of gene target due to high decay rate (e.g., precursors or mRNA), or unpredictable cross-reactions (e.g., when incubating a complex community with radioactive substrates or stable isotopes). However, irrespective of these drawbacks that may eventually be overcome in future, it is crucial to keep in mind that our greatest hindrance for successful, unequivocal interpretations of FISH experiments in the environment is the unknown biodiversity and multitude facets of dynamics and interactions, which paradoxically seem to grow with each advancement in the science of microbiology. Thus, it appears likely that the development of novel FISH technologies will continue in parallel with the development of novel tools and our increased understanding of microbial diversity.

Different FISH applications in geomicrobiology

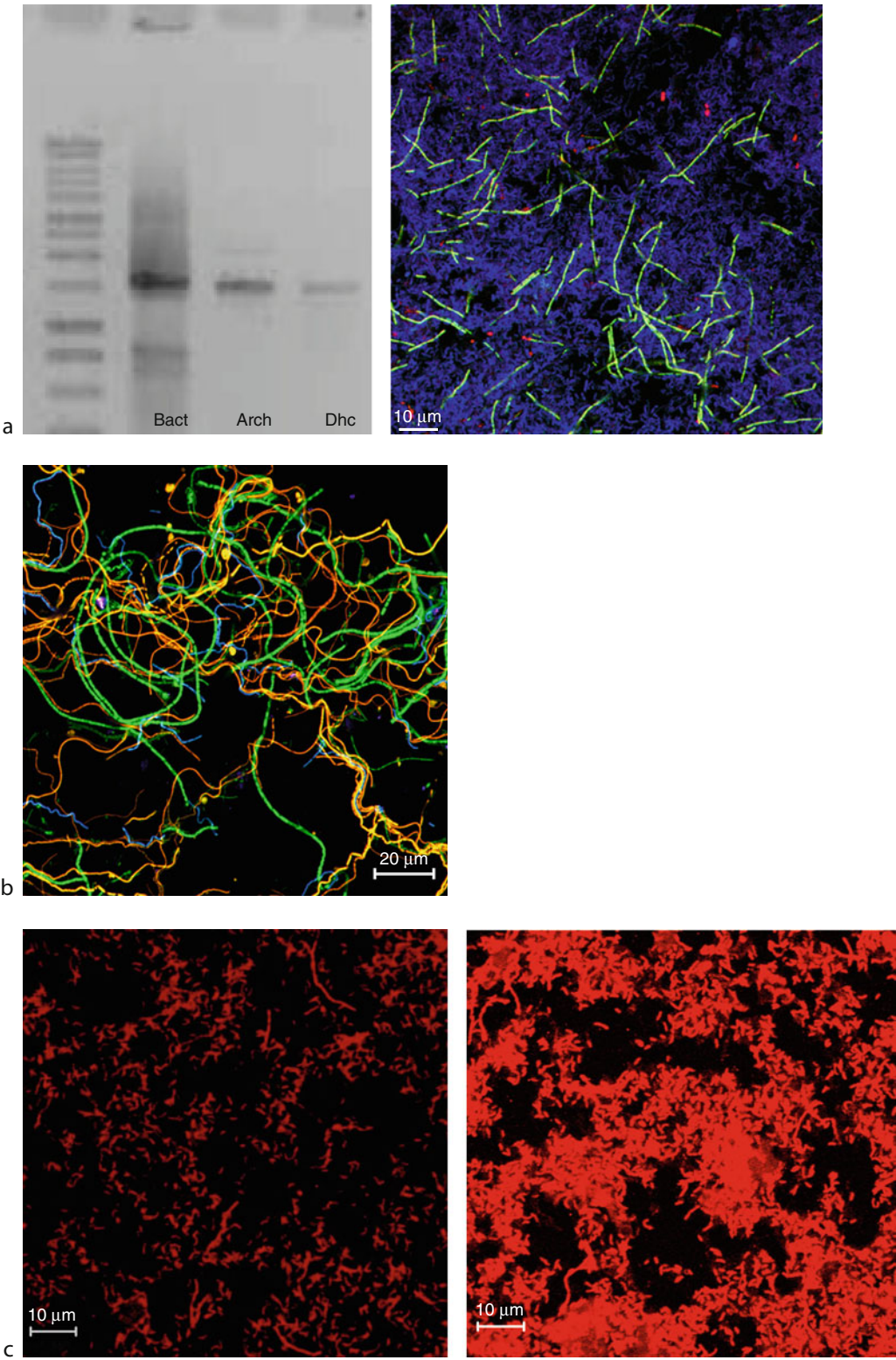
The fields explored in geomicrobiology are extremely diverse, ranging from the deep biosphere to the atmosphere surrounding our planet Earth, and from there to the outer space. The conditions for the different ecosystems will therefore vary tremendously so that different sampling technologies and modifications of FISH protocols must be employed in order to achieve successful FISH results. So far, FISH has not been employed in geomicrobiology as often and as successfully as in other microbiological fields like environmental biotechnology (e.g., wastewater treatment plants, Nielsen et al., 2009) or in medical sciences (e.g., Bridger et al., 2010). This is because many of the ecosystems explored in geomicrobiology are often exposed to extreme conditions and generally contain low amounts of usually novel microorganisms with slow or unknown growth rates. Furthermore, special considerations must be made in terms of interpreting results with regard to the risk of contamination of samples during sampling (e.g., during drilling experiments) and to the impact of geochemical conditions on so far published FISH protocols. Because of these obstacles, many of the recent advanced developments of FISH protocols have actually been driven by microbial ecologists and geomicrobiologists (Orphan, 2009). Below follow some examples of how FISH has been employed for some of the most widely explored ecosystems in geomicrobiology (see also images in Figure 2):

Aquatic systems: In general, successful FISH experiments can be performed on aquatic systems, since at least one of the main parameters for good microbial growth and dispersion of cells and nutrients, water, is abundant. Nevertheless, several parameters may introduce several biases, e.g., low nutrient status and/or naturally occurring

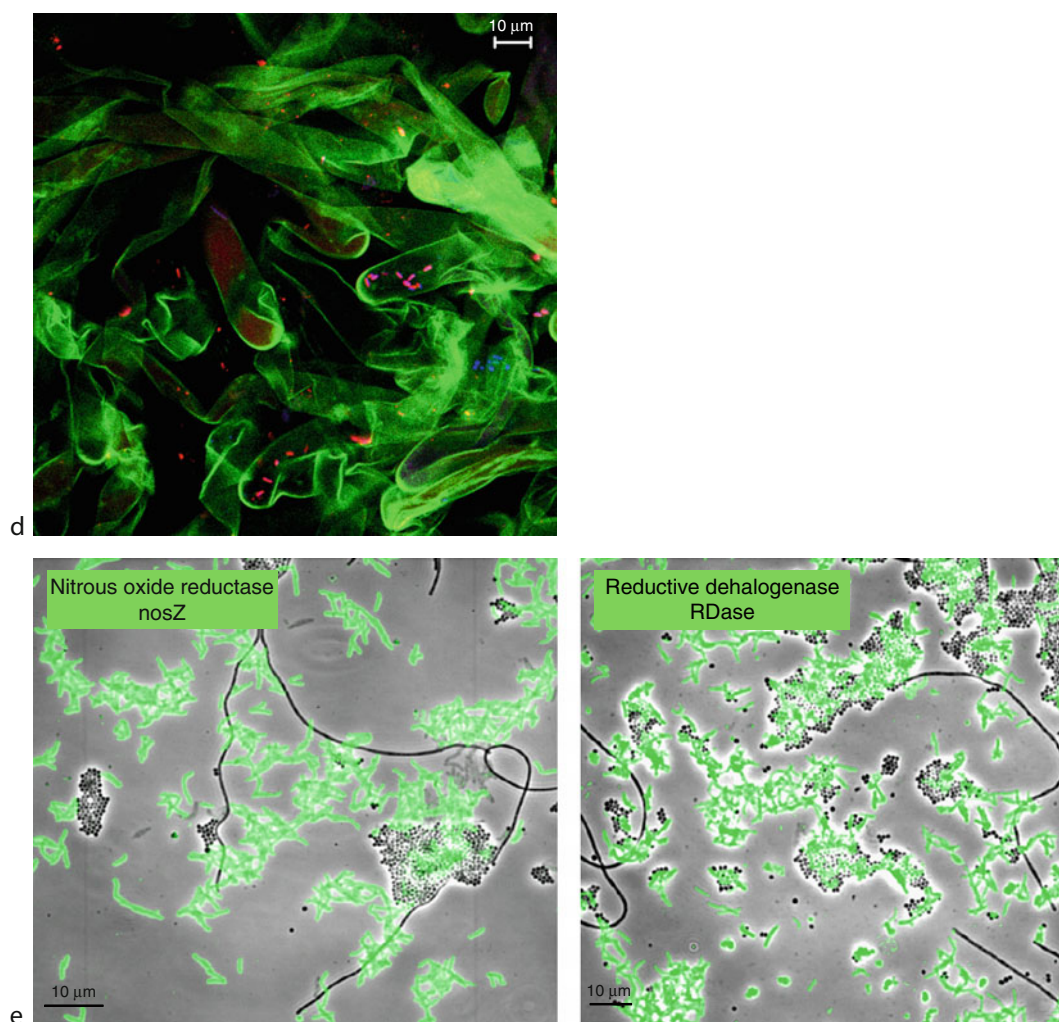
autofluorescing particles (Vesey et al., 1997). Aquatic systems with high nutrient content such as contaminated lakes or even anthropogenic systems like wastewater treatment plants will contain greater amounts of highly active bacteria than oligotrophic systems like aquifers and thus produce stronger FISH probe signals. For improved FISH on oligotrophic systems, different approaches have been undertaken:

Aquifers/ground water/drinking water: These ecosystems are generally dominated by planctonic cells in rather low cell numbers due to extreme conditions like oligotrophy, different redox states (e.g., anaerobic conditions), or toxic compounds (e.g., heavy metals, chlorinated drinking water or contaminated aquifers). Due to this, the general turn-over activities and thus also the ribosome content in the cells are generally extremely low. For adequate FISH evaluation, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris removed (e.g., by cell extraction, Caracciolo et al., 2005). Early studies reported that standard FISH protocols may work well on certain aquatic samples like lakes and bottled water (Pernthaler et al., 1998; Glöckner et al., 2000; Watanabe et al., 2000; Flies et al., 2005; Loy et al., 2005); however, more recent studies report that either rainbow FISH (Sunamura and Maruyama, 2006) or CARD-FISH are more recommendable on drinking water or groundwater (Sekar et al., 2003; Nielsen et al., 2006; Wilhartitz et al., 2007; Meisinger et al., 2010a).

Ocean: Similar to the freshwater systems mentioned above, oceanic ecosystems are generally dominated by planctonic cells in rather low cell numbers. However, in contrast to the conditions in the aquifers in the subsurface, the cells in oceans are often exposed to more dynamic conditions. Despite this, the ribosome content in the cells and the turn-over activities may be rather low at least for certain species – depending on location and other conditions such as oxygen concentration, light frequencies, redox conditions, nutrient conditions, flux rates. For adequate FISH evaluation of cells, samples often need to be concentrated (e.g., by filtration) and confounding precipitates or debris must be removed (e.g., by cell extraction). Early studies demonstrated that standard FISH protocols may work well (Murray et al., 1998; Glöckner, 1999; Cottrell and Kirchman, 2000; Morris et al., 2004); however during the last decade, most studies have tried to increase probe signal intensities by modifying standard FISH protocols or applying other FISH tools like polynucleotide FISH (DeLong et al., 1999; Pernthaler et al., 2002; Preston et al., 2002) or CARD-FISH (Pernthaler et al., 2002a; b; Ishii et al., 2004; Teira et al., 2004; Herndl et al., 2005). In addition to this, various combinations with different activity targeted studies based on incubation with chloramphenicol (Ouverney and Fuhrman, 1997), radioactive substrates like thymidine (Pernthaler et al., 2002) or bromodeoxyuridine (BrdU, Pernthaler and Pernthaler, 2005), or other substrates such as amino acids



Fluorescence In Situ Hybridization (FISH), Figure 2 (Continued)



Fluorescence In Situ Hybridization (FISH), Figure 2 Demonstration of different images from different ecosystems produced by different FISH techniques.

(Ouverney and Fuhrman, 2000; Cottrell, 2000; Cottrell, 2003), have been undertaken.

Soil/sediments/subseafloor: Soil and sediments are rather difficult environments for successful FISH studies, as these habitats are extremely diverse, complex, and subject to harsh and fluctuating conditions in terms of, e.g., water content and redox conditions. Thus, more than most other ecosystems, the biosphere of microorganisms in soil is often characterized by micro-niches which may be exposed to dramatic changes with regard to space and time. In addition to this, many substances in soil or sediments produce strongly confounding background such as humic compounds, mineral precipitates or other microscopic particles of unknown character. For successful FISH experiments, two different main approaches have been made (for a review, see Schmid et al., 2006; Schmid et al., 2007): (1) Spatial separations of cells or micro-niches by approaches such as embedding and

sectioning of soil columns or by confocal laser scanning microscopy of soil aggregates, different cell extraction procedures such as density centrifugation or cation exchange membranes (McDonald, 1986; Smith and Stribley, 1994; Caracciolo et al., 2005; Kurola et al., 2005; Lunau et al., 2005; Bertaux et al., 2007), or flow cytometry (Kalyuzhnaya et al., 2006; Podar et al., 2007). (2) The second approach employed a variety of different FISH protocols. The early studies reported generally only partial success or even contradictory results between FISH and other analytical tools (Hahn et al., 1992; Ludwig et al., 1997; Zarda et al., 1997; Chatzinotas, 1998; Llobet-Brossa et al., 1998; Christensen, 1999; Hristova et al., 2000; Ravensschlag et al., 2000; Janvier et al., 2003; Kobabe et al., 2004; Rusch and Amend, 2004; Flies et al., 2005; Knittel et al., 2005). However, during the last years, most studies have now replaced the standard FISH tool with CARD-FISH and nanoSIMS (Ishii et al., 2004;

Mussmann et al., 2005; Schippers et al., 2005; Tal et al., 2005; Orphan 2009).

Karst regions/rock/deep biosphere: During the last decade, our understanding of the role of microorganisms and their interactions with rocks and minerals over geological timescales has expanded considerably. The interaction of minerals with water and microbes do not only influence soil fertility, transport compounds, and pollutants through the subsurface, but they also contribute to dissolution of rocks or precipitation of various mineral formations like speleothems in caves and may thus even play a key role in global climate change (Northup and Lavoie, 2001; Brown and Lee, 2007; Cockell and Herrera, 2008). Several attempts have been made to screen for microbial life in rocks and set up validated guidelines for this (Barton et al., 2001; Barton et al., 2006; Tobin et al., 1999). The reoccurring concern is to not only prove extant microbial processes (Orphan and House, 2009) but also current cellular-based in situ activities in often carbon-poor systems. Since it may be difficult to retrieve sufficient amounts of RNA or other activity biomarkers from these systems due to low cell counts and activities, FISH, in particular in combination with advanced tools like nanoSIMS, may play a crucial role for demonstrating whole cells with targetable genes (Orphan, 2009). Despite these obstacles, standard FISH as well as CARD-FISH protocols have been successfully applied on, e.g., microbial mats in association with water and energy-rich substances for life based on chemolithoautotrophy in rocky environments or caves (Engel et al., 2003; Macalady et al., 2006; Meisinger et al., 2007; Meisinger et al., 2010b). However, certain types of microbial mats may be strongly hampered by confounding background caused by various mineral precipitates (Engel et al., 2010; Koeberich, 2008). Rigid environments like calcified biofilms or speleothems thus demand sophisticated modifications of standard FISH protocols as well as CARD-FISH (e.g., Shiraishi et al., 2008).

Extremophiles in various geological environments: Extremophiles thrive in extreme ecosystems which are characterized by extreme physical or geochemical conditions. Such conditions may pose a particular challenge for FISH experiments. So far, FISH has only been practiced in certain types of extremophilic ecosystems with mixed success, e.g., in hot springs (Nubel et al., 2002; Simbahan et al., 2005; Nakagawa et al., 2006; Weidler et al., 2008), polar regions (Junge et al., 2004), halophilic systems (Rossello-Mora et al., 2003; Maturano et al., 2006), mines/bioleaching systems (Schrenk et al., 1998; Edwards et al., 1999; Bond et al., 2000; Takai, 2002; Baker et al., 2004), hydrothermal vents (Harmsen et al., 1997; Schrenk et al., 2003; Nakagawa et al., 2006), and methane hydrate seeps (Boetius and al, 2000).

Summary

FISH (fluorescence in situ hybridization) is a valuable tool for in situ identification of microbial community structure, cell morphology, dynamics and spatial distribution.

If combined with different analytical tools additional information of activity, function and interactions of different species on single cell level can be performed. However, for successful FISH experiments, it is crucial to optimize and validate all steps throughout the FISH protocol, ranging from proper sampling technique and fixation, selection of appropriate gene markers, hybridization conditions and relevant controls, to optimal detection and evaluation strategies.

Bibliography

- Ainsworth, T. D., Fine, M., Blackall, L. L., and Hoegh-Guldberg, O., 2006. Fluorescence *in situ* hybridization and spectral imaging of coral-associated bacterial communities. *Applied and Environmental Microbiology*, **72**, 3016–3020.
- Alm, E., Oerther, D., Larsen, N., Stahl, D., and Raskin, L., 1996. The oligonucleotide probe database. *Applied and Environmental Microbiology*, **62**, 3557–3559.
- Amann, R., and Fuchs, B. M., 2008. Single-cell identification in microbial communities by improved fluorescence *in situ* hybridization techniques. *Nature Reviews Microbiology*, **6**, 339–348.
- Amann, R., Ludwig, W., and Schleifer, K.-H., 1994. Identification of uncultured bacteria: a challenging task for molecular taxonomists. *ASM News*, **60**, 360–365.
- Amann, R., Ludwig, W., and Schleifer, K.-H., 1995. Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Amann, R., and Schleifer, K.-H., 2001. Nucleic acid probes and their application in environmental microbiology. In Garrity, G. M. (ed.), *Bergey's Manual of Systematic Bacteriology*. New York: Springer, 2nd edn. pp. 67–82.
- Amann, R., Snaird, J., Wagner, M., Ludwig, W., and Schleifer, K. H., 1996. *In situ* visualization of high genetic diversity in a natural microbial community. *Journal of Bacteriology*, **178**, 3496–3500.
- Amann, R. I., 1995. *In situ* identification of micro-organisms by whole cell hybridization with rRNA-targeted nucleic acid probes. In *Molecular Microbial Ecology Manual*. Vol. 3.3.6, pp. 1–15.
- Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R., and Stahl, D., 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Applied and Environmental Microbiology*, **56**, 1919–1925.
- Amann, R. I., Krumholz, L., and Stahl, D. A., 1990. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *Journal of Bacteriology*, **172**, 762–770.
- Amann, R. I., Zarda, B., Stahl, D. A., and Schleifer, K. H., 1992. Identification of individual prokaryotic cells by using enzyme-labeled, rRNA-targeted oligonucleotide probes. *Applied and Environmental Microbiology*, **58**, 3007–3011.
- Amaral-Zettler, L., Peplies, J., Ramette, A., Fuchs, B., Ludwig, W., and Glöckner, F. O., 2008. Proceedings of the international workshop on Ribosomal RNA technology, April 7–9, 2008, Bremen, Germany. *Systematic and Applied Microbiology*, **31**, 258–268.
- Anderson, R., 2010. Multiplex fluorescence in situ hybridization (M-FISH). *Methods Molecular Biology*, **659**, 83–97.
- Baker, B. J., Lutz, M. A., Dawson, S. C., Bond, P. L., and Banfield, J. F., 2004. Metabolically active eukaryotic communities in extremely acidic mine drainage. *Applied and Environmental Microbiology*, **70**, 6264–6271.

- Barton, H. A., Spear, J. R., and Pace, N. R., 2001. Microbial life in the underworld: biogenicity in secondary mineral formations. *Geomicrobiology Journal*, **18**, 359–368.
- Barton, H. A., Taylor, N. M., Lubbers, B. R., and Pemberton, A. C., 2006. DNA extraction from low-biomass carbonate rock: an improved method with reduced contamination and the low-biomass contaminant database. *Journal of Microbiological Methods*, **66**, 21–31.
- Baschien, C., Manz, W., Neu, T. R., and Szewzyk, U., 2001. I. Decomposition and microbial activities fluorescence *in situ* hybridization of freshwater fungi. *International Review of Hydrobiology*, **86**, 371–381.
- Behrens, S., Fuchs, B. M., and Amann, R., 2004. The effect of nucleobase-specific fluorescence quenching on *in situ* hybridization with rRNA-targeted oligonucleotide probes. *Systematic and Applied Microbiology*, **27**, 565–572.
- Behrens, S., Fuchs, B. M., Mueller, F., and Amann, R., 2003. Is the *in situ* accessibility of the 16S rRNA of *Escherichia coli* for Cy3-labeled oligonucleotide probes predicted by a three-dimensional structure model of the 30S ribosomal subunit? *Applied and Environmental Microbiology*, **69**, 4935–4941.
- Behrens, S., Losekann, T., Pett-Ridge, J., Weber, P. K., Ng, W.-O., Stevenson, B. S., Hutcheon, I. D., Relman, D. A., and Spormann, A. M., 2008. Linking microbial phylogeny to metabolic activity at the single-cell level by using enhanced element labeling-catalyzed reporter deposition fluorescence *in situ* hybridization (EL-FISH) and NanoSIMS. *Applied and Environmental Microbiology*, **74**, 3143–3150.
- Behrens, S., Rühland, C., Inácio, J., Huber, H., Fonseca, Á., Spencer-Martins, I., Fuchs, B. M., and Amann, R., 2003. *In situ* accessibility of small-subunit rRNA of members of the domains bacteria, archaea, and eucarya to Cy3-labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **69**, 1748–1758.
- Bentolila, I. A., and Weiss, S., 2006. Single-step multicolor fluorescence *in situ* hybridization using semiconductor quantum dot–DNA conjugates. *Cell Biochemistry and Biophysics*, **45**, 59–70.
- Bertaux, J., Gloger, U., Schmid, M., Hartmann, A., and Scheu, S., 2007. Routine fluorescence *in situ* hybridization in soil. *Journal of Microbiological Methods*, **69**, 451–460.
- Boetius, A., et al., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623.
- Bond, P. L., Druschel, G. K., and Banfield, J. F., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Bridger, J. M., and Volpi, E. V., 2010. *Fluorescence In Situ Hybridization. Methods in Molecular Biology*. Totowa: Humana Press, Vol. 659.
- Brown, D. J., and Lee, M., 2007. From microscopic minerals to global climate change? *Geology Today*, **23**, 172–177.
- Caracciolo, A. B., Grenni, P., Cupo, C., and Rossetti, S., 2005. *In situ* analysis of native microbial communities in complex samples with high particulate loads. *FEMS Microbiology Letters*, **253**, 55–58.
- Chatzinotas, A. S. R., Schönhuber, W., Amann, R., Daae, F. L., Torsvik, V., Zeyer, J., and Hahn, D., 1998. Analysis of broad-scale differences in microbial community composition of two pristine forest soils. *Systematic and Applied Microbiology*, **21**, 579–587.
- Christensen, H., Hansen, M., and Sørensen, J., 1999. Counting and size classification of active soil bacteria by fluorescence *in situ* hybridization with an rRNA oligonucleotide probe. *Applied and Environmental Microbiology*, **65**, 1753–1761.
- Cockell, C. S., and Herrera, A., 2008. Why are some microorganisms boring? *Trends in Microbiology*, **16**, 101–106.
- Coleman, J. R., Culley, D. E., Chrisler, W. B., and Brockman, F. J., 2007. mRNA-targeted fluorescent *in situ* hybridization (FISH) of gram-negative bacteria without template amplification or tyramide signal amplification. *Journal of Microbiological Methods*, **71**, 246–255.
- Cottrell, M. T., and Kirchman, D. L., 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnology and Oceanography*, **48**, 168–178.
- Cottrell, M. T., and Kirchman, D. L., 2000. Natural assemblages of marine proteobacteria and members of the cytophaga-flavobacter cluster consuming low- and high-molecular-weight dissolved organic matter. *Applied and Environmental Microbiology*, **66**, 1692–1697.
- Cottrell, M. T., and Kirchman, D. L., 2000. Community composition of marine bacterioplankton determined by 16S rRNA gene clone libraries and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **66**, 5116–5122.
- Currin, C. A., Pearl, H. W., Suba, G. K., and Alberte, R. S., 1990. Immunofluorescence detection and characterization of N₂-fixing microorganisms from aquatic environments. *Limnology and Oceanography*, **35**, 59–71.
- Daims, H., 2009. Use of fluorescence *in situ* hybridization and the daim image analysis program for the cultivation-independent quantification of microorganisms in environmental and medical samples. *Cold Spring Harbor Protocols*, pdb.prot5253.
- Daims, H., and Wagner, M., 2007. Quantification of uncultured microorganisms by fluorescence microscopy and digital image analysis. *Applied Microbiology and Biotechnology*, **75**, 237–248.
- Daims, H., Bruhl, A., Amann, R., Schleifer, K. H., and Wagner, M., 1999. The domain-specific probe EUB338 is insufficient for the detection of all Bacteria: development and evaluation of a more comprehensive probe set. *Systematic and Applied Microbiology*, **22**, 434–444.
- Daims, H., Ramsing, N. B., Schleifer, K.-H., and Wagner, M., 2001. Cultivation-independent, semiautomatic determination of absolute bacterial cell numbers in environmental samples by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **67**, 5810–5818.
- de los Reyes, F., Ritter, W., and Raskin, L., 1997. Group-specific small-subunit rRNA hybridization probes to characterize filamentous foaming in activated sludge systems. *Applied and Environmental Microbiology*, **63**, 1107–1117.
- DeLong, E. F., Taylor, L. T., Marsh, T. L., and Preston, C. M., 1999. Visualization and enumeration of marine planktonic archaea and bacteria by using polyribonucleotide probes and fluorescent *in situ* hybridization. *Applied and Environmental Microbiology*, **65**, 5554–5563.
- DeLong, E. F., Wickham, G. S., and Pace, N. R., 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. *Science*, **243**, 1360–1363.
- DeSantis, T. Z., Stone, C. E., Murray, S. R., Moberg, J. P., and Andersen, G. L., 2005. Rapid quantification and taxonomic classification of environmental DNA from both prokaryotic and eukaryotic origins using a microarray. *FEMS Microbiology Letters*, **245**, 271–278.
- Edwards, K. J., Gihring, T. M., and Banfield, J. F., 1999. Seasonal variations in microbial populations and environmental conditions in an extreme acid mine drainage environment. *Applied and Environmental Microbiology*, **65**, 3627–3632.
- Engel, A. S., Lee, N., Porter, M. L., Stern, L. A., Bennett, P. C., and Wagner, M., 2003. Filamentous “Epsilonproteobacteria” dominate microbial mats from sulfidic cave springs. *Applied and Environmental Microbiology*, **69**, 5503–5511.

- Engel, A. S., Meisinger, D. B., Porter, M. L., Payn, R. A., Schmid, M., Stern, L. A., Schleifer, K. H., and Lee, N. M., 2010. Linking phylogenetic and functional diversity to nutrient spiraling in microbial mats from Lower Kane Cave (USA). *The ISME Journal*, **4**, 98–110.
- Flies, C. B., Peplies, J., and Schuler, D., 2005. Combined approach for characterization of uncultivated magnetotactic bacteria from various aquatic environments. *Applied and Environmental Microbiology*, **71**, 2723–2731.
- Fried, J., and Foissner, W., 2007. Differentiation of two very similar glaucocid ciliate morphospecies (ciliophora, tetrahymenida) by fluorescence *in situ* hybridization with 18S rRNA targeted oligonucleotide probes. *Journal of Eukaryotic Microbiology*, **54**, 381–387.
- Fried, J., Ludwig, W., Psenner, R., and Schleifer, K. H., 2002. Improvement of ciliate identification and quantification: a new protocol for fluorescence *in situ* hybridization (FISH) in combination with silver stain techniques. *Systematic and Applied Microbiology*, **25**, 555–571.
- Fuchs, B., Wallner, G., Beisker, W., Schwiapl, I., Ludwig, W., and Amann, R., 1998. Flow cytometric analysis of the *in situ* accessibility of *Escherichia coli* 16S rRNA for fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **64**, 4973–4982.
- Fuchs, B. M., Glöckner, F. O., Wulf, J., and Amann, R., 2000. Unlabeled helper oligonucleotides increase the *in situ* accessibility to 16S rRNA of fluorescently labeled oligonucleotide probes. *Applied and Environmental Microbiology*, **66**, 3603–3607.
- Gerard, E., Guyot, F., Philippot, P., and Purificacion, L.-G., 2005. Fluorescence *in situ* hybridisation coupled to ultra small immunogold detection to identify prokaryotic cells using transmission and scanning electron microscopy. *Journal of Microbiological Methods*, **63**, 20–28.
- Giovannoni, S. J., DeLong, E. F., Olsen, G. J., and Pace, N. R., 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. *Journal of Bacteriology*, **170**, 720–726.
- Glöckner, F. O., Fuchs, B. M., and Amann, R., 1999. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **65**, 3721–3726.
- Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A., and Amann, R., 2000. Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Applied and Environmental Microbiology*, **66**, 5053–5065.
- Gmür, R., and Lüthi-Schaller, H., 2007. A combined immunofluorescence and fluorescent *in situ* hybridization assay for single cell analyses of dental plaque microorganisms. *Journal of Microbiological Methods*, **69**, 402–405.
- Hahn, D., Amann, R. I., Ludwig, W., Akkermans, A. D. L., and Schleifer, K. H., 1992. Detection of micro-organisms in soil after *in situ* hybridization with rRNA-targeted, fluorescently labelled oligonucleotides. *Journal of General Microbiology*, **138**, 879–887.
- Harmsen, H., Prieur, D., and Jeanthon, C., 1997. Group-specific 16S rRNA-targeted oligonucleotide probes to identify thermophilic bacteria in marine hydrothermal vents. *Applied and Environmental Microbiology*, **63**, 4061–4068.
- Hauer, K., Pavlekovic, M., Chee-Sanford, J., Sanford, R., A., Löffler, F. E., and Lee, N. M., *In situ* detection of functional genes by RING-FISH in *Anaeromyxobacter* sp. (in press)
- Herndl, G. J., Reinthaler, T., Teira, E., van Aken, H., Veth, C., Pernthaler, A., and Pernthaler, J., 2005. Contribution of archaea to total prokaryotic production in the deep Atlantic ocean. *Applied and Environmental Microbiology*, **71**, 2303–2309.
- Heydorn, A., Ersboll, B. K., Hentzer, M., Parsek, M. R., Givskov, M., and Molin, S., 2000. Experimental reproducibility in flow-chamber biofilms. *Microbiology*, **146**, 2409–2415.
- Hodson, R. E., Dustman, W. A., Garg, R. P., and Moran, M. A., 1995. *In situ* PCR for visualization of microscale distribution of specific genes and gene products in prokaryotic communities. *Applied and Environmental Microbiology*, **61**, 4074–4082.
- Hoshino, T., Yilmaz, L. S., Noguera, D. R., Daims, H., and Wagner, M., 2008. Quantification of target molecules needed to detect microorganisms by fluorescence *in situ* hybridization (FISH) and catalyzed reporter deposition-FISH. *Applied and Environmental Microbiology*, **74**, 5068–5077.
- Hoshino, T., and Schramm, A., 2010. Detection of denitrification genes by *in situ* rolling circle amplification-fluorescence *in situ* hybridization to link metabolic potential with identity inside bacterial cells. *Environmental Microbiology*, **12**, 2508–2517.
- Hristova, K. R., Mau, M., Zheng, D., Aminov, R. I., Mackie, R. I., Gaskins, H. R., and Raskin, L., 2000. Desulfotomaculum genus- and subgenus-specific 16S rRNA hybridization probes for environmental studies. *Environmental Microbiology*, **2**, 143–159.
- Huang, J., Ma, L., Sundararajan, S., Fei, S.-z., and Li, L., 2009. Visualization by atomic force microscopy and FISH of the 45S rDNA gaps in mitotic chromosomes of *Lolium perenne*. *Protoplasma*, **236**, 59–65.
- Hugenholtz, P., Tyson, G. W., and Blackall, L. L., 2001. Design and evaluation of 16S rRNA-targeted oligonucleotide probes for fluorescence *in situ* hybridization. In *Gene Probes: Principles and Protocols*. Vol. 176. London: Humana Press.
- Ishii, K., Mu[ss]mann, M., MacGregor, B. J., and Amann, R., 2004. An improved fluorescence *in situ* hybridization protocol for the identification of bacteria and archaea in marine sediments. *FEMS Microbiology Ecology*, **50**, 203–212.
- Janvier, M., Regnault, B., and Grimont, P., 2003. Development and use of fluorescent 16S rRNA-targeted probes for the specific detection of *Methylophaga* species by *in situ* hybridization in marine sediments. *Research in Microbiology*, **154**, 483–490.
- Junge, K., Eicken, H., and Deming, J. W., 2004. Bacterial activity at -2 to -20°C in Arctic wintertime sea ice. *Applied and Environmental Microbiology*, **70**, 550–557.
- Kalmbach, S., Manz, W., and Szwezyk, U., 1997. Dynamics of biofilm formation in drinking water: phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and *in situ* hybridization. *FEMS Microbiology Ecology*, **22**, 265–279.
- Kalyuzhnaya, M. G., Zabinsky, R., Bowerman, S., Baker, D. R., Lidstrom, M. E., and Chistoserdova, L., 2006. Fluorescence *in situ* hybridization-flow cytometry-cell sorting-based method for separation and enrichment of type I and type II methanotroph populations. *Applied and Environmental Microbiology*, **72**, 4293–4301.
- Kenzaka, T., Ishidoshiro, A., Yamaguchi, N., Tani, K., and Nasu, M., 2005. rRNA sequence-based scanning electron microscopic detection of bacteria. *Applied and Environmental Microbiology*, **71**, 5523–5531.
- Kenzaka, T., Tamaki, S., Yamaguchi, N., Tani, K., and Nasu, M., 2005. Recognition of individual genes in diverse microorganisms by cycling primed *in situ* amplification. *Applied and Environmental Microbiology*, **71**, 7236–7244.
- Knittel, K., Losekann, T., Boetius, A., Kort, R., and Amann, R., 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology*, **71**, 467–479.
- Kobabe, S., Wagner, D., and Pfeiffer, E.-M., 2004. Characterisation of microbial community composition of a Siberian tundra soil by fluorescence *in situ* hybridisation. *FEMS Microbiology Ecology*, **50**, 13–23.

- Koebberich, M., 2008. Methodenevaluierung zur fluoreszenz in situ hybridisierung des eisenoxidierenden bakteriums *Gallionella ferruginea*. Bachelor of science thesis of Georg August University of Göttingen, Germany. (Supervisors: J. Reitner and N. V. Queric, Geobiology Center).
- Konstantinidis, K. T., Serres, M. H., Romine, M. F., Rodrigues, J. L. M., Auchtung, J., McCue, L.-A., Lipton, M. S., Obraztsova, A., Giometti, C. S., Nealson, K. H., Fredrickson, J. K., and Tiedje, J. M., 2009. Comparative systems biology across an evolutionary gradient within the *Shewanella* genus. *Proceedings of the National Academy of Sciences*, **106**, 15909–15914.
- Kubota, K., Ohashi, A., Imachi, H., and Harada, H., 2006a. Improved *in situ* hybridization efficiency with locked-nucleic-acid-incorporated DNA probes. *Applied and Environmental Microbiology*, **72**, 5311–5317.
- Kubota, K., Ohashi, A., Imachi, H., and Harada, H., 2006b. Visualization of *mcr* mRNA in a methanogen by fluorescence *in situ* hybridization with an oligonucleotide probe and two-pass tyramide signal amplification (two-pass TSA-FISH). *Journal of Microbiological Methods*, **66**, 521–528.
- Kumar, Y., Westram, R., Behrens, S., Fuchs, B., Glockner, F., Amann, R., Meier, H., and Ludwig, W., 2005. Graphical representation of ribosomal RNA probe accessibility data using ARB software package. *BMC Bioinformatics*, **6**, 61.
- Kumar, Y., Westram, R., Kipfer, P., Meier, H., and Ludwig, W., 2006. Evaluation of sequence alignments and oligonucleotide probes with respect to three-dimensional structure of ribosomal RNA using ARB software package. *BMC Bioinformatics*, **7**, 240.
- Kurola, J., Wittmann, C., Salkinoja-Salonen, M., Aarnio, T., and Romantschuk, M., 2005. Application of cation-exchange membranes for characterisation and imaging ammonia-oxidising bacteria in soils. *FEMS Microbiology Ecology*, **53**, 463–472.
- Lim, E., Amaral, L., Caron, D., and DeLong, E., 1993. Application of rRNA-based probes for observing marine nanoplanktonic protists. *Applied and Environmental Microbiology*, **59**, 1647–1655.
- Llobet-Brossa, E., Rossello-Mora, R., and Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **64**, 2691–2696.
- Loy, A., Arnold, R., Tischler, P., Rattei, T., Wagner, M., and Horn, M., 2008. Probecheck – a central resource for evaluating oligonucleotide probe coverage and specificity. *Environmental Microbiology*, **10**, 2894–2898.
- Loy, A., Beisker, W., and Meier, H., 2005. Diversity of bacteria growing in natural mineral water after bottling. *Applied and Environmental Microbiology*, **71**, 3624–3632.
- Loy, A., and Bodrossy, L., 2006. Highly parallel microbial diagnostics using oligonucleotide microarrays. *Clinica Chimica Acta (From Real-Time PCR to Nanotechnology: Rapid and/or High-Throughput Diagnostic Methods for Nucleic Acids)*, **363**, 106–119.
- Loy, A., Lehner, A., Lee, N., Adamczyk, J., Meier, H., Ernst, J., Schleifer, K.-H., and Wagner, M., 2002. Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. *Applied and Environmental Microbiology*, **68**, 5064–5081.
- Loy, A., Maixner, F., Wagner, M., and Horn, M., 2007. Probebase – an online resource for rRNA-targeted oligonucleotide probes: new features 2007. *Nucleic Acids Research*, **35**, D800–04.
- Lubeck, P. S., Hansen, M., and Sorensen, J., 2000. Simultaneous detection of the establishment of seed-inoculated *Pseudomonas fluorescens* strain DR54 and native soil bacteria on sugar beet root surfaces using fluorescence antibody and *in situ* hybridization techniques. *FEMS Microbiology Ecology*, **33**, 11–19.
- Ludwig, W., Bauer, S. H., Bauer, M., Held, I., Kirchhof, G., Schulze, R., Huber, I., Spring, S., Hartmann, A., and Schleifer, K. H., 1997. Detection and *in situ* identification of representatives of a widely distributed new bacterial phylum. *FEMS Microbiology Letters*, **153**, 181–190.
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M., and Schleifer, K. H., 1998. Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis*, **19**, 554–568.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Forster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lussmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K.-H., 2004. ARB: a software environment for sequence data. *Nucleic Acids Research*, **32**, 1363–1371.
- Lunau, M., Lemke, A., Walther, K., Martens-Habbena, W., and Simon, M., 2005. An improved method for counting bacteria from sediments and turbid environments by epifluorescence microscopy. *Environmental Microbiology*, **7**, 961–968.
- Macalady, J. L., Lyon, E. H., Koffman, B., Albertson, L. K., Meyer, K., Galdenzi, S., and Mariani, S., 2006. Dominant microbial populations in limestone-corroding stream biofilms, Frasassi Cave System, Italy. *Applied and Environmental Microbiology*, **72**, 5596–5609.
- Manz, W., Amann, R., Ludwig, W., Wagner, M., and Schleifer, K.-H., 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: problems and solutions. *Systematic and Applied Microbiology*, **15**, 593–600.
- Maruyama, F., Kanzaka, T., Yamaguchi, N., Tani, K., and Nasu, M., 2003. Detection of bacteria carrying the *stx2* gene by *in situ* loop-mediated isothermal amplification. *Applied and Environmental Microbiology*, **69**, 5023–5028.
- Maruyama, F., Kanzaka, T., Yamaguchi, N., Tani, K., and Nasu, M., 2005. Visualization and enumeration of bacteria carrying a specific gene sequence by *in situ* rolling circle amplification. *Applied and Environmental Microbiology*, **71**, 7933–7940.
- Maturrano, L., Santos, F., Rossello-Mora, R., and Anton, J., 2006. Microbial diversity in Maras Salterns, a hypersaline environment in the Peruvian Andes. *Applied and Environmental Microbiology*, **72**, 3887–3895.
- McDonald, R. M., 1986. Sampling soil microfloras: dispersion of soil by ion exchange and extraction of specific micro organisms form suspension by elutriation. *Soil Biology. Biochemistry*, **18**, 399–406.
- Meisinger, D. B., Pavlekovic, M., Löffler, F. E., and Lee, N. M., 2010a. Comparison of FISH and PCR applications on contaminated groundwater. (in press).
- Meisinger, D. B., Spring, S., Schmid, M., Löffler, F. E., Porter, M. L., Engel, A. S., and Lee, N. M., 2010b. Novel *Chloroflexi* lineages of subclasses I and VI in chemolithoautotrophic microbial mats in the sulphidic Lower Kane Cave, USA. (in press).
- Meisinger, D. B., Zimmermann, J., Ludwig, W., Schleifer, K.-H., Wanner, G., Schmid, M., Stern, L. A., Bennett, P. C., Engel, A. S., and Lee, N., 2007. *In situ* detection of novel *Acidobacteria* in microbial mats from a chemolithoautotrophically-based cave ecosystem (Lower Kane Cave, WY, USA). *Environmental Microbiology*, **9**, 1523–1534.
- Metfies, K., and Medlin, L. K., 2008. Feasibility of transferring fluorescent *in situ* hybridization probes to an 18S rRNA gene phylochip and mapping of signal intensities. *Applied and Environmental Microbiology*, **74**, 2814–2821.
- Molin, S., and Givskov, M., 1999. Application of molecular tools for *in situ* monitoring of bacterial growth activity. *Environmental Microbiology*, **1**, 383–391.

- Moraru, C., Lam, P., Fuchs, B. H., Kuypers, M. M. M., and Amann, R., 2010. GeneFISH – an *in situ* technique for linking gene presence and cell identity in environmental microorganisms. *Environmental Microbiology*, doi:10.1111/j.1462-2920.2010.02281.x
- Morris, R. M., Rappe, M. S., Connon, S. A., Vergin, K. L., Siebold, W. A., Carlson, C. A., and Giovannoni, S. J., 2002. SAR11 clade dominates ocean surface bacterioplankton communities. *Nature*, **420**, 806–810.
- Morris, R. M., Rappe, M. S., Urbach, E., Connon, S. A., and Giovannoni, S. J., 2004. Prevalence of the chloroflexi-related SAR202 bacterioplankton cluster throughout the Mesopelagic zone and deep ocean. *Applied and Environmental Microbiology*, **70**, 2836–2842.
- Moter, A., Schmiedel, J., Petrich, A., Schmiedel, D., Mallmann, C., Musci, M., Hetzer, R., and Göbel, U., 2010. In situ measurement of biofilm activity: implications for diagnosis of infective endocarditis. In Poster presentation at *ISME 13 International Symposium on Microbial Ecology Microbes – Stewards of a Changing Planet*, Seattle, WA, USA, 22–27 August 2010. Abstract Category 31 Methodological Developments.
- Murray, A. E., Preston, C. M., Massana, R., Taylor, L. T., Blakis, A., Wu, K., and DeLong, E. F., 1998. Seasonal and spatial variability of bacterial and archaeal assemblages in the coastal waters near Anvers Island, Antarctica. *Applied and Environmental Microbiology*, **64**, 2585–2595.
- Musmann, M., Ishii, K., Rabus, R., and Amann, R., 2005. Diversity and vertical distribution of cultured and uncultured Deltaproteobacteria in an intertidal mud flat of the Wadden Sea. *Environmental Microbiology*, **7**, 405–418.
- Nakagawa, T., Takai, K., Suzuki, Y., Hirayama, H., Konno, U., Tsunogai, U., and Horikoshi, K., 2006. Geomicrobiological exploration and characterization of a novel deep-sea hydrothermal system at the TOTO caldera in the Mariana Volcanic Arc. *Environmental Microbiology*, **8**, 37–49.
- Nakamura, K., Terada, T., Sekiguchi, Y., Shinzato, N., Meng, X.-Y., Enoki, M., and Kamagata, Y., 2006. Application of pseudomurein endoisopeptidase to fluorescence *in situ* hybridization of methanogens within the family methanobacteriaceae. *Applied and Environmental Microbiology*, **72**, 6907–6913.
- Nielsen, J. L., Aquino de Muro, M., and Nielsen, P. H., 2003. Evaluation of the redox dye 5-cyano-2,3-tolyl-tetrazolium chloride for activity studies by simultaneous use of microautoradiography and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **69**, 641–643.
- Nielsen, J. L., Klausen, C., Nielsen, P. H., Burford, M., and Jorgensen, N. O. G., 2006. Detection of activity among uncultured Actinobacteria in a drinking water reservoir. *FEMS Microbiology Ecology*, **55**, 432–438.
- Nielsen, J. L., and Nielsen, P. H., 2005. Advantages in microscopy: microautoradiography of single cells. In Leadbetter, J. R. (ed.), *Methods in Enzymology*. San Diego: Elsevier Academic Press, Vol. 397, pp. 237–56.
- Northup, D. E., and Lavoie, K. H., 2001. Geomicrobiology of caves: a review. *Geomicrobiology Journal*, **18**, 199–222.
- Nubel, U., Bateson, M. M., Vandieken, V., Wieland, A., Kuhl, M., and Ward, D. M., 2002. Microscopic examination of distribution and phenotypic properties of phylogenetically diverse chloroflexaceae-related bacteria in hot spring microbial mats. *Applied and Environmental Microbiology*, **68**, 4593–4603.
- Nielsen, P. H., Daims, H., and Lemmer, H., 2009. *FISH handbook for Biological Wastewater Treatment*. London: IWA Publishing.
- Oerther, D. B., Pernthaler, J., Schramm, A., Amann, R., and Raskin, L., 2000. Monitoring precursor 16S rRNAs of acinetobacter spp. in activated sludge wastewater treatment systems. *Applied and Environmental Microbiology*, **66**, 2154–2165.
- Orphan, V. J., 2009. Methods for unveiling cryptic microbial partnerships in nature. *Current Opinion in Microbiology*, **12**, 231–237.
- Orphan, V. J., and House, C. H., 2009. Geobiological investigations using secondary ion mass spectrometry: microanalysis of extant and paleo-microbial processes. *Geobiology*, **7**, 360–372.
- Ouverney, C., and Fuhrman, J., 1997. Increase in fluorescence intensity of 16S rRNA *in situ* hybridization in natural samples treated with chloramphenicol. *Applied and Environmental Microbiology*, **63**, 2735–2740.
- Ouverney, C. C., and Fuhrman, J. A., 2000. Marine planktonic archaea take up amino acids. *Applied and Environmental Microbiology*, **66**, 4829–4833.
- Rodriguez-castaño, G. P., 2008. *Sequence diversity and expression of novel bacterial nitrous oxide reductase (nosZ) genes in tropical environments*. Master of Science Thesis in Biology. Mayagüez Campus, USA, University of Puerto Rico.
- Pavlekovic, M., Schmid, M. C., Schmider-Poignee, N., Spring, S., Pilhofer, M., Gaul, T., Fiandaca, M., Löffler, F. E., Jetten, M., Schleifer, K.-H., and Lee, N. M., 2009. Optimization of three FISH procedures for *in situ* detection of anaerobic ammonium oxidizing bacteria in biological wastewater treatment. *Journal of Microbiological Methods*, **78**, 119–126.
- Peplies, J., Glockner, F. O., Amann, R., and Ludwig, W., 2004. Comparative sequence analysis and oligonucleotide probe design based on 23S rRNA genes of alphaproteobacteria from North Sea Bacterioplankton. *Systematic and Applied Microbiology*, **27**, 573–580.
- Pernthaler, A., and Amann, R., 2004. Simultaneous fluorescence *in situ* hybridization of mRNA and rRNA in environmental bacteria. *Applied and Environmental Microbiology*, **70**, 5426–5433.
- Pernthaler, A., and Pernthaler, J., 2005. Diurnal variation of cell proliferation in three bacterial taxa from coastal North Sea waters. *Applied and Environmental Microbiology*, **71**, 4638–4644.
- Pernthaler, J., Glockner, F.-O., Unterholzner, S., Alfreider, A., Psenner, R., and Amann, R., 1998. Seasonal community and population dynamics of pelagic bacteria and archaea in a high mountain lake. *Applied and Environmental Microbiology*, **64**, 4299–4306.
- Pernthaler, A., Pernthaler, J., and Amann, R., 2002a. Fluorescence *in situ* hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Applied and Environmental Microbiology*, **68**, 3094–3101.
- Pernthaler, A., Pernthaler, J., Schattnerhofer, M., and Amann, R., 2002b. Identification of DNA-synthesizing bacterial cells in coastal North Sea Plankton. *Applied and Environmental Microbiology*, **68**, 5728–5736.
- Pernthaler, A., Preston, C. M., Pernthaler, J., DeLong, E. F., and Amann, R., 2002c. Comparison of fluorescently labeled oligonucleotide and polynucleotide probes for the detection of pelagic marine bacteria and archaea. *Applied and Environmental Microbiology*, **68**, 661–667.
- Perry-O'Keefe, H., Stender, H., Broomer, A., Oliveira, K., Coull, J., and Hyldig-Nielsen, J. J., 2001. Filter-based PNA *in situ* hybridization for rapid detection, identification and enumeration of specific micro-organisms. *Journal of Applied Microbiology*, **90**, 180–189.
- Petroni, G., Rosati, G., Vannini, C., Modeo, L., Dini, F., and Verni, F., 2003. *In Situ* Identification by fluorescently labeled oligonucleotide probes of morphologically similar, closely related ciliate species. *Microbial Ecology*, **45**, 156–162.
- Pilhofer, M., Pavlekovic, M., Lee, N. M., Ludwig, W., and Schleifer, K.-H., 2009. Fluorescence *in situ* hybridization for intracellular localization of nifH mRNA. *Systematic and Applied Microbiology*, **32**, 186–192.
- Podar, M., Abulencia, C. B., Walcher, M., Hutchison, D., Zengler, K., Garcia, J. A., Holland, T., Cotton, D., Hauser, L.,

- and Keller, M., 2007. Targeted access to the genomes of low-abundance organisms in complex microbial communities. *Applied and Environmental Microbiology*, **73**, 3205–3214.
- Polerecky, L., Bissett, A., Al-Najjar, M., Faerber, P., Osmer, H., Suci, P. A., Stoodley, P., and de Beer, D., 2009. Modular spectral imaging system for discrimination of pigments in cells and microbial communities. *Applied and Environmental Microbiology*, **75**, 758–771.
- Pratscher, J., Stichternoth, C., Fichtl, K., Schleifer, K.-H., and Braker, G., 2009. Application of recognition of individual genes-fluorescence *in situ* hybridization (RING-FISH) to detect nitrite reductase genes (*nirK*) of denitrifiers in pure cultures and environmental samples. *Applied and Environmental Microbiology*, **75**, 802–810.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J., and Glockner, F. O., 2007. SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Research*, **35**, 7188–7196.
- Ravenschlag, K., Sahm, K., Knoblauch, C., Jorgensen, B. B., and Amann, R., 2000. Community structure, cellular rRNA content, and activity of sulfate-reducing bacteria in marine arctic sediments. *Applied and Environmental Microbiology*, **66**, 3592–3602.
- Rossello-Mora, R., Lee, N., Anton, J., and Wagner, M., 2003. Substrate uptake in extremely halophilic microbial communities revealed by microautoradiography and fluorescence *in situ* hybridization. *Extremophiles*, **7**, 409–413.
- Rossetti, S., Tomei, M. C., Blackall, L. L., and Tandoi, V., 2007. Bacterial growth kinetics estimation by fluorescence *in situ* hybridization and spectrofluorometric quantification. *Letters in Applied Microbiology*, **44**, 643–648.
- Rusch, A., and Amend, J. P., 2004. Order-specific 16S rRNA-targeted oligonucleotide probes for (hyper)thermophilic archaea and bacteria. *Extremophiles*, **8**, 357–366.
- Sando, S., and Kool, E. T., 2002. Imaging of RNA in bacteria with self-ligating quenched probes. *Journal of the American Chemical Society*, **124**, 9686–9687.
- Schatz, M. C., Phillippy, A. M., Gajer, P., DeSantis, T. Z., Andersen, G. L., Ravel, J., 2010. Integrated microbial survey analysis of prokaryotic communities for the PhyloChip microarray. *Applied and Environmental Microbiology*, **76**, 5636–5638.
- Schippers, A., Neretin, L. N., Kallmeyer, J., Ferdelman, T. G., Cragg, B. A., John Parkes, R., and Jorgensen, B. B., 2005. Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. *Nature*, **433**, 861–864.
- Schmid, M., Schmitz-Esser, S., Jetten, M., and Wagner, M., 2001. 16S-23S rDNA intergenic spacer and 23S rDNA of anaerobic ammonium-oxidizing bacteria: implications for phylogeny and *in situ* detection. *Environmental Microbiology*, **3**, 450–459.
- Schmid, M., Selesi, D., Rothballer, M., Schlöter, M., Hartmann, A., Lee, N., and Kandeler, E., 2006. Localization and visualization of microbial community structure and activity in soil microhabitats. In König, H., and Varma, A., (ed.), *Soil Biology*, Berlin Heidelberg: Springer, Vol. 6.
- Schmid, M., Rothballer, M., and Hartmann, A., 2007. Analysis of microbial communities in soil microhabitats using fluorescence *in situ* hybridization. In van Elsas, J. D., Jansson, J. K., and Trevors, J. (eds.), *Modern Soil Microbiology*, 2nd edn. Boca Raton: CRC-Press, Chap. 12, pp. 317–335.
- Schonhuber, W., Le Bourhis, G., Tremblay, J., Amann, R., and Kulakauskas, S., 2001. Utilization of tmRNA sequences for bacterial identification. *BMC Microbiology*, **1**, 20.
- Schramm, A., Fuchs, B. M., Nielsen, J. L., Tonolla, M., and Stahl, D. A., 2002. Fluorescence *in situ* hybridization of 16S rRNA gene clones (Clone-FISH) for probe validation and screening of clone libraries. *Environmental Microbiology*, **4**, 713–720.
- Schrenk, M. O., Edwards, K. J., Goodman, R. M., Hamers, R. J., and Banfield, J. F., 1998. Distribution of thiobacillus ferrooxidans and Leptospirillum ferrooxidans: implications for generation of acid mine drainage. *Science*, **279**, 1519–1522.
- Schrenk, M. O., Kelley, D. S., Delaney, J. R., and Baross, J. A., 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology*, **69**, 3580–3592.
- Sekar, R., Pernthaler, A., Pernthaler, J., Warnecke, F., Posch, T., and Amann, R., 2003. An improved protocol for quantification of freshwater actinobacteria by fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **69**, 2928–2935.
- Shiraishi, F., Zippel, B., Neu, T. R., and Arp, G., 2008. *In situ* detection of bacteria in calcified biofilms using FISH and CARD-FISH. *Journal of Microbiological Methods*, **75**, 103–108.
- Simbahan, J., Kurth, E., Schelert, J., Dillman, A., Moriyama, E., Jovanovich, S., and Blum, P., 2005. Community analysis of a mercury hot spring supports occurrence of domain-specific forms of mercuric reductase. *Applied and Environmental Microbiology*, **71**, 8836–8845.
- Smith, N. C., and Stribley, D. P., 1994. A new approach to direct extraction of microorganisms from soil. In Dighton, K. R. J., and Giller, K. E. (ed.), *Beyond the Biomass*. Chichester: British Society of Soil Science (BSSS). A Wiley-Sayce Publication, Vol. 1194.
- Smolina, I., Lee, C., and Frank-Kamenetskii, M., 2007. Detection of low-copy-number genomic DNA sequences in individual bacterial cells by using peptide nucleic acid assisted rolling-circle amplification and fluorescence *in situ* hybridization. *Applied and Environmental Microbiology*, **73**, 2324–2328.
- Stahl, D. A., and Amann, R., 2001. Development and application of nucleic acid probes. In Stackebrandt, E., and Goodfellow, M. (ed.), *Nucleic Acid Techniques in Bacterial Systematics*. Chichester: Wiley.
- Stoecker, K., Dorninger, C., Daims, H., and Michael, W., 2010. Double-labeling of oligonucleotide probes for fluorescence *in situ* hybridization (DOPE-FISH) improves signal intensity and increases rRNA accessibility. *Applied and Environmental Microbiology*, **76**(3), 922–926.
- Stoffels, M., Ludwig, W., and Schleifer, K. H., 1999. rRNA probe-based cell fishing of bacteria. *Environmental Microbiology*, **1**, 259–271.
- Sunamura, M., and Maruyama, A., 2006. A digital imaging procedure for seven-probe-labeling FISH (Rainbow-FISH) and its application to estuarine microbial communities. *FEMS Microbiology Ecology*, **55**, 159–166.
- Tal, Y., Watts, J. E. M., and Schreier, H. J., 2005. Anaerobic ammonia-oxidizing bacteria and related activity in Baltimore Inner Harbor Sediment. *Applied and Environmental Microbiology*, **71**, 1816–1821.
- Tani, K., Kurokawa, K., and Nasu, M., 1998. Development of a direct *in situ* PCR method for detection of specific bacteria in natural environments. *Applied and Environmental Microbiology*, **64**, 1536–1540.
- Taylor, M. W., Loy, A., and Wagner, M., 2010. Microarrays for studying the composition and function of microbial communities. In Seviour Robert, J., and Nielsen, P.H. (ed.), *The Microbiology of Activated Sludge*. London: IWA Publishing.
- Teira, E., Reinthaler, T., Pernthaler, A., Pernthaler, J., and Herndl, G. J., 2004. Combining catalyzed reporter deposition-fluorescence *in situ* hybridization and microautoradiography to detect substrate utilization by bacteria and archaea in the deep ocean. *Applied and Environmental Microbiology*, **70**, 4411–4414.
- Tobin, K. J., Onstott, T. C., DeFlaun, M. F., Colwell, F. S., and Fredrickson, J., 1999. *In situ* imaging of microorganisms in geologic material. *Journal of Microbiological Methods*, **37**, 201–213.

- Trebesius, K., Amann, R., Ludwig, W., Muhlegger, K., and Schleifer, K., 1994. Identification of whole fixed bacterial cells with nonradioactive 23S rRNA-targeted polynucleotide probes. *Applied and Environmental Microbiology*, **60**, 3228–3235.
- Tsuchiya, D., and Taga, M., 2001., Application of fibre-FISH (fluorescence *in situ* hybridization) to filamentous fungi: visualization of the rRNA gene cluster of the ascomycete *Cochliobolus heterostrophus*. *Microbiology*, **147**, 1183–1187.
- Vesey, G., Deere, D., Gauci, M. R., Griffiths, K. R., Williams, K. L., and Veal, D. A., 1997. Evaluation of fluorochromes and excitation sources for immunofluorescence in water samples. *Cytometry*, **29**, 147–154.
- Wagner, M., 2009. Single-cell ecophysiology of microbes as revealed by Raman microspectroscopy or secondary ion mass spectrometry imaging. *Annual Review of Microbiology*, **63**, 411–429.
- Wagner, M., Erhardt, R., Manz, W., Amann, R., Lemmer, H., Wedi, D., and Schleifer, K.-H., 1994. Development of an rRNA-targeted oligonucleotide probe specific for the genus *acinetobacter* and its application for *in situ* monitoring in activated sludge. *Applied and Environmental Microbiology*, **60**, 792–800.
- Wagner, M., Horn, M., and Daims, H., 2003. Fluorescence *in situ* hybridisation for the identification and characterisation of prokaryotes. *Current Opinion in Microbiology*, **6**, 302–309.
- Wagner, M., Hutzler, P., and Amann, R., 1998. 3-D Analysis of complex microbial communities by combining confocal laser scanning microscopy and fluorescence *in situ* hybridization (FISH). In Wilkinson, M. H. F. and Schut, F. (ed.), *Digital Image Analysis of Microbes*. Chichester: Wiley, pp. 467–486.
- Wagner, M., Nielsen, P. H., Loy, A., Nielsen, J. L., and Daims, H., 2006. Linking microbial community structure with function: fluorescence *in situ* hybridization-microautoradiography and isotope arrays. In van der Meer, J. R., and Murrel, J. C. (eds.), *Current Opinion in Biotechnology, Analytical biotechnology*, **17**, 83–91.
- Wagner, M., Rath, G., Amann, R., Koops, H.-P., and Schleifer, K.-H., 1995. *In situ* identification of ammonia-oxidizing bacteria. *Systematic Applied Microbiology*, **18**, 251–264.
- Wagner, M., Schmid, M., Juretschko, S., Trebesius, K.-H., Bubert, A., Goebel, W., and Schleifer, K.-H., 1998. *In situ* detection of a virulence factor mRNA and 16S rRNA in *Listeria monocytogenes*. *FEMS Microbiology Letters*, **160**, 159–168.
- Wallner, G., Amann, R., and Beisker, W., 1993. Optimizing fluorescent *in situ* hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. *Cytometry*, **14**, 136–143.
- Watanabe, K., Watanabe, K., Kodama, Y., Sytsubo, K., and Harayama, S., 2000. Molecular characterization of bacterial populations in petroleum-contaminated groundwater discharged from underground crude oil storage cavities. *Applied and Environmental Microbiology*, **66**, 4803–4809.
- Weber, S. D., Ludwig, W., Schleifer, K.-H., and Fried, J., 2007. Microbial composition and structure of aerobic granular sewage biofilms. *Applied and Environmental Microbiology*, **73**, 6233–6240.
- Wendeberg, A., 2010. Fluorescence *in situ* hybridization for the identification of environmental microbes. *Cold Spring Harbor Protocols*, doi:10.1101/pdb.prot5366.
- Weidler, G. W., Gerbl, F. W., and Stan-Lotter, H., 2008. Crenarchaeota and their role in the nitrogen cycle in a subsurface radioactive thermal spring in the Austrian Central Alps. *Applied and Environmental Microbiology*, **74**: 5934–5942.
- Wilhartitz, I., Mach, R. L., Teira, E., Reinthaler, T., Herndl, G. J., and Farnleitner, A. H., 2007. Prokaryotic community analysis with CARD-FISH in comparison with FISH in ultra-oligotrophic ground- and drinking water. *Journal of Applied Microbiology*, **103**, 871–881.
- Xia, Y., Kong, Y., and Nielsen, P. H., 2007. *In situ* detection of protein-hydrolysing microorganisms in activated sludge. *FEMS Microbiology Ecology*, **60**, 156–165.
- Yarza, P., Richter, M., Peplies, J., Euzéby, J., Amann, R., Schleifer, K.-H., Ludwig, W., Glöckner, F. O., and Rosselló-Móra, R., 2008. The all-species living tree project: a 16S rRNA-based phylogenetic tree of all sequenced type strains. *Systematic and Applied Microbiology*, **31**, 241–250.
- Yilmaz, L. S., Okten, H. E., and Noguera, D. R., 2006. Making all parts of the 16S rRNA of *Escherichia coli* accessible *in situ* to single DNA Oligonucleotides. *Applied and Environmental Microbiology*, **72**, 733–744.
- Yilmaz, L., Parnerkar, S., and Noguera, D., 2010a. Introducing mathfish: a website that runs mathematical models of fluorescent *in situ* hybridization for thermodynamics-based probe design. In Poster presentation at *ISME 13 International Symposium on Microbial Ecology Microbes - Stewards of a Changing Planet*, Seattle, WA, USA, 22–27 August 2010. Abstract Category 31 Methodological Developments.
- Yilmaz, S., Haroon, M., Rabkin, B., Tyson, G., and Hugenholtz, P., 2010b. Fixation-free fluorescence *in situ* hybridization for targeted enrichment of microbial populations. *The ISME Journal*, doi:10.1038/ismej.2010.73.
- Zarda, B., Amann, R., Wallner, G., and Schleifer, K.-H., 1991. Identification of single bacterial cells using digoxigenin-labelled, rRNA-targeted oligonucleotides. *Journal of General Microbiology*, **137**, 2823–2830.
- Zarda, B., Hahn, D., Chatzinotas, A., Schönhuber, W., Neef, A., Amann, R. I., and Zeyer, J., 1997. Analysis of bacterial community structure in bulk soil by *in situ* hybridization. *Archives of Microbiology*, **168**, 185–192.
- Zhang, D., Wu, J., Ye, F., Feng, T., Lee, I., and Yin, B., 2006. Amplification of circularizable probes for the detection of target nucleic acids and proteins. *Clinica Chimica Acta (From Real-Time PCR to Nanotechnology: Rapid and/or High-Throughput Diagnostic Methods for Nucleic Acids)*, **363**, 61–70.
- Zhou, Z., Pons, M. N., Raskin, L., and Zilles, J. L., 2007. Automated image analysis for quantitative fluorescence *in situ* hybridization with environmental samples. *Applied and Environmental Microbiology*, **73**, 2956–2962.
- Zimmermann, J., Ludwig, W., and Schleifer, K. H., 2001. DNA polynucleotide probes generated from representatives of the genus *acinetobacter* and their application in fluorescence *in situ* hybridization of environmental samples. *Systematic and Applied Microbiology*, **24**, 238–244.
- Zwirgmaier, K., Ludwig, W., and Schleifer, K.-H., 2004a. Improved method for polynucleotide probe-based cell sorting, using DNA-coated microplates. *Applied and Environmental Microbiology*, **70**, 494–497.
- Zwirgmaier, K., Ludwig, W., and Schleifer, K.-H., 2004b. Recognition of individual genes in a single bacterial cell by fluorescence *in situ* hybridization - RING-FISH. *Molecular Microbiology*, **51**, 89–96.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Basalt \(Glass, Endoliths\)](#)
[Biogeochemical Cycles](#)
[Biomarkers \(Molecular Fossils\)](#)
[Immunolocalization](#)
[Isotopes and Geobiology](#)
[Karst Ecosystems](#)
[Metagenomics](#)
[Microbial Communities, Structure, and Function](#)

Microbial Ecology of Submarine Caves
 Protozoa (Heterotroph, Eukaryotic)
 RNA World
 Scanning Probe Microscopy
 Speleothems
 Terrestrial Deep Biosphere

FORAMINIFERA

Alexander V. Altenbach
 Ludwig-Maximilians-University Munich, Munich,
 Germany

Synonyms

Forams (for short)

Definition

Foraminifera (from ancient Greek “hole bearers”, refers to the pores that early observers recognized in the calcitic tests) are amoeboid, eukaryotic protists with a large network of very thin cytoplasm extrusions (reticulopodia). Most species produce a genetically fixed shell (test), made of organic matter, agglutinated particles, calcite, aragonite, high-magnesium calcite, or opaline silica. The tests may be either simple bowls or tubes, or branched, coiled, substructured, and ornamented in diverse complexity. Several thousand species are found living in modern oceans, and this number still increases. Several times more extinct species are recovered from the fossil record. Foraminifera are considered an order (sometimes a class) in paleontology, subdivided by the structure and composition of modern and fossil tests. They are ranked among the “Granuloreticulosa” in biology, characterized by (1) their branching and anastomosing networks of reticulopodia, (2) a rapid bidirectional transport of intracellular granules and membrane domains driven by specific tubulin assemblies, and (3) a complex alternation of sexual and asexual reproductive cycles. Genetic investigations led to the definition of the first rank group “Foraminifera” within the Supergroup “Rhizaria”. Independent from such rankings, they range among the most common, diverse, and ubiquitous groups of single celled, test building eukaryotes on earth.

Introduction

Numerous foraminiferal species indicate specific environmental conditions, and the first and last appearance of extinct species exactly indicates time intervals in earth history. Investigations on the chemical and isotopical composition of their calcitic tests reveal environmental drifts in global ice volumes, palaeo-temperatures and -salinities of marine water masses, and marine palaeo-productivity (Hemleben et al., 1989; Murray, 2006). Despite the long term and extensive attention on foraminifera in

taxonomy (Loeblich and Tappan, 1988) and environmental sciences (Sen Gupta, 1999), our comprehensions on their evolution and ecophysiology are still in transition. Molecular ecology and metagenomics applied to the study of microbial biodiversity are changing our view of the biosphere. An impressive extension of foraminiferal clades has been uncovered by such molecular tools: naked freshwater amoebians, naked marine giant amoebians, and giant deep-sea protists formerly considered of unknown origin, where added to the first rank group Foraminifera (Adl et al., 2005; Pawlowski and Burki, 2009). Even amoeboid parasites of terrestrial plants are supposed to reach out from this lineage (Archibald and Keeling, 2004). Efforts to couple the growing phylogenetic diversity of single-celled organisms with ecological function lead to the discovery of novel metabolisms, processes, and symbiosis, and thus to a reevaluation of the geobiochemical impact of pro- and eukaryotes (Moya et al., 2008).

Earth history

The earliest fossils presumed for the foraminiferal lineage reach back into the Mesoproterozoic, despite severe problems in defining even Ediacaran remainders (Dong et al., 2008). The Cambrian provides already several foraminiferal families with organic-walled or agglutinated tests (Benton, 1993). A significant eukaryote radiation occurs in the Ordovician, coincident with increasing nutrient supply, enhanced primary production, and the propagation of planktonic metazoan stages. Such amplified planktonic food chains sponsored increased flux rates of organic matter at the sea floor, and thus the radiation of planktotrophy (Nützel et al., 2006). The calcitic fusulinids start in the Silurian (Loeblich and Tappan, 1988), and extend into the first rhizarian, rock forming mass deposition of biogenic shallow water carbonates, from the Pennsylvanian to the Permian. The fusulinid clade develops all principal morphological adaptations known from modern benthic foraminifera sheltering phototrophic symbionts, thus these mass deposits should result from host–symbiont interactions (Anderson and Lee, 1991). The structure of modern marine primary production was based on the evolution of diatoms, coccoliths, and modern dinoflagellates in the Triassic to Jurassic (Falkowski et al., 2004). Restructuring and enhancing marine primary productivity to an elevated level is recorded by the boost of rotaliid foraminifera, modern metazoans, and increasing bioturbation (Martin et al., 2008). One tribe of calcitic foraminifera, the globigerinids, completely adapted to this new planktonic food chain, and started to enter a purely pelagic live style in the Middle Jurassic. The boost of rock forming biogenic carbonate (chalk) and silica (diatomite) production from these new planktonic systems, however, was delayed to the Cretaceous in the marine realm, and to the Eocene for lacustrine diatomites. The first major radiation of planktonic foraminifera occurred during the mid-Cretaceous. The density structure of the upper water

column and the formation of deeper water masses were altered by plate tectonic activities, a major long-term rise of global sea level, and an overall increase of global temperatures (Leckie, 1989; Martin et al., 2008). Following the massive mass extinction of planktonic foraminifera at the K/T boundary, symbiosis with phototrophic endosymbionts was one key factor for the radiation of Cenozoic lineages (Norris, 1996). The diversity of benthic foraminifera, and thus presumably their ecological demands and adaptations, were influenced more modestly. The first appearance of foraminiferal tests build up by opaline silica occurs in the Miocene, by the benthic foraminiferal suborder Silicoloculinina (Loeblich and Tappan, 1988). Enhanced terrestrial silica weathering and riverine silica input by grassland expansion offers a causally driven correlation, similar to marine diatom diversification (Falkowski et al., 2004).

Modern oceans

Planktonic foraminifera are most influential for the open marine calcite budget. Their annual flux rate of calcitic tests at 100 m water depth accounts for 1.3–3.2 Gt, equivalent to 23–56% of the global, open marine CaCO_3 flux. During most of the year, dissolution takes place within the upper water masses (>700 m water depth), and only 1–3% of the initially exported CaCO_3 reaches the seafloor. This biogeochemical pump is most influential for the alkalinity of surface and intermediate water masses. During short pulsed flux events, mass dumps of fast settling particles yield the major contribution to the formation of deep sea sediments. This pathway transfers 0.4–0.9 Gt CaCO_3 annually, accounting for 32–80% of the total deep-marine calcite budget (Schiebel, 2002). Maximum carbonate production appears in open ocean realms where symbiotic and non-symbiotic planktonic foraminifera occur syntopic (Zaric et al., 2006). The contribution of coccolithophores (mean 12%) and calcareous dinophytes (0–3.5%) is comparably small (Schiebel, 2002; Sarmiento et al., 2002). Planktic gastropods (pteropods) may add another 10% only locally (Schiebel, 2002). Phototrophic endosymbionts play a major role in the carbonate production of planktic foraminifera. Such endosymbionts are the key factor for the foraminiferal successes in nutrient depleted environments, and for their enhanced turnover (Hemleben et al., 1989; Lee, 2006).

One sixth of the global carbonate production is shared by reef complexes, dominated by symbiont-bearing corals, foraminifera, and coralline algae. With an annual production of 130 million tons of CaCO_3 , benthic foraminifera bearing phototrophic symbionts contribute nearly 5% of the annual present-day carbonate production in the world's reef and upper shelf areas (Langer, 2008). Marine benthic foraminifera without phototrophic symbionts are much more diverse, inhabiting all ocean realms from estuaries to deep ocean trenches. But their share in marine biogenic carbonate production ranges only near 1% (Langer, 2008). This is due to the rapidly decreasing

flux rate and nutritional value of organic matter reaching the deeper ocean floors (Altenbach and Struck, 2001). Deep-sea foraminifera have a metabolic turnover comparable to bacteria (Linke, 1992; Moodley et al., 2002), and their grazing rate exceeds that of common meiofaunal groups (Pascal et al., 2008). They have a considerable share in abyssal respiration rates (Witte et al., 2003), but this does not sum up to a significant impact in biogenic carbonate production.

Complete denitrification was observed as an endogenic metabolic pathway for benthic foraminifera in laboratory cultures (Risgaard-Petersen et al., 2006). The species facultatively switch to denitrification when high loads of organic matter are available in oxygen-depleted environments. Field observations in the upwelling region off Chile revealed that benthic denitrification is dominated by foraminifera, and not by bacteria (Høgslund et al., 2008). In view of the large benthic foraminiferal populations recovered from other oxygen minimum zones, and the large uncertainties in the global nitrogen budget (Gruber and Galloway, 2008), these findings may challenge our considerations on marine denitrification cycles (Høgslund et al., 2008). Several species related to denitrification acquire chloroplasts from settled algae in the deep ocean, which are kept functional in the foraminifers cytoplasm for at least one year (Grzymski et al., 2002). Such kleptoplasts produce hydroxyl radicals, which offer an alternative source of oxygen for the hosts metabolism by H_2O_2 breakdown (Bernhard and Bowser, 2008). Sulfur-oxidizing bacterial endosymbionts were considered for benthic foraminifera recovered from long-term sulfidic environments in the deep sea (Bernhard et al., 2006) and from temporal anoxic shelf areas (Bernhard, 2003). However, the definition of oxic or anoxic conditions is a problem by itself near the redox cline, as the interwoven, submillimetric structures of differing redox conditions build up a foamy, three dimensional space. Contrasting microbial turnover takes place in smallest pore water compartments. The interlacement may be so dense that foraminiferal inhabitants can not be attributed to either oxic or anoxic conditions (Bernhard et al., 2003).

Xenophyophores and Komokiaceans are very common deep-sea foraminifera with a comparatively large and loosely agglutinated test. They may cover up to 50% of hadal seafloors (Lemche et al., 1976). Early research on the role of Xenophyophores and Komokiaceans for biogenic metal dissolution and precipitation considered a significant impact for the formation of manganese nodules (Riemann, 1985). The microscopic barite crystals, commonly found in the endoplasm of Xenophyophores (Gooday and Nott, 1982), were considered a proof for their endogenic barite precipitation (Bertram and Cowen, 1997). However, these rhizarians ingest considerable amounts of highly refractory organic matter and produce large amounts of waste pellets (stercomata), offering an attractive food source for deep ocean microbial communities (Nozawa et al., 2006). Gardening of a specialized

bacterial flora growing on the stercomes of Xenophyophores was made plausible by Laureillard et al. (2004). Such affiliated microbial communities will move the redox state by degrading the stercomata, a factor that should interplay with the observed metal reactions.

Gathering refractory material that is transferred to higher levels of the food chain by bacterial counterparts is highly effective. Parallel to the increasing number of bacteria available as food, it can enhance the metabolic pathways of the host. Limited endogenous proteolytic capacities of benthic organisms are surpassed by enzyme sharing with bacteria, in most cases combined with the production of an agglutinated detrital lump or burrow (Riemann and Helmke, 2002). Benthic foraminifera from the earliest tribes to most recent lineages retain the ability to rapidly produce such detrital lumps or cysts, in addition to the genetically fixed test (Heinz et al., 2005). Most researchers make reasonable tropical adaptations and bacterial gardening for the common production of such cysts (Heinz et al., 2005).

Conclusions

The general outline of foraminiferal ecology and their overall impact on the marine food chains and calcite budgets is well documented and understood. Recently discovered, endogenic denitrification offers a facultative metabolic pathway for benthic foraminifera with potential impact for the marine nitrogen cycle. Additional complexity in synecology and ecophysiology is also indicated by observations on chemotrophic bacterial endosymbionts and bacterial gardening, not fully understood at present. Chemotrophic symbionts and functional kleptoplasts seem to play a key role for anoxic and sulfidic microenvironments. We may presume that foraminiferal interactions with bacteria play a more significant role in geobiology and biogeochemical cycles than has previously been suspected.

Bibliography

- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., Mccourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *The Journal of Eukaryotic Microbiology*, **52**, 399–451.
- Altenbach, A. V., and Struck, U., 2001. On the coherence of organic carbon flux and benthic foraminiferal biomass. *Journal of Foraminiferal Research*, **31**, 79–85.
- Anderson, O. R., and Lee, J. J., 1991. Symbiosis in foraminifera. In Lee, J. J., and Anderson, O. R. (eds.), *Biology of Foraminifera*. London: Academic Press, pp. 157–220.
- Archibald, J. M., and Keeling, P. J., 2004. Actin and ubiquitin protein sequences support a Cercocoan/Foraminiferan ancestry for the Plasmodiophorid plant pathogenes. *Journal of Eukaryotic Microbiology*, **51**, 113–118.
- Benton, M. J., 1993. *The Fossil Record 2*. London: Chapman & Hall.
- Bernhard, J. M., 2003. Potential symbionts in bathyal foraminifera. *Science*, **299**, 861.
- Bernhard, J. M., and Bowser, S. S., 2008. Peroxisome proliferation in foraminifera inhabiting the chemocline: An adaptation to reactive oxygen species exposure? *Journal of Eukaryotic Microbiology*, **55**, 135–144.
- Bernhard, J. M., Visscher, P. T., and Bowser, S. S., 2003. Submillimeter life positions of bacteria, protists, and metazoans in laminated sediments of the Santa Barbara Basin. *Limnology and Oceanography*, **48**, 813–828.
- Bernhard, J. M., Habura, A., and Bowser, S. S., 2006. An endobiont-bearing allogromiid from the Santa Barbara Basin: implications for the early diversification of foraminifera. *Journal of Geophysical Research*, **111**, G03002, DOI:10.1029/2005JG000158.
- Bertram, M. A., and Cowen, J. P., 1997. Morphological and compositional evidence for biotic precipitation of marine barite. *Journal of Marine Research*, **55**, 577–593.
- Dong, L., Xiao, S., Shen, B., and Zhou, C., 2008. Silicified Horodyskia and Palaeopascichnus from upper Ediacaran cherts in South China: tentative phylogenetic interpretation and implications for evolutionary stasis. *Journal of the Geological Society U. K.*, **165**, 367–378.
- Falkowski, P. G., Katz, M. E., Knoll, A. H., Quigg, A., Raven, J. A., Schofield, O., and Taylor, F. J. R., 2004. The evolution of modern eukaryotic phytoplankton. *Science*, **305**, 354–360.
- Gooday, A. J., and Nott, A. J., 1982. Intracellular barite crystals in two Xenophyophores, *Aschemonella ramuliformis* and *Galathea minima* sp. (Protozoa, Rhizopoda) with comments on the taxonomy of *A. ramuliformis*. *Journal of the Marine Biological Association U. K.*, **62**, 595–605.
- Gruber, N., and Galloway, J. N., 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*, **451**, 293–296.
- Grzymski, J., Schofield, O. M., Falkowski, P. G., and Bernhard, J. M., 2002. The function of plastids in the deep-sea benthic foraminifer, *Nonionella stella*. *Limnology and Oceanography*, **47**, 1569–1580.
- Heinz, P., Geslin, E., and Hemleben, C., 2005. Laboratory observations of benthic foraminiferal cysts. *Marine Biology Research*, **1**, 149–159.
- Hemleben, C., Spindler, M., and Anderson, O. R., 1989. *Modern Planktonic Foraminifera*. New York: Springer.
- Høgslund, S., Revsbech, N., Cedhagen, T., Nielsen, L., and Gallardo, V., 2008. Denitrification, nitrate turnover, and aerobic respiration by benthic foraminiferans in the oxygen minimum zone off Chile. *Journal of Experimental Marine Biology and Ecology*, **359**, 85–91.
- Langer, M. R., 2008. Assessing the contribution of foraminiferan protists to global ocean carbonate production. *Journal of Eukaryotic Microbiology*, **55**, 163–169.
- Laureillard, J., Mejanelle, L., and Sibuet, M., 2004. Use of lipids to study the trophic ecology of deep-sea xenophyophores. *Marine Ecology Progress Series*, **270**, 129–140.
- Leckie, R. M., 1989. A paleoceanographic model for the early evolution history of planktonic foraminifera. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **73**, 107–138.
- Lee, J. J., 2006. Algal symbiosis in larger Foraminifera. *Symbiosis*, **42**, 63–75.
- Lemche, H., Hansen, B., Madsen, E. J., Tendal, O. S., and Wolf, T., 1976. Hadal life as analyzed from photographs. *Videnskabelige meddelelser fra Dansk naturhistorisk forening*, **139**, 263–336.
- Linke, P., 1992. Metabolic adaptations of deep-sea benthic foraminifera to seasonally varying food input. *Marine Ecology Progress Series*, **81**, 51–63.

- Loeblich, A. R., and Tappan, H., 1988. *Foraminiferal Genera and their Classification*. New York: Van Nostrand Reinhold. [year of publication often cited erroneously as 1987; see Loeblich, A. R., and Tappan, H., 1989. Publication date of foraminiferal genera and their classification. *Journal of Paleontology*, **63**, 253.]
- Martin, R. E., Quigg, A., and Podkovyrov, V., 2008. Marine Biodiversification in response to evolving phytoplankton stoichiometry. *Palaeogeography Palaeoclimatology Palaeoecology*, **258**, 277–291.
- Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M. J., and Heip, C. H. R., 2002. Bacteria and foraminifera: key players in a short-term deep-sea response to phytodetritus. *Marine Ecology Progress Series*, **236**, 23–29.
- Moya, A., Pereto, J., Gil, R., and Latorre, A., 2008. Learning how to live together: genomic insight into prokaryote-animal symbiosis. *Nature Reviews Genetics*, **9**, 218–229.
- Murray, J. W., 2006. *Ecology and Applications of Benthic Foraminifera*. Cambridge: Cambridge University Press.
- Norris, R. D., 1996. Symbiosis as an evolutionary innovation in the radiation of Paleocene planktic foraminifera. *Paleobiology*, **22**, 461–480.
- Nozawa, F., Kitazato, H., Tsuchiya, M., and Gooday, A. J., 2006. 'Live' benthic foraminifera at a abyssal site in the equatorial Pacific nodule province: abundance, diversity and taxonomic composition. *Deep-Sea Research I*, **53**, 1406–1422.
- Nützel, A., Lehnert, O., and Fryda, J., 2006. Origin of planktotrophy - evidence from early molluscs. *Evolution and Development*, **8**, 325–330.
- Pascal, P., Dupuy, C., Mallet, C., Richard, P., and Niquil, N., 2008. Bacterivory in benthic organisms in sediment: quantification using ¹⁵N-enriched bacteria. *Journal of Experimental Biology and Ecology*, **355**, 18–26.
- Pawlowski, J., and Burki, F., 2009. Untangling the phylogeny of amoeboid protists. *Journal of Eukaryotic Microbiology*, **56**(1), 16–25.
- Riemann, F., 1985. Eisen und Mangan in pazifischen Tiefsee-Rhizopoden und Beziehungen zur Manganknollen-Genese. *Internationale Revue der gesamten Hydrobiologie*, **70**(1), 165–172.
- Riemann, F., and Helmke, E., 2002. Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: the enzyme sharing concept. *Marine Ecology*, **23**, 93–113.
- Risgaard-Petersen, N., Langezaal, A. M., Ingvarsdén, S., Schmid, M. C., Jetten, M. S. M., Op den Camp, H. J. M., Derksen, J. W. M., Pina-Ochoa, E., Eriksson, S. P., Nielsen, L. P., Revsbech, N. P., Cedhagen, T., and van der Zwaan, G. J., 2006. Evidence for complete denitrification in a benthic foraminifer. *Nature*, **443**, 93–96.
- Sarmiento, J. L., Dunne, J., Gnanadesikan, A., Key, R. M., Matsumoto, K., and Slater, R., 2002. A new estimate of the CaCO₃ to organic carbon export ratio. *Global Biogeochemical Cycles*, **16**(4), no 1107. doi:10.1029/2002/GB001919.
- Schiebel, R., 2002. Planktic foraminiferal sedimentation and the marine calcite budget. *Global Biogeochemical Cycles*, **16**(4), no 1065, doi:10.1029/2001GB001459.
- Sen Gupta, B., 1999. *Modern Foraminifera*. Dordrecht: Kluwer Academic Press.
- Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P. N. A., Sand, M., Cremer, A., Abraham, W. B. J. B., and Olaf, P., 2003. In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, **424**, 763–766.
- Zaric, S., Schulz, M., and Mulitza, S., 2006. Global prediction of planktonic foraminiferal fluxes from hydrographic and productivity data. *Biogeosciences*, **3**, 187–207.

Cross-references

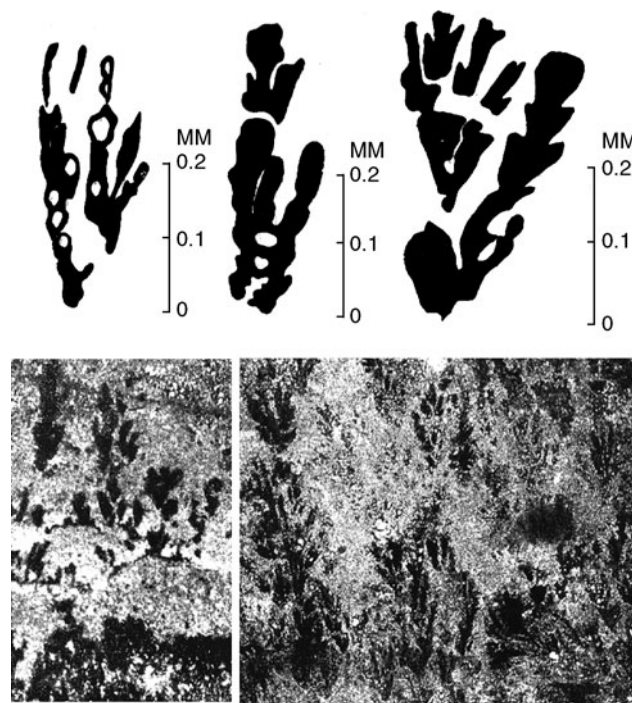
Algae (Eukaryotic)
 Bioerosion
 Carbonate Environments
 Carbonates
 Chemolithotrophy
 Divalent Earth Alkaline Cations in Seawater
 Isotopes and Geobiology
 Protozoa (Heterotroph, Eukaryotic)
 Symbiosis

FRUTEXITES

Marta Rodríguez-Martínez¹, Christine Heim²,
 Nadia-Valérie Quéric², Joachim Reitner²
¹Universidad de Alcalá, Alcalá de Henares, Madrid, Spain
²University of Göttingen, Göttingen, Germany

Synonyms

Colloform limonitic crusts; Frutexites crusts; Frutexites-like forms; Frutexites microstromatolite; Frutexites tuffs; Haematitic/ferruginous/iron microstromatolites; Iron dendritic aggregates; Iron shrubs; Pillar-shaped microstromatolites



Frutexites, Figure 1 Original figures of genus of *Frutexites* described by Maslov (1960).

Definition

Frutexites is a problematic microfossil rich in iron. From a taxonomic point of view, only five species have been figured (*Frutexites arboriformis*, Maslov, 1960; *F. microstroma*, Walter and Awramik, 1979; *Frutexites* sp. 1, *Frutexites* sp. 2, and *New gen.* 3, Tsien, 1979), although the authors mostly use the term *Frutexites sensu lato*. The genus *Frutexites* was coined by Maslov (1960) in order to describe submillimeter-sized, iron-rich, and subordinate calcite microfossils (Figure 1). *Frutexites* have a dendritic shape formed by divergently branched microcolumns. The height and width of microcolumns as well as their composition and microstructure can vary (Table 1). The preservation of microstructure is strongly controlled by its dominant mono- or polymineral

character. Microstructure is formed by convex-upward laminae, which sometimes show radially arranged fibers.

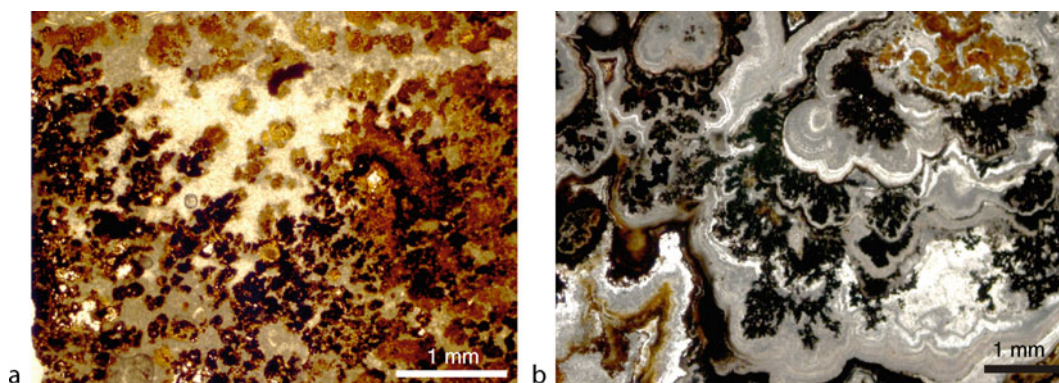
Frutexites can occur mainly as monomineral as well as polymineral structures with: iron-rich and/or manganese-rich (hematites, iron hydroxides, Fe-Mn oxyhydroxides), and/or carbonate-rich (calcite, ferroan calcite, dolomite), and/or siliciclastic-rich (argillite, microquartz), and/or phosphatic-rich zones.

Depositional environmental occurrences

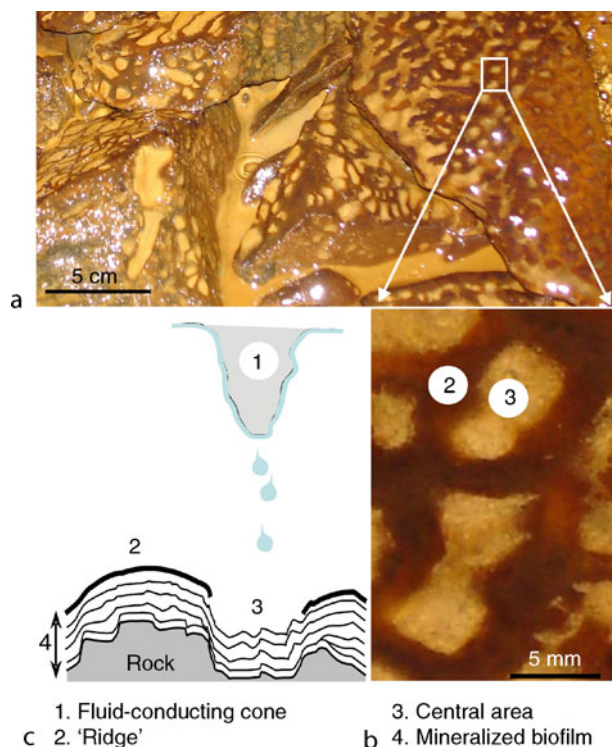
Frutexites has been described in marine environments such as shallow and deep water stromatolites, microbial limestones, hardgrounds, and condensed pelagic limestones as well as in cavities, sheet-cracks, veins, and Neptunian dikes. However, comparisons with

Frutexites, Table 1 Sizes, morphological and compositional parameters of *Frutexites* according to different authors

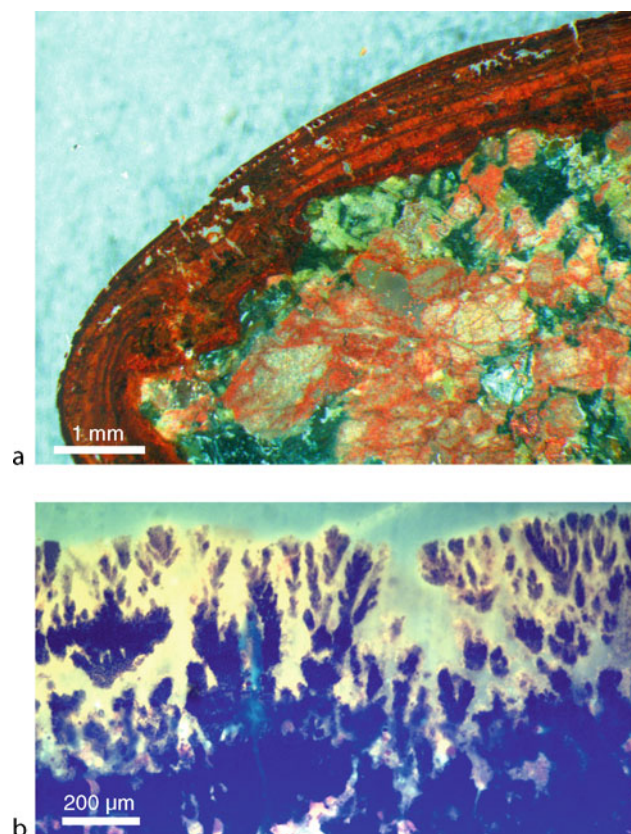
Authors	Height	Width	Microstructure	Morphology	Composition
Maslov (1960)	Up to 400 μm (see Figure 1)	25–30 μm , more of 50 μm	Sheets with occasionally circular spaces	Radially diverging and branched sheets	Iron hydroxide and carbonate
Horodyski (1975)	20–500 μm	10–200 μm	2–10 μm convex-upward laminae	Pillar-shaped branched and not	Hematite, calcite, and argillite
Walter and Awramik (1979)	Up to 450 μm	5–120 μm	0.7–2.7 μm convex upward laminae and axial tube (trichome?)	Undulose layers, laminae with protruding pustules and erect branching microcolumns	Organic matter permineralized by silica
Myrow and Coniglio (1991)	250 μm –4 mm, most <1 mm	75–600 μm , 250 μm average	Chambers, laminae, fibers, and projections	Unbranching and branching columns	Calcite, ferroan calcite, hematite, and microquartz
Böhm and Brachert (1993)	Up to 5 mm, average 1–2 mm		Convex laminae with radially arranged fibers		Fe-Mn oxides, calcite, and phosphates
Woods and Baud (2008)	Up to 800 μm	50–200 μm	Lighter and darker colored layers resulting chambered appearance		Hematite and/or Fe-Mn minerals, and calcite



Frutexites, Figure 2 (a) Marine Upper Cenomanian/Lower Turonian *Frutexites* colonies from a deepwater hardground environment (Lienres coast, northern Spain; Reitner et al., 1995). The *Frutexites* facies is located on top of the hardground sequence and marks a fundamental change of the oceanographic conditions. (b) Deep subterranean *Frutexites* colonies growing on calcareous microstromatolites from tectonic fractures in the so-called “sole dolomite” at the base of the Naukluft Nappe Complex (NNC) in southern Namibia. The colonies are growing upside down from the fracture ceiling. The black ones are rich in manganese oxides, the ochre parts are enriched in iron oxides.



Frutexites, Figure 3 (a) Net-like, mineralized microbial mat covering the surface of granitic rocks (Åspö Hard Rock Laboratory, Sweden). (b) Scheme of the Åspö-dropping microsystem, comprising a pending mineral cone as a fluid-conduit (1) and the evolving, net-like microstructure beneath, displaying a dark mineralized ridge (2) covering the laminated biofilm (3) and the central area directly exposed to the fluid dropping (4). (c) Close-up of the net-like, mineralized microbial mat, pointing to the dark mineralized ridge (2) and the bright central area (4) according to scheme b.



Frutexites, Figure 4 (a) Thick section of the mineralized, net-like, recent biofilm from the tunnel of Åspö (Sweden) under reflected light, revealing the typical laminated structure. These laminae predominantly consist of iron oxides and hydroxides. (b) UV-fluorescence microphotography exhibits *Frutexites*-like colonies within the laminae of the mineralized biofilm.

continental black shrubs are common, too. *Frutexites* structures are also frequently found in veins and fractures of deep subterranean environments (Figures 2b, 3, and 4).

Stromatolites

The main record of *Frutexites* has commonly been recognized from shallow to deep calcareous stromatolites from Proterozoic to recent examples (see Caldera lake stromatolites at Tonga, by Kazmierczak and Kempe, 2006). *Frutexites* structures grow upward perpendicular to the stromatolite laminae; however, they can sometimes cross them (Horodyski, 1975) and even destroy them. For this reason, Böhm and Brachert (1993) interpreted that stromatolite accretion was independent of *Frutexites*, which would act as dweller or secondary binder in the Jurassic deep water stromatolites from Germany and Austria. Descriptions about the associated macro- and micro-fauna in the *Frutexites*-bearing stromatolites are not

abundant. In the Canning Basin, the late Devonian *Frutexites*-bearing stromatolites show abundant meta-zoans in life position as crinoids and coral holdfasts encrusting the successive laminae (Playford et al., 1976), which would indicate normal oxygenated water conditions (Nicoll and Playford, 1993). In Jurassic stromatolites, *Frutexites* occur with shells of *Bositra* and *Lenticulina* foraminifers.

Cavities, sheet cracks, veins, and Neptunian dikes

The second most frequent occurrence of *Frutexites* in the geologic record is related to cavity walls and fissures. On the horizontal surfaces *Frutexites* could display a dominant upward growth, but, in general, their growth is normally perpendicular to the substrate where they were nucleated. *Frutexites* occur interbedded with fibrous calcite cements as well as with internal sediment. The oldest described record of *Frutexites* is in the Paleoproterozoic Gunflint chert (Walter and Awramik, 1979) and from sheet

cracks within stromatolites of the Upper Vendian to Lower Cambrian Chapel Island Formation, Canada (Myrow and Coniglio, 1991). *Frutexites* have been found in cavities of Devonian deep water mud mounds and grouped with *Renalcis-Epiphyton* calcimicrobes, and with deep water stromatolites assemblage (Tsien, 1979) as well as in stromatactoid cavities from Viséan microbial limestones where they occur interbedded with marine isopachous crusts of fibrous and botryoidal calcite cements (Gischler, 1996). The presence of *Frutexites* in Neptunian dikes has been described only in Devonian records from the Harz Mountains in Germany (Gischler, 1996) and mud mounds of the Hamar Laghdad Ridge in Morocco (Cavalazzi et al., 2007). Similar structures as *Frutexites* have also been described in voids from Toarcian Mn-rich layer at Tatra Mountains in Poland (Jach and Dudek, 2005) as well as in synsedimentary karstic cavities in Pleistocene travertines in Germany (Koban and Schweigert, 1993).

Condensed pelagic limestones and hardgrounds

The last most extended occurrence of *Frutexites* is associated with condensed hemipelagic and pelagic red to grey limestones like Griotte and Hallstatt Limestone as well as Rosso Ammonitico facies from Devonian up to Jurassic. These facies occur during very low sedimentation rates, from deep to relatively shallow water depths, during fast transgressive and/or drowning episodes. Nektonic organisms are the dominant fauna (goniatites, nautiloids, ammonoids) and *Frutexites* occur in (a) water-sediment interfaces colonizing sessile fauna, reworked bioclasts as well as ferromanganese hardgrounds (see below) and (b) within the micritic sediment. The first type of *Frutexites* growth in condensed pelagic limestones was described in Matagne Formation (Devonian of Belgium) by Tsien (1979) and in current-swept shallow pelagic ridge (Tafilalt Platform, Devonian of Morocco) by Wendt (1988). No less representative examples have been shown from the Triassic Hallstatt facies of Austria (Wendt, 1969; Rodríguez-Martínez et al., in press) and Oman Mountains (Woods and Baud, 2008). In the Oman Mountains, the sea-floor was directly colonized by *Frutexites*-bearing microbialites and synsedimentary cements. However, in the Northern Calcareous Alps, multiple ferromanganese crusts were colonized by epibenthonic sessile agglutinated foraminifers which were successively encrusted by *Frutexites* forming pillar-like structures above the seawater-sediment interface.

A similar situation has been described from deep-water hardgrounds during Mid-Cretaceous times in Spain (Reitner et al., 1995) (Figure 2). In this case, a previous benthic community dominated by coralline sponges was replaced by thick limonitic stromatolites with encrusting foraminifera and colonies of *Frutexites*.

The growth of *Frutexites* within the sediment was firstly pointed out by Böhm and Brachert (1993). They described the changes in composition and shapes of

Frutexites as a result of their growth in open spaces or in interstitial environments. Mamet and Pr  at (2006) found *Frutexites* associated to other hematite microstructures in condensed Griotte facies (Coumiac Limestones, Montagne Noire, Baleas Limestone Spain) and Rosso Ammonitico Limestone (Subbetic Cordillera Spain).

Continental environments

Some authors (Myrow and Coniglio, 1991; B  hm and Brachert, 1993) have compared the marine records of *Frutexites* with similar arborescent, dendritic forms found in speleothems, desert varnish, and travertines. Also, continental manganese and iron-rich black shrubs have been compared with the marine *Frutexites* records (Koban and Schweigert, 1993; Chafetz et al., 1998). Shapes, sizes, and polymineral character are similar in both marine and continental records.

Subterranean environments

Frutexites structures are sometimes common in light-independent, deep continental caves, fractures, and veins forming small microbial crusts often associated with calcareous stromatolitic structures. A characteristic representative was found by J. Reitner in southern Namibia (Figure 2b) at the base of the Naukluft Nappe Complex (NNC), which is part of the early Cambrian Damara orogeny (Miller, 2008). The deep base of the NNC is formed by the so-called "Sole Dolomite" (Korn and Martin, 1959) which is often heavily fractured. Within these cryptic fractures *Frutexites* is very common and intergrows with calcareous stromatolitic structures. Thin-shelled freshwater type ostracodes are also abundant in this fracture system.

A modern example of *Frutexites* structures has been found in the deep tunnel of   sp   in southern Sweden (Heim et al., 2007), as part of a highly oxygenated system. Wherever groundwater drops from the tunnel ceiling, a net-like, mineralized micro-system is formed on any rock surface beneath (Figure 3). In general, the dropping system is associated to pending mineral cones which are dominated by the iron-oxidizing, chemolithotrophic bacterium *Gallionella ferruginea*.

The evolving net-like structures feature semi-solid ridges, harboring a particular, highly diverse microbial community. The cross-section of this mineralized biofilm shows laminae with *Frutexites*-like structures (Figure 4). These are mainly composed of iron hydroxides, iron oxides, and less abundant manganese oxides. Small amounts of siderite, calcite, and siliceous material occur side by side with iron and manganese oxides.

Interpretation

Holotypes of *Frutexites* spp. (*Frutexites arboriformis*, Maslov, 1960; *Frutexites microstroma*, Walter and Awramik, 1979) were originally described from stromatolites; thus, they were genetically linked to different types of cyanobacteria (Playford et al., 1976, 1984; Scytonematacean – Walter and Awramik, 1979;

Rivulariacean – Hofmann and Grotzinger, 1985; Angulocellularia group – Riding, 1991). Hofmann and Grotzinger (1985) discussed the affinity of *Frutexites* to different cyanobacteria and proposed further alternatives (purely physicochemical accretion and/or iron bacteria due to the ferruginous character of stromatolite). The occurrence of *Frutexites* in cavities, fissures, and dykes was interpreted as a cryptobiont role (Myrow and Coniglio, 1991). However, Tsien (1979) was the first suggesting a non-phototrophic character, sometimes linked to chemoheterotrophic cyanobacteria (Gischler, 1996) or to chemohetero and autotrophic bacteria (Cavalazzi et al., 2007). Böhm and Brachert (1993) emphasized the roles of *Frutexites* as a cryptobiontic as well as a cryptoendopelitic organisms (living in interstitial habitats). Based on these aspects, the authors proposed the preference of *Frutexites* for oxygen-deficient environments (dysaerobic to anaerobic conditions). Furthermore, they suggested a bacterial precursor for the formation of *Frutexites* but did not exclude a possible fungal and/or physicochemical origin. According to previous publications (Maslov, 1960; Myrow and Coniglio, 1991), Böhm and Brachert (1993) explained the ferromanganese or phosphate mineralogy of *Frutexites* as a replacement of primary carbonate mineralogy. However, a primary iron mineralization of *Frutexites* was postulated by Horodyski (1975) and Hurley and Van der Voo (1990). Others like Hofmann and Grotzinger (1985) believed that microbiota involved in *Frutexites* could regulate the local water chemistry and iron oxyhydroxides as well as aragonite could coprecipitate.

Finally, the occurrence of *Frutexites* in condensed pelagic limestones and hardgrounds has been linked to a physicochemical origin (Wendt, 1969) as well as to iron bacteria (Reitner et al., 1995; Mamet and Pr  at, 2006). Reitner et al. (1995) interpreted the *Frutexites*–foraminifer assemblage as an R-strategic community which replaced the previous K-strategic community (sponges–microbes) due to fundamental changes in oceanographic conditions (from oligotrophic to more eutrophic conditions). Mamet and Pr  at discussed the origin of red pigmentation in some Phanerozoic limestones, where some condensed pelagic limestones with *Frutexites* are included. They argued that under anoxic to dysoxic conditions, ferrous iron may be available for oxidation by microaerophilic iron microbes growing at the sediment–water interface. In contrast, the recent *Frutexites*-like structures found in the deep biosphere in fact grow in air-exposed environments under aerobic conditions. However, the presence of different mineral phases within these structures could be associated to changing redox conditions and/or to a different microbial community composition at micrometer-range.

Conclusions

The different modes of growth of *Frutexites* have been recognized within shallow to deep water stromatolites,

on the seawater–sediment interface, marine micritic sediments, continental cavities, and fractures of deep subterranean environments. Such wider environmental distribution has been taken into consideration for paleoecological interpretations. According to the occurrence of *Frutexites*, some general aspects can be summarized: (a) its distribution does not show bathymetric control (although the dominant record is in deep waters facies), (b) it is formed in environments with very low sedimentation rate (in quiet as well as in agitated waters), (c) in marine environments, *Frutexites* mainly encrusts heterozoan assemblages (crinoids, sponges, solitary corals, and foraminifers), and finally, (d) there is no proof for an exclusive occurrence of *Frutexites* under anoxic and aphotic conditions.

Most of the authors considered a bacterially induced origin for *Frutexites*, but the final assessment strongly depends on which mineralogical composition they interpreted as primary (timing of iron mineralization) as well as its *loci* of growth.

Bibliography

- B  hm, F., and Brachert, T. C., 1993. Deep-water stromatolites and *Frutexites* Maslov from the Early and Middle Jurassic of S-Germany and Austria. *Facies*, **28**, 145–168.
- Cavalazzi, B., Barbieri, R., and Ori, G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chafetz, H. S., Akdim, B., Julia, R., and Reid, A., 1998. Mn- and Fe-rich black travertine shrubs: bacterially (and nanobacterially) induced precipitates. *Journal of Sedimentary Research*, **68**, 404–412.
- Gischler, E., 1996. Late Devonian–Early Carboniferous deep-water coral assemblages and sedimentation on a Devonian seamount: Iberg Reef, Harz Mts., Germany. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **123**, 297–322.
- Heim, C., Simon, K., Qu  ric, N. -V., and Thiel, V., 2007. Mineralized microbial mats with extreme lanthanum enrichments in the tunnel of   sp  , Sweden. *Geochimica et Cosmochimica Acta*, Goldschmidt Abstracts 2007 – H, A390.
- Hofmann, J., and Grotzinger, J. P., 1985. Shelf-facies microbiotas from the Odjick and Rocknest formations (Epworth Group; 1.89 Ga), northwestern Canada. *Canadian Journal of Earth Sciences*, **22**, 1781–1792.
- Horodyski, R. J., 1975. Stromatolites of the Lower Missoula Group (middle Proterozoic), Belt Supergroup, Glacier National Park, Montana. *Precambrian Research*, **2**, 215–254.
- Hurley, N. F., and Van der Voo, R., 1990. Magnetostratigraphy, Late Devonian iridium anomaly, and impact hypotheses. *Geology*, **18**, 291–294.
- Jach, R., and Dudek, T., 2005. Origin of a Toarcian manganese carbonate/silicate deposit from the Kri  zna unit, Tatra Mountains, Poland. *Chemical Geology*, **224**, 136–152.
- Kazmierczak, J., and Kempe, S., 2006. Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo‘ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Koban, C. G., and Schweigert, G., 1993. Microbial origin of travertine fabrics – two examples from southern Germany (Pleistocene Stuttgart travertines and Miocene Riedoschingen travertine). *Facies*, **29**, 251–264.

- Korn, H., and Martin, H., 1959. Gravity tectonics in the Naukluft Mountains of South West Africa. *Bulletin of Geological Society of America*, **70**, 1047–1078.
- Mamet, B., and Pr  at, A., 2006. Iron-bacterial mediation in Phanerozoic red limestones: State of the art. *Sedimentary Geology*, **185**, 147–157.
- Maslov, V. P., 1960. Stromatolites. *Trudy Instituta geologicheskikh nauk Akademii nauk SSR*, **41**, 188.
- Miller, R. McG., 2008. *The Geology of Namibia*. Windhoek: Geological Survey of Namibia, Vol. 2.
- Myrow, P. M., and Coniglio, M. 1991. Origin and diagenesis of cryptobiontic *Frutixites* in the Chapel island Formation (Vendian to Early Cambrian) of Southeast Newfoundland, Canada. *Palaios*, **6**, 572–585.
- Nicoll, R. S., and Playford, P. E., 1993. Upper Devonian iridium anomalies, conodont zonation and the Frasnian-Famennian boundary in the Canning Basin, Western Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **104**, 105–113.
- Playford, P. E., Cockbain, A. E., Druce, E. C., and Wray, J. L., 1976. Devonian stromatolites from the Canning Basin, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 543–563.
- Playford, P. E., McLaren, D. J., Orth, C. J., Gilmore, J. S., and Goodfellow, W. D., 1984. Iridium anomaly in the Upper Devonian of the Canning Basin, Western Australia. *Science*, **226**, 437–439.
- Reitner, J., Wilmsen, M., and Neuweiler, F., 1995. Cenomanian/Turonian sponge microbialite deep-water hardground community (Liencres, Northern Spain). *Facies*, **32**, 203–212.
- Riding, R., 1991. Calcified cyanobacteria. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 55–87.
- Rodr  guez-Mart  nez, M., Heim, C., Simon, K., Reitner, J., and Zilla, T., (in press). *Tolypammina gregaria* Wendt-*Frutixites* assemblage and ferromanganese crusts: a coupled nutrient-metal interplay in the Carnian sedimentary condensed record of Hallstatt Facies (Austria). *Lecture Notes in Earth Sciences*
- Tsien, H. H., 1979. Paleocology of algal-bearing facies in the Devonian (Cuvirtian to Frasnian) Reef complexes of Belgium. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **27**, 103–127.
- Walter, M. R., and Awramik, S. M., 1979. *Frutixites* from stromatolites of the Gunflint iron formation of Canada, and its biological affinities. *Precambrian Research*, **9**, 23–33.
- Wendt, J., 1969. Foraminiferen-‘Riffe’ im karnischen Hallst  tter Kalk des Feuerkogels (Steiermark,   sterreich). *Pal  ontologische Zeitschrift*, **43**, 177–193.
- Wendt, J., 1988. Condensed carbonate sedimentation in the late Devonian of the eastern anti-Atlas (Morocco). *Eclogae geologicae Helvetica*, **81**, 155–173.
- Woods, A. D., and Baud, A., 2008. Anachronistic facies from a drowned Lower Triassic carbonate platform: Lower member of the Alwa Formation (Ba’id Exotic), Oman Mountains. *Sedimentary Geology*, **209**, 1–14.

Cross-references

Bacteria
 Calcified Cyanobacteria
 Cyanobacteria
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Stromatactis
 Stromatolites

FUNGI AND LICHENS

Bettina Weber, Burkhard B  del
 University of Kaiserslautern, Kaiserslautern, Germany

Definition

Fungi. Saprophytic, parasitic, or symbiotic living organisms, mainly filamentous (hyphae), rarely unicellular. Cell walls are of chitin or other non-cellulose compounds.
Lichens. Symbiotic organisms composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont.

Characteristics

Fungi

Fungi are a group of eukaryotic, heterotrophic organisms with their thallus on or in the substratum. The thallus can be unicellular (rarely) or filamentous (mostly), the latter being either composed of septate or nonseptate hyphae and often forming a more or less defined web of filaments (mycelium). The cell wall is typically chitinized or composed of other non-cellulose compounds. The spore forming thallus (sporocarp) can be microscopic or macroscopic with limited tissue differentiation. Fungi are cosmopolitan and ubiquitous, saprotrophic, mutualistic (mycorrhizal, lichen-forming), or parasitic. A number of rock-inhabiting fungi belong to the Deuteromycetes, a heterogenic group of fungi that develop no or only asexual sporocarp types. The vast majority of rock fungi are dark brown to blackish pigmented and are filamentous or forming microcolonies (B  del et al., 2000).

Lichens

Symbiotic mode of living

Lichens are mutualistic organisms consisting of a fungal partner, the mycobiont, and of one or more photosynthetic partners, the photobiont. Taxonomically speaking, they belong to the kingdom of fungi and represent here a special group, the lichenized fungi (Honegger, 1996). The lichen-forming fungi are polyphyletic, meaning that they have developed several times during the evolution of fungi. This brought about a taxonomically heterogeneous group with the large majority of them belonging to the Ascomycetes and only few Basidiomycetes and Deuteromycetes (= Fungi Imperfecti; Nash III, 1996a, Miadlikowska et al., 2006).

As photobiont of lichens, green algae with the most frequent genera *Trebouxia* and *Trentepohlia* make up about 90% of the photosynthetic partners, whereas cyanobacteria with the prevalent genus *Nostoc* account for the remaining 10%. The green algae, also referred to as “chlorobionts” (Lange and Wagenitz, 2003), belong to the eucaryotic algae which share many cytological features and their pigmentation (e.g., chlorophylls a and b) with land plants (Bold and Wynne, 1985; van den Hoek et al., 1993). Cyanobacteria (“cyanobionts”) are of

procaryotic nature, lacking a nucleus, mitochondria and chloroplasts, all of which are found in eucaryotic cells. The DNA, which in eucaryotic cells is stored within the nucleus, is found as circular DNA in the cytoplasm and also the thylacoids, which occur in the chloroplasts of eucaryotes, are here embedded into the cytoplasm (Friedl and Büdel, 2008).

The interactions between the mycobiont and photobiont are not completely balanced. There is metabolic transport from the autotrophic photobiont to the heterotrophic mycobiont in the form of carbohydrates, which passively leak from the photobiont cells. Metabolic transport processes from the mycobiont to the photobiont are not known (Schöller and Mollenhauer, 1997). However, the photobiont often also profits from growth within the mycelium of hyphae, since more balanced water and light conditions are granted within the thallus. Besides that, the mycobionts produce a variety of secondary substances that might be beneficial for the photobiont (Schöller and Mollenhauer, 1997). Therefore, the lichenization of the photobiont is probably most adequately described as a controlled parasitism by lichen-forming fungi (Ahmadjian, 1993).

The thallus of a lichen is formed by a more or less dense network of fungal hyphae, in which the photobionts are either evenly distributed (homoiomorous thalli), or are concentrated within a defined layer (heteromorous thalli). Homoiomorous thallus construction mainly occurs in gelatinous, crustose lichens (e.g., *Collema*, *Pyrenopsis*) whereas heteromorous thallus construction is typically formed by an upper cortex followed by a photobiont layer, a medulla and a lower cortex (Büdel and Scheidegger, 2008). A surface tissue, like the epidermis with stomata and a protective cuticle as in higher plants, does not exist in lichens, neither do they have water and nutrient-transporting structures.

Lichen morphology

The appearance of a lichen thallus is determined primarily by the mycobiont and only in few cases the photobiont determines, to a certain extent, the form of a thallus (e.g., in the filamentous genera *Coenogonium*, *Ephebe*, *Cystocoleus*, and *Rhacodium*). However, only after the establishment of the symbiosis, under the influence of the photobiont, the characteristic thallus of a lichen is developed (Büdel and Scheidegger, 2008).

Based on their overall growth form, lichens are traditionally divided into three main morphological types: crustose, foliose, and fruticose. Crustose lichens are tightly attached to the substrate with their lower surface and cannot be removed without destruction of the thallus. Foliose lichens form leaf-like thalli that are partly attached to the substrate and have a dorsiventral organization (Büdel and Scheidegger, 2008). Thallus lobes of fruticose lichens are hairlike, strap-shaped, flat, or cylindrical, forming erect or hanging thalli. Some fruticose genera like *Baeomyces* and *Cladonia* develop a twofold thallus with

a foliose horizontal and a fruticose vertical thallus part. Endolithic lichens represent a special form of crustose lichens, where the thallus partly or completely grows within the rock. According to Golubic et al. (1981), the endolithic growth form can be further separated into cryptoendolithic (= growing within the rock), chasmoendolithic (= growing along cracks or fissures of the rock), and euendolithic (= actively boring into the rock) organisms.

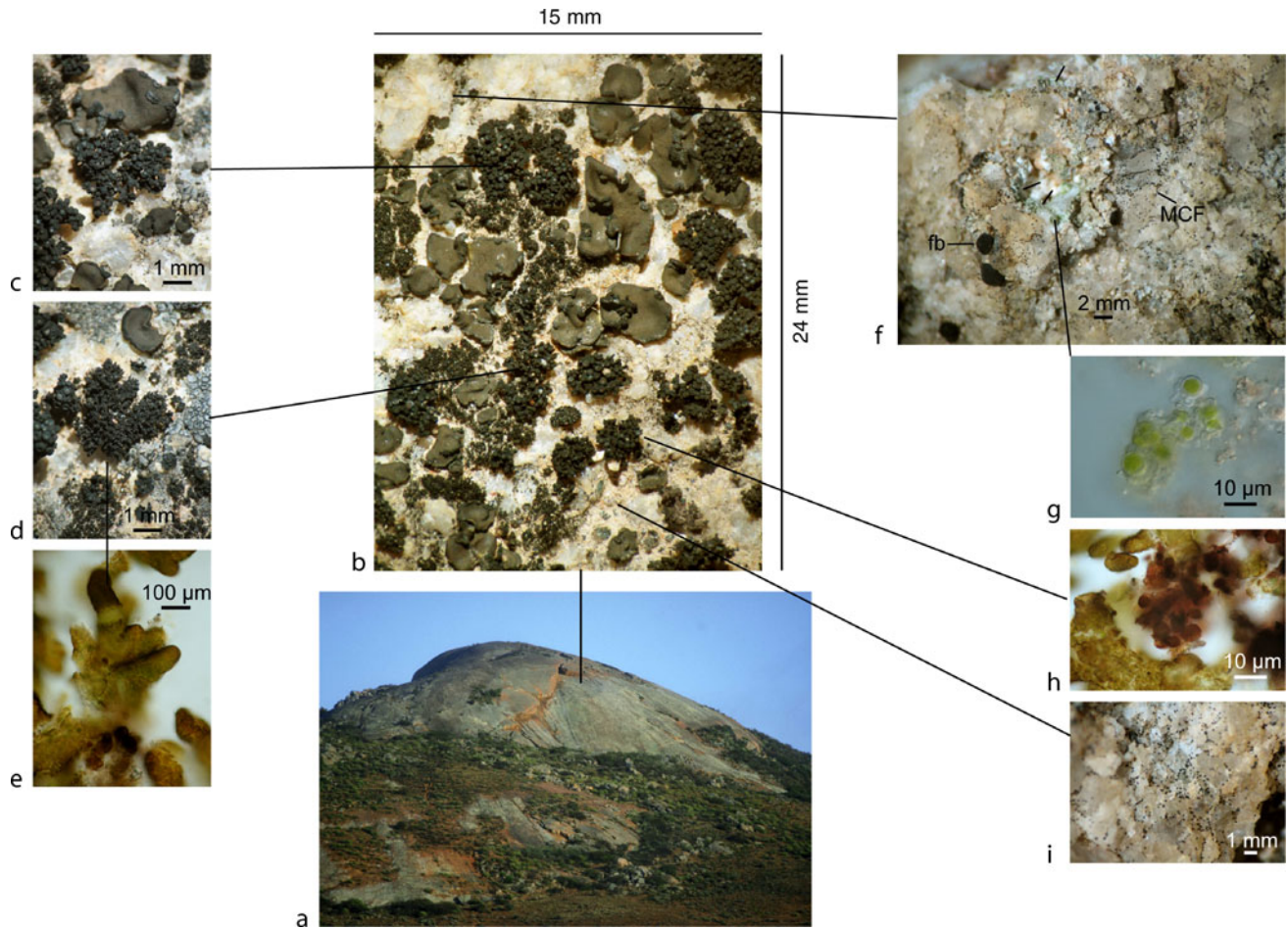
Diversity

Fungi

Rock-inhabiting fungi belong to major groups of fungi: the hyphomycetes and the “black fungi,” the latter named because of the strong production of melanoid pigments that causes dark-brown or blackish pigmented hyphae. Formation of microcolonies is a universal feature of the latter group, and they are therefore named “microcolonial fungi” (= MCF; Figure 1f and i; Gorbushina, 2003). Hyphomycetes form a mycelium but lack a sporocarp and the conidia (asexual spores) are borne on hyphae. They occur on soil or epiphytic habitats and dominate on rock surfaces under milder climate regimes (e.g., high air humidity, abundant nutrients). The black MCF prevail under harsh climatic conditions and are slow-growing. They represent a diverse assemblage of non-lichenized ascomycetes. Because of a convergent microcolonial morphology and their morphological plasticity, they are extremely difficult to identify by classical methods. Identification procedures based on molecular methods are just in progress and have been applied to a few strains only. So far, identified samples and strains have been placed in the mostly anamorphic (asexual) genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Coniosporium*, *Cryomyces*, *Friedmanniomyces*, *Lichenothelia*, *Phaeococcomyces*, *Phaeosclera*, *Phoma*, *Sarcinomyces*, and *Scolecobasidium* (Sigler et al., 1981; Diakumaku et al., 1995; Wollenzien et al., 1997; Sterflinger et al., 1997; Büdel et al., 2000; Selbmann et al., 2005; Onofri et al., 2007). Most of them belong to the ascomycete order Pleosporales, Capnodiales, and Dothideales, summarized in the class Dothideomycetes (Schoch et al., 2006), few belong to the Chaetothyriales in the class Eurotiomycetes (Geiser et al., 2006).

Lichens

Lichens grow on a wide variety of substrates including rocks (= epi- or endolithic), soil (= epigeic), detritus, and the branches, stems (= epiphytic), and leaves of trees (= epiphyllic). Most lichen species are specialized to one type of substrate comprising certain habitat characteristics. There are species with a wide ecological amplitude (euryoecious as compared to stenoecious species), able to colonize different types of substrate, like e.g., *Caloplaca citrina* (Hoffm.) Th.Fr., *Candelariella vitellina* (Hoffm.) Müll.Arg., and *Physcia adscendens* (Fr.) Oliv.,



Fungi and Lichens, Figure 1 (a) Granite inselberg at Soebatsfontein, Succulent Karoo, South Africa. (b) Close-up of the rock surface, showing dense colonization by the microlichens *Peltula bolanderi* (large olive-colored thalli), *Peccania* sp., *Synalissa* sp., cyanobacteria, and microcolonial fungi. (c) Detail with *Peccania* sp. (d) Detail with *Synalissa* sp. (e) Microscopical view on the lobes of *Synalissa* sp., showing the incorporation of the yellow pigment scytonemin (light and UV protectant) in the gelatinous sheath of the lichen. (f) Endolithic lecidoid lichen, thallus parts visible; where the rock surface was removed (arrows), the sporocarps (fruit bodies) are exposed on the rock surface (fb); MCF indicates the presence of numerous microcolonial fungi. (g) Microscopical view on a part of the algal layer of the endolithic lecidoid lichen showing the *Trebouxia*-like chlorobionts in an envelope of lichen hyphae. (h) Colonies of the red-sheathed cyanobacterium *Gloeocapsa* sp. growing on the rock surface between the lichens. (i) Microcolonial fungi on the rock surface.

and there are nutrient-tolerant species that occur epiphytically as well as epilithically (Figure 2b and c; Wirth, 1995).

On soils, lichens often represent part of a biological soil crust (BSC). BSC are formed by an association between soil particles and different proportions of cyanobacteria, algae, microfungi, bryophytes, and lichens (Belnap et al., 2003). In arid and semiarid regions, which make up more than 30% of the land surface of the earth, BSC cover up to 25% of the soil surface (Weber et al., 2008), but also in temperate regions like Central Europe, BSC occur in various habitats (Büdel, 2003).

Rock-inhabiting lichens come from most taxonomic groups of lichens as, for example, members of the class Lichinomycetes (e.g., *Anema*, *Digitothyrea*, *Heppia*, *Lichinella*, *Peccania*, *Peltula*, *Phyllisciella*, *Synalissa*, *Thyrea*; Figure 1b–e) that exclusively have cyanobacteria as photobionts. Although these lichens can be most abundant in arid and semiarid landscapes, the vast majority of lichens (more than 200 species) come from the largest class of lichenized fungi, the Lecanoromycetes. Within the Lecanoromycetes, green algal photobionts dominate (e.g., *Acarospora*, *Buellia*, *Caloplaca*, *Dermatiscum*, *Dimelaena*, *Hymenelia*,



Fungi and Lichens, Figure 2 (a) Vegetation pattern of lichens on a natural rock surface (dolomite, Untersberg, Austria). *Hymenelia coerulea* (grayish blue areas), *Hymenelia prevostii* (white, arrows), both endolithic growing lichens. (b) Lichen community colonizing mortar of medieval churches (built in the thirteenth to fifteenth centuries, Mecklenburg-Western Pomerania, Germany) consisting of *Caloplaca citrina* (Hoffm.) Th.Fr., *Caloplaca saxicola* (Hoffm.) Nordin, and *Lecanora dispersa* (Pers.) Sommerf. (c) Damp lower part of north-exposed wall of rural church built on a field stone foundation and brick walls joined with mortar. Lichen community consisting of *Enterographa zonata* (Körb.) Torrente & Egea, *Haematomma ochroleucum* (Neck.) J.R.Laundon, *Ramalina pollinaria* (Westr.) Ach., *Xanthoria calcicola* Oxner and *Lepraria incana* (L.) Ach. (Mecklenburg-Western Pomerania, Germany). (d) Endolithic growing lichen *Caloplaca* sp., growing on sandstone of the Clarens formation, Golden Gate Highlands National Park, South Africa. This lichen is actively weathering the rock; note the rim of the rock at the margin of the lichen, indicating the original rock surface.

Lecidea, *Lecanora*, *Lepraria*, *Rinodina*, *Parmelia*, *Parmotrema*, *Usnea*, *Verrucaria*, *Xanthoparmelia*; Figure 2a).

Apart from natural habitats, lichens also grow on artificial substrates like mortar and building stone (Figure 2b and c; e.g., Ariño and Saiz-Jimenez, 1996; Weber and Büdel, 2001), wooden material like fences and benches, and even glass, bone, tyres, and metal under favorable climatic conditions (Schöller, 1997).

Biogeography

Fungi

Since determination on the species level is a challenging task and not easily possible without culturing or before more molecular data are available, biogeographical information is very limited at present. For some species of the genus *Lichenothelia*, however, information on distribution patterns are available. While *L. intermedia* occurs on almost every granite rock surface throughout Africa, the species *L. gigantea* and *L. radiata* are restricted to Australia, and *L. globulifera* was only found on rock outcrops of the Seychelle island La Digue (Henssen 1987). On the basis of morphological and molecular data (SSU phylogeny and ITS sequence data), Selbmann et al. (2005) recently described two new genera of MCF, *Cryomyces*, and *Friedmanniomyces*, only known from Antarctic rocks so far. The authors provide good evidence that at least the new genus *Cryomyces* might be endemic to Antarctica. Since MCF diaspores are easily spread over large distances by the wind, many of them might have a cosmopolitan distribution (airborne fungi).

Lichens

Lichens have developed during evolutionary radiation of ascomycete fungi within the last 600–400 million years, possibly contemporaneous to the emergence of land plants (Galloway, 1996). Due to this long-lasting evolutionary process, their distribution patterns show similarities to other groups of organisms and can be explained utilizing the theory of plate tectonics (Galloway, 1996). Compared to vascular plants, lichens comprise a higher number of cosmopolitan species within different climate zones of the earth and endemism within smaller geographic regions is normally lower. Exemplarily, on the Kap Verde Islands 13.4% of vascular plants were endemics, whereas only 2.5% of the lichen species were found to be endemic (Schöller, 1997). Dispersal of both symbiotic partners is facilitated by the fact that fungal spores as well as algal cells are transported over vast distances within the air (Brown et al., 1964).

At least 16 major distribution patterns of lichens can be distinguished (Galloway, 1996). Besides lichen species that are constrained to one single continent, like austral and neotropical taxa, there are distribution patterns that comprise spatially contiguous regions, like the paleotropics that include most of Africa, the Arabian Peninsula, most of the Indian subcontinent, the Malaysian archipelago, and the Islands of the Pacific Ocean (Galloway, 1996). A third type of distribution pattern embraces disjunct distributions over continents, which have been close by or unified in history, but were separated by plate tectonic movements, like pantropical and American–Asian distribution patterns (Grotzinger et al., 2006). Taxa with bipolar distribution represent the last pattern, which results from climate change through earth history. These organisms occur at high latitudes of the

northern and southern hemisphere, but often also occur in alpine regions, forming relict habitats of the last ice age (Galloway, 1996).

Physiology

Fungi

There is not much known of the specific physiology of rock-inhabiting fungi except that they are able to tolerate extremely high (70–80°C; Palmer et al., 1987) and low temperatures (–40°C; Selbmann et al., 2005) due to their poikilohydric nature and related to that, their ability to withstand long periods (in the magnitude of tens of years) in the dry state. Many fungi are heavily melanized, most probably as a shelter against high radiation of both visible and UV light.

Lichens

Lichens are poikilohydric organisms, meaning that they cannot actively take up or lose water. Moistening of the thallus can be achieved by rain/snow precipitation, dew-fall, and equilibrium with high air humidity. This clearly distinguishes them from vascular plants that actively regulate water uptake and loss.

Water

Although water uptake, storage, and loss are modified by differences in thallus structure and morphology, these mechanisms are predominantly controlled by physical processes (Rundel, 1988). Therefore, the physiological activity of lichens is highly dependent on the prevailing water conditions. In general, the photosynthetic response of lichens to increasing water content can be described as an optimum curve, which, however, reveals large variability (Lange et al., 2001). Air-dried lichens with a water content below 15–30% show no photosynthetic response at all (Nash III, 1996b). With increasing water content, lichens first reveal net respiration rates before the compensation point is reached, above which positive photosynthetic activity is recorded. In general, cyanolichens commence positive net photosynthesis (NP) at higher water contents and are generally able to perform positive NP under higher water content than chlorolichens (especially the homoimerous species; Lange et al., 2001).

Above an optimum water content, at which maximum NP rates are reached, free water is present and the lichens have a water potential close to zero (Green and Snelgar, 1982). These periods of water supra-saturation cause a depression in photosynthesis by increased thallus diffusive resistances; CO₂ pathways within the thallus are blocked by water (Lange et al., 1997). Water supra-saturation can occur in chloro- and cyanolichens of all growth forms (Lange et al., 1996, 2000, 2004; Lange and Green, 1997), and also endolithic lichens of various substrates have to cope with this problem (Tretiach and Pecchiari, 1995; Tretiach and Geletti, 1997; Winkler and Kappen, 1997). In a recent study, the photosynthetic

performance of *Hymenelia prevostii* and *H. coerulea*, two endolithic green algal lichens growing on steep limestone outcrops in the eastern alpine mountains, have been investigated in detail (Weber et al., 2007). Both species were saturated at extremely low water content (*H. prevostii*: 0.26 mm, *H. coerulea*: 0.07 mm rainfall equivalent) and revealed very narrow ranges of positive NP values upon increasing thallus water content (0.2 mm vs. 0.1 mm). Their growth on steep rock surfaces therefore represents an adaptation to avoid excessively high water contents or the formation of CO₂ blocking water films.

A crucial difference in the physiology of cyano- and chlorolichens lies in the capability to use water vapor for photosynthetic activation. While air-dry chlorolichens gain positive NP in equilibrium with air of about 80% relative humidity and above, all cyanobacterial lichens investigated so far need liquid water for activation of NP (Büdel and Lange, 1991; Lange et al., 1993). This fundamental difference also affects the natural habitats of lichens with cyanolichens preferably occurring at moist habitats with frequent liquid water (e.g., *Collema* spp., *Lichinella* spp.), whereas green algal lichens often also occur in rain-shaded habitats of high air humidity (e.g., *Lepraria* spp., *Usnea* spp.). The endolithic *Hymenelia* species mentioned above were also shown to perform positive NP at relative air humidities of 90% and above (Weber et al., 2007).

Light

The capture of radiant energy is essential for photosynthesis. Only light of wavelengths between 400 and 780 nm, the photosynthetically active radiation (PAR), is utilized for photosynthesis (Nash III, 1996b). Under constant and favorable abiotic conditions, photosynthetic response of lichens to increasing light intensities exhibits a saturation curve with saturation for most species occurring between 100 and 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Demmig-Adams et al., 1990). However, extremely shade-tolerant species have saturation values as low as 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Green et al., 1991), whereas others, like *Ramalina maciformis* (Del.) Bory reveal values above 1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Demmig-Adams et al., 1990). At increasing temperatures, light saturation is achieved at higher levels (Kershaw, 1985) and also water content has an influence (Lange, 1969), probably due to alterations of light penetration within the thallus (Ertl, 1951; Dietz et al., 2000). Exposure of fully hydrated lichens to excess light can induce a depression of photosynthesis, both in chloro- and cyanolichens (Demmig-Adams et al., 1990).

As a protection against excessive light, lichens in general possess a cortex made of fungal tissue, and some species even form a so-called epinecral layer consisting of dead, collapsed, and often gelatinized hyphae and photobiont cells (Büdel and Scheidegger, 2008). *Peltigera rufescens* (Weiss) Humb., for example, has a cortical organization that causes a decrease of light transmittance to

40% at the upper boundary of the algal layer (Dietz et al., 2000).

The endolithic growth form (Figure 1f) can also be interpreted as a protection from excess light. Winkler and Kappen (1997) found that only 0.1–0.2% of the ambient light reaches the upper part of the algal layer in *Sarcogyne* cf. *austroafricana* (Zahlbr.) H. Magn. Light reduction within the sandstone on top of the photosynthetically active layer is also reflected by light saturation values above 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ measured for *Sarcogyne* cf. *austroafricana*, *Lecidea* aff. *sarcogynoides* Körb., *Lecidea confluenta* Müll. Arg. and *Lithoglypha aggregata* Brusse (Winkler and Kappen, 1997). In limestone, *Hymenelia prevostii* and *H. coerulea* were saturated at high light intensities (1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ vs. 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; Weber et al., 2007).

Temperature

Lichens are generally known to survive a wide range of temperatures. Being dominant terrestrial organisms of Antarctica, they must be able to tolerate extremely low temperatures (Nash III, 1996b), whereas in sun-exposed microhabitats, lichens experience temperatures of up to 60°C (Hahn et al., 1989). However, lichens in general reveal a vast difference in temperature tolerance depending on whether the thalli are hydrated or dry. Whereas dry thalli of some lichen species are able to tolerate a stepwise cooling to temperatures as low as –196°C (Kappen, 1973) and heating up to 90 or 100°C (Lange, 1953), wet thalli are by far more sensitive. Review of field measurements of lichen photosynthesis revealed that many lichens are primarily active at temperatures between 5°C and 20°C, although 0–10°C may better characterize some Antarctic species (Kappen, 1988; Kappen and Friedmann, 1983).

Photosynthetic capacity of some lichen species is known to reveal seasonal changes that involve an adjustment to higher summer temperatures (Kershaw, 1985). Recently, Lange and Green (2005) proved that dark respiration (DR) of lichens does exhibit seasonal changes of temperature sensitivity. At 5°C, for example, the respiration rates of *Cladonia convoluta* (Lam.) P.Cout., *Lecanora muralis* (Schreber) Rabenh., and *Diploschistes muscorum* (Scop.) R.Sant. were several times higher in winter than in summer. This acclimation of DR implies that maximal net carbon fixation rates remain similar throughout the year and are not depressed by increased carbon loss due to higher temperatures during the warmer season (Lange and Green, 2005).

CO₂

The photosynthetic CO₂-fixation rate of lichens at optimal water content generally increases with increasing CO₂-concentrations, typically reaching saturation around 600–1,300 ppm (Nash III, 1996b; Tretiach and Geletti, 1997; Lange et al., 1999; Lange, 2002). At natural CO₂ concentrations (around 360 ppm), many species only

reach about 60–80% of maximal NP under optimal water conditions (Lange et al., 1999; Lange, 2002).

Under supra-saturated water conditions, NP of lichens is often depressed at natural CO₂ concentrations. Increased CO₂ values, however, in most cases result in attenuation of this depression (e.g., Lange, 2002). In some species, as for the epigeic species *Fulgensia fulgens* (Sw.) Elekin, a severe depression in CO₂ uptake can even be completely counterbalanced by a CO₂ concentration of 4,500 ppm (Lange et al., 1999).

The CO₂ compensation point (CO₂ concentration, at which respiration equals photosynthesis) in many lichen species is very low as compared to vascular plants (Nash III, 1996b). This often results from a CO₂ concentrating mechanism (CCM), by which CO₂ is actively pumped into the photobiont cells (Badger et al., 1993). Such a CCM is also present within the photobiont *Trebouxia irregularis* Hildreth and Ahmadjian of the endolithically growing *Hymenelia* species, which makes them less susceptible to elevated CO₂ concentrations (Weber et al., 2007).

Interactions with the substrate

Bare natural rock surfaces are extremely rare on earth and might only be found under extreme climatic conditions of hot or cold deserts. Normally, rock surfaces are covered by a dense layer (film, crust; Figure 1a) composed of cyanobacteria (Figure 1h), algae, lichens (Figure 1b–f), and fungi (Figure 1f and i). Cyanobacteria, lichens, and fungi also can grow inside the rock itself, in the upper few millimetres (= endolithic, Figure 1f and g). On inselbergs of tropical savannas, these biofilms are even responsible for the characteristic black (cyanobacteria dominated) or brown color (lichen dominated) of the rock surface (Büdel et al., 2000).

Fungi

Fungi grow on rock surfaces (epilithic), inside the rock (endolithic) or are part of the desert varnish produced by microorganisms growing inside the up to 1 cm thick black layer (e.g., Krumbein and Jens, 1981). Also MCF have been shown to be involved in desert varnish formation in Arizona and Australia (Taylor-George et al., 1983). Microbial growth on rock surfaces follows the microscopic landscape formed by mineral grains, pores, and fissures of the substratum (Figure 1f and i). Characteristically, microorganisms of the resulting biofilms colonize in the way that fungal hyphae and some cyanobacteria penetrate the substrate, while algae, bacteria, and microcolonies of yeast-like fungi clump together (Gorbushina, 2007). They secrete extracellular polymeric substances (EPS), which envelope the microcolonies, keep them together and adhere them to the substratum.

Biogenic rock weathering is surely a multifactorial process. The role of fungi in the wear-down of rocks is difficult to separate from the activity of other microorganisms of biofilms. Generally, there are two main groups of weathering that can be distinguished. (1) Either the growth of cells forces separation of mineral grains (Dornieden

et al., 1997), or desiccation/hydration cycles of cell walls and EPS cause their separation (Friedmann and Weed, 1987; Warscheid et al., 1991). (2) Dissolution of rock minerals can be achieved by organic acids released from cells, chemical impact of respiratory CO₂, H⁺, and redox reactions with metal-ions (Gadd, 1999; Burford et al., 2003).

Lichens

During the 1980s and 1990s, a huge variety of literature on the patterns and effects of biogenic weathering by lichens has been published. However, a comprehensive understanding on the overall mechanisms, effects, and magnitude of effects could not yet be accomplished (Chen et al., 2000).

As for the fungi, the impact of lichens can be subdivided into physical and chemical effects. The physical effects comprise: (1) penetration of hyphae through intergranular cavities and along mineral cleavage planes; (2) thallus expansion and contraction due to varying water content; (3) freezing and thawing of the lichen thallus; (4) swelling of organic and inorganic salts originating from lichen activity; and (5) incorporation of mineral fragments into the thallus (Chen et al., 2000).

The chemical effects comprise the solubilization and the insolubilization of inorganic elements, resulting in the weathering of minerals (silicates, phosphates, carbonates, sulfides, oxides) and the formation of new deposits (carbonates, oxides, sulfides, phosphates; Benthelin, 1988). The solubilization processes, as induced by lichens include (1) the release of metal ions by acid and complexing compounds, and (2) the acidification or alkalization by uptake or release of alkaline compounds. Insolubilization processes are oxidation of Fe²⁺ and Mn²⁺, and uptake of elements by the organisms in different environments (Benthelin, 1988).

Besides the alkalization activity of endolithic cyanobacteria (Büdel et al., 2004), it is also expected that lichens perform a similar role on/in rocks. Wessels and Schoeman (1988) describe an exfoliation process initiated by the endolithic lichen *Lecidea* aff. *sarcogynoides* Koerb. In SEM imagery, they perceived a dissolution of the binding material along the growth zone of the hyphae and also pitting marks on the quartz crystals were observed, where hyphae had attached to the surface of the crystals (Wessels and Schoeman, 1988). Besides *L. aff. sarcogynoides*, also lichens of the genus *Caloplaca* are found to contribute to rock weathering in sandstones of South Africa (Figure 2d). Friedmann (1982) and Friedmann and Weed (1987) report on cryptoendolithic microorganisms in the Antarctic cold desert (Ross Desert) being dominated by lichens that cause a exfoliative weathering pattern along the lichen growth zone very similar to the pattern described by Büdel et al. (2004).

Conclusions

Identification of lichens on the basis of their external and internal morphology, as well as chemical characteristics, is well-established and only in some cases, where lichens

do not express a typical morphology or where they are often sterile, e.g., in the endolithic habitat, additional identification methods, using a molecular approach are necessary. Thus, lichen diversity of different rock types is quite well-known. Rock-inhabiting fungi, on the other hand, are much more difficult to identify. They often only express a reduced set of morphological features, insufficient for save identifications. Here, the use of molecular techniques is a must and already lead to a considerable increase in knowledge (e.g., Selbmann et al., 2005).

The interaction of fungi and lichens with their rock substratum is manifold. Biofilms with fungi and lichens might either increase weathering rates of their substratum, or in some cases, protect it from weathering. However, these crusts and films on rocks are a complex mixture of bacteria, cyanobacteria, algae, fungi, and microlichens embedded in the common structure of an EPS. Macrolichens rising above the biofilm form a separate layer, comparable to that of emergent trees that rise above the canopy of rain forests.

Microbial biodiversity of rock surfaces and the complex interaction of these organisms among themselves and with the rock substratum, for example, their role in bioweathering, is still poorly understood at present and will surely be a field of intensive and fascinating research in future.

Bibliography

- Ahmadjian, V., 1993. *The Lichen Symbiosis*. New York: Wiley.
- Ariño, X., and Saiz-Jimenez, C., 1996. Lichen deterioration of consolidants used in the conservation of stone monuments. *Lichenologist*, **28**(4), 391–394.
- Badger, M. R., Pfanz, H., Büdel, B., Heber, U., and Lange, O. L., 1993. Evidence for the functioning of photosynthetic CO₂ concentration mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta*, **191**, 57–70.
- Belnap, J., Büdel, B., and Lange, O. L., 2003. Biological soil crusts: characteristics and distribution. In Belnap, J., and Lange, O. L. (eds.), *Ecological Studies 150: Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, pp. 3–30.
- Benthelin, J., 1988. Microbial weathering processes in natural environments. In Lerman, A., and Meybeck, M. (eds.), *Physical and Chemical Weathering in Geochemical Cycles*. Berlin: Springer, pp. 33–60.
- Bold, H. C., and Wynne, M. J., 1985. *Introduction to the Algae. Structure and Reproduction*, 2nd edn. Englewood Cliffs: Prentice-Hall.
- Brown, R. M., Jr., Larson, D. A., and Bold, H. C., 1964. Airborne algae: their abundance and heterogeneity. *Science*, **143**, 583–585.
- Büdel, B., 2003. Biological soil crusts in european temperate and mediterranean regions. In Belnap, J., and Lange, O. L. (eds.), *Ecological Studies 150: Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, pp. 75–86.
- Büdel, B., and Lange, O. L., 1991. Water status of green and blue-green phycobionts in lichen thalli after hydration by water vapor uptake: do they become turgid? *Botanica Acta*, **104**, 361–366.
- Büdel, B., and Scheidegger, C., 2008. Thallus morphology and anatomy. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 40–68.
- Büdel, B., Becker, U., Follmann, G., and Sterflinger, K., 2000. Algae, fungi, and lichens on inselbergs. In Porembski, S., and Barthlott, W. (eds.), *Inselbergs*. Berlin: Springer, pp. 69–90.

- Büdel, B., Weber, B., Köhl, M., Pfanz, H., Sültemeyer, D., and Wessels, D., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268.
- Burford, E. P., Fomina, M., and Gadd, G. M., 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine*, **67**, 1127–1155.
- Chen, J., Blume, H. P., and Beyer, L., 2000. Weathering of rocks induced by lichen colonization - a review. *Catena*, **39**, 121–146.
- Demmig-Adams, B., Máguas, C., Adams, III, W. W., Meyer, A., Kilian, E., and Lange, O. L., 1990. Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. *Planta*, **180**, 400–409.
- Diakumaku, E., Gorbushina, A. A., Krumbein, W. E., Panina, L., and Soukharjevski, S., 1995. Black fungi in marble and limestones - an aesthetical, chemical and physical problem for the conservation of monuments. *Science of the Total Environment*, **167**, 295–304.
- Dietz, S., Büdel, B., Lange, O. L., and Bilger, W., 2000. Transmittance of light through the cortex of lichens from contrasting habitats. *Bibliotheca Lichenologica*, **75**, 171–182.
- Dornieden, T., Gorbushina, A. A., and Krumbein, W. E., 1997. Änderungen von physikalischen Eigenschaften von Marmor durch Pilzbewuchs. *International Journal for Restoration of Buildings and Monuments*, **3**, 441–456.
- Ertl, L., 1951. Über die Lichtverhältnisse in Laubflechten. *Planta*, **39**, 245–270.
- Friedl, T., and Büdel, B., 2008. Photobionts. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 9–26.
- Friedmann, E. I., 1982. Endolithic microorganisms in the Antarctic cold desert. *Science*, **193**, 1247–1249.
- Friedmann, E. I., and Weed, R., 1987. Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic cold desert. *Science*, **236**, 703–705.
- Gadd, G. M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Advances in Microbial Physiology*, **41**, 47–92.
- Galloway, D. J., 1996. Lichen biogeography. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 199–216.
- Geiser, D. M., Gueidan, C., Miadlikowska, J., Lutzoni, F., Kauff, F., Hofstetter, V., Fraker, E., Schoch, C. L., Tibell, L., Untereiner, W. A., and Aptroot, A., 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. *Mycologia*, **98**, 1053–1064.
- Golubic, S., Friedman, I., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**(2), 475–478.
- Gorbushina, A. A., 2003. Microcolonial fungi: survival potential of terrestrial vegetative structures. *Astrobiology*, **3**, 543–554.
- Gorbushina, A. A., 2007. Life on the rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Green, T. G. A., and Snelgar, W. P., 1982. Carbon dioxide exchange in lichens: relationship between the diffusive resistance of carbon dioxide and water vapour. *Lichenologist*, **14**, 255–260.
- Green, T. G. A., Kilian, E., and Lange, O. L., 1991. Pseudocyphellaria dissimilis: a desiccation-sensitive, highly shade-adapted lichen from New Zealand. *Oecologia*, **85**, 498–503.
- Grotzinger, J., Jordan, T. H., Press, F., and Siever, R., 2006. Understanding Earth. 5th edn. New York: W.H. Freeman.
- Hahn, S., Speer, M., Meyer, A., and Lange, O. L., 1989. Photosynthetische Primärproduktion von epigäischen Flechten im "Mainfränkischen Trockenrasen". I. Tagesläufe von Mikroklima, Wassergehalt und CO₂-Gaswechsel zu den verschiedenen Jahreszeiten. *Flora*, **182**, 313–339.
- Henssen, A., 1987. Lichenothelia, a genus of microfungi on rocks. *Bibliotheca Lichenologica*, **25**, 257–293.
- Honegger, R., 1996. Mycobionts. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 24–36.
- Kappen, L., 1973. Responses to extreme environments. In Ahmadjan, V., and Hale, M. (eds.), *The Lichens*. London: Academic, pp. 311–380.
- Kappen, L., 1988. Ecophysiological relationships in different climatic regions. In Galun, M. (ed.), *Handbook of Lichenology*. Boca Raton: CRC, Vol. 2, pp. 39–99.
- Kappen, L., and Friedmann, E. I., 1983. Ecophysiology of lichens in the dry valleys of Southern Victoria Land, Antarctica. II. CO₂ gas exchange in cryptoendolithic lichens. *Polar Biology*, **1**, 227–232.
- Kershaw, K. A., 1985. *Physiological Ecology of Lichens*. Cambridge: Cambridge University Press.
- Krumbein, W. E., and Jens, J., 1981. Biogenic rock varnishes of the Negev Desert (Israel): an ecological study of iron and manganese transformation by cyanobacteria and fungi. *Oecologia*, **50**, 25–38.
- Lange, O. L., 1953. Hitze- und Trockenresistenz der Flechten in Beziehung zu ihrer Verbreitung. *Flora*, **140**, 39–97.
- Lange, O. L., 1969. Experimentell-ökologische Untersuchungen an Flechten der Negev-Wüste. I. CO₂-Gaswechsel von *Ramalina maciformis* (Del.) Bory unter kontrollierten Bedingungen im Laboratorium. *Flora*, **158**, 324–359.
- Lange, O. L., 2002. Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO₂ exchange and its physiological interpretation. *Flora*, **197**, 233–249.
- Lange, O. L., and Green, T. G. A., 1997. High thallus water contents can limit photosynthetic productivity of crustose lichens in the field. *Bibliotheca Lichenologica*, **68**, 81–99.
- Lange, O. L., and Green, T. G. A., 2005. Lichens show that fungi can acclimate their respiration to seasonal changes in temperature. *Oecologia*, **142**, 11–19.
- Lange, O. L., and Wagenitz, G., 2003. What is a "phycolichen"? differences and changes in the meaning of an old lichenological term. *Lichenologist*, **35**(4), 341–345.
- Lange, O. L., Büdel, B., Meyer, A., and Kilian, E., 1993. Further evidence that activation of net photosynthesis by dry cyanobacterial lichens requires liquid water. *Lichenologist*, **25**(2), 175–189.
- Lange, O. L., Green, T. G. A., Reichenberger, H., and Meyer, A., 1996. Photosynthetic depression at high water contents in lichens: concurrent use of gas exchange and fluorescence techniques with a cyanobacterial and a green algal *Peltigera* species. *Botanica Acta*, **109**, 43–50.
- Lange, O. L., Belnap, J., Reichenberger, H., and Meyer, A., 1997. Photosynthesis of green algal soil crust lichens from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO₂ exchange. *Flora*, **192**, 1–15.
- Lange, O. L., Green, T. G. A., and Reichenberger, H., 1999. The response of lichen photosynthesis to external CO₂ concentration and its interaction with thallus water-status. *Journal of Plant Physiology*, **154**, 157–166.
- Lange, O. L., Büdel, B., Meyer, A., Zellner, H., and Zotz, G., 2000. Lichen carbon gain under tropical conditions: water relations and CO₂ exchange of three *Leptogium* species of a lower montane rainforest in Panama. *Flora*, **195**, 172–190.
- Lange, O. L., Green, T. G. A., and Heber, U., 2001. Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance? *Journal of Experimental Botany*, **52**(363), 2033–2042.
- Lange, O. L., Büdel, B., Meyer, A., Zellner, H., and Zotz, G., 2004. lichen carbon gain under tropical conditions: water

- relations and CO₂ exchange of *Lobariaceae* species of a lower montane rainforest in Panama. *Lichenologist*, **36**(5), 329–342.
- Miadlikowska, J., Kauff, F., Hofstetter, V., Fraker, E., Grube, M., Hafellner, J., Reeb, V., Hodkinson, B. P., Kukwa, M., Lücking, R., Hestmark, G., Garcia-Otalora, M., Rauhut, A., Büdel, B., Scheidegger, C., Timdal, E., Stenroos, S., Brodo, I., Perlmutter, G. B., Ertz, D., Diederich, P., Lendemer, J. C., Tripp, E., Yahr, R., May, P., Gueidan, C., Spatafora, J. W., Schoch, C., Arnold, A. E., Robertson, C., and Lutzoni, F., 2006. New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia*, **98**, 1088–1103.
- Nash, T. H., 1996a. Introduction. In Nash III, T. H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 1–7.
- Nash, T. H., 1996b. Photosynthesis, respiration, productivity and growth. In Nash III, T. H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 88–120.
- Onofri, S., Barreca, D., Rabbow, E., de Vera, J. P., Selbmann, L., and Zucconi, L., 2007. Antarctic rock fungi in space and mars simulated conditions. *Geophysical Research Abstracts*, **9**, 9782–9783.
- Palmer, F. E., Emery, D. R., Stemmler, J., and Staley, J. T., 1987. Survival and growth of microcolonial rock fungi as affected by temperature and humidity. *New Phytologist*, **107**, 155–162.
- Rundel, P. W., 1988. Water relations. In Galun, M. (ed.), *Handbook of Lichenology*. Boca Raton: CRC, Vol. 2, 17–36.
- Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W., and Crous, P. W., 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia*, **98**, 1041–1052.
- Schöller, H., 1997. Ökologie und Verbreitung. In Schöller, H. (ed.), *Flechten: Geschichte, Biologie, Systematik, Ökologie, Naturschutz und kulturelle Bedeutung*. Frankfurt am Main: Senckenbergische Naturforschende Gesellschaft, pp. 83–109.
- Schöller, H., and Mollenhauer, D., 1997. Flechtensymbiose und Flechtenthallus. In Schöller, H. (ed.), *Flechten: Geschichte, Biologie, Systematik, Ökologie, Naturschutz und kulturelle Bedeutung*. Frankfurt am Main: Senckenbergische Naturforschende Gesellschaft, pp. 15–47.
- Selbmann, L., de Hoog, G. S., Mazzaglia, A., Friedmann, E. I., and Onofri, S., 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology*, **51**, 1–32.
- Sigler, L., Tsuneda, A., and Carmichael, J. W., 1981. *Phaeotheca* and *Phaeosclera*, two new genera of dematiaceous hyphomycetes and a redescription of *Sarcinomyces lindneri*. *Mycotaxon*, **12**, 449–467.
- Sterflinger, K., De Baere, R., de Hoog, G. S., De Wachter, R., Krumbein, W. E., and Haase, G., 1997. *Coniosporium Perforans* and *C. apollinis*, two new rock-inhabiting fungi isolated from marble in the sanctuary of Delos (Cyclades, Greece). *Antonie van Leeuwenhoek*, **72**, 349–363.
- Taylor-George, S., Palmer, F., Staley, J. T., Borns, D. J., Curtiss, B., and Adams, J. B., 1983. Fungi and bacteria involved in desert varnish formation. *Microbial Ecology*, **9**, 227–245.
- Tretiach, M., and Geletti, A., 1997. CO₂ exchange of the endolithic lichen *Verrucaria baldensis* from karst habitats in Northern Italy. *Oecologia*, **111**, 515–522.
- Tretiach, M., and Pecchiari, M., 1995. Gas exchange rates and chlorophyll content of epi- and endolithic lichens from the Trieste Karst (NE Italy). *New Phytologist*, **130**, 585–592.
- van den Hoek, C., Jahns, H. M., and Mann, D. G., 1993. *Algen*. 3. Auflage. Stuttgart: Thieme.
- Warscheid, T., Oelting, M., and Krumbein, W. E., 1991. Physicochemical aspects of biodeterioration processes on rocks with special regard to organic pollutants. *International Biodeterioration*, **28**, 37–48.
- Weber, B., and Büdel, B., 2001. Mapping and analysis of distribution patterns of lichens on rural medieval churches in North-Eastern Germany. *Lichenologist*, **33**(3), 231–248.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Weber, B., Olechowski, C., Knerr, T., Hill, J., Deutschewitz, K., Wessels, D. C. J., Eitel, B., and Büdel, B., 2008. A new approach for mapping of biological soil crusts in semidesert areas with hyperspectral imagery. *Remote Sensing of Environment*, **112**, 2187–2201.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Winkler, J. B., and Kappen, L., 1997. Photosynthetic capacity of endolithic lichens from South Africa. *Bibliotheca Lichenologica*, **67**, 165–181.
- Wirth, V., 1995. *Die Flechten Baden-Württembergs*. Teil 1–2. Stuttgart: Eugen Ulmer GmbH.
- Wollenzien, U., de Hoog, G. S., Krumbein, W. E., and Uijthof, J. M. J., 1997. *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. *Antonie van Leeuwenhoek*, **71**, 281–288.

Cross-references

[Algae \(Eukaryotic\)](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococcidiopsis](#)
[Cyanobacteria](#)
[Endoliths](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbiocorrosion](#)

- Loeblich, A. R., and Tappan, H., 1988. *Foraminiferal Genera and their Classification*. New York: Van Nostrand Reinhold. [year of publication often cited erroneously as 1987; see Loeblich, A. R., and Tappan, H., 1989. Publication date of foraminiferal genera and their classification. *Journal of Paleontology*, **63**, 253.]
- Martin, R. E., Quigg, A., and Podkovyrov, V., 2008. Marine Biodiversification in response to evolving phytoplankton stoichiometry. *Palaeogeography Palaeoclimatology Palaeoecology*, **258**, 277–291.
- Moodley, L., Middelburg, J. J., Boschker, H. T. S., Duineveld, G. C. A., Pel, R., Herman, P. M. J., and Heip, C. H. R., 2002. Bacteria and foraminifera: key players in a short-term deep-sea response to phytodetritus. *Marine Ecology Progress Series*, **236**, 23–29.
- Moya, A., Pereto, J., Gil, R., and Latorre, A., 2008. Learning how to live together: genomic insight into prokaryote-animal symbiosis. *Nature Reviews Genetics*, **9**, 218–229.
- Murray, J. W., 2006. *Ecology and Applications of Benthic Foraminifera*. Cambridge: Cambridge University Press.
- Norris, R. D., 1996. Symbiosis as an evolutionary innovation in the radiation of Paleocene planktic foraminifera. *Paleobiology*, **22**, 461–480.
- Nozawa, F., Kitazato, H., Tsuchiya, M., and Gooday, A. J., 2006. 'Live' benthic foraminifera at a abyssal site in the equatorial Pacific nodule province: abundance, diversity and taxonomic composition. *Deep-Sea Research I*, **53**, 1406–1422.
- Nützel, A., Lehnert, O., and Fryda, J., 2006. Origin of planktotrophy - evidence from early molluscs. *Evolution and Development*, **8**, 325–330.
- Pascal, P., Dupuy, C., Mallet, C., Richard, P., and Niquil, N., 2008. Bacterivory in benthic organisms in sediment: quantification using ¹⁵N-enriched bacteria. *Journal of Experimental Biology and Ecology*, **355**, 18–26.
- Pawlowski, J., and Burki, F., 2009. Untangling the phylogeny of amoeboid protists. *Journal of Eukaryotic Microbiology*, **56**(1), 16–25.
- Riemann, F., 1985. Eisen und Mangan in pazifischen Tiefsee-Rhizopoden und Beziehungen zur Manganknollen-Genese. *Internationale Revue der gesamten Hydrobiologie*, **70**(1), 165–172.
- Riemann, F., and Helmke, E., 2002. Symbiotic relations of sediment-agglutinating nematodes and bacteria in detrital habitats: the enzyme sharing concept. *Marine Ecology*, **23**, 93–113.
- Risgaard-Petersen, N., Langezaal, A. M., Ingvarsdén, S., Schmid, M. C., Jetten, M. S. M., Op den Camp, H. J. M., Derksen, J. W. M., Pina-Ochoa, E., Eriksson, S. P., Nielsen, L. P., Revsbech, N. P., Cedhagen, T., and van der Zwaan, G. J., 2006. Evidence for complete denitrification in a benthic foraminifer. *Nature*, **443**, 93–96.
- Sarmiento, J. L., Dunne, J., Gnanadesikan, A., Key, R. M., Matsumoto, K., and Slater, R., 2002. A new estimate of the CaCO₃ to organic carbon export ratio. *Global Biogeochemical Cycles*, **16**(4), no 1107. doi:10.1029/2002/GB001919.
- Schiebel, R., 2002. Planktic foraminiferal sedimentation and the marine calcite budget. *Global Biogeochemical Cycles*, **16**(4), no 1065, doi:10.1029/2001GB001459.
- Sen Gupta, B., 1999. *Modern Foraminifera*. Dordrecht: Kluwer Academic Press.
- Witte, U., Wenzhöfer, F., Sommer, S., Boetius, A., Heinz, P. N. A., Sand, M., Cremer, A., Abraham, W. B. J. B., and Olaf, P., 2003. In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, **424**, 763–766.
- Zaric, S., Schulz, M., and Mulitza, S., 2006. Global prediction of planktonic foraminiferal fluxes from hydrographic and productivity data. *Biogeosciences*, **3**, 187–207.

Cross-references

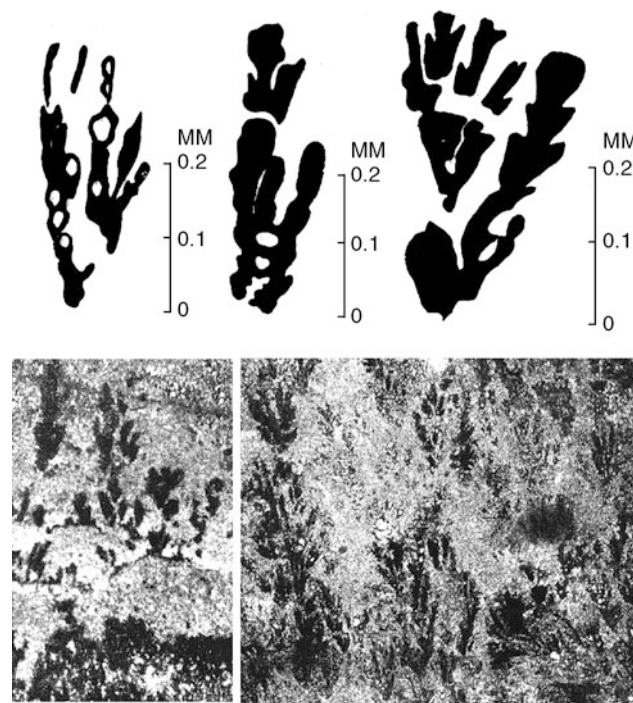
Algae (Eukaryotic)
 Bioerosion
 Carbonate Environments
 Carbonates
 Chemolithotrophy
 Divalent Earth Alkaline Cations in Seawater
 Isotopes and Geobiology
 Protozoa (Heterotroph, Eukaryotic)
 Symbiosis

FRUTEXITES

Marta Rodríguez-Martínez¹, Christine Heim²,
 Nadia-Valérie Quéric², Joachim Reitner²
¹Universidad de Alcalá, Alcalá de Henares, Madrid, Spain
²University of Göttingen, Göttingen, Germany

Synonyms

Colloform limonitic crusts; Frutexites crusts; Frutexites-like forms; Frutexites microstromatolite; Frutexites tuffs; Haematitic/ferruginous/iron microstromatolites; Iron dendritic aggregates; Iron shrubs; Pillar-shaped microstromatolites



Frutexites, Figure 1 Original figures of genus of *Frutexites* described by Maslov (1960).

Definition

Frutexites is a problematic microfossil rich in iron. From a taxonomic point of view, only five species have been figured (*Frutexites arboriformis*, Maslov, 1960; *F. microstroma*, Walter and Awramik, 1979; *Frutexites* sp. 1, *Frutexites* sp. 2, and *New gen.* 3, Tsien, 1979), although the authors mostly use the term *Frutexites sensu lato*. The genus *Frutexites* was coined by Maslov (1960) in order to describe submillimeter-sized, iron-rich, and subordinate calcite microfossils (Figure 1). *Frutexites* have a dendritic shape formed by divergently branched microcolumns. The height and width of microcolumns as well as their composition and microstructure can vary (Table 1). The preservation of microstructure is strongly controlled by its dominant mono- or polymineral

character. Microstructure is formed by convex-upward laminae, which sometimes show radially arranged fibers.

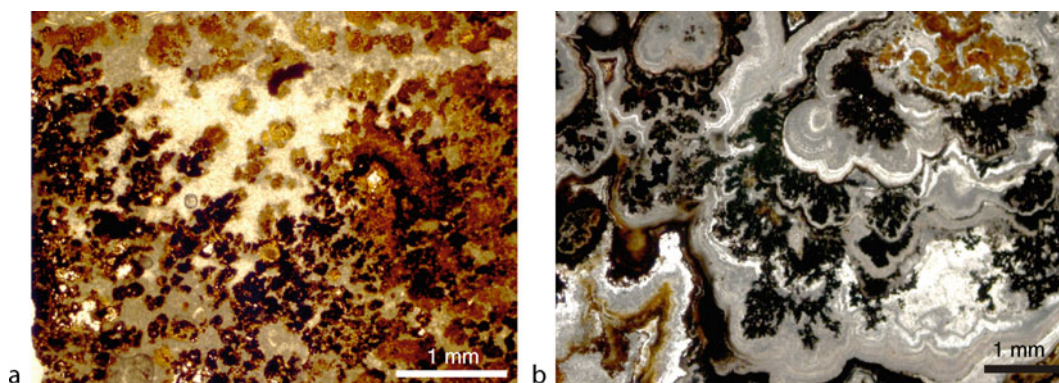
Frutexites can occur mainly as monomineral as well as polymineral structures with: iron-rich and/or manganese-rich (hematites, iron hydroxides, Fe-Mn oxyhydroxides), and/or carbonate-rich (calcite, ferroan calcite, dolomite), and/or siliciclastic-rich (argillite, microquartz), and/or phosphatic-rich zones.

Depositional environmental occurrences

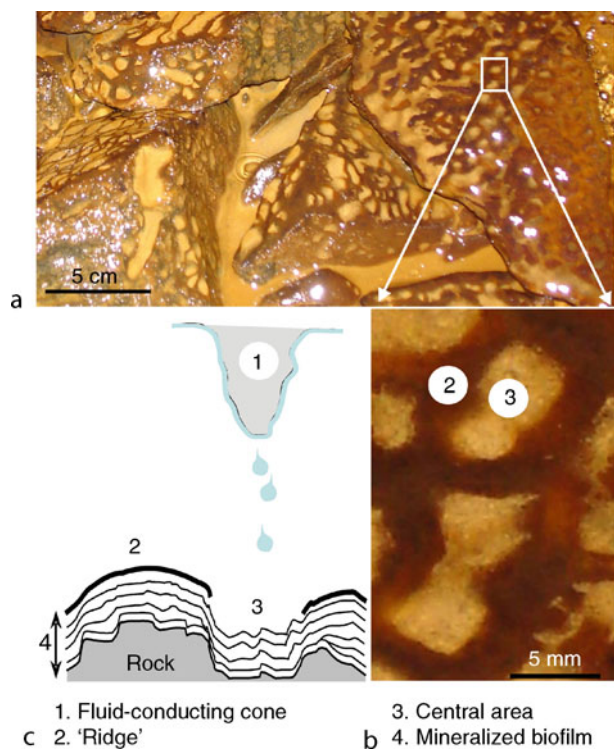
Frutexites has been described in marine environments such as shallow and deep water stromatolites, microbial limestones, hardgrounds, and condensed pelagic limestones as well as in cavities, sheet-cracks, veins, and Neptunian dikes. However, comparisons with

Frutexites, Table 1 Sizes, morphological and compositional parameters of *Frutexites* according to different authors

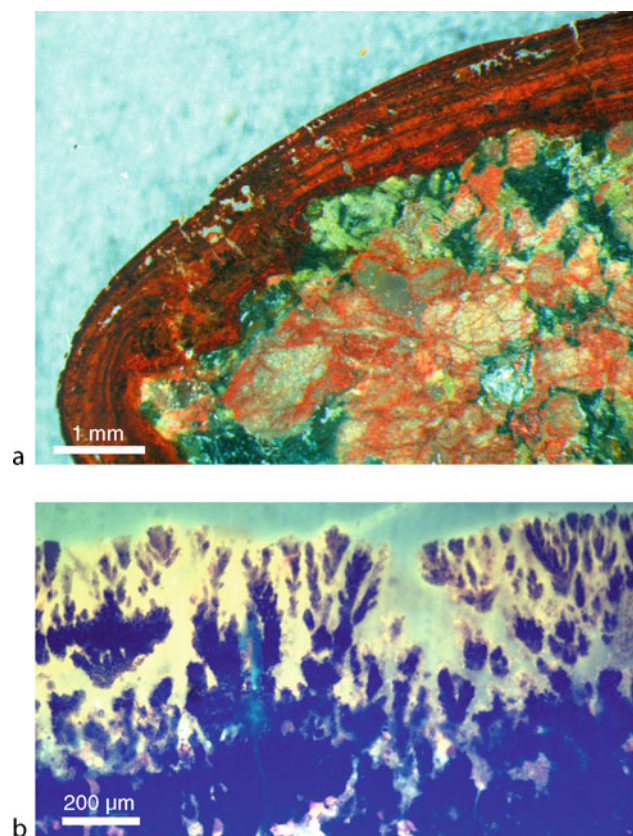
Authors	Height	Width	Microstructure	Morphology	Composition
Maslov (1960)	Up to 400 μm (see Figure 1)	25–30 μm , more of 50 μm	Sheets with occasionally circular spaces	Radially diverging and branched sheets	Iron hydroxide and carbonate
Horodyski (1975)	20–500 μm	10–200 μm	2–10 μm convex-upward laminae	Pillar-shaped branched and not	Hematite, calcite, and argillite
Walter and Awramik (1979)	Up to 450 μm	5–120 μm	0.7–2.7 μm convex upward laminae and axial tube (trichome?)	Undulose layers, laminae with protruding pustules and erect branching microcolumns	Organic matter permineralized by silica
Myrow and Coniglio (1991)	250 μm –4 mm, most <1 mm	75–600 μm , 250 μm average	Chambers, laminae, fibers, and projections	Unbranching and branching columns	Calcite, ferroan calcite, hematite, and microquartz
Böhm and Brachert (1993)	Up to 5 mm, average 1–2 mm		Convex laminae with radially arranged fibers		Fe-Mn oxides, calcite, and phosphates
Woods and Baud (2008)	Up to 800 μm	50–200 μm	Lighter and darker colored layers resulting chambered appearance		Hematite and/or Fe-Mn minerals, and calcite



Frutexites, Figure 2 (a) Marine Upper Cenomanian/Lower Turonian *Frutexites* colonies from a deepwater hardground environment (Liencrees coast, northern Spain; Reitner et al., 1995). The *Frutexites* facies is located on top of the hardground sequence and marks a fundamental change of the oceanographic conditions. (b) Deep subterranean *Frutexites* colonies growing on calcareous microstromatolites from tectonic fractures in the so-called “sole dolomite” at the base of the Naukluft Nappe Complex (NNC) in southern Namibia. The colonies are growing upside down from the fracture ceiling. The black ones are rich in manganese oxides, the ochre parts are enriched in iron oxides.



Frutexites, Figure 3 (a) Net-like, mineralized microbial mat covering the surface of granitic rocks (Åspö Hard Rock Laboratory, Sweden). (b) Scheme of the Åspö-dropping microsystem, comprising a pending mineral cone as a fluid-conduit (1) and the evolving, net-like microstructure beneath, displaying a dark mineralized ridge (2) covering the laminated biofilm (3) and the central area directly exposed to the fluid dropping (4). (c) Close-up of the net-like, mineralized microbial mat, pointing to the dark mineralized ridge (2) and the bright central area (4) according to scheme b.



Frutexites, Figure 4 (a) Thick section of the mineralized, net-like, recent biofilm from the tunnel of Åspö (Sweden) under reflected light, revealing the typical laminated structure. These laminae predominantly consist of iron oxides and hydroxides. (b) UV-fluorescence microphotography exhibits *Frutexites*-like colonies within the laminae of the mineralized biofilm.

continental black shrubs are common, too. *Frutexites* structures are also frequently found in veins and fractures of deep subterranean environments (Figures 2b, 3, and 4).

Stromatolites

The main record of *Frutexites* has commonly been recognized from shallow to deep calcareous stromatolites from Proterozoic to recent examples (see Caldera lake stromatolites at Tonga, by Kazmierczak and Kempe, 2006). *Frutexites* structures grow upward perpendicular to the stromatolite laminae; however, they can sometimes cross them (Horodyski, 1975) and even destroy them. For this reason, Böhm and Brachert (1993) interpreted that stromatolite accretion was independent of *Frutexites*, which would act as dweller or secondary binder in the Jurassic deep water stromatolites from Germany and Austria. Descriptions about the associated macro- and micro-fauna in the *Frutexites*-bearing stromatolites are not

abundant. In the Canning Basin, the late Devonian *Frutexites*-bearing stromatolites show abundant meta-zoans in life position as crinoids and coral holdfasts encrusting the successive laminae (Playford et al., 1976), which would indicate normal oxygenated water conditions (Nicoll and Playford, 1993). In Jurassic stromatolites, *Frutexites* occur with shells of *Bositra* and *Lenticulina* foraminifers.

Cavities, sheet cracks, veins, and Neptunian dikes

The second most frequent occurrence of *Frutexites* in the geologic record is related to cavity walls and fissures. On the horizontal surfaces *Frutexites* could display a dominant upward growth, but, in general, their growth is normally perpendicular to the substrate where they were nucleated. *Frutexites* occur interbedded with fibrous calcite cements as well as with internal sediment. The oldest described record of *Frutexites* is in the Paleoproterozoic Gunflint chert (Walter and Awramik, 1979) and from sheet

cracks within stromatolites of the Upper Vendian to Lower Cambrian Chapel Island Formation, Canada (Myrow and Coniglio, 1991). *Frutexites* have been found in cavities of Devonian deep water mud mounds and grouped with *Renalcis-Epiphyton* calcimicrobes, and with deep water stromatolites assemblage (Tsien, 1979) as well as in stromatactoid cavities from Viséan microbial limestones where they occur interbedded with marine isopachous crusts of fibrous and botryoidal calcite cements (Gischler, 1996). The presence of *Frutexites* in Neptunian dikes has been described only in Devonian records from the Harz Mountains in Germany (Gischler, 1996) and mud mounds of the Hamar Laghdad Ridge in Morocco (Cavalazzi et al., 2007). Similar structures as *Frutexites* have also been described in voids from Toarcian Mn-rich layer at Tatra Mountains in Poland (Jach and Dudek, 2005) as well as in synsedimentary karstic cavities in Pleistocene travertines in Germany (Koban and Schweigert, 1993).

Condensed pelagic limestones and hardgrounds

The last most extended occurrence of *Frutexites* is associated with condensed hemipelagic and pelagic red to grey limestones like Griotte and Hallstatt Limestone as well as Rosso Ammonitico facies from Devonian up to Jurassic. These facies occur during very low sedimentation rates, from deep to relatively shallow water depths, during fast transgressive and/or drowning episodes. Nektonic organisms are the dominant fauna (goniatites, nautiloids, ammonoids) and *Frutexites* occur in (a) water-sediment interfaces colonizing sessile fauna, reworked bioclasts as well as ferromanganese hardgrounds (see below) and (b) within the micritic sediment. The first type of *Frutexites* growth in condensed pelagic limestones was described in Matagne Formation (Devonian of Belgium) by Tsien (1979) and in current-swept shallow pelagic ridge (Tafilalt Platform, Devonian of Morocco) by Wendt (1988). No less representative examples have been shown from the Triassic Hallstatt facies of Austria (Wendt, 1969; Rodríguez-Martínez et al., in press) and Oman Mountains (Woods and Baud, 2008). In the Oman Mountains, the sea-floor was directly colonized by *Frutexites*-bearing microbialites and synsedimentary cements. However, in the Northern Calcareous Alps, multiple ferromanganese crusts were colonized by epibenthonic sessile agglutinated foraminifers which were successively encrusted by *Frutexites* forming pillar-like structures above the seawater-sediment interface.

A similar situation has been described from deep-water hardgrounds during Mid-Cretaceous times in Spain (Reitner et al., 1995) (Figure 2). In this case, a previous benthic community dominated by coralline sponges was replaced by thick limonitic stromatolites with encrusting foraminifera and colonies of *Frutexites*.

The growth of *Frutexites* within the sediment was firstly pointed out by Böhm and Brachert (1993). They described the changes in composition and shapes of

Frutexites as a result of their growth in open spaces or in interstitial environments. Mamet and Pr  at (2006) found *Frutexites* associated to other hematite microstructures in condensed Griotte facies (Coumiac Limestones, Montagne Noire, Baleas Limestone Spain) and Rosso Ammonitico Limestone (Subbetic Cordillera Spain).

Continental environments

Some authors (Myrow and Coniglio, 1991; B  hm and Brachert, 1993) have compared the marine records of *Frutexites* with similar arborescent, dendritic forms found in speleothems, desert varnish, and travertines. Also, continental manganese and iron-rich black shrubs have been compared with the marine *Frutexites* records (Koban and Schweigert, 1993; Chafetz et al., 1998). Shapes, sizes, and polymineral character are similar in both marine and continental records.

Subterranean environments

Frutexites structures are sometimes common in light-independent, deep continental caves, fractures, and veins forming small microbial crusts often associated with calcareous stromatolitic structures. A characteristic representative was found by J. Reitner in southern Namibia (Figure 2b) at the base of the Naukluft Nappe Complex (NNC), which is part of the early Cambrian Damara orogeny (Miller, 2008). The deep base of the NNC is formed by the so-called "Sole Dolomite" (Korn and Martin, 1959) which is often heavily fractured. Within these cryptic fractures *Frutexites* is very common and intergrows with calcareous stromatolitic structures. Thin-shelled freshwater type ostracodes are also abundant in this fracture system.

A modern example of *Frutexites* structures has been found in the deep tunnel of   sp   in southern Sweden (Heim et al., 2007), as part of a highly oxygenated system. Wherever groundwater drops from the tunnel ceiling, a net-like, mineralized micro-system is formed on any rock surface beneath (Figure 3). In general, the dropping system is associated to pending mineral cones which are dominated by the iron-oxidizing, chemolithotrophic bacterium *Gallionella ferruginea*.

The evolving net-like structures feature semi-solid ridges, harboring a particular, highly diverse microbial community. The cross-section of this mineralized biofilm shows laminae with *Frutexites*-like structures (Figure 4). These are mainly composed of iron hydroxides, iron oxides, and less abundant manganese oxides. Small amounts of siderite, calcite, and siliceous material occur side by side with iron and manganese oxides.

Interpretation

Holotypes of *Frutexites* spp. (*Frutexites arboriformis*, Maslov, 1960; *Frutexites microstroma*, Walter and Awramik, 1979) were originally described from stromatolites; thus, they were genetically linked to different types of cyanobacteria (Playford et al., 1976, 1984; Scytonematacean – Walter and Awramik, 1979;

Rivulariacean – Hofmann and Grotzinger, 1985; Angulocellularia group – Riding, 1991). Hofmann and Grotzinger (1985) discussed the affinity of *Frutexites* to different cyanobacteria and proposed further alternatives (purely physicochemical accretion and/or iron bacteria due to the ferruginous character of stromatolite). The occurrence of *Frutexites* in cavities, fissures, and dykes was interpreted as a cryptobiont role (Myrow and Coniglio, 1991). However, Tsien (1979) was the first suggesting a non-phototrophic character, sometimes linked to chemoheterotrophic cyanobacteria (Gischler, 1996) or to chemohetero and autotrophic bacteria (Cavalazzi et al., 2007). Böhm and Brachert (1993) emphasized the roles of *Frutexites* as a cryptobiontic as well as a cryptoendopelitic organisms (living in interstitial habitats). Based on these aspects, the authors proposed the preference of *Frutexites* for oxygen-deficient environments (dysaerobic to anaerobic conditions). Furthermore, they suggested a bacterial precursor for the formation of *Frutexites* but did not exclude a possible fungal and/or physicochemical origin. According to previous publications (Maslov, 1960; Myrow and Coniglio, 1991), Böhm and Brachert (1993) explained the ferromanganese or phosphate mineralogy of *Frutexites* as a replacement of primary carbonate mineralogy. However, a primary iron mineralization of *Frutexites* was postulated by Horodyski (1975) and Hurley and Van der Voo (1990). Others like Hofmann and Grotzinger (1985) believed that microbiota involved in *Frutexites* could regulate the local water chemistry and iron oxyhydroxides as well as aragonite could coprecipitate.

Finally, the occurrence of *Frutexites* in condensed pelagic limestones and hardgrounds has been linked to a physicochemical origin (Wendt, 1969) as well as to iron bacteria (Reitner et al., 1995; Mamet and Pr  at, 2006). Reitner et al. (1995) interpreted the *Frutexites*–foraminifer assemblage as an R-strategic community which replaced the previous K-strategic community (sponges–microbes) due to fundamental changes in oceanographic conditions (from oligotrophic to more eutrophic conditions). Mamet and Pr  at discussed the origin of red pigmentation in some Phanerozoic limestones, where some condensed pelagic limestones with *Frutexites* are included. They argued that under anoxic to dysoxic conditions, ferrous iron may be available for oxidation by microaerophilic iron microbes growing at the sediment–water interface. In contrast, the recent *Frutexites*-like structures found in the deep biosphere in fact grow in air-exposed environments under aerobic conditions. However, the presence of different mineral phases within these structures could be associated to changing redox conditions and/or to a different microbial community composition at micrometer-range.

Conclusions

The different modes of growth of *Frutexites* have been recognized within shallow to deep water stromatolites,

on the seawater–sediment interface, marine micritic sediments, continental cavities, and fractures of deep subterranean environments. Such wider environmental distribution has been taken into consideration for paleoecological interpretations. According to the occurrence of *Frutexites*, some general aspects can be summarized: (a) its distribution does not show bathymetric control (although the dominant record is in deep waters facies), (b) it is formed in environments with very low sedimentation rate (in quiet as well as in agitated waters), (c) in marine environments, *Frutexites* mainly encrusts heterozoan assemblages (crinoids, sponges, solitary corals, and foraminifers), and finally, (d) there is no proof for an exclusive occurrence of *Frutexites* under anoxic and aphotic conditions.

Most of the authors considered a bacterially induced origin for *Frutexites*, but the final assessment strongly depends on which mineralogical composition they interpreted as primary (timing of iron mineralization) as well as its *loci* of growth.

Bibliography

- B  hm, F., and Brachert, T. C., 1993. Deep-water stromatolites and *Frutexites* Maslov from the Early and Middle Jurassic of S-Germany and Austria. *Facies*, **28**, 145–168.
- Cavalazzi, B., Barbieri, R., and Ori, G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chafetz, H. S., Akdim, B., Julia, R., and Reid, A., 1998. Mn- and Fe-rich black travertine shrubs: bacterially (and nanobacterially) induced precipitates. *Journal of Sedimentary Research*, **68**, 404–412.
- Gischler, E., 1996. Late Devonian–Early Carboniferous deep-water coral assemblages and sedimentation on a Devonian seamount: Iberg Reef, Harz Mts., Germany. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **123**, 297–322.
- Heim, C., Simon, K., Qu  ric, N. -V., and Thiel, V., 2007. Mineralized microbial mats with extreme lanthanum enrichments in the tunnel of   sp  , Sweden. *Geochimica et Cosmochimica Acta*, Goldschmidt Abstracts 2007 – H, A390.
- Hofmann, J., and Grotzinger, J. P., 1985. Shelf-facies microbiotas from the Odjick and Rocknest formations (Epworth Group; 1.89 Ga), northwestern Canada. *Canadian Journal of Earth Sciences*, **22**, 1781–1792.
- Horodyski, R. J., 1975. Stromatolites of the Lower Missoula Group (middle Proterozoic), Belt Supergroup, Glacier National Park, Montana. *Precambrian Research*, **2**, 215–254.
- Hurley, N. F., and Van der Voo, R., 1990. Magnetostratigraphy, Late Devonian iridium anomaly, and impact hypotheses. *Geology*, **18**, 291–294.
- Jach, R., and Dudek, T., 2005. Origin of a Toarcian manganese carbonate/silicate deposit from the Kri  zna unit, Tatra Mountains, Poland. *Chemical Geology*, **224**, 136–152.
- Kazmierczak, J., and Kempe, S., 2006. Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafo‘ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Koban, C. G., and Schweigert, G., 1993. Microbial origin of travertine fabrics – two examples from southern Germany (Pleistocene Stuttgart travertines and Miocene Riedoschingen travertine). *Facies*, **29**, 251–264.

- Korn, H., and Martin, H., 1959. Gravity tectonics in the Naukluft Mountains of South West Africa. *Bulletin of Geological Society of America*, **70**, 1047–1078.
- Mamet, B., and Pr  at, A., 2006. Iron-bacterial mediation in Phanerozoic red limestones: State of the art. *Sedimentary Geology*, **185**, 147–157.
- Maslov, V. P., 1960. Stromatolites. *Trudy Instituta geologicheskikh nauk Akademii nauk SSR*, **41**, 188.
- Miller, R. McG., 2008. *The Geology of Namibia*. Windhoek: Geological Survey of Namibia, Vol. 2.
- Myrow, P. M., and Coniglio, M. 1991. Origin and diagenesis of cryptobiontic *Frutixites* in the Chapel island Formation (Vendian to Early Cambrian) of Southeast Newfoundland, Canada. *Palaios*, **6**, 572–585.
- Nicoll, R. S., and Playford, P. E., 1993. Upper Devonian iridium anomalies, conodont zonation and the Frasnian-Famennian boundary in the Canning Basin, Western Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **104**, 105–113.
- Playford, P. E., Cockbain, A. E., Druce, E. C., and Wray, J. L., 1976. Devonian stromatolites from the Canning Basin, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 543–563.
- Playford, P. E., McLaren, D. J., Orth, C. J., Gilmore, J. S., and Goodfellow, W. D., 1984. Iridium anomaly in the Upper Devonian of the Canning Basin, Western Australia. *Science*, **226**, 437–439.
- Reitner, J., Wilmsen, M., and Neuweiler, F., 1995. Cenomanian/Turonian sponge microbialite deep-water hardground community (Liencre, Northern Spain). *Facies*, **32**, 203–212.
- Riding, R., 1991. Calcified cyanobacteria. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 55–87.
- Rodr  guez-Mart  nez, M., Heim, C., Simon, K., Reitner, J., and Zilla, T., (in press). *Tolypammina gregaria* Wendt-*Frutixites* assemblage and ferromanganese crusts: a coupled nutrient-metal interplay in the Carnian sedimentary condensed record of Hallstatt Facies (Austria). *Lecture Notes in Earth Sciences*
- Tsien, H. H., 1979. Paleocology of algal-bearing facies in the Devonian (Cuvirtian to Frasnian) Reef complexes of Belgium. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **27**, 103–127.
- Walter, M. R., and Awramik, S. M., 1979. *Frutixites* from stromatolites of the Gunflint iron formation of Canada, and its biological affinities. *Precambrian Research*, **9**, 23–33.
- Wendt, J., 1969. Foraminiferen-‘Riffe’ im karnischen Hallst  tter Kalk des Feuerkogels (Steiermark,   sterreich). *Pal  ontologische Zeitschrift*, **43**, 177–193.
- Wendt, J., 1988. Condensed carbonate sedimentation in the late Devonian of the eastern anti-Atlas (Morocco). *Eclogae geologicae Helvetica*, **81**, 155–173.
- Woods, A. D., and Baud, A., 2008. Anachronistic facies from a drowned Lower Triassic carbonate platform: Lower member of the Alwa Formation (Ba’id Exotic), Oman Mountains. *Sedimentary Geology*, **209**, 1–14.

Cross-references

Bacteria
 Calcified Cyanobacteria
 Cyanobacteria
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Stromatactis
 Stromatolites

FUNGI AND LICHENS

Bettina Weber, Burkhard B  del
 University of Kaiserslautern, Kaiserslautern, Germany

Definition

Fungi. Saprophytic, parasitic, or symbiotic living organisms, mainly filamentous (hyphae), rarely unicellular. Cell walls are of chitin or other non-cellulose compounds.
Lichens. Symbiotic organisms composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont.

Characteristics

Fungi

Fungi are a group of eukaryotic, heterotrophic organisms with their thallus on or in the substratum. The thallus can be unicellular (rarely) or filamentous (mostly), the latter being either composed of septate or nonseptate hyphae and often forming a more or less defined web of filaments (mycelium). The cell wall is typically chitinized or composed of other non-cellulose compounds. The spore forming thallus (sporocarp) can be microscopic or macroscopic with limited tissue differentiation. Fungi are cosmopolitan and ubiquitous, saprotrophic, mutualistic (mycorrhizal, lichen-forming), or parasitic. A number of rock-inhabiting fungi belong to the Deuteromycetes, a heterogenic group of fungi that develop no or only asexual sporocarp types. The vast majority of rock fungi are dark brown to blackish pigmented and are filamentous or forming microcolonies (B  del et al., 2000).

Lichens

Symbiotic mode of living

Lichens are mutualistic organisms consisting of a fungal partner, the mycobiont, and of one or more photosynthetic partners, the photobiont. Taxonomically speaking, they belong to the kingdom of fungi and represent here a special group, the lichenized fungi (Honegger, 1996). The lichen-forming fungi are polyphyletic, meaning that they have developed several times during the evolution of fungi. This brought about a taxonomically heterogeneous group with the large majority of them belonging to the Ascomycetes and only few Basidiomycetes and Deuteromycetes (= Fungi Imperfecti; Nash III, 1996a, Miadlikowska et al., 2006).

As photobiont of lichens, green algae with the most frequent genera *Trebouxia* and *Trentepohlia* make up about 90% of the photosynthetic partners, whereas cyanobacteria with the prevalent genus *Nostoc* account for the remaining 10%. The green algae, also referred to as “chlorobionts” (Lange and Wagenitz, 2003), belong to the eucaryotic algae which share many cytological features and their pigmentation (e.g., chlorophylls a and b) with land plants (Bold and Wynne, 1985; van den Hoek et al., 1993). Cyanobacteria (“cyanobionts”) are of

- Korn, H., and Martin, H., 1959. Gravity tectonics in the Naukluft Mountains of South West Africa. *Bulletin of Geological Society of America*, **70**, 1047–1078.
- Mamet, B., and Pr  at, A., 2006. Iron-bacterial mediation in Phanerozoic red limestones: State of the art. *Sedimentary Geology*, **185**, 147–157.
- Maslov, V. P., 1960. Stromatolites. *Trudy Instituta geologicheskikh nauk Akademii nauk SSR*, **41**, 188.
- Miller, R. McG., 2008. *The Geology of Namibia*. Windhoek: Geological Survey of Namibia, Vol. 2.
- Myrow, P. M., and Coniglio, M. 1991. Origin and diagenesis of cryptobiontic *Frutixites* in the Chapel island Formation (Vendian to Early Cambrian) of Southeast Newfoundland, Canada. *Palaios*, **6**, 572–585.
- Nicoll, R. S., and Playford, P. E., 1993. Upper Devonian iridium anomalies, conodont zonation and the Frasnian-Famennian boundary in the Canning Basin, Western Australia. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **104**, 105–113.
- Playford, P. E., Cockbain, A. E., Druce, E. C., and Wray, J. L., 1976. Devonian stromatolites from the Canning Basin, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 543–563.
- Playford, P. E., McLaren, D. J., Orth, C. J., Gilmore, J. S., and Goodfellow, W. D., 1984. Iridium anomaly in the Upper Devonian of the Canning Basin, Western Australia. *Science*, **226**, 437–439.
- Reitner, J., Wilmsen, M., and Neuweiler, F., 1995. Cenomanian/Turonian sponge microbialite deep-water hardground community (Liencres, Northern Spain). *Facies*, **32**, 203–212.
- Riding, R., 1991. Calcified cyanobacteria. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 55–87.
- Rodr  guez-Mart  nez, M., Heim, C., Simon, K., Reitner, J., and Zilla, T., (in press). *Tolypammina gregaria* Wendt-*Frutixites* assemblage and ferromanganese crusts: a coupled nutrient-metal interplay in the Carnian sedimentary condensed record of Hallstatt Facies (Austria). *Lecture Notes in Earth Sciences*
- Tsien, H. H., 1979. Paleocology of algal-bearing facies in the Devonian (Cuvirtian to Frasnian) Reef complexes of Belgium. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **27**, 103–127.
- Walter, M. R., and Awramik, S. M., 1979. *Frutixites* from stromatolites of the Gunflint iron formation of Canada, and its biological affinities. *Precambrian Research*, **9**, 23–33.
- Wendt, J., 1969. Foraminiferen-‘Riffe’ im karnischen Hallst  tter Kalk des Feuerkogels (Steiermark,   sterreich). *Pal  ontologische Zeitschrift*, **43**, 177–193.
- Wendt, J., 1988. Condensed carbonate sedimentation in the late Devonian of the eastern anti-Atlas (Morocco). *Eclogae geologicae Helvetica*, **81**, 155–173.
- Woods, A. D., and Baud, A., 2008. Anachronistic facies from a drowned Lower Triassic carbonate platform: Lower member of the Alwa Formation (Ba’id Exotic), Oman Mountains. *Sedimentary Geology*, **209**, 1–14.

Cross-references

Bacteria
 Calcified Cyanobacteria
 Cyanobacteria
 Fe(II)-Oxidizing Prokaryotes
 Gallionella
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Stromatactis
 Stromatolites

FUNGI AND LICHENS

Bettina Weber, Burkhard B  del
 University of Kaiserslautern, Kaiserslautern, Germany

Definition

Fungi. Saprophytic, parasitic, or symbiotic living organisms, mainly filamentous (hyphae), rarely unicellular. Cell walls are of chitin or other non-cellulose compounds.
Lichens. Symbiotic organisms composed of a fungal partner, the mycobiont, and one or more photosynthetic partners, the photobiont.

Characteristics

Fungi

Fungi are a group of eukaryotic, heterotrophic organisms with their thallus on or in the substratum. The thallus can be unicellular (rarely) or filamentous (mostly), the latter being either composed of septate or nonseptate hyphae and often forming a more or less defined web of filaments (mycelium). The cell wall is typically chitinized or composed of other non-cellulose compounds. The spore forming thallus (sporocarp) can be microscopic or macroscopic with limited tissue differentiation. Fungi are cosmopolitan and ubiquitous, saprotrophic, mutualistic (mycorrhizal, lichen-forming), or parasitic. A number of rock-inhabiting fungi belong to the Deuteromycetes, a heterogenic group of fungi that develop no or only asexual sporocarp types. The vast majority of rock fungi are dark brown to blackish pigmented and are filamentous or forming microcolonies (B  del et al., 2000).

Lichens

Symbiotic mode of living

Lichens are mutualistic organisms consisting of a fungal partner, the mycobiont, and of one or more photosynthetic partners, the photobiont. Taxonomically speaking, they belong to the kingdom of fungi and represent here a special group, the lichenized fungi (Honegger, 1996). The lichen-forming fungi are polyphyletic, meaning that they have developed several times during the evolution of fungi. This brought about a taxonomically heterogeneous group with the large majority of them belonging to the Ascomycetes and only few Basidiomycetes and Deuteromycetes (= Fungi Imperfecti; Nash III, 1996a, Miadlikowska et al., 2006).

As photobiont of lichens, green algae with the most frequent genera *Trebouxia* and *Trentepohlia* make up about 90% of the photosynthetic partners, whereas cyanobacteria with the prevalent genus *Nostoc* account for the remaining 10%. The green algae, also referred to as ‘‘chlorobionts’’ (Lange and Wagenitz, 2003), belong to the eucaryotic algae which share many cytological features and their pigmentation (e.g., chlorophylls a and b) with land plants (Bold and Wynne, 1985; van den Hoek et al., 1993). Cyanobacteria (‘‘cyanobionts’’) are of

procaryotic nature, lacking a nucleus, mitochondria and chloroplasts, all of which are found in eucaryotic cells. The DNA, which in eucaryotic cells is stored within the nucleus, is found as circular DNA in the cytoplasm and also the thylacoids, which occur in the chloroplasts of eucaryotes, are here embedded into the cytoplasm (Friedl and Büdel, 2008).

The interactions between the mycobiont and photobiont are not completely balanced. There is metabolic transport from the autotrophic photobiont to the heterotrophic mycobiont in the form of carbohydrates, which passively leak from the photobiont cells. Metabolic transport processes from the mycobiont to the photobiont are not known (Schöller and Mollenhauer, 1997). However, the photobiont often also profits from growth within the mycelium of hyphae, since more balanced water and light conditions are granted within the thallus. Besides that, the mycobionts produce a variety of secondary substances that might be beneficial for the photobiont (Schöller and Mollenhauer, 1997). Therefore, the lichenization of the photobiont is probably most adequately described as a controlled parasitism by lichen-forming fungi (Ahmadjian, 1993).

The thallus of a lichen is formed by a more or less dense network of fungal hyphae, in which the photobionts are either evenly distributed (homoiomorous thalli), or are concentrated within a defined layer (heteromorous thalli). Homoiomorous thallus construction mainly occurs in gelatinous, crustose lichens (e.g., *Collema*, *Pyrenopsis*) whereas heteromorous thallus construction is typically formed by an upper cortex followed by a photobiont layer, a medulla and a lower cortex (Büdel and Scheidegger, 2008). A surface tissue, like the epidermis with stomata and a protective cuticle as in higher plants, does not exist in lichens, neither do they have water and nutrient-transporting structures.

Lichen morphology

The appearance of a lichen thallus is determined primarily by the mycobiont and only in few cases the photobiont determines, to a certain extent, the form of a thallus (e.g., in the filamentous genera *Coenogonium*, *Ephebe*, *Cystocoleus*, and *Rhacodium*). However, only after the establishment of the symbiosis, under the influence of the photobiont, the characteristic thallus of a lichen is developed (Büdel and Scheidegger, 2008).

Based on their overall growth form, lichens are traditionally divided into three main morphological types: crustose, foliose, and fruticose. Crustose lichens are tightly attached to the substrate with their lower surface and cannot be removed without destruction of the thallus. Foliose lichens form leaf-like thalli that are partly attached to the substrate and have a dorsiventral organization (Büdel and Scheidegger, 2008). Thallus lobes of fruticose lichens are hairlike, strap-shaped, flat, or cylindrical, forming erect or hanging thalli. Some fruticose genera like *Baeomyces* and *Cladonia* develop a twofold thallus with

a foliose horizontal and a fruticose vertical thallus part. Endolithic lichens represent a special form of crustose lichens, where the thallus partly or completely grows within the rock. According to Golubic et al. (1981), the endolithic growth form can be further separated into cryptoendolithic (= growing within the rock), chasmoendolithic (= growing along cracks or fissures of the rock), and euendolithic (= actively boring into the rock) organisms.

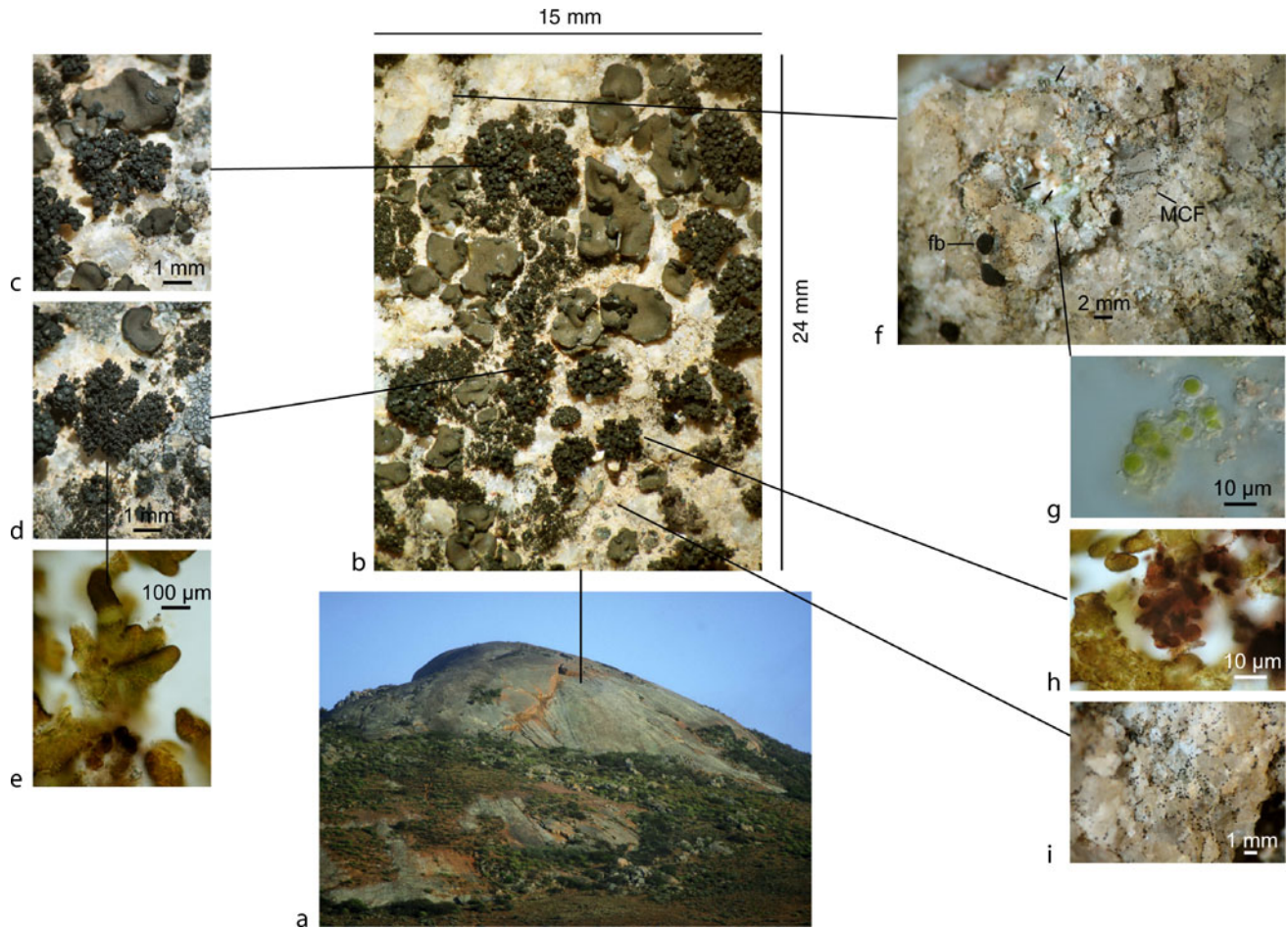
Diversity

Fungi

Rock-inhabiting fungi belong to major groups of fungi: the hyphomycetes and the “black fungi,” the latter named because of the strong production of melanoid pigments that causes dark-brown or blackish pigmented hyphae. Formation of microcolonies is a universal feature of the latter group, and they are therefore named “microcolonial fungi” (= MCF; Figure 1f and i; Gorbushina, 2003). Hyphomycetes form a mycelium but lack a sporocarp and the conidia (asexual spores) are borne on hyphae. They occur on soil or epiphytic habitats and dominate on rock surfaces under milder climate regimes (e.g., high air humidity, abundant nutrients). The black MCF prevail under harsh climatic conditions and are slow-growing. They represent a diverse assemblage of non-lichenized ascomycetes. Because of a convergent microcolonial morphology and their morphological plasticity, they are extremely difficult to identify by classical methods. Identification procedures based on molecular methods are just in progress and have been applied to a few strains only. So far, identified samples and strains have been placed in the mostly anamorphic (asexual) genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Coniosporium*, *Cryomyces*, *Friedmanniomyces*, *Lichenothelia*, *Phaeococcomyces*, *Phaeosclera*, *Phoma*, *Sarcinomyces*, and *Scolecobasidium* (Sigler et al., 1981; Diakumaku et al., 1995; Wollenzien et al., 1997; Sterflinger et al., 1997; Büdel et al., 2000; Selbmann et al., 2005; Onofri et al., 2007). Most of them belong to the ascomycete order Pleosporales, Capnodiales, and Dothideales, summarized in the class Dothideomycetes (Schoch et al., 2006), few belong to the Chaetothyriales in the class Eurotiomycetes (Geiser et al., 2006).

Lichens

Lichens grow on a wide variety of substrates including rocks (= epi- or endolithic), soil (= epigeic), detritus, and the branches, stems (= epiphytic), and leaves of trees (= epiphyllic). Most lichen species are specialized to one type of substrate comprising certain habitat characteristics. There are species with a wide ecological amplitude (euryoecious as compared to stenoecious species), able to colonize different types of substrate, like e.g., *Caloplaca citrina* (Hoffm.) Th.Fr., *Candelariella vitellina* (Hoffm.) Müll.Arg., and *Physcia adscendens* (Fr.) Oliv.,



Fungi and Lichens, Figure 1 (a) Granite inselberg at Soebatsfontein, Succulent Karoo, South Africa. (b) Close-up of the rock surface, showing dense colonization by the microlichens *Peltula bolanderi* (large olive-colored thalli), *Peccania* sp., *Synalissa* sp., cyanobacteria, and microcolonial fungi. (c) Detail with *Peccania* sp. (d) Detail with *Synalissa* sp. (e) Microscopical view on the lobes of *Synalissa* sp., showing the incorporation of the yellow pigment scytonemin (light and UV protectant) in the gelatinous sheath of the lichen. (f) Endolithic lecidoid lichen, thallus parts visible; where the rock surface was removed (arrows), the sporocarps (fruit bodies) are exposed on the rock surface (fb); MCF indicates the presence of numerous microcolonial fungi. (g) Microscopical view on a part of the algal layer of the endolithic lecidoid lichen showing the *Trebouxia*-like chlorobionts in an envelope of lichen hyphae. (h) Colonies of the red-sheathed cyanobacterium *Gloeocapsa* sp. growing on the rock surface between the lichens. (i) Microcolonial fungi on the rock surface.

and there are nutrient-tolerant species that occur epiphytically as well as epilithically (Figure 2b and c; Wirth, 1995).

On soils, lichens often represent part of a biological soil crust (BSC). BSC are formed by an association between soil particles and different proportions of cyanobacteria, algae, microfungi, bryophytes, and lichens (Belnap et al., 2003). In arid and semiarid regions, which make up more than 30% of the land surface of the earth, BSC cover up to 25% of the soil surface (Weber et al., 2008), but also in temperate regions like Central Europe, BSC occur in various habitats (Büdel, 2003).

Rock-inhabiting lichens come from most taxonomic groups of lichens as, for example, members of the class Lichinomycetes (e.g., *Anema*, *Digitothyrea*, *Heppia*, *Lichinella*, *Peccania*, *Peltula*, *Phyllisciella*, *Synalissa*, *Thyrea*; Figure 1b–e) that exclusively have cyanobacteria as photobionts. Although these lichens can be most abundant in arid and semiarid landscapes, the vast majority of lichens (more than 200 species) come from the largest class of lichenized fungi, the Lecanoromycetes. Within the Lecanoromycetes, green algal photobionts dominate (e.g., *Acarospora*, *Buellia*, *Caloplaca*, *Dermatiscum*, *Dimelaena*, *Hymenelia*,



Fungi and Lichens, Figure 2 (a) Vegetation pattern of lichens on a natural rock surface (dolomite, Untersberg, Austria). *Hymenelia coerulea* (grayish blue areas), *Hymenelia prevostii* (white, arrows), both endolithic growing lichens. (b) Lichen community colonizing mortar of medieval churches (built in the thirteenth to fifteenth centuries, Mecklenburg-Western Pomerania, Germany) consisting of *Caloplaca citrina* (Hoffm.) Th.Fr., *Caloplaca saxicola* (Hoffm.) Nordin, and *Lecanora dispersa* (Pers.) Sommerf. (c) Damp lower part of north-exposed wall of rural church built on a field stone foundation and brick walls joined with mortar. Lichen community consisting of *Enterographa zonata* (Körb.) Torrente & Egea, *Haematomma ochroleucum* (Neck.) J.R.Laundon, *Ramalina pollinaria* (Westr.) Ach., *Xanthoria calcicola* Oxner and *Lepraria incana* (L.) Ach. (Mecklenburg-Western Pomerania, Germany). (d) Endolithic growing lichen *Caloplaca* sp., growing on sandstone of the Clarens formation, Golden Gate Highlands National Park, South Africa. This lichen is actively weathering the rock; note the rim of the rock at the margin of the lichen, indicating the original rock surface.

Lecidea, *Lecanora*, *Lepraria*, *Rinodina*, *Parmelia*, *Parmotrema*, *Usnea*, *Verrucaria*, *Xanthoparmelia*; Figure 2a).

Apart from natural habitats, lichens also grow on artificial substrates like mortar and building stone (Figure 2b and c; e.g., Ariño and Saiz-Jimenez, 1996; Weber and Büdel, 2001), wooden material like fences and benches, and even glass, bone, tyres, and metal under favorable climatic conditions (Schöller, 1997).

Biogeography

Fungi

Since determination on the species level is a challenging task and not easily possible without culturing or before more molecular data are available, biogeographical information is very limited at present. For some species of the genus *Lichenothelia*, however, information on distribution patterns are available. While *L. intermedia* occurs on almost every granite rock surface throughout Africa, the species *L. gigantea* and *L. radiata* are restricted to Australia, and *L. globulifera* was only found on rock outcrops of the Seychelle island La Digue (Henssen 1987). On the basis of morphological and molecular data (SSU phylogeny and ITS sequence data), Selbmann et al. (2005) recently described two new genera of MCF, *Cryomyces*, and *Friedmanniomyces*, only known from Antarctic rocks so far. The authors provide good evidence that at least the new genus *Cryomyces* might be endemic to Antarctica. Since MCF diaspores are easily spread over large distances by the wind, many of them might have a cosmopolitan distribution (airborne fungi).

Lichens

Lichens have developed during evolutionary radiation of ascomycete fungi within the last 600–400 million years, possibly contemporaneous to the emergence of land plants (Galloway, 1996). Due to this long-lasting evolutionary process, their distribution patterns show similarities to other groups of organisms and can be explained utilizing the theory of plate tectonics (Galloway, 1996). Compared to vascular plants, lichens comprise a higher number of cosmopolitan species within different climate zones of the earth and endemism within smaller geographic regions is normally lower. Exemplarily, on the Kap Verde Islands 13.4% of vascular plants were endemics, whereas only 2.5% of the lichen species were found to be endemic (Schöller, 1997). Dispersal of both symbiotic partners is facilitated by the fact that fungal spores as well as algal cells are transported over vast distances within the air (Brown et al., 1964).

At least 16 major distribution patterns of lichens can be distinguished (Galloway, 1996). Besides lichen species that are constrained to one single continent, like austral and neotropical taxa, there are distribution patterns that comprise spatially contiguous regions, like the paleotropics that include most of Africa, the Arabian Peninsula, most of the Indian subcontinent, the Malaysian archipelago, and the Islands of the Pacific Ocean (Galloway, 1996). A third type of distribution pattern embraces disjunct distributions over continents, which have been close by or unified in history, but were separated by plate tectonic movements, like pantropical and American–Asian distribution patterns (Grotzinger et al., 2006). Taxa with bipolar distribution represent the last pattern, which results from climate change through earth history. These organisms occur at high latitudes of the

northern and southern hemisphere, but often also occur in alpine regions, forming relict habitats of the last ice age (Galloway, 1996).

Physiology

Fungi

There is not much known of the specific physiology of rock-inhabiting fungi except that they are able to tolerate extremely high (70–80°C; Palmer et al., 1987) and low temperatures (–40°C; Selbmann et al., 2005) due to their poikilohydric nature and related to that, their ability to withstand long periods (in the magnitude of tens of years) in the dry state. Many fungi are heavily melanized, most probably as a shelter against high radiation of both visible and UV light.

Lichens

Lichens are poikilohydric organisms, meaning that they cannot actively take up or lose water. Moistening of the thallus can be achieved by rain/snow precipitation, dew-fall, and equilibrium with high air humidity. This clearly distinguishes them from vascular plants that actively regulate water uptake and loss.

Water

Although water uptake, storage, and loss are modified by differences in thallus structure and morphology, these mechanisms are predominantly controlled by physical processes (Rundel, 1988). Therefore, the physiological activity of lichens is highly dependent on the prevailing water conditions. In general, the photosynthetic response of lichens to increasing water content can be described as an optimum curve, which, however, reveals large variability (Lange et al., 2001). Air-dried lichens with a water content below 15–30% show no photosynthetic response at all (Nash III, 1996b). With increasing water content, lichens first reveal net respiration rates before the compensation point is reached, above which positive photosynthetic activity is recorded. In general, cyanolichens commence positive net photosynthesis (NP) at higher water contents and are generally able to perform positive NP under higher water content than chlorolichens (especially the homoiomerous species; Lange et al., 2001).

Above an optimum water content, at which maximum NP rates are reached, free water is present and the lichens have a water potential close to zero (Green and Snelgar, 1982). These periods of water supra-saturation cause a depression in photosynthesis by increased thallus diffusive resistances; CO₂ pathways within the thallus are blocked by water (Lange et al., 1997). Water supra-saturation can occur in chloro- and cyanolichens of all growth forms (Lange et al., 1996, 2000, 2004; Lange and Green, 1997), and also endolithic lichens of various substrates have to cope with this problem (Tretiach and Pecchiari, 1995; Tretiach and Geletti, 1997; Winkler and Kappen, 1997). In a recent study, the photosynthetic

performance of *Hymenelia prevostii* and *H. coerulea*, two endolithic green algal lichens growing on steep limestone outcrops in the eastern alpine mountains, have been investigated in detail (Weber et al., 2007). Both species were saturated at extremely low water content (*H. prevostii*: 0.26 mm, *H. coerulea*: 0.07 mm rainfall equivalent) and revealed very narrow ranges of positive NP values upon increasing thallus water content (0.2 mm vs. 0.1 mm). Their growth on steep rock surfaces therefore represents an adaptation to avoid excessively high water contents or the formation of CO₂ blocking water films.

A crucial difference in the physiology of cyano- and chlorolichens lies in the capability to use water vapor for photosynthetic activation. While air-dry chlorolichens gain positive NP in equilibrium with air of about 80% relative humidity and above, all cyanobacterial lichens investigated so far need liquid water for activation of NP (Büdel and Lange, 1991; Lange et al., 1993). This fundamental difference also affects the natural habitats of lichens with cyanolichens preferably occurring at moist habitats with frequent liquid water (e.g., *Collema* spp., *Lichinella* spp.), whereas green algal lichens often also occur in rain-shaded habitats of high air humidity (e.g., *Lepraria* spp., *Usnea* spp.). The endolithic *Hymenelia* species mentioned above were also shown to perform positive NP at relative air humidities of 90% and above (Weber et al., 2007).

Light

The capture of radiant energy is essential for photosynthesis. Only light of wavelengths between 400 and 780 nm, the photosynthetically active radiation (PAR), is utilized for photosynthesis (Nash III, 1996b). Under constant and favorable abiotic conditions, photosynthetic response of lichens to increasing light intensities exhibits a saturation curve with saturation for most species occurring between 100 and 400 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Demmig-Adams et al., 1990). However, extremely shade-tolerant species have saturation values as low as 20 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Green et al., 1991), whereas others, like *Ramalina maciformis* (Del.) Bory reveal values above 1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Demmig-Adams et al., 1990). At increasing temperatures, light saturation is achieved at higher levels (Kershaw, 1985) and also water content has an influence (Lange, 1969), probably due to alterations of light penetration within the thallus (Ertl, 1951; Dietz et al., 2000). Exposure of fully hydrated lichens to excess light can induce a depression of photosynthesis, both in chloro- and cyanolichens (Demmig-Adams et al., 1990).

As a protection against excessive light, lichens in general possess a cortex made of fungal tissue, and some species even form a so-called epinecral layer consisting of dead, collapsed, and often gelatinized hyphae and photobiont cells (Büdel and Scheidegger, 2008). *Peltigera rufescens* (Weiss) Humb., for example, has a cortical organization that causes a decrease of light transmittance to

40% at the upper boundary of the algal layer (Dietz et al., 2000).

The endolithic growth form (Figure 1f) can also be interpreted as a protection from excess light. Winkler and Kappen (1997) found that only 0.1–0.2% of the ambient light reaches the upper part of the algal layer in *Sarcogyne* cf. *austroafricana* (Zahlbr.) H. Magn. Light reduction within the sandstone on top of the photosynthetically active layer is also reflected by light saturation values above 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ measured for *Sarcogyne* cf. *austroafricana*, *Lecidea* aff. *sarcogynoides* Körb., *Lecidea confluenta* Müll. Arg. and *Lithoglypha aggregata* Brusse (Winkler and Kappen, 1997). In limestone, *Hymenelia prevostii* and *H. coerulea* were saturated at high light intensities (1,000 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ vs. 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$; Weber et al., 2007).

Temperature

Lichens are generally known to survive a wide range of temperatures. Being dominant terrestrial organisms of Antarctica, they must be able to tolerate extremely low temperatures (Nash III, 1996b), whereas in sun-exposed microhabitats, lichens experience temperatures of up to 60°C (Hahn et al., 1989). However, lichens in general reveal a vast difference in temperature tolerance depending on whether the thalli are hydrated or dry. Whereas dry thalli of some lichen species are able to tolerate a stepwise cooling to temperatures as low as –196°C (Kappen, 1973) and heating up to 90 or 100°C (Lange, 1953), wet thalli are by far more sensitive. Review of field measurements of lichen photosynthesis revealed that many lichens are primarily active at temperatures between 5°C and 20°C, although 0–10°C may better characterize some Antarctic species (Kappen, 1988; Kappen and Friedmann, 1983).

Photosynthetic capacity of some lichen species is known to reveal seasonal changes that involve an adjustment to higher summer temperatures (Kershaw, 1985). Recently, Lange and Green (2005) proved that dark respiration (DR) of lichens does exhibit seasonal changes of temperature sensitivity. At 5°C, for example, the respiration rates of *Cladonia convoluta* (Lam.) P.Cout., *Lecanora muralis* (Schreber) Rabenh., and *Diploschistes muscorum* (Scop.) R.Sant. were several times higher in winter than in summer. This acclimation of DR implies that maximal net carbon fixation rates remain similar throughout the year and are not depressed by increased carbon loss due to higher temperatures during the warmer season (Lange and Green, 2005).

CO₂

The photosynthetic CO₂-fixation rate of lichens at optimal water content generally increases with increasing CO₂-concentrations, typically reaching saturation around 600–1,300 ppm (Nash III, 1996b; Tretiach and Geletti, 1997; Lange et al., 1999; Lange, 2002). At natural CO₂ concentrations (around 360 ppm), many species only

reach about 60–80% of maximal NP under optimal water conditions (Lange et al., 1999; Lange, 2002).

Under supra-saturated water conditions, NP of lichens is often depressed at natural CO₂ concentrations. Increased CO₂ values, however, in most cases result in attenuation of this depression (e.g., Lange, 2002). In some species, as for the epigeic species *Fulgensia fulgens* (Sw.) Elekin, a severe depression in CO₂ uptake can even be completely counterbalanced by a CO₂ concentration of 4,500 ppm (Lange et al., 1999).

The CO₂ compensation point (CO₂ concentration, at which respiration equals photosynthesis) in many lichen species is very low as compared to vascular plants (Nash III, 1996b). This often results from a CO₂ concentrating mechanism (CCM), by which CO₂ is actively pumped into the photobiont cells (Badger et al., 1993). Such a CCM is also present within the photobiont *Trebouxia irregularis* Hildreth and Ahmadjian of the endolithically growing *Hymenelia* species, which makes them less susceptible to elevated CO₂ concentrations (Weber et al., 2007).

Interactions with the substrate

Bare natural rock surfaces are extremely rare on earth and might only be found under extreme climatic conditions of hot or cold deserts. Normally, rock surfaces are covered by a dense layer (film, crust; Figure 1a) composed of cyanobacteria (Figure 1h), algae, lichens (Figure 1b–f), and fungi (Figure 1f and i). Cyanobacteria, lichens, and fungi also can grow inside the rock itself, in the upper few millimetres (= endolithic, Figure 1f and g). On inselbergs of tropical savannas, these biofilms are even responsible for the characteristic black (cyanobacteria dominated) or brown color (lichen dominated) of the rock surface (Büdel et al., 2000).

Fungi

Fungi grow on rock surfaces (epilithic), inside the rock (endolithic) or are part of the desert varnish produced by microorganisms growing inside the up to 1 cm thick black layer (e.g., Krumbein and Jens, 1981). Also MCF have been shown to be involved in desert varnish formation in Arizona and Australia (Taylor-George et al., 1983). Microbial growth on rock surfaces follows the microscopic landscape formed by mineral grains, pores, and fissures of the substratum (Figure 1f and i). Characteristically, microorganisms of the resulting biofilms colonize in the way that fungal hyphae and some cyanobacteria penetrate the substrate, while algae, bacteria, and microcolonies of yeast-like fungi clump together (Gorbushina, 2007). They secrete extracellular polymeric substances (EPS), which envelope the microcolonies, keep them together and adhere them to the substratum.

Biogenic rock weathering is surely a multifactorial process. The role of fungi in the wear-down of rocks is difficult to separate from the activity of other microorganisms of biofilms. Generally, there are two main groups of weathering that can be distinguished. (1) Either the growth of cells forces separation of mineral grains (Dornieden

et al., 1997), or desiccation/hydration cycles of cell walls and EPS cause their separation (Friedmann and Weed, 1987; Warscheid et al., 1991). (2) Dissolution of rock minerals can be achieved by organic acids released from cells, chemical impact of respiratory CO₂, H⁺, and redox reactions with metal-ions (Gadd, 1999; Burford et al., 2003).

Lichens

During the 1980s and 1990s, a huge variety of literature on the patterns and effects of biogenic weathering by lichens has been published. However, a comprehensive understanding on the overall mechanisms, effects, and magnitude of effects could not yet be accomplished (Chen et al., 2000).

As for the fungi, the impact of lichens can be subdivided into physical and chemical effects. The physical effects comprise: (1) penetration of hyphae through intergranular cavities and along mineral cleavage planes; (2) thallus expansion and contraction due to varying water content; (3) freezing and thawing of the lichen thallus; (4) swelling of organic and inorganic salts originating from lichen activity; and (5) incorporation of mineral fragments into the thallus (Chen et al., 2000).

The chemical effects comprise the solubilization and the insolubilization of inorganic elements, resulting in the weathering of minerals (silicates, phosphates, carbonates, sulfides, oxides) and the formation of new deposits (carbonates, oxides, sulfides, phosphates; Benthelin, 1988). The solubilization processes, as induced by lichens include (1) the release of metal ions by acid and complexing compounds, and (2) the acidification or alkalization by uptake or release of alkaline compounds. Insolubilization processes are oxidation of Fe²⁺ and Mn²⁺, and uptake of elements by the organisms in different environments (Benthelin, 1988).

Besides the alkalization activity of endolithic cyanobacteria (Büdel et al., 2004), it is also expected that lichens perform a similar role on/in rocks. Wessels and Schoeman (1988) describe an exfoliation process initiated by the endolithic lichen *Lecidea* aff. *sarcogynoides* Koerb. In SEM imagery, they perceived a dissolution of the binding material along the growth zone of the hyphae and also pitting marks on the quartz crystals were observed, where hyphae had attached to the surface of the crystals (Wessels and Schoeman, 1988). Besides *L. aff. sarcogynoides*, also lichens of the genus *Caloplaca* are found to contribute to rock weathering in sandstones of South Africa (Figure 2d). Friedmann (1982) and Friedmann and Weed (1987) report on cryptoendolithic microorganisms in the Antarctic cold desert (Ross Desert) being dominated by lichens that cause a exfoliative weathering pattern along the lichen growth zone very similar to the pattern described by Büdel et al. (2004).

Conclusions

Identification of lichens on the basis of their external and internal morphology, as well as chemical characteristics, is well-established and only in some cases, where lichens

do not express a typical morphology or where they are often sterile, e.g., in the endolithic habitat, additional identification methods, using a molecular approach are necessary. Thus, lichen diversity of different rock types is quite well-known. Rock-inhabiting fungi, on the other hand, are much more difficult to identify. They often only express a reduced set of morphological features, insufficient for save identifications. Here, the use of molecular techniques is a must and already lead to a considerable increase in knowledge (e.g., Selbmann et al., 2005).

The interaction of fungi and lichens with their rock substratum is manifold. Biofilms with fungi and lichens might either increase weathering rates of their substratum, or in some cases, protect it from weathering. However, these crusts and films on rocks are a complex mixture of bacteria, cyanobacteria, algae, fungi, and microlichens embedded in the common structure of an EPS. Macrolichens rising above the biofilm form a separate layer, comparable to that of emergent trees that rise above the canopy of rain forests.

Microbial biodiversity of rock surfaces and the complex interaction of these organisms among themselves and with the rock substratum, for example, their role in bioweathering, is still poorly understood at present and will surely be a field of intensive and fascinating research in future.

Bibliography

- Ahmadjian, V., 1993. *The Lichen Symbiosis*. New York: Wiley.
- Ariño, X., and Saiz-Jimenez, C., 1996. Lichen deterioration of consolidants used in the conservation of stone monuments. *Lichenologist*, **28**(4), 391–394.
- Badger, M. R., Pfanz, H., Büdel, B., Heber, U., and Lange, O. L., 1993. Evidence for the functioning of photosynthetic CO₂ concentration mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta*, **191**, 57–70.
- Belnap, J., Büdel, B., and Lange, O. L., 2003. Biological soil crusts: characteristics and distribution. In Belnap, J., and Lange, O. L. (eds.), *Ecological Studies 150: Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, pp. 3–30.
- Benthelin, J., 1988. Microbial weathering processes in natural environments. In Lerman, A., and Meybeck, M. (eds.), *Physical and Chemical Weathering in Geochemical Cycles*. Berlin: Springer, pp. 33–60.
- Bold, H. C., and Wynne, M. J., 1985. *Introduction to the Algae. Structure and Reproduction*, 2nd edn. Englewood Cliffs: Prentice-Hall.
- Brown, R. M., Jr., Larson, D. A., and Bold, H. C., 1964. Airborne algae: their abundance and heterogeneity. *Science*, **143**, 583–585.
- Büdel, B., 2003. Biological soil crusts in european temperate and mediterranean regions. In Belnap, J., and Lange, O. L. (eds.), *Ecological Studies 150: Biological Soil Crusts: Structure, Function, and Management*. Berlin: Springer, pp. 75–86.
- Büdel, B., and Lange, O. L., 1991. Water status of green and blue-green phycobionts in lichen thalli after hydration by water vapor uptake: do they become turgid? *Botanica Acta*, **104**, 361–366.
- Büdel, B., and Scheidegger, C., 2008. Thallus morphology and anatomy. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 40–68.
- Büdel, B., Becker, U., Follmann, G., and Sterflinger, K., 2000. Algae, fungi, and lichens on inselbergs. In Porembski, S., and Barthlott, W. (eds.), *Inselbergs*. Berlin: Springer, pp. 69–90.

- Büdel, B., Weber, B., Köhl, M., Pfanz, H., Sültemeyer, D., and Wessels, D., 2004. Reshaping of sandstone surfaces by cryptoendolithic cyanobacteria: bioalkalization causes chemical weathering in arid landscapes. *Geobiology*, **2**, 261–268.
- Burford, E. P., Fomina, M., and Gadd, G. M., 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine*, **67**, 1127–1155.
- Chen, J., Blume, H. P., and Beyer, L., 2000. Weathering of rocks induced by lichen colonization - a review. *Catena*, **39**, 121–146.
- Demmig-Adams, B., Máguas, C., Adams, III, W. W., Meyer, A., Kilian, E., and Lange, O. L., 1990. Effect of high light on the efficiency of photochemical energy conversion in a variety of lichen species with green and blue-green phycobionts. *Planta*, **180**, 400–409.
- Diakumaku, E., Gorbushina, A. A., Krumbein, W. E., Panina, L., and Soukharjevski, S., 1995. Black fungi in marble and limestones - an aesthetical, chemical and physical problem for the conservation of monuments. *Science of the Total Environment*, **167**, 295–304.
- Dietz, S., Büdel, B., Lange, O. L., and Bilger, W., 2000. Transmittance of light through the cortex of lichens from contrasting habitats. *Bibliotheca Lichenologica*, **75**, 171–182.
- Dornieden, T., Gorbushina, A. A., and Krumbein, W. E., 1997. Änderungen von physikalischen Eigenschaften von Marmor durch Pilzbewuchs. *International Journal for Restoration of Buildings and Monuments*, **3**, 441–456.
- Ertl, L., 1951. Über die Lichtverhältnisse in Laubflechten. *Planta*, **39**, 245–270.
- Friedl, T., and Büdel, B., 2008. Photobionts. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 9–26.
- Friedmann, E. I., 1982. Endolithic microorganisms in the Antarctic cold desert. *Science*, **193**, 1247–1249.
- Friedmann, E. I., and Weed, R., 1987. Microbial trace-fossil formation, biogenous, and abiotic weathering in the Antarctic cold desert. *Science*, **236**, 703–705.
- Gadd, G. M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Advances in Microbial Physiology*, **41**, 47–92.
- Galloway, D. J., 1996. Lichen biogeography. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 199–216.
- Geiser, D. M., Gueidan, C., Miadlikowska, J., Lutzoni, F., Kauff, F., Hofstetter, V., Fraker, E., Schoch, C. L., Tibell, L., Untereiner, W. A., and Aptroot, A., 2006. Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. *Mycologia*, **98**, 1053–1064.
- Golubic, S., Friedman, I., and Schneider, J., 1981. The lithobiontic ecological niche, with special reference to microorganisms. *Journal of Sedimentary Petrology*, **51**(2), 475–478.
- Gorbushina, A. A., 2003. Microcolonial fungi: survival potential of terrestrial vegetative structures. *Astrobiology*, **3**, 543–554.
- Gorbushina, A. A., 2007. Life on the rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Green, T. G. A., and Snelgar, W. P., 1982. Carbon dioxide exchange in lichens: relationship between the diffusive resistance of carbon dioxide and water vapour. *Lichenologist*, **14**, 255–260.
- Green, T. G. A., Kilian, E., and Lange, O. L., 1991. Pseudocyphellaria dissimilis: a desiccation-sensitive, highly shade-adapted lichen from New Zealand. *Oecologia*, **85**, 498–503.
- Grotzinger, J., Jordan, T. H., Press, F., and Siever, R., 2006. Understanding Earth. 5th edn. New York: W.H. Freeman.
- Hahn, S., Speer, M., Meyer, A., and Lange, O. L., 1989. Photosynthetische Primärproduktion von epigäischen Flechten im "Mainfränkischen Trockenrasen". I. Tagesläufe von Mikroklima, Wassergehalt und CO₂-Gaswechsel zu den verschiedenen Jahreszeiten. *Flora*, **182**, 313–339.
- Henssen, A., 1987. Lichenothelia, a genus of microfungi on rocks. *Bibliotheca Lichenologica*, **25**, 257–293.
- Honegger, R., 1996. Mycobionts. In Nash III, T.H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 24–36.
- Kappen, L., 1973. Responses to extreme environments. In Ahmadjan, V., and Hale, M. (eds.), *The Lichens*. London: Academic, pp. 311–380.
- Kappen, L., 1988. Ecophysiological relationships in different climatic regions. In Galun, M. (ed.), *Handbook of Lichenology*. Boca Raton: CRC, Vol. 2, pp. 39–99.
- Kappen, L., and Friedmann, E. I., 1983. Ecophysiology of lichens in the dry valleys of Southern Victoria Land, Antarctica. II. CO₂ gas exchange in cryptoendolithic lichens. *Polar Biology*, **1**, 227–232.
- Kershaw, K. A., 1985. *Physiological Ecology of Lichens*. Cambridge: Cambridge University Press.
- Krumbein, W. E., and Jens, J., 1981. Biogenic rock varnishes of the Negev Desert (Israel): an ecological study of iron and manganese transformation by cyanobacteria and fungi. *Oecologia*, **50**, 25–38.
- Lange, O. L., 1953. Hitze- und Trockenresistenz der Flechten in Beziehung zu ihrer Verbreitung. *Flora*, **140**, 39–97.
- Lange, O. L., 1969. Experimentell-ökologische Untersuchungen an Flechten der Negev-Wüste. I. CO₂-Gaswechsel von *Ramalina maciformis* (Del.) Bory unter kontrollierten Bedingungen im Laboratorium. *Flora*, **158**, 324–359.
- Lange, O. L., 2002. Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO₂ exchange and its physiological interpretation. *Flora*, **197**, 233–249.
- Lange, O. L., and Green, T. G. A., 1997. High thallus water contents can limit photosynthetic productivity of crustose lichens in the field. *Bibliotheca Lichenologica*, **68**, 81–99.
- Lange, O. L., and Green, T. G. A., 2005. Lichens show that fungi can acclimate their respiration to seasonal changes in temperature. *Oecologia*, **142**, 11–19.
- Lange, O. L., and Wagenitz, G., 2003. What is a "phycolichen"? differences and changes in the meaning of an old lichenological term. *Lichenologist*, **35**(4), 341–345.
- Lange, O. L., Büdel, B., Meyer, A., and Kilian, E., 1993. Further evidence that activation of net photosynthesis by dry cyanobacterial lichens requires liquid water. *Lichenologist*, **25**(2), 175–189.
- Lange, O. L., Green, T. G. A., Reichenberger, H., and Meyer, A., 1996. Photosynthetic depression at high water contents in lichens: concurrent use of gas exchange and fluorescence techniques with a cyanobacterial and a green algal *Peltigera* species. *Botanica Acta*, **109**, 43–50.
- Lange, O. L., Belnap, J., Reichenberger, H., and Meyer, A., 1997. Photosynthesis of green algal soil crust lichens from arid lands in southern Utah, USA: role of water content on light and temperature responses of CO₂ exchange. *Flora*, **192**, 1–15.
- Lange, O. L., Green, T. G. A., and Reichenberger, H., 1999. The response of lichen photosynthesis to external CO₂ concentration and its interaction with thallus water-status. *Journal of Plant Physiology*, **154**, 157–166.
- Lange, O. L., Büdel, B., Meyer, A., Zellner, H., and Zotz, G., 2000. Lichen carbon gain under tropical conditions: water relations and CO₂ exchange of three *Leptogium* species of a lower montane rainforest in Panama. *Flora*, **195**, 172–190.
- Lange, O. L., Green, T. G. A., and Heber, U., 2001. Hydration-dependent photosynthetic production of lichens: what do laboratory studies tell us about field performance? *Journal of Experimental Botany*, **52**(363), 2033–2042.
- Lange, O. L., Büdel, B., Meyer, A., Zellner, H., and Zotz, G., 2004. lichen carbon gain under tropical conditions: water

- relations and CO₂ exchange of *Lobariaceae* species of a lower montane rainforest in Panama. *Lichenologist*, **36**(5), 329–342.
- Miadlikowska, J., Kauff, F., Hofstetter, V., Fraker, E., Grube, M., Hafellner, J., Reeb, V., Hodkinson, B. P., Kukwa, M., Lücking, R., Hestmark, G., Garcia-Otalora, M., Rauhut, A., Büdel, B., Scheidegger, C., Timdal, E., Stenroos, S., Brodo, I., Perlmutter, G. B., Ertz, D., Diederich, P., Lendemer, J. C., Tripp, E., Yahr, R., May, P., Gueidan, C., Spatafora, J. W., Schoch, C., Arnold, A. E., Robertson, C., and Lutzoni, F., 2006. New insights into classification and evolution of the Lecanoromycetes (Pezizomycotina, Ascomycota) from phylogenetic analyses of three ribosomal RNA- and two protein-coding genes. *Mycologia*, **98**, 1088–1103.
- Nash, T. H., 1996a. Introduction. In Nash III, T. H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 1–7.
- Nash, T. H., 1996b. Photosynthesis, respiration, productivity and growth. In Nash III, T. H. (ed.), *Lichen Biology*. Cambridge, Cambridge University Press, pp. 88–120.
- Onofri, S., Barreca, D., Rabbow, E., de Vera, J. P., Selbmann, L., and Zucconi, L., 2007. Antarctic rock fungi in space and mars simulated conditions. *Geophysical Research Abstracts*, **9**, 9782–9783.
- Palmer, F. E., Emery, D. R., Stemmler, J., and Staley, J. T., 1987. Survival and growth of microcolonial rock fungi as affected by temperature and humidity. *New Phytologist*, **107**, 155–162.
- Rundel, P. W., 1988. Water relations. In Galun, M. (ed.), *Handbook of Lichenology*. Boca Raton: CRC, Vol. 2, 17–36.
- Schoch, C. L., Shoemaker, R. A., Seifert, K. A., Hambleton, S., Spatafora, J. W., and Crous, P. W., 2006. A multigene phylogeny of the Dothideomycetes using four nuclear loci. *Mycologia*, **98**, 1041–1052.
- Schöller, H., 1997. Ökologie und Verbreitung. In Schöller, H. (ed.), *Flechten: Geschichte, Biologie, Systematik, Ökologie, Naturschutz und kulturelle Bedeutung*. Frankfurt am Main: Senckenbergische Naturforschende Gesellschaft, pp. 83–109.
- Schöller, H., and Mollenhauer, D., 1997. Flechtensymbiose und Flechtenthallus. In Schöller, H. (ed.), *Flechten: Geschichte, Biologie, Systematik, Ökologie, Naturschutz und kulturelle Bedeutung*. Frankfurt am Main: Senckenbergische Naturforschende Gesellschaft, pp. 15–47.
- Selbmann, L., de Hoog, G. S., Mazzaglia, A., Friedmann, E. I., and Onofri, S., 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. *Studies in Mycology*, **51**, 1–32.
- Sigler, L., Tsuneda, A., and Carmichael, J. W., 1981. *Phaeotheca* and *Phaeosclera*, two new genera of dematiaceous hyphomycetes and a redescription of *Sarcinomyces lindneri*. *Mycotaxon*, **12**, 449–467.
- Sterflinger, K., De Baere, R., de Hoog, G. S., De Wachter, R., Krumbein, W. E., and Haase, G., 1997. *Coniosporium Perforans* and *C. apollinis*, two new rock-inhabiting fungi isolated from marble in the sanctuary of Delos (Cyclades, Greece). *Antonie van Leeuwenhoek*, **72**, 349–363.
- Taylor-George, S., Palmer, F., Staley, J. T., Borns, D. J., Curtiss, B., and Adams, J. B., 1983. Fungi and bacteria involved in desert varnish formation. *Microbial Ecology*, **9**, 227–245.
- Tretiach, M., and Geletti, A., 1997. CO₂ exchange of the endolithic lichen *Verrucaria baldensis* from karst habitats in Northern Italy. *Oecologia*, **111**, 515–522.
- Tretiach, M., and Pecchiari, M., 1995. Gas exchange rates and chlorophyll content of epi- and endolithic lichens from the Trieste Karst (NE Italy). *New Phytologist*, **130**, 585–592.
- van den Hoek, C., Jahns, H. M., and Mann, D. G., 1993. *Algen*. 3. Auflage. Stuttgart: Thieme.
- Warscheid, T., Oelting, M., and Krumbein, W. E., 1991. Physicochemical aspects of biodeterioration processes on rocks with special regard to organic pollutants. *International Biodeterioration*, **28**, 37–48.
- Weber, B., and Büdel, B., 2001. Mapping and analysis of distribution patterns of lichens on rural medieval churches in North-Eastern Germany. *Lichenologist*, **33**(3), 231–248.
- Weber, B., Scherr, C., Reichenberger, H., and Büdel, B., 2007. Fast reactivation by high air humidity and photosynthetic performance of alpine lichens growing endolithically in limestone. *Arctic, Antarctic and Alpine Research*, **39**(2), 309–317.
- Weber, B., Olechowski, C., Knerr, T., Hill, J., Deutschewitz, K., Wessels, D. C. J., Eitel, B., and Büdel, B., 2008. A new approach for mapping of biological soil crusts in semidesert areas with hyperspectral imagery. *Remote Sensing of Environment*, **112**, 2187–2201.
- Wessels, D. C. J., and Schoeman, P., 1988. Mechanisms and rate of weathering of Clarens sandstone by an endolithic lichen. *South African Journal of Science*, **84**, 274–277.
- Winkler, J. B., and Kappen, L., 1997. Photosynthetic capacity of endolithic lichens from South Africa. *Bibliotheca Lichenologica*, **67**, 165–181.
- Wirth, V., 1995. *Die Flechten Baden-Württembergs*. Teil 1–2. Stuttgart: Eugen Ulmer GmbH.
- Wollenzien, U., de Hoog, G. S., Krumbein, W. E., and Uijthof, J. M. J., 1997. *Sarcinomyces petricola*, a new microcolonial fungus from marble in the Mediterranean basin. *Antonie van Leeuwenhoek*, **71**, 281–288.

Cross-references

[Algae \(Eukaryotic\)](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biofilms](#)
[Chroococcidiopsis](#)
[Cyanobacteria](#)
[Endoliths](#)
[Geomycology](#)
[Microbial Communities, Structure, and Function](#)
[Microbiocorrosion](#)

G

GALLIONELLA

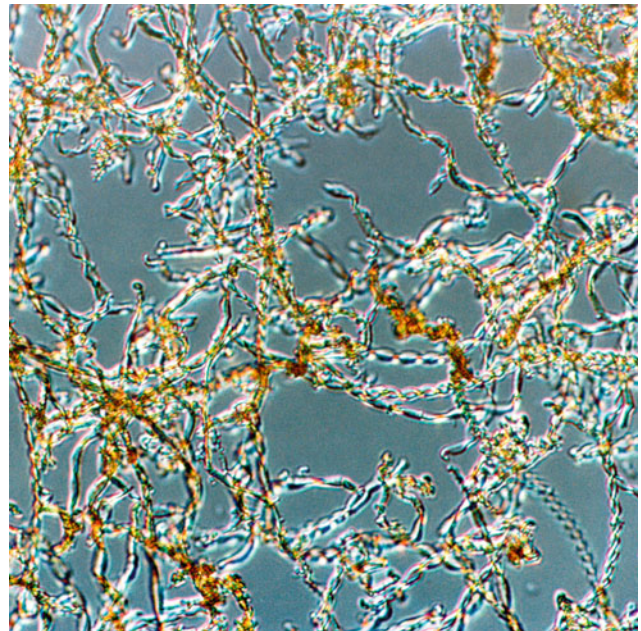
Karsten Pedersen
Göteborg University, Göteborg, Sweden

Definition

Gram-negative, microaerophilic, and chemolithotrophic bean-shaped cells that secrete an extracellular twisted stalk and can grow with ferrous iron as source of energy and carbon dioxide as the sole source of carbon.

The genus *Gallionella* is characterized by its chemolithotrophic growth with ferrous iron as source of electrons and energy and carbon dioxide as source of carbon. Mixotrophic metabolism has been demonstrated with glucose, fructose, and sucrose (Hallbeck and Pedersen, 1991). The family *Gallionellaceae* has one known species, *Gallionella ferruginea* (Hallbeck et al., 1993; Hallbeck and Pedersen, 2005). The organism produces a twisted stalk that consists of a bundle of numerous organic fibers, which makes *Gallionella* very easy to identify (Figure 1). The potential functions of the stalk is to act as a holdfast (Figure 2) and possibly also to act as a sorbent for the iron oxides produced by the organism during its oxidation of ferrous iron to ferric iron. This stalk allows easy identification not only in modern systems but it also allows use as fossil to trace the activity of *Gallionellaceae* in the past. The stalk production appears to be stimulated by pH values above 6 (Hallbeck and Pedersen, 1990). Optimal growth and stalk formation under in situ conditions occur under microaerophilic conditions (0.4–1.5 mg oxygen L⁻¹) in an E_h range between +150 and +250 mV (Hallbeck and Pedersen, 1995; Anderson and Pedersen, 2003). The environment where stalk-forming *Gallionella* can be observed, commonly attached to surfaces, is slowly flowing groundwater that is rich in ferrous iron

but has low organic carbon and oxygen concentrations. Typical places to find *Gallionella* are in drain pipes, storage basins for groundwater from deep wells, in tunnels, and on rock walls with seeping groundwater. Colonies of *Gallionella* rapidly develop to visible structures in



Gallionella, Figure 1 *Gallionella ferruginea* produces a twisted stalk that consists of numerous thin organic fibers. As the stalks age, iron oxides precipitate on the stalks and many trace elements co-precipitate (Anderson and Pedersen, 2003). The attachment of the cell to the stalk is very brittle and they are, therefore, commonly detached during the preparation of specimens for the microscope. Diameter of the stalks is typically 0.3–0.5 µm in width and up to 400 µm or more in length. Photo: Karsten Pedersen.



Gallionella, Figure 2 Colonies of *Gallionella ferruginea* growing in a shallow pond. The twisted stalks make their way up through the water. In this way, they reach the energy-rich ferrous iron in the water, while escaping the waste they produce, iron oxides. Diameter of the moss-like colonies was 1–2 cm. Photo: Karsten Pedersen.

water (Figure 2). Growth of *Gallionella* in groundwater seeping out from fractures in rock walls develops to compact mats of cells, stalks, and iron oxides. Such mats can reach a thickness of several centimeters in less than a year and they may enrich trace elements up to 10^6 times their respective concentrations in the seeping groundwater (Anderson and Pedersen, 2003).

Bibliography

- Anderson, C. R., and Pedersen, K., 2003. In situ growth of *Gallionella* biofilms and partitioning of lanthanids and actinides between biological material and ferric oxyhydroxides. *Geobiology*, **1**, 169–178.
- Hallbeck, L., and Pedersen, K., 1990. Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. *Journal of General Microbiology*, **136**, 1675–1680.
- Hallbeck, L., and Pedersen, K., 1991. Autotrophic and mixotrophic growth of *Gallionella ferruginea*. *Journal of General Microbiology*, **13**, 2657–2661.
- Hallbeck, L., and Pedersen, K., 1995. Benefits associated with the stalk of *Gallionella ferruginea*, evaluated by comparison of a stalk-forming and a non-stalk-forming strain and biofilm studies in situ. *Microbial Ecology*, **30**, 257–268.
- Hallbeck, L. E., and Pedersen, K., 2005. Genus I. *Gallionella* Ehrenberg 1838 166AL. In Brenner, D. J., Krieg, N. R., and Staley, J. T. (eds.), *Bergey's Manual of Systematic Bacteriology. The Proteobacteria. Part C. The Alpha-, Beta-, Delta and Epsilonproteobacteria*. New York: Springer, Vol. 2, pp. 880–886.
- Hallbeck, L., Ståhl, F., and Pedersen, K., 1993. Phylogeny and phenotypic characterization of the stalk-forming and iron-oxidizing bacterium *Gallionella ferruginea*. *Journal of General Microbiology*, **139**, 1531–1535.

Cross-references

[Banded Iron Formations](#)
[Chemolithotrophy](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)

[Fe\(III\)-Reducing Prokaryotes](#)
[Geobacter](#)
[Microbial Biomineralization](#)

GEOBACTER

Kristina L. Straub
 University of Vienna, Vienna, Austria

Definition

Geobacter is the name of a genus that is located within the *Deltaproteobacteria* comprising several species of Fe(III)-reducing bacteria (Lovley et al., 1993). The genus *Geobacter* represents the type genus of the family Geobacteraceae which includes also the genera *Desulfuromonas*, *Desulfuromusa*, *Geoalkalibacter*, *Geopsychrobacter*, *Geothermobacter*, *Malonomonas*, and *Pelobacter*.

The type species of the genus *Geobacter* is *Geobacter metallireducens* strain GS-15, which was enriched and isolated from pristine freshwater sediments of the Potomac River with acetate as the electron donor and poorly crystalline ferric iron as the electron acceptor. In addition to pristine aquatic habitats, *Geobacter* species were isolated from deep aquifer sediments (e.g., *G. chapellei*), subsurface sediments (e.g., *G. bemidjensis*), or various contaminated sites (e.g., *G. sulfurreducens*). Cells of *Geobacter* species stain Gram-negative and are non-spore-forming. All *Geobacter* species have midrange temperature optima (25–35°C) and grow only under strictly anoxic conditions. Alternative electron acceptors for *Geobacter* species include nitrate, elemental sulfur, fumarate, malate, Mn (IV), U(VI), Co(III), anthraquinone-2,6-disulfonic acid,

- Wood, R. A., Grotzinger, J. P., and Dickson, J. A. D., 2002. Proterozoic modular biomineralized metazoan from the Nama Group, Namibia. *Science*, **296**, 2383–2386.
- Wooldbridge, S. A., 2008. Mass extinctions past and present: a unifying hypothesis. *Biogeosciences Discussion*, **5**, 2401–2423.
- Xian-guang, H., Aldridge, R. J., Bergström, J., Sivetter, D. J., Siveter, D. J., and Xiang-hong, F., 2004. *The Cambrian Fossils Of Chengjiang, China. The Flowering Of Early Animal Life*. Oxford: Blackwell.
- Xiao, S., and Kaufman, A. J. (eds.), 2007. Neoproterozoic geobiology and paleobiology. *Topics in Geobiology*, **27**, 300.
- Xiao, S., Yuan, X., and Knoll, A. H., 2000. Eumetazoan fossils in terminal Proterozoic phosphorites? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 13684–13689.
- Young, S. A., Saltzman, M. R., Foland, K. A., Linder, J. J., and Kump, L. R., 2009. A major drop in seawater $^{87}\text{Sr}/^{86}\text{Sr}$ during the Middle Ordovician (Darrivilian): links to volcanism and climate? *Geology*, **37**, 951–954.
- Zhuravlev, A. Y., and Riding, R. (eds.), 2001. *The Ecology of the Cambrian Radiation*. New York: Columbia University Press.
- Zhuravlev, A. Y., and Wood, R. A., 2008. Eve of biomineralization: controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Animal Biocalcification, Evolution
 Animal Skeletons, Advent
 Archaea
 Asteroids and Comet Impacts
 Bacteria
 Banded Iron Formations
 Early Precambrian Eukaryotes
 Ediacaran Biota
 Isotope Fractionation (Metal)
 Mass Extinctions, Phanerozoic
 Mat-Related Sedimentary Structures
 Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Nickel, Biology
 Origin of Life
 Photosynthesis
 Salinity History of the Earth's Ocean
 Snowball Earth
 Soda Ocean Hypothesis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Trace Fossils: Neoproterozoic

CRYOBIOSPHERE

The term “cryobiosphere” refers to organisms living in extremely cold environments. For detailed reading, see entries “*Permafrost Microbiology*” and “*Extreme Environments*.”

CYANOBACTERIA

Kathrin I. Mohr^{1,2}, Nicole Brinkmann¹, Thomas Friedl¹
¹University of Göttingen, Göttingen, Germany
²Helmholtz Centre for Infection Research, Braunschweig, Germany

Synonyms

Blue-green algae; Chloroxybacteria; Cyanophyta; Cyanoprokaryota

Definition

Cyanobacteria are a large and morphologically rather diverse group of photoautotrophic prokaryotes and are classified as a monophyletic phylum within Domain Bacteria. Cyanobacteria share oxygenic photosynthesis with the eukaryotic algae. Cyanobacteria belong to the oldest organisms on earth. Their broad environmental tolerance range makes cyanobacteria well suited to live in habitats of extreme and fluctuating conditions where they can outcompete eukaryotic algae.

Many cyanobacteria have large cells like the eukaryotic algae with which they share the same life-style: Consequently, they are often treated as prokaryotic algae or, formerly, blue-green algae. Some cyanobacteria even form macroscopic colonies from the size of a few millimeters to up to 30 cm (Dodds and Castenholz, 1988). Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony-forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. Photosynthetic cyanobacteria produced earth's first oxygen atmosphere, which fostered the rise of eukaryotes. In addition, ancient cyanobacteria produced massive undersea carbonate deposits, thereby reducing atmospheric carbon dioxide levels and influencing global climate. The first plastids arose from cyanobacteria through a process called primary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003; Reyes-Prieto et al., 2007). Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Nitrogen-fixing cyanobacteria increase soil and water fertility and foster the growth of certain plants and fungi in symbiotic associations. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years (Rasmussen and Willemotte, 2008) and with an evolutionary history stretching over at least 1.5 and possibly 2.7 billion years (Blank, 2004). Cyanobacteria are able to live in some of the most extreme habitats on earth (e.g., Seckbach, 2007 for an overview). The broad environmental tolerance range of cyanobacteria in extreme habitats is in contrast to most other prokaryotic and eukaryotic organisms from extreme environments that occur only within a small window of almost constant conditions. From an evolutionary point of view, it appears that cyanobacteria which are



Gallionella, Figure 2 Colonies of *Gallionella ferruginea* growing in a shallow pond. The twisted stalks make their way up through the water. In this way, they reach the energy-rich ferrous iron in the water, while escaping the waste they produce, iron oxides. Diameter of the moss-like colonies was 1–2 cm. Photo: Karsten Pedersen.

water (Figure 2). Growth of *Gallionella* in groundwater seeping out from fractures in rock walls develops to compact mats of cells, stalks, and iron oxides. Such mats can reach a thickness of several centimeters in less than a year and they may enrich trace elements up to 10^6 times their respective concentrations in the seeping groundwater (Anderson and Pedersen, 2003).

Bibliography

- Anderson, C. R., and Pedersen, K., 2003. In situ growth of *Gallionella* biofilms and partitioning of lanthanids and actinides between biological material and ferric oxyhydroxides. *Geobiology*, **1**, 169–178.
- Hallbeck, L., and Pedersen, K., 1990. Culture parameters regulating stalk formation and growth rate of *Gallionella ferruginea*. *Journal of General Microbiology*, **136**, 1675–1680.
- Hallbeck, L., and Pedersen, K., 1991. Autotrophic and mixotrophic growth of *Gallionella ferruginea*. *Journal of General Microbiology*, **13**, 2657–2661.
- Hallbeck, L., and Pedersen, K., 1995. Benefits associated with the stalk of *Gallionella ferruginea*, evaluated by comparison of a stalk-forming and a non-stalk-forming strain and biofilm studies in situ. *Microbial Ecology*, **30**, 257–268.
- Hallbeck, L. E., and Pedersen, K., 2005. Genus I. *Gallionella* Ehrenberg 1838 166AL. In Brenner, D. J., Krieg, N. R., and Staley, J. T. (eds.), *Bergey's Manual of Systematic Bacteriology. The Proteobacteria. Part C. The Alpha-, Beta-, Delta and Epsilonproteobacteria*. New York: Springer, Vol. 2, pp. 880–886.
- Hallbeck, L., Ståhl, F., and Pedersen, K., 1993. Phylogeny and phenotypic characterization of the stalk-forming and iron-oxidizing bacterium *Gallionella ferruginea*. *Journal of General Microbiology*, **139**, 1531–1535.

Cross-references

[Banded Iron Formations](#)
[Chemolithotrophy](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)

[Fe\(III\)-Reducing Prokaryotes](#)
[Geobacter](#)
[Microbial Biomineralization](#)

GEOBACTER

Kristina L. Straub
 University of Vienna, Vienna, Austria

Definition

Geobacter is the name of a genus that is located within the *Deltaproteobacteria* comprising several species of Fe(III)-reducing bacteria (Lovley et al., 1993). The genus *Geobacter* represents the type genus of the family Geobacteraceae which includes also the genera *Desulfuromonas*, *Desulfuromusa*, *Geoalkalibacter*, *Geopsychrobacter*, *Geothermobacter*, *Malonomonas*, and *Pelobacter*.

The type species of the genus *Geobacter* is *Geobacter metallireducens* strain GS-15, which was enriched and isolated from pristine freshwater sediments of the Potomac River with acetate as the electron donor and poorly crystalline ferric iron as the electron acceptor. In addition to pristine aquatic habitats, *Geobacter* species were isolated from deep aquifer sediments (e.g., *G. chapellei*), subsurface sediments (e.g., *G. bemidjensis*), or various contaminated sites (e.g., *G. sulfurreducens*). Cells of *Geobacter* species stain Gram-negative and are non-spore-forming. All *Geobacter* species have midrange temperature optima (25–35°C) and grow only under strictly anoxic conditions. Alternative electron acceptors for *Geobacter* species include nitrate, elemental sulfur, fumarate, malate, Mn (IV), U(VI), Co(III), anthraquinone-2,6-disulfonic acid,

and humics. Furthermore, *G. metallireducens*, *G. psychrophilus*, and *G. sulfurreducens* were shown to transfer electrons from the oxidation of organic compounds onto the surface of electrodes. All *Geobacter* species utilize acetate as electron donor and carbon source; other electron donors that are utilized by the majority of *Geobacter* species include ethanol, pyruvate, lactate, hydrogen, and formate. *Geobacter* species are unable to grow by fermentation. Ecological studies indicate that *Geobacter* species predominate under iron-reducing conditions. Furthermore, the combination of ecological and physiological studies implies that *Geobacter* species might play important roles in bioremediation processes such as the oxidation of aromatic compounds, the reduction of tetrachloroethene, or the immobilization of U(VI).

Species: *G. argillaceus*, *G. bemidjensis*, *G. bremensis*, *G. chapellei*, *G. grbiciae*, *G. humireducens*, *G. hydrogenophilus*, *G. lovleyi*, *G. metallireducens*, *G. pelophilus*, *G. pickeringii*, *G. psychrophilus*, *G. sulfurreducens*, *G. thiogenes*, *G. uraniireducens*

Among the validly described *Geobacter* species, *G. metallireducens* strain GS-15 and *G. sulfurreducens* strain PCA are the most intensively studied in terms of physiology, biochemistry, and genetics. The genomes of both species were sequenced and a genetic system was established for *G. sulfurreducens* (Méthé et al., 2003).

Bibliography

- Lovley, D. R., Giovannoni, S. J., White, D. C., Champine, J. E., Phillips, E. J., Gorby, Y. A., and Goodwin, S., 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Archives of Microbiology*, **159**, 336–344.
- Méthé, B. A., Nelson, K. E., Eisen, J. A., Paulsen, I. T., Nelson, W., Heidelberg, J. F., Wu, D., Wu, M., Ward, N., Beanan, M. J., Dodson, R. J., Madupu, R., Brinkac, L. M., Daugherty, S. C., DeBoy, R. T., Durkin, A. S., Gwinn, M., Kolonay, J. F., Sullivan, S. A., Haft, D. H., Selengut, J., Davidsen, T. M., Zafar, N., White, O., Tran, B., Romero, C., Forberger, H. A., Weidman, J., Khouri, H., Feldblyum, T. V., Utterback, T. R., Van Aken, S. E., Lovley, D. R., and Fraser, C. M., 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science*, **302**, 1967–1969.

Cross-references

Fe(II)-Oxidizing Prokaryotes
Fe(III)-Reducing Prokaryotes
Gallionella

GEOCHRONOLOGY

Volker Liebetrau
Leibniz Institute of Marine Sciences, IFM-GEOMAR,
Kiel, Germany

Synonyms

Age determination; Dating

Definition

Geochronology. Determination of ages and time intervals for geologic materials and processes on geologic, archeologic, and historic time scales. The science of investigating and reflecting the chronology of the earth constituents as induced from geologic data, based on absolute and relative dating methods.

Age, absolute. Age determination based on radioactive elements, their rates of decay and physical measurements, resulting in an actual age given in years for the analyzed geologic material (e.g., mineral, rock, fossil, fluid).

Age, relative. Age information based on stratigraphic anomalies (e.g., lithostratigraphic and biostratigraphic principles, field observations) or utilizing chemical reaction rates (e.g., diffusion, exchange, oxidation, hydration) as a measure of time.

Units. Geochronologic data are presented as yr BP (years before present), as ka (age date in thousands of years), as kyr (time interval in thousands of years, e.g., between distinct age data), following the same principle as Ma and Myr (millions of years), respectively. Alternatively, the mathematical expression in years, times order of magnitude is used and favored for age data and time intervals in the range of billions of years and the description of half-lives (e.g., 4.47×10^9 years, half-life of the ^{238}U isotope).

Introduction

The term “geochronology” may reasonably be used in reference to all principles and methods employed in geology to determine the relative and numerical ages of all kinds of rocks (Walsh, 2001). The immense age of the Earth and the long-term duration of processes changing the morphology and composition of the Earth’s crust and interior were progressively recognized throughout the last centuries. Geoscientists developed and established a relative chronological systematic based on lithostratigraphic and biostratigraphic principles, including global correlations and ongoing refinement. The actual state of the art is presented by the International Commission on Stratigraphy (ICS, 2009; <http://www.stratigraphy.org/upload/ISChart2009.pdf>).

In contrast, the direct determination of absolute ages required fundamental new physical approaches and analytical methods in the field of geochemistry. The basis for “geochronology” as a new geoscientific discipline was provided by the early description of radioactive transformations more than 100 years ago (Rutherford, 1906). An actual status in combining detailed knowledge of relative age successions with precise absolute ages and the impact of resulting chronostratigraphy on the understanding of the Earth’s geologic evolution is presented in concise manner by Ogg et al. (2008). A still valid and systematic overview about the broad variety, principles, scopes, potentials, and limitations of physical and chemical dating methods is given in a comprehensive way by Geyh and Schleicher (1990). This work is especially

ICP-MS. Unfortunately, this in situ approach is still hampered by the high uncertainties of low-counting statistics on samples younger than 10 ka. Meanwhile, recent efforts in conventional data precision and sample selection pushed the maximum limit of age determination with the U-Th method to the successful dating of extraordinarily well preserved 600-ka-old corals (Andersen et al., 2008).

Summary

Geochronology in geobiologic approaches offers detailed insights into the time scales of biological processes, changes in biochemical fluxes, and the identification of driving mechanisms. Still open challenges are the rather low sample throughput of conventional clean-lab preparation procedures, the necessary achievements in precision for in situ approaches, and the methodical extension toward reliable dating tools covering the last millions of years.

Bibliography

- Allègre, C. J., 2008. *Isotope Geology*. New York: Cambridge University Press.
- Andersen, M. B., Stirling, C. H., Potter, E.-K., Halliday, A. N., Blake, S. G., McCulloch, M. T., Ayling, B. F., and O'Leary, M., 2008. High precision U-series measurements of more than 500,000 year old fossil corals. *Earth and Planetary Science Letters*, **265**, 229–245.
- Bourdon, B., Turner, S., Henderson, G. M., and Lundstrom, C. C., 2003. *Reviews in Mineralogy and Geochemistry 52: Uranium Series Geochemistry*. Washington: The Mineralogical Society of America.
- Dickin, A. P., 2005. *Radiogenic Isotope Geology*, 2nd edn. New York: Cambridge University Press.
- Faure, G., and Mensing, T. M., (2005). *Isotopes: Principles and Applications*, 3rd edn. New Jersey: Wiley.
- Fietzke, J., Liebetrau, V., Eisenhauer, A., and Dullo, Chr., (2005). Determination of uranium isotope ratios by multi-static MIC-ICP-MS: Method and implementation for precise U- and Th-series isotope measurements. *Journal of Analytical Atomic Spectrometry*, **20**, 395–401.
- Geyh, M. A., and Schleicher H., 1990. *Absolute Age Determination: Physical and Chemical Dating Methods and Their Application*. Berlin, Heidelberg, New York: Springer.
- International Commission on Stratigraphy, 2009. International Stratigraphic Chart <http://www.stratigraphy.org/upload/ISChart2009.pdf>
- Mann, M. E., 2001. Little ice age. In MacCracken, M. C., and Perry, J. S. (eds.), *Encyclopedia of Global Environmental Change*. Chichester: Wiley.
- Ogg, J. G., Ogg, G., and Gradstein, F. M., 2008. *The Concise Geological Time Scale*. Cambridge, New York, Melbourne: Cambridge University Press. doi:10.1017/S0016756809006207.
- Potter, E.-K., Stirling, C. H., Wichert, U. H., Halliday, A. N., and Spötl, C., 2005. Uranium-series dating of corals in situ using laser-ablation MC-ICPMS. *International Journal of Mass Spectrometry*, **240**, 27–35.
- Rutherford, E., 1906. *Radioactive Transformations*. New York: Scribner.
- Walsh, S. L., 2001. Notes on geochronologic and chronostratigraphic units. *Geological Society of America*, **113**, 704–713. doi: 10.1130/0016–7606.

Cross-references

Anaerobic Oxidation of Methane with Sulfate
Chemolithotrophy
Cold Seep
Reefs

GEOMYCOLOGY

Geoffrey M. Gadd
University of Dundee, Dundee, Scotland, UK

Definition

Geomycology. The scientific study of the roles of fungi in processes of fundamental importance to geology

Introduction

Fungi are chemoorganotrophic (heterotrophic) organisms relying on organic carbon sources for energy and metabolism, and ubiquitous in aquatic and terrestrial environments. They are important as decomposers, animal and plant mutualistic symbionts and pathogens, and spoilage organisms of natural and manufactured materials (Gadd, 1993a, 1999, 2006; Burford et al., 2003a). They also have a role in the maintenance of soil structure due to their predominantly filamentous branching growth habit (each filament is called a hypha; plural = hyphae; all the hyphae constitute the mycelium) and frequent exopolymer production. Fungi have important roles in the biogeochemical cycling of the elements (e.g., C, N, P, S, metals), and this is linked with their ability to adopt a variety of growth, metabolic, and morphological strategies, their adaptive capabilities to environmental extremes and, their mutualistic associations with animals, plants, algae, and cyanobacteria (Gadd and Griffiths, 1980; Burford et al., 2003a; Gadd, 2004a, 2007a, b; Braissant et al., 2004; Gadd et al., 1984, 2005; Fomina et al., 2005a, 2006a, b) (Table 1). Fungal polymorphism and reproduction by huge numbers of spores underpins successful colonization of many different environments. Most fungi exhibit a filamentous growth habit which enables explorative or exploitative growth strategies, and many form linear organs of aggregated hyphae for protected translocation of nutrients (Jacobs et al., 2002, 2004; Boswell et al., 2002, 2003, 2006; Fomina et al., 2005a, b). Some fungi are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells, as in the black microcolonial fungi colonizing rocks (Gadd and Griffiths, 1980; Sterflinger, 2000; Gorbushina et al., 2002a, b, 2003). Some fungi are predominantly unicellular such as the yeasts, exemplified by *Saccharomyces cerevisiae*.

A broad appreciation of fungi as agents of biogeochemical change is lacking within geobiology, and apart from obvious connections with the carbon cycle, they are frequently neglected within broader geomicrobiological and geochemical contexts. While the geochemical activities

Geomycology, Table 1 Summary of important roles and activities of fungi in geomycological processes. These may take place in aquatic and terrestrial ecosystems, as well as in artificial and man-made systems, their relative importance depending on the species and active biomass present and physicochemical factors. The terrestrial environment is the main site of fungal-mediated biogeochemical changes, especially in mineral soils and the plant root zone, decomposing vegetation, and on exposed rocks and mineral surfaces. There is rather a limited amount of knowledge on fungal geobiology in freshwater and marine systems, sediments, and the deep subsurface. In this table, fungal roles have been arbitrarily split into categories based on growth, organic and inorganic metabolism, physicochemical attributes, and symbiotic relationships. It should be noted that many if not all of these are linked, and almost all directly or indirectly depend on the mode of fungal growth (including symbiotic relationships) and accompanying chemoorganotrophic metabolism, in turn dependent on a utilizable C source for biosynthesis and energy, and other essential elements, such as N, O, P, S, and many metals, for structural and cellular components. Mineral dissolution and formation are detailed separately although these processes clearly depend on metabolic activity and growth form (adapted from Gadd, 2007a)

Fungal attribute or activity	Geomycological consequences
Growth	
Growth and mycelium development; fruiting body development; hyphal differentiation; melanization	Stabilization of soil structure Penetration of rocks and minerals Biomechanical disruption of solid substrates, building stone, cement, plaster, concrete, etc. Plant, animal and microbial colonization, symbiosis and/or infection; mycorrhizas, lichens, pathogens Nutrient and water translocation Surfaces for bacterial growth, transport, and migration Mycelium acting as a reservoir of N and/or other elements
Metabolism	
Carbon and energy metabolism	Organic matter decomposition and cycling of component elements, e.g., C, H, O, N, P, S, metals, metalloids, radionuclides Altered geochemistry of local environment, e.g., changes in redox, O ₂ , pH Production of inorganic and organic metabolites, e.g., H ⁺ , respiratory CO ₂ , organic acids, siderophores Exopolymer production Organometal formation and/or degradation Degradation of xenobiotics and other complex compounds
Inorganic nutrition	Altered distribution and cycling of inorganic nutrient elements, e.g., N, S, P, essential and inessential metals, metalloids, organometals, and radionuclides Transport, accumulation, incorporation of elements into macromolecules Redox transformations of metal(loid)s and radionuclides Translocation of water, N, P, Ca, Mg, K, etc. through mycelium and/or to plant hosts Fe(III) capture by siderophores MnO ₂ reduction Element mobilization or immobilization including metals, metalloids, radionuclides, C, P, S, etc.
Mineral dissolution	Mineral and rock bioweathering Leaching/solubilization of metals and other components, e.g., phosphate Element redistributions including transfer from terrestrial to aquatic systems Altered bioavailability of, e.g., metals, P, S, Si, and Al Altered plant and microbial nutrition or toxicity Mineral formation, e.g., carbonates, oxalates, clays Altered metal and nutrient distribution, toxicity, and bioavailability Mineral soil formation
Mineral formation	Biodeterioration of building stone, cement, plaster, concrete etc. Element immobilization including metals and radionuclides, C, P, and S Mycogenic carbonate formation Limestone calcrete cementation Mycogenic metal oxalate formation Metal detoxification Contribution to patinas on rocks (e.g., "desert varnish")
Physicochemical properties	
Sorption of soluble and particulate metal species, soil colloids, clay minerals, etc.	Altered metal distribution and bioavailability Metal detoxification Metal-loaded food source for invertebrates Prelude to secondary mineral nucleation and formation

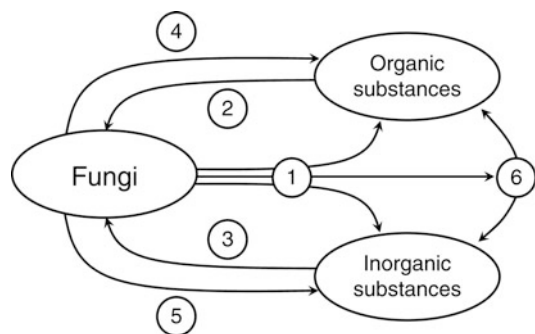
Geomycology, Table 1 (Continued)

Fungal attribute or activity	Geomycological consequences
Exopolymer production	Complexation of cations Provision of hydrated matrix for mineral formation Enhanced adherence to substrate Clay mineral binding Stabilization of soil aggregates Matrix for bacterial growth Chemical interactions of exopolymers with mineral substrates
Symbiotic associations Mycorrhizas	Altered mobility and bioavailability of nutrient and inessential metals, N, P, S, etc. Altered C flow and transfer between plant, fungus, and rhizosphere organisms Altered plant productivity Mineral dissolution and metal and nutrient release from bound and mineral sources Altered biogeochemistry in soil-plant root region Altered microbial activity in plant root region Altered metal distributions between plant and fungus Water transport to and from the plant
Lichens	Pioneer colonizers of rocks and minerals, and other surfaces Bioweathering Mineral dissolution and/or formation Metal accumulation by dry or wet deposition, particulate entrapment, metal sorption, transport, etc. Enrichment of C, N, P, etc. in thallus and alteration of elemental concentrations and distribution in local microenvironment Early stages of mineral soil formation Development and stimulation of geochemically active microbial populations Mineral dissolution by metabolites including "lichen acids" Biomechanical disruption of substrate
Insects and invertebrates	Fungal populations in gut aid degradation of plant material Invertebrates mechanically render plant residues more amenable for decomposition Cultivation of fungal gardens by certain insects (organic matter decomposition and recycling) Transfer of fungi between plant hosts by insect vectors (aiding infection and disease)
Pathogenic effects Plant and animal pathogenicity	Plant infection and colonization Animal predation (e.g., nematodes) and infection (e.g., insects, etc.) Redistribution of elements and nutrients Increased supply of organic material for decomposition Stimulation of other geochemically active microbial populations

of bacteria and archaea receive considerable attention, especially in relation to carbon-limited and/or anaerobic environments, in aerobic terrestrial environments fungi are of great importance, especially when considering rock surfaces, soil, and the plant root–soil interface (Table 1). For example, symbiotic mycorrhizal fungi are associated with most plant species and are involved in major mineral transformations and redistributions of inorganic nutrients, e.g., essential metals and phosphate, as well as carbon flow (Fomina and Gadd, 2007) (Figure 1). Free-living fungi have major roles in the decomposition of plant and other organic materials, including xenobiotics, as well as in mineral solubilization. Lichens (a fungal growth form comprising a mutualistic symbiosis between an alga and/

or cyanobacterium and a fungus) commonly inhabit exposed rock (and other) substrates, and play fundamental roles in early stages of rock colonization and mineral soil formation (Haas and Purvis, 2006). Fungi are also major biodeterioration agents of stone, wood, plaster, cement, and other building materials, and they are important components of the rock-inhabiting microbial communities with involvement in mineral dissolution and secondary mineral formation (Burford et al., 2003a, b, 2006; Fomina et al., 2005a, b; Gadd, 2007a; Gadd et al., 2005, 2007).

The earliest fossil filamentous fungal remains appear to be from the mid- to late Precambrian (1430–1542 Myr ago), and they were extremely diverse by Devonian times, when forms belonging to major groups and even some



Geomycology, Figure 1 Simple model of fungal action on naturally occurring and/or anthropogenically derived organic and inorganic substrates. (1) organic and inorganic transformations mediated by enzymes and metabolites, e.g., H^+ , CO_2 , and organic acids, and physicochemical changes occurring as a result of metabolism; (2) uptake, metabolism, or degradation of organic substrates; (3) uptake, accumulation, sorption, metabolism, or degradation of inorganic substrates; (4) biosynthesis and production of organic metabolites, exopolymers, macromolecules, and biomass; (5) production of inorganic metabolites, secondary minerals, and transformed (organo)metal(loid)s; and (6) chemical interactions between organic and inorganic substances, e.g., complexation and chelation. (Adapted from Gadd, 2004a, 2007a.)

genera present today were found (Taylor and Osborn, 1996; Taylor et al., 1994, 1997, 2005; Heckman et al., 2001; Butterfield, 2005). Since that time fungi have been ubiquitous components of the microbial communities of any terrestrial environment (Hawksworth, 2001), including such hostile locations as the arctic and Antarctic, hot deserts, and metal-rich and hypersaline soils (Burford et al., 2003a). One of the most successful mechanisms that enables fungi to survive in extreme subaerial environments is by the formation of mutualistic symbioses with algae and/or cyanobacteria as lichens, where the phototrophs provide a source of carbon and are protected to some degree from light and other external factors (Gorbushina et al., 1993; Sterflinger, 2000). The majority of terrestrial fungi inhabit soil environments which are perhaps more hospitable than bare rock surfaces. Fungal communities in soil include free-living and symbiotic fungi, as well as plant and animal pathogens, and unicellular yeasts.

Fungi encounter metals as components of the natural environment as well as those introduced or redistributed by human activities. Like other organisms, fungi possess a variety of properties that can influence interactions with metals, while “normal” growth and metabolism is dependent on metal and metal–mineral interactions to satisfy essential metal and associated nutrient requirements (Gadd and Griffiths, 1978). At potentially toxic metal concentrations, a variety of resistance mechanisms may be expressed: sensitive organisms may be vulnerable and population changes can result. Metal toxicity can be

influenced by the physicochemical attributes of the environment, while fungi possess a variety of properties that can ensure survival. It seems fungi can be isolated from any habitats polluted by toxic metals.

This article will outline important fungal roles and functions in rock, mineral, metal, and soil transformations, and will emphasize the importance of fungi as agents of geochemical change. It will also include fungal-mediated metal transformations, the role of fungi in the geochemistry of metal cycling, and the applied significance of these processes in environmental biotechnology. Such roles can all be included under the term “geomycology,” which can be simply defined as “the scientific study of the role of fungi in processes of fundamental importance to geology.” This includes past, current, and potential future fungal activities and such roles include the alteration and weathering of rocks and minerals, soil formation, the transformation and accumulation of metals, decomposition, and nutrient cycling. In this article, the decomposition of organic substances is included under the heading of “geomycology” since this process results in major geochemical cycling of elements in the biosphere, and, as carbon and energy sources, the metabolism of organic compounds underpins all fungal activities and interactions with environmental components. “Geomycology” can be considered to be a subset of “geomicrobiology,” namely the role of microorganisms and microbial processes in geological and geochemical processes, and is itself a subset of “microbiology,” the scientific study of microorganisms. “Geomycology” can also be considered to be a subset of “mycology” which is the scientific study of fungi. Such definitions are clearly imprecise and important topics dealt with under “geomycology” and “geomicrobiology” headings are also relevant to and also important to such research areas as geochemistry, mineralogy, hydrometallurgy, and biogeochemistry, for example. Some geomycological activity involves enzymatic oxidation or reduction of inorganic substances, although prokaryotes are more usually associated with such reactions, and these may contribute to mineral formation, transformation, and degradation. Other activities involve the biosynthesis of inorganic and organic compounds and metabolites, and the degradation of natural and anthropogenically produced carbon compounds in which both prokaryotes and eukaryotes participate. Some geomicrobial activity involves reactions where inorganic or organic products of metabolism serve as chemical reagents in reactions such as metal precipitation, metal solubilization, and mineral weathering and dissolution. Some geomicrobial activity depends on physical effects exerted on the environment by growing microorganisms which can depend on growth form and metabolism, e.g., biomechanical pressure exerted by growing fungi in rock fissures, and pH reduction due to acid excretion. Some processes may be the result of a combination of several of these general activities. While the majority of processes discussed pertain to the terrestrial environment, it should be noted that the same processes may also occur in aquatic

environments and sediments, though their significance may be different as well as influenced strongly by spatial and environmental factors. It should also be noted that detailed discussion of fungal roles and the mechanisms involved in the cycling and transformations of elements like N, P, and S is precluded here because of space limitations.

Organic matter degradation and biogeochemical cycling

The ability of fungi to utilize a wide spectrum of organic compounds is well-known. These range from simple compounds such as sugars, organic acids, and amino acids to more complex molecules which are broken down by extracellular enzymes before cellular entry. These latter compounds include natural substances like cellulose, pectin, lignin, lignocellulose, chitin and starch, but also anthropogenic products such as hydrocarbons, pesticides, and other xenobiotics. Utilization of these substances results in redistribution of component elements, primarily C, H, and O, but also N, P, S, and others (see later).

Some fungi have remarkable degradative properties, and lignin-degrading white rot fungi like *Phanerochaete chrysosporium* can degrade aromatic hydrocarbons, chlorinated organics, polychlorinated biphenyls, nitrogen-containing aromatics, and many other pesticides, dyes, and xenobiotics. Such activities are relevant to bioremediation where ligninolytic fungi have been used to treat soil contaminated with, e.g., pentachlorophenol (PCP) and polycyclic aromatic hydrocarbons (PAHs), the latter being constituents of creosote. Treatment generally involves inoculation of contaminated soil, nutrient addition, irrigation and aeration, and maintenance by land-farming procedures (Singleton, 2001). Xenobiotic-transforming fungi may need additional utilizable carbon sources because they cannot utilize these complex substrates. Inexpensive utilizable lignocellulosic wastes, such as corn cobs, straw, and sawdust are therefore supplied as nutrients. Wood-rotting and other fungi are also receiving attention for the bleaching of dyes and industrial effluents, and the biotreatment of agricultural wastes, such as forestry, pulp and paper by-products, sugarcane bagasse, coffee pulp, sugar beet pulp, apple and tomato pulp, and cyanide.

Polycyclic aromatic hydrocarbons (PAHs) enter the environment via fossil-fuel combustion, vehicle exhaust emissions, gas and coal tar manufacture, wood-preservation processes, and waste incineration (Harvey, 1997; Cerniglia and Sutherland, 2001, 2006). Aerobic biodegradation of PAHs by soil microorganisms depends on monooxygenase, peroxidase, and dioxygenase pathways; the first and third of these pathways are employed by bacteria while the first and second are found in fungi. Many fungi can metabolize PAHs but since fungi cannot generally use PAHs as their sole carbon and energy source, they must be supplemented with other carbon sources to allow co-metabolism (Cerniglia and Sutherland, 2001, 2006; Sutherland, 2004).

The transformation of PAHs by ligninolytic wood-decaying fungi involves several enzymes. Those produced by white-rot fungi include lignin peroxidase, manganese peroxidase, laccase, cytochrome P450, and epoxide hydrolase. Ligninolytic fungi metabolize PAHs via reactions involving reactive oxygen species to phenols and quinones and these may be further degraded by ring-fission enzymes. Several wood-decaying fungi (e.g., *Bjerkandera*, *Coriolopsis*, *Irpex*, *Phanerochaete*, *Pleurotus*, and *Trametes* spp.), have been investigated for bioremediation of PAH-contaminated soils. Non-ligninolytic fungi, including *Cunninghamella*, *Mucor*, *Fusarium*, and *Penicillium* spp., have also been considered for PAH bioremediation. Complete biodegradation may require the presence of mixed bacterial and fungal communities.

Fungi are also important in the degradation of naturally occurring complex molecules in the soil, an environment where the filamentous hyphal mode of growth provides several advantages, and also in aquatic habitats. Since 95% of plant tissue is composed of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulphur, the decomposition activities of fungi are important in redistribution of these elements between organisms and environmental compartments (Table 2). As well as C, H, O, N, P, and S, another 15 elements are typically found in living plant tissues – K, Ca, Mg, B, Cl, Fe, Mn, Zn, Cu, Mo, Ni, Co, Se, Na, Si. However, all 90 or so naturally occurring elements may be found in plants, mostly at low concentrations, although this may be highly dependent on environmental conditions. These include Au, As, Hg, Pb, and U, and there are some plants that accumulate relatively high concentrations of metals Ni and Cd. Animals likewise contain a spectrum of elements in varying amounts. For example, the human body is mostly water, and 99% of the mass comprises oxygen, carbon, hydrogen, nitrogen, calcium, and phosphorus. However, many other elements are present in lower amounts including those in substances taken up as contaminants in food, air, and water. A similar situation occurs throughout the plant, animal, and microbial worlds. Therefore, any decomposition, degradative and pathogenic activities of fungi are linked to the cycling of all these constituent elements, some of which may be radionuclides accumulated from environmental sources (Table 2). This simple perspective on organic matter decomposition illustrates the global significance of fungi in geochemical element cycling and their involvement in almost all elemental cycles. Space considerations preclude detailed discussion of the mechanisms involved in fungal transformations and cycling of elements like N, P, and S and their compounds.

Organometals (compounds with at least one metal-carbon bond) can be attacked by fungi. Degradation of organometallic (and organometalloid) compounds, which are still widely used in agriculture and industry, can be carried out by fungi, either by direct biotic action (enzymes), or by the fungi facilitating abiotic degradation, for instance by alteration of pH and excretion of metabolites

Geomycology, Table 2 Fungal roles in key biogeochemical cycles of the elements. Some of the major or representative roles of fungi in elemental cycles are indicated without reference to their global significance. Major elemental movements relate to decomposition activities reliant on metabolism and the hyphal mode of growth. Note that only representative elements are shown here: virtually all elements in the Periodic Table (including actinides, lanthanides, and radionuclides) can be accumulated within or associated with fungal biomass depending on the environment. Fungi possess transport systems for essential metals; inessential metal species can also be taken up. Fungi are also capable of mediating metal bioprecipitation by, e.g., metabolite production, changing the physicochemical microenvironmental conditions around the biomass, and indirect release of metal-precipitating substances from other activities, e.g., phosphate. Fungal walls and exopolymers can sorb, bind, or entrap many substances. Redox transformations are also widespread in fungal metabolism. While most roles occur in the terrestrial aerobic environment, similar transformations may occur in aquatic environments and in sediments where fungal populations occur

Element(s)	Fungal roles in elemental cycles
C	Decomposition of organic substances; respiration (CO ₂ production); synthesis of polymers, organic metabolites, etc.; humus formation; CN ⁻ production; carbonate formation; oxalate formation; oxalate-carbonate cycle; dissolution of carbonates
H, O	Uptake, assimilation, degradation, and metabolism of organic and inorganic compounds; water uptake, transport, translocation, and conduction; respiration (CO ₂); organic and inorganic metabolite excretion
N	Decomposition of nitrogenous compounds; assimilation and transformations of organic and inorganic N compounds; fungal nitrification and denitrification; biosynthesis of N-containing biopolymers, e.g., chitin; production of N-containing metabolites and gases, e.g., N ₂ O; ammonia fermentation under anaerobic conditions; mycorrhizal N transfer to plants
P	Dissolution of inorganic phosphates and P-containing minerals in soils and rocks; decomposition of organic P-containing organic compounds; formation of insoluble P, e.g., polyphosphate, secondary phosphate minerals; release of organically bound P by phosphatases; assimilation and transformation of inorganic P species; oxidation of reduced forms of phosphate, e.g., phosphate; transformations of soil organic P; production of diphosphates and phosphonates; mycorrhizal P transfer to plants
S	Degradation of organic S-containing compounds; organic-inorganic S transformations; uptake and assimilation of organic and inorganic S compounds; SO ₄ ²⁻ reduction and assimilation; oxidation of reduced S compounds, e.g., S(0), thiosulfate, tetrathionate; sulfide production; oxidation of H ₂ S to S(0); reduction of S(0) to H ₂ S; dissolution of S-containing minerals in soils and rocks, e.g., sulfides, sulfates
Fe	Bioweathering of iron-containing minerals in rocks and soils; iron solubilization by siderophores, organic acids, metabolites, etc.; Fe(III) reduction to Fe(II)
Mn	Mn(II) oxidation and immobilization as Mn oxides; Mn(IV) reduction; indirect Mn(IV)O ₂ reduction by metabolites, e.g., oxalate; bioaccumulation of Mn oxides to surfaces, exopolymers; contribution to desert varnish formation; biosorption; accumulation; intracellular precipitation
Cr	Cr(VI) reduction to Cr(III); accumulation of Cr oxyanions
Mg, Ca, Co, Ni, Zn, Cd, Sr	Bioweathering of minerals in rocks and soil; biosorption; uptake and accumulation; bioprecipitation, e.g., oxalates, sulfides, phosphates, carbonate
Ag	Reduction of Ag(I) to Ag(0); biosorption; accumulation
K, Na, Cs	Uptake and accumulation; translocation through mycelium; concentration in fruit bodies; mobilization from minerals in soil
Cu	Mobilization from Cu-containing minerals in rocks and soils; CuS formation; biosorption; uptake and accumulation; bioprecipitation, e.g., oxalates
Se	Reductive transformation of Se oxyanions, e.g., Se(VI) to Se(IV) to Se(0); Biomethylation of Se compounds; assimilation of organic and inorganic Se compounds
Te	Reductive transformation of Te oxyanions, e.g., Te(VI) to Te(IV) to Te(0); Biomethylation of Te compounds; assimilation of organic and inorganic Te compounds
Pb	Biosorption; lead oxalate formation
Cl, Br, I	Methylation
Sn	Organotin degradation; sorption and accumulation of soluble Sn species
Au	Reduction of soluble Au species to Au(0)
As	Methylation of arsenic species, e.g., arsenite to trimethylarsine; reduction of As oxyanions, e.g., arsenate to arsenite; oxidation of As oxyanions, e.g., arsenite to arsenate
Hg	Hg methylation; reduction of Hg(II) to Hg(0); Hg volatilization as Hg(0); degradation of organomercurials; biosorption; accumulation
Al	Al mobilization from Al-containing minerals in soils and rocks; aluminosilicate dissolution; Al precipitation as oxides (early stage of bauxitization); biosorption
Si	Uptake of soluble Si species; organic Si complex formation from inorganic silicates; organic siloxane formation; silica, silicate, and aluminosilicate degradation; Si mobilization through production of chelators, acids, bases, exopolymers
U, Th	Biosorption; deposition of hydrolysis products; intracellular precipitation

(Gadd, 1993b). Organotin compounds, such as tributyltin oxide and tributyltin naphthenate, may be degraded to mono- and dibutyltins by fungal action, inorganic Sn(II) being the ultimate product (Gadd, 2000a). Organomercury compounds may be detoxified by fungal organomercury lyase, the resultant Hg(II) being subsequently reduced to less toxic, diffusible, and volatile Hg(0) by mercuric reductase (Gadd, 1993b). Degradation of persistent carbon sources, such as charcoal and black shale, can be accelerated by fungal activity, which in turn may accelerate the release of toxic metals as organic metal complexes (Wengel et al., 2006).

Transformations of rocks and minerals

Minerals are naturally occurring inorganic solids of definite chemical composition with an ordered internal structure; rocks can be considered to be any solid mass of mineral or mineral-like material. Silicates are the most common minerals with non-silicates constituting <10% of the Earth's crust, the most common being carbonates, oxides, sulfides, and phosphates. Rocks and minerals are a vast reservoir of elements, many essential for life, and such elements must be released in forms that may be assimilated by the biota. These include essential metals as well as nutrient elements like S and P (Gadd, 2007a; Gadd et al., 2005, 2007).

Microbial processes influenced by minerals

Many important microbial processes can be influenced by minerals including energy generation, nutrient acquisition, cell adhesion, and biofilm formation (Hochella, 2002), and such effects are also likely to be found within fungal populations. Essential nutrients may be acquired from mineral surfaces and this concentrates these substances above surrounding environmental levels (e.g., C, N, P, Fe, essential metals, various organic compounds) (Vaughan et al., 2002). Environmental contaminants may also be sorbed to mineral surfaces and these can be displaced by microbial activity (Kraemer et al., 1999). Potentially toxic metals released from minerals as a result of physicochemical and biological processes, may also affect microbial communities (Gadd, 2005). Such properties of mineral surfaces as microtopography, surface composition, surface charge and hydrophobicity play an important role in microbial attachment and detachment, and are therefore critical for colonization and biofilm formation, and the ecology of microbial populations, including fungi, associated with mineral substrates.

Bioweathering by fungi

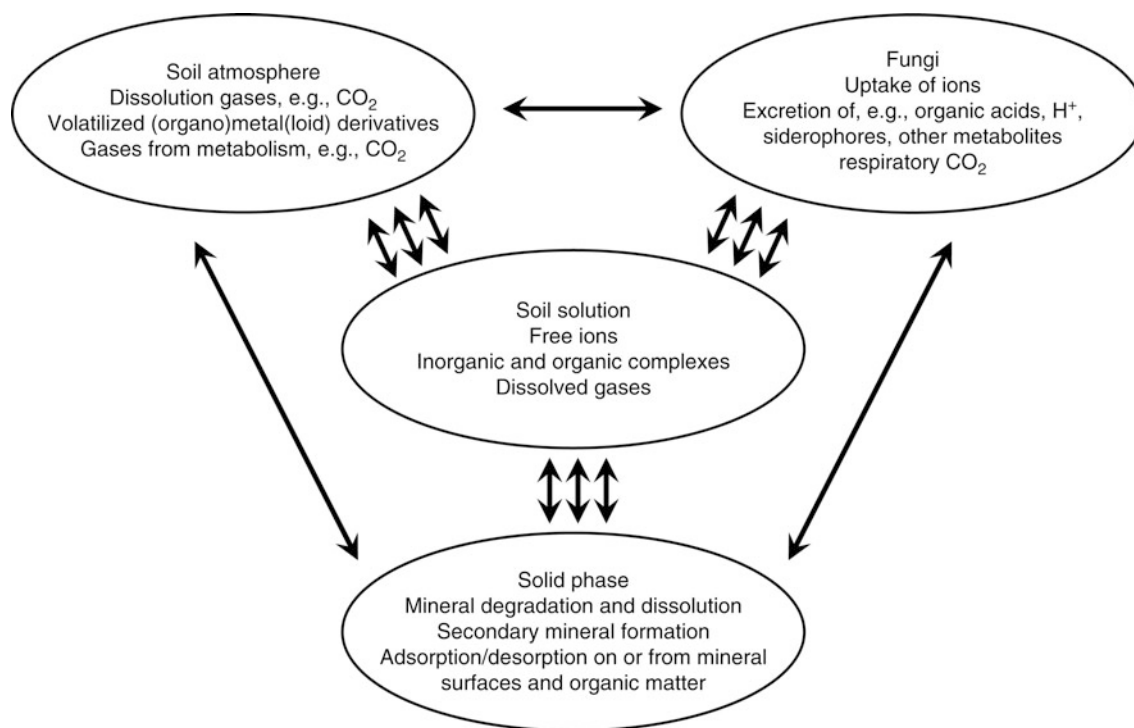
Bioweathering can be defined as the erosion, decay, and decomposition of rocks and minerals mediated by living organisms. Fungi are well-suited as biological weathering agents since they can be highly resistant to extreme environmental conditions such as metal toxicity, UV radiation, and desiccation; they can adopt a variety of growth, metabolic, and morphological strategies; they can exude protons and metal-complexing metabolites, and form

mutualistic associations with animals, plants, algae, and cyanobacteria (Sterflinger, 2000; Burford et al., 2003a, b; Gadd, 2007a). Most fungi exhibit a filamentous growth habit, while some are polymorphic occurring as filamentous mycelium and unicellular yeasts or yeast-like cells, e.g., black microcolonial rock-dwelling fungi (Gorbushina, 2007). The ability of fungi to translocate water, nutrients, and organelles within the mycelial network is another important feature for exploiting heterogeneous environments (Boswell et al., 2002, 2003, 2006).

Rock surfaces may be an inhospitable habitat due to moisture deficit and nutrient limitation although many fungi can tolerate extremes in such factors as light, salinity, pH, and water potential (Gorbushina et al., 1993; Sterflinger, 2000; Verrecchia, 2000; Burford et al., 2003a, b). Many fungi can scavenge nutrients from the atmosphere and rainwater. In the subaerial rock environment, fungi can also use organic and inorganic residues on surfaces or within cracks and fissures, waste products of other microorganisms, decaying plants and insects, dust particles, aerosols, and animal feces as nutrient sources (Sterflinger, 2000). Fungi may receive protection from the presence of melanin pigments and mycosporines in their cell walls, and by the production of mucilaginous exopolymeric slime that may entrap clay particles providing extra protection (Gadd, 1993a; Gorbushina, 2007). Fungi appear to be ubiquitous components of the microbiota of all rocks, building stone, and concrete, and have been reported from a wide range of rock types, e.g., limestone, marble, granite, sandstone, basalt, gneiss, dolerite and quartz, even from the most extreme environments (Burford et al., 2003a, b; Gorbushina, 2007).

Elements found in soil largely reflect the composition of the Earth's crust, though some modification occurs by weathering, biogenic and anthropogenic activities: chemical changes include mineral dissolution while biological activity causes enrichment of C, N, and S. Elements and minerals that remain can reform as secondary minerals. In soil, fungus–mineral interactions are an integral component of environmental cycling (Figure 2). Mycorrhizal fungi are particularly important in mineral weathering and dissolution of insoluble metal compounds. Fungi are also important components of lithobiotic biofilm communities at mineral–microbe interfaces where they interact with the substrate both geophysically and geochemically: this can result in the formation of patinas, films, varnishes, crusts, and stromatolites (Gadd, 2007a; Gorbushina, 2007).

Biomechanical deterioration of rocks can occur through hyphal penetration and burrowing into decaying material and along crystal planes in, e.g., calcitic and dolomitic rocks (Sterflinger, 2000; Gadd, 2007a). Cleavage penetration can also occur with lichens (Banfield et al., 1999). Spatial exploration of the environment to locate and exploit new substrates is facilitated by a range of sensory responses that determine the direction of hyphal growth. For example, thigmotropism (or contact guidance) is a well-known property of fungi that grow on and within solid substrates with the direction of fungal growth being influenced by grooves,



Geomycology, Figure 2 Indicated role of fungi (as well as other biota) in soil solution reactions relevant to metal(loid) speciation and partitioning between solid, liquid, and gaseous phases (derived from Cotter-Howells and Paterson, 2000). The soil solution has a pivotal role in most reactions of metabolic and geochemical significance in the soil. The arrows linking the outer components of the diagram that represent the fungi, soil atmosphere, and solid phase indicate those direct reactions that may occur with little or no significant involvement of the soil solution. These could include utilization or production of gases (e.g., O_2 and CO_2 respectively), direct attack of solid phases, and adsorption of gases onto solid phase surfaces. However, in the absence of water, these reactions may be limited, especially those dependent on metabolism.

ridges, and pores (Bowen et al., 2007a, b). However, biochemical actions are believed to be more important processes than mechanical degradation. Biochemical weathering of rocks and minerals can occur through excretion of, e.g., H^+ , organic acids and other metabolites. This can result in changes in the mineral microtopography through pitting and etching of surfaces to complete dissolution of mineral grains (Ehrlich, 1998; Gharieb et al., 1998; Kumar and Kumar, 1999; Adeyemi and Gadd, 2005). Fungi generally acidify their microenvironment via a number of mechanisms, which include the excretion of protons and organic acids, while respiratory CO_2 can result in carbonic acid formation. In addition, fungi excrete a variety of other metal-complexing metabolites (e.g., siderophores, carboxylic acids, amino acids, and phenolic compounds) (Burgstaller and Schinner, 1993). The weathering of sandstone monuments by fungi has been attributed to the production of, e.g., acetic, oxalic, citric, formic, fumaric, glyoxylic, gluconic, succinic, and tartaric acids.

Concrete biodeterioration in radioactive waste disposal

Cement and concrete are used as barriers in all kinds of nuclear waste repositories. Despite the theoretical service

life of concrete reaching up to one million years, biocorrosion is an important factor to take into account. All types of building and ceramic materials, concrete, and cement can be deteriorated by microorganisms and in some environments, fungi dominate the microbiota and play an important role in biodeterioration of concrete (Gu et al., 1998; Nica et al., 2000; Zhdanova et al., 2000). Fungal attack on concrete can be strongly and mildly aggressive caused by protons and organic acids and production of hydrophilic slimes leading to biochemical and biophysical/biomechanical deterioration (Fomina et al., 2007a). Fungal degradation may proceed more rapidly than bacterial degradation with complexolysis suggested as the main mechanism of calcium mobilization. Microfungi from the genera *Aspergillus*, *Alternaria*, and *Cladosporium* were able to colonize samples of the concrete used as the radioactive waste barrier in the Chernobyl reactor and leached iron, aluminium, silicon, and calcium, and re-precipitated silicon and calcium oxalate in their microenvironment (Fomina et al., 2007a). Fungi are also important members of the microbial communities (including lichens) that colonize and deteriorate “normal” concrete and cement used in buildings and other structures.

Geomycology, Table 3 Some examples of biomineralization of fungal hyphae and lichen thalli with secondary minerals. The hydration state of some minerals is unclear and only stated when this is specifically identified. The list is not exhaustive and many other mycogenic minerals are possible, as well as many other fungal species capable of mediating their formation (taken from Gadd, 2007a; Burford et al., 2003a, b, and also adapted from a number of sources including Grote and Krumbein, 1992; de la Torre and Gomez-Alarcon, 1994; Easton, 1997; Verrecchia, 2000; Haas and Purvis, 2006; Burford et al., 2006)

Mineral	Fungal hyphae	Lichen thalli	Organism(s)
Birnessite ((Na, Ca, K) Mn ₇ O ₁₄ ·3H ₂ O)	Fungi on siderite boulder and Natraqualf soil		<i>Alternaria</i> spp. <i>Cladosporium</i> spp.
Cadmium oxalate (CdC ₂ O ₄)	Fungi cultured with, e.g., cadmium phosphate, or other cadmium compounds and minerals		<i>Beauveria caledonica</i>
Calcite (CaCO ₃)	Fungi on stalactites, Quaternary eolianites and calcretes; fungi grown in limestone cement microcosms and laboratory media containing insoluble calcium compounds, e.g., calcite, or other Ca-containing compounds and minerals	Lichens on roofing tiles, andesite, volcaniclastite and exposed caliche plates in weathered basaltic and rhyolitic rocks	<i>Caloplaca aurantia</i> <i>Doratomyces</i> sp. <i>Penicillium coryphilum</i> <i>Penicillium simplicissimum</i> <i>Verrucaria</i> spp.
Cobalt oxalate (CoC ₂ O ₄)	Fungi cultured with Co compounds		<i>Aspergillus niger</i>
Desert Varnish (MnO and FeO)	Fungal action on siderite and rhodochrosite in desert regions and sandstone limestone and granite monuments		<i>Alternaria alternata</i> <i>Cladosporium cladosporoides</i> <i>Lichenothelia</i> spp. <i>Penicillium frequentans</i> <i>Penicillium steckii</i> <i>Phoma glomerata</i>
Ferrihydrite (Fe ₂ HO ₈ ·H ₂ O or 5Fe ₂ O ₃ ·9H ₂ O)		Lichen on recent lava flow, on olivine of basalt, gabbro and augite	<i>Pertusaria corallina</i> <i>Stereocaulon vulcani</i>
Glushinskite (MgC ₂ O ₄ ·2H ₂ O)	Fungi cultured with hydromagnesite	<i>Lichen/rock interface on serpentinite</i>	<i>Lecanora atra</i> <i>Penicillium simplicissimum</i>
Goethite (FeO(OH))		Lichen on metamorphic rocks, feldspars, granite, and gneiss	<i>Parmelia conspersa</i> <i>Parmelia tiliacea</i>
Halloysite (Al ₂ Si ₂ O ₅ (OH) ₄ ·2H ₂ O)		Action of lichens on cave deposits and waters	<i>Lasallia</i> spp. <i>Mucor</i> spp. <i>Parmelia</i> spp. <i>Penicillium</i> spp. <i>Rhizocarpon</i> spp. <i>Rhizopus</i> spp.
Humboldtine (FeC ₂ O ₄ ·2H ₂ O)		Lichens on Fe rich crystalline limestone and cupriferous rocks	<i>Acarospora smargdula</i> <i>Aspicila alpina</i> <i>Lecidea lactea</i>
Hydrocerussite (Pb ₃ (CO ₃) ₂ (OH) ₂)		Mycobiont of lichen in ruins of a lead-smelting mill	<i>Stereocaulon vesuvianum</i>
Hydromagnesite (Mg ₅ (CO ₃) ₄ (OH) _{2.4} ·H ₂ O)	Fungi cultured with hydromagnesite		<i>Penicillium simplicissimum</i>
Lead oxalate, lead oxalate dehydrate (PbC ₂ O ₄ , PbC ₂ O ₄ ·2H ₂ O)	Fungi cultured with pyromorphite, or in laboratory media containing Pb compounds		<i>Aspergillus niger</i> <i>Beauveria caledonica</i>
Mn-oxalate (MnC ₂ O ₄ ·2H ₂ O)	Fungi cultured in laboratory media containing Mn compounds	Lichen on Mn ore	<i>Aspergillus niger</i> <i>Pertusaria corallina</i>
Montmorillonite (X _{0.33} Al ₂ Si ₂ O ₁₀ (OH) ₂ ·nH ₂ O) [X = Na ⁺ , K ⁺ , Ca ²⁺ , Mg ²⁺]		Action of lichens on cave deposits and waters	<i>Lasallia</i> spp. <i>Mucor</i> spp. <i>Parmelia</i> spp. <i>Penicillium</i> spp. <i>Rhizocarpon</i> spp. <i>Rhizopus</i> spp.
Moolooite (CuC ₂ O ₄ ·nH ₂ O (n < 1))	Fungi cultured with, e.g., copper phosphate, or other Cu-containing compounds and minerals	Lichens on cupriferous rocks	<i>Acarospora rugulosa</i> <i>Aspergillus niger</i> <i>Beauveria caledonica</i> <i>Lecidea inops</i> <i>Lecidea lactea</i> <i>Rhizopogon rubescens</i> <i>Serpula himantioides</i>

Geomycology, Table 3 (Continued)

Mineral	Fungal hyphae	Lichen thalli	Organism(s)
Strontium oxalate hydrate ($\text{SrC}_2\text{O}_4 \cdot \text{H}_2\text{O}$; $\text{SrC}_2\text{O}_4 \cdot 2.5\text{H}_2\text{O}$)	Fungi cultured with strontianite (SrCO_3), or other Sr-containing compounds and minerals		<i>Penicillium simplicissimum</i> <i>Pseudallescheria boydii</i> <i>Serpula himantoides</i>
Todorokite ((Mn, Ca, Mg) $\text{Mn}_3\text{O}_7 \cdot \text{H}_2\text{O}$)	Fungi in cave deposits and waters		<i>Mucor</i> spp. <i>Penicillium</i> spp. <i>Rhizopus</i> spp.
Uramphite ($\text{NH}_4(\text{UO}_2)$ (PO_4) $\cdot 3\text{H}_2\text{O}$) and Chernikovite ($(\text{H}_3\text{O})_2(\text{UO}_2)_2(\text{PO}_4)_2 \cdot 6\text{H}_2\text{O}$)	Fungi cultured with uranium oxides, metallic depleted uranium, or other U- containing compounds and minerals		<i>Beauveria caledonica</i> <i>Hymenoscyphus ericae</i> <i>Rhizopogon rubescens</i> <i>Serpula himantoides</i>
Weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$)	In leaf litter and soils; fungi grown on limestone cement microcosms, and laboratory media containing insoluble calcium compounds, e.g., calcite, or other Ca-containing compounds and minerals	On serpentinite, cupriferous rocks, andesite and volcaniclastite	<i>Acarospora rugulosa</i> <i>Aphylllophorales</i> spp. <i>Aspicilia calcarea</i> <i>Caloplaca aurantia</i> <i>Caloplaca flavescens</i> <i>Gastrum</i> spp. <i>Hypogymnia physodes</i> <i>Hysterangium crassum</i> <i>Lecanora atra</i> <i>Lecanora rupicola</i> <i>Lecidea inops</i> <i>Lecidea lactea</i> <i>Ochrolechia parella</i> <i>Penicillium coryliphilum</i> <i>Penicillium simplicissimum</i> <i>Pseudallescheria boydii</i> <i>Serpula</i> <i>himantoides</i>
Whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$)	In Nari limecrusts, Quaternary calcretes, forest leaf litter and soils; fungi grown on limestone cement microcosms, and laboratory media containing insoluble calcium compounds, e.g., calcite, or other Ca-containing compounds and minerals	On basalt, serpentinite, cupriferous rocks, gabbro, dolerite, andesite and volcaniclastite	<i>Acarospora rugulosa</i> <i>Acarospora smargdula</i> <i>Aspicilia alpina</i> <i>Aspicilia calcarea</i> <i>Aspicilia radiosa</i> <i>Caloplaca flavescens</i> <i>Doratomyces</i> sp. <i>Hypogymnia physodes</i> <i>Lecanora atra</i> <i>Lecanora rupicola</i> <i>Lecidea inops</i> <i>Lecidea lactea</i> <i>Ochrolechia parella</i> <i>Parmelia conspersa</i> <i>Parmelia subrudecta</i> <i>Penicillium coryliphilum</i> <i>Penicillium simplicissimum</i> <i>Pertusaria corallina</i> <i>Pseudallescheria boydii</i> <i>Serpula</i> <i>himantoides</i> <i>Xanthoria ectaneoides</i> <i>Aspergillus niger</i> <i>Beauveria caledonica</i> <i>Rhizopogon rubescens</i> <i>Suillus collimitus</i>
Zinc oxalate ($\text{ZnC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$)	Fungi exposed to, e.g., zinc oxide, zinc phosphate		

Formation of secondary mycogenic minerals

Formation of secondary minerals by fungi can occur through metabolism-independent and -dependent processes (Gadd, 2007a) (Table 3). Precipitation, nucleation, and deposition of crystalline material on and within cell walls are influenced by factors such as pH and wall

composition. This process may be important in soil as the precipitation of carbonates, phosphates, and hydroxides increases soil aggregation. Cationic species of Si(IV), Fe(III), Al(III), and Ca(II) that may be released through dissolution mechanisms, stimulate precipitation of compounds that may bond soil particles. Hyphae and any

associated exopolymeric material can also enmesh soil particles, and also release organic metabolites that enhance aggregate stability (Bronick and Lal, 2005).

Carbonates

Microbial carbonate precipitation coupled with silicate weathering could provide an important sink for CO_2 in terrestrial environments. In limestone, fungi and lichens are considered to be important agents of mineral deterioration. In contrast, many near-surface limestones (calcretes), calcic, and petrocalcic horizons in soils are secondarily cemented with calcite (CaCO_3) and whewellite (calcium oxalate monohydrate, $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$). The presence of fungal filaments mineralized with calcite (CaCO_3), together with whewellite (calcium oxalate monohydrate, $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$), has been reported in limestone and calcareous soils from a range of localities (Verrecchia, 2000). Calcium oxalate can also be degraded to calcium carbonate, and this may again cement preexisting limestones (Verrecchia et al., 2006). During the decomposition of fungal hyphae, calcite crystals can act as sites of further secondary calcite precipitation. Calcite will also readily nucleate on chitin, the major component of fungal cell walls. Other experimental work has demonstrated fungal precipitation of secondary calcite, whewellite, and glushkinskite ($\text{MgC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) (Figure 3) (Burford et al., 2003b, 2006; Gadd, 2007a).

Oxalates

Many fungi can produce metal oxalates by interaction with a variety of different metals and metal-bearing minerals (Ca, Cd, Co, Cu, Mg, Mn, Sr, Zn, Ni, and Pb) (Figure 4) (Sayer and Gadd, 1997; Gadd, 2007a) (Table 3). Calcium oxalate dihydrate (weddelite) and the more stable calcium oxalate monohydrate (whewellite) are the most common forms of oxalate associated with fungi: biotic fungal calcium oxalate can exhibit a variety

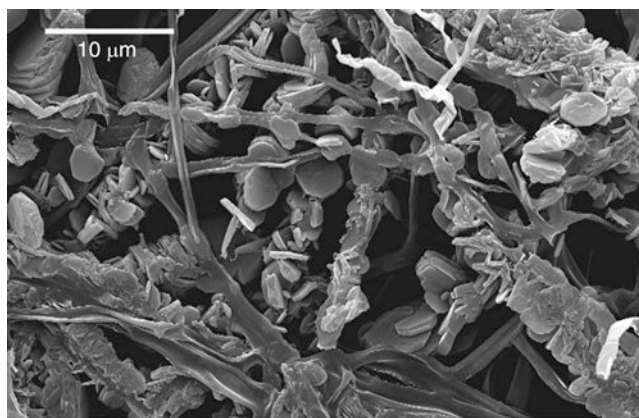
of crystalline forms (tetragonal, bipyramidal, plate-like, rhombohedral or needles) (Arnott, 1995). Precipitation of calcium oxalate can act as a reservoir for calcium and also influences phosphate availability. The formation of toxic metal oxalates may also provide a mechanism enabling fungi to tolerate high concentrations of toxic metals (Gadd, 1993a; Jarosz-Wilkolazka and Gadd, 2003).

Reductive and oxidative precipitation

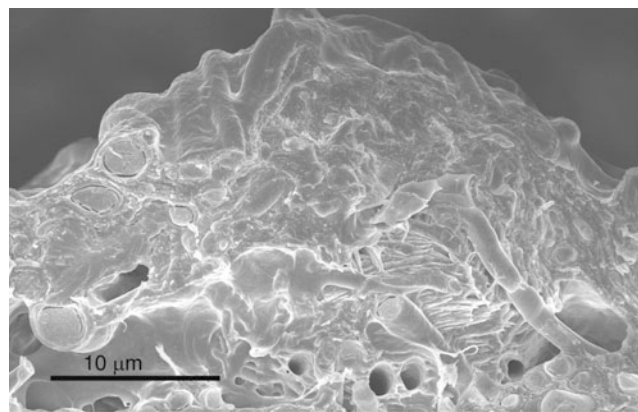
Reduced forms of metals and metalloids (e.g., elemental silver, gold, selenium, tellurium) can be precipitated by many fungi (Kierans et al., 1991; Gharieb et al., 1995, 1999; Gadd, 1993b). The reductive ability of fungi is manifest by black coloration of fungal colonies precipitating elemental Ag or Te, or a red coloration for those precipitating elemental Se. An oxidized metal layer (patina) a few millimetres thick found on rocks and in soils of arid and semi-arid regions, called desert varnish, is also believed to be of microbial origin with some proposed fungal involvement. Fungi can oxidize Mn(II) and Fe(II) in metal-bearing minerals such as siderite (FeCO_3) and rhodochrosite (MnCO_3) and precipitate them as oxides (Grote and Krumbein, 1992).

Other mycogenic minerals

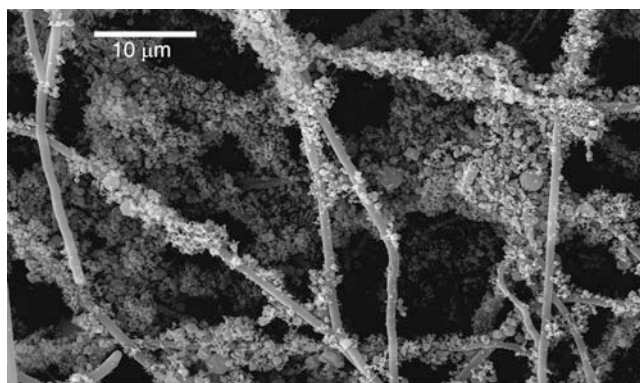
Combinations of biotic and abiotic factors can lead to deposition of a variety of other secondary minerals associated with fungal biomass, e.g., birnessite, MnO and FeO, ferrihydrite, iron gluconate, calcium formate, forsterite, goethite, halloysite, hydrocerussite, todorokite, moolooite, montmorillonite, and uranium phosphates (Figure 5) (Gadd, 2007a) (Table 3). Such biomineralization immobilizes metals and therefore limits metal bioavailability.



Geomycology, Figure 3 Glushkinskite ($\text{MgC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) and hydromagnesite ($\text{Mg}_5(\text{CO}_3)_4(\text{OH})_2 \cdot 4\text{H}_2\text{O}$) deposition around hyphae of *Penicillium simplicissimum* cultured on media containing hydromagnesite. (Burford E. P., and Gadd G. M., 2004 unpublished data.)



Geomycology, Figure 4 Moolooite (copper oxalate, $\text{CuC}_2\text{O}_4 \cdot n\text{H}_2\text{O}$ ($n < 1$)) formation around hyphae of *Beauveria caledonica* grown on media containing copper phosphate. The moolooite has been fractured showing the extensive precipitation around hyphae, and the resulting holes or tunnels. (Fomina M., and Gadd G. M., 2005 unpublished data; see also Fomina et al., 2005a.)



Geomycology, Figure 5 Extensive precipitation of uranium phosphate minerals, uramphite ($\text{NH}_4(\text{UO}_2)(\text{PO}_4) \cdot 3\text{H}_2\text{O}$) and chernikovite ($(\text{H}_3\text{O})_2(\text{UO}_2)_2(\text{PO}_4)_2 \cdot 6\text{H}_2\text{O}$) around hyphae of *Beauveria caledonica* cultured on media containing uranium oxide (U_3O_8). (Fomina M., et al., 2007 unpublished data; see also Fomina et al., 2007b.)

Fungal–clay interactions

Clay mineral formation and impact on soil properties

Silicon dioxide, when combined with oxides of Mg, Al, Ca, and Fe, forms the silicate minerals in rocks and soil. Silicates are the largest class of minerals comprising 30% of all minerals and making up 90% of the Earth's crust (Ehrlich, 2002). These minerals are unstable in the biosphere and break down to form clays. Microorganisms, including fungi, play a fundamental role in the dissolution of silicates in rock weathering, and therefore in the formation of clay minerals, and soil and sediment formation (Banfield et al., 1999). The presence of clay minerals can be a typical symptom of biogeochemically weathered rocks, and this has been observed for symbiotic fungal associations (lichens and ectomycorrhizas) (Barker and Banfield, 1998; Arocena et al., 1999). Some studies have shown that the transformation rate of mica and chlorite to 2:1 expandable clays was predominant in the ectomycorrhizosphere compared to non-ectomycorrhizosphere soils, likely to be a result of the high production of organic acids and direct extraction of K and Mg by the fungal hyphae (Arocena et al., 1999).

Soil can be considered to be a biologically active loose mass of weathered rock fragments mixed with organic matter, and is the ultimate product of rock weathering, i.e., interactions between the biota, climate, and rocks. Clay minerals are generally present in soil in larger amounts than organic matter and because of their adsorptive and ion-exchange capacity, they perform a significant buffering function in mineral soils and are important reservoirs of cations and organic molecules.

Biological effects of clay minerals

Fungi are generally in close proximity to clay minerals in soils and sediments. Several studies have shown that interactions of microorganisms with solid adsorbents lead to an increase in biomass, growth rate, and production of

various enzymes and metabolites. Stimulatory effects may arise from the abilities of different clays to serve as pH buffers, as a source of metal cationic nutrients, as specific adsorbents of metabolic inhibitors, other nutrients and growth stimulators; and modifiers of the microbial microenvironment because of their adsorptive capacity. However, some clays may inhibit fungal growth and metabolism (Stotzky, 1966).

Fungal–clay mineral interactions in soil aggregation

Fungal–clay mineral interactions play an important role in soil development, aggregation and stabilization (Burford et al., 2003a). Fungi entangle soil particles in their hyphae forming stable microaggregates and also take part in exopolysaccharide-mediated aggregation. Interactions between hyphae and solid particles are subject to complex forces of both a physicochemical (electrostatic, ionic, hydrophobic effects, etc.) and biological nature (chemotropism, production of specific enzymes, polysaccharides, lectins and other adhesins, etc.) (Ritz and Young, 2004). Interactions between clay minerals and fungi alter the adsorptive properties of both clays and hyphae (Morley and Gadd, 1995; Fomina and Gadd, 2002).

Clay and silicate weathering by fungi

Fungi and bacteria play an important role in the mobilization of silica and silicates. Their action is mainly indirect, either through the production of chelates or the production of acids, mineral or organic (including oxalic), or, as for certain bacteria, the production of ammonia or amines (Ehrlich, 2002).

Metal, metalloid, radionuclide, and organometal transformations

Metals and their compounds interact with fungi in various ways depending on the metal species, organism, and environment, while fungal metabolism also influences metal speciation and mobility (Gadd and Griffiths, 1978; Gadd, 1992, 1993a, 2004a, b, 2005, 2007a). Many metals are essential for life, e.g., Na, K, Cu, Zn, Co, Ca, Mg, Mn, and Fe, but all can exert toxicity when present above certain threshold concentrations. Other metals, e.g., Cs, Al, Cd, Hg, and Pb, have no known metabolic function in fungi but all can be accumulated. Metal toxicity is affected by environmental conditions and the chemical behavior of the particular metal species. Despite potential toxicity, many fungi survive and grow in metal-rich locations and a variety of mechanisms, both active and incidental, contribute to tolerance. Fungi have many properties which influence metal mobility and toxicity including the production of metal-binding proteins, organic and inorganic precipitation, active transport, and intracellular compartmentalization, while major constituents of cell walls, e.g., chitin and melanin, have significant metal-binding abilities (Gadd, 2004a, b, 2005, 2006). Other properties lead to metal solubilization from organic and inorganic sources (Gadd, 2007a).

Fungi can also transform certain metals, metalloids (elements with properties intermediate between those of metals and non-metals, e.g., arsenic, selenium, and tellurium), organometallic compounds and metal radionuclides by, e.g., oxidation, reduction, methylation, and dealkylation (Gadd, 1993b, 2004a, b). Such transformations alter chemical speciation and may modify mobility and toxicity. For example, methylated selenium derivatives are volatile and less toxic than inorganic forms while reduction of metalloid oxyanions, such as selenite or tellurite to amorphous elemental selenium or tellurium respectively, results in immobilization and detoxification. All the mechanisms by which fungi (and other microorganisms) effect changes in metal speciation and mobility are survival determinants but also components of biogeochemical cycles for metals, and many other associated elements including carbon, nitrogen, sulfur, and phosphorus (Gadd, 2004a, 2006, 2007a, 2008). They may be simply considered in terms of metal mobilization or immobilization mechanisms.

Metal mobilization

Metal mobilization from rocks, minerals, soil, and other substrates can be achieved by protonolysis, resulting in carbonic acid formation from respiratory CO₂, complexation by excreted metabolites and Fe(III)-binding siderophores, and methylation which can result in volatilization. In addition, other excreted metabolites with metal-complexing properties, e.g., amino acids, phenolic compounds, and organic acids may also play a role. Fungal-derived carboxylic acids can play an important role in chemical attack of mineral surfaces and these provide protons as well as a metal-chelating anion (Burgstaller and Schinner, 1993). Oxalic acid can act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe (Strasser et al., 1994). Solubilization mechanisms can also have consequences for mobilization of metals from toxic metal containing minerals, e.g., pyromorphite (Pb₅(PO₄)₃Cl), contaminated soil, and other solid wastes (Sayer et al., 1999; Fomina et al., 2004, 2005b, c). Fungi can also mobilize metals and attack mineral surfaces by redox processes: Fe(III) and Mn(IV) solubility is increased by reduction to Fe(II) and Mn(II) respectively. Reduction of Hg(II) to volatile elemental Hg(0) can also be mediated by fungi (Gadd, 1993b).

Removal of metals from industrial wastes and by-products, low grade ores and metal-bearing minerals by fungal “heterotrophic leaching” is relevant to metal recovery and recycling and/or bioremediation of contaminated solid wastes (Burgstaller and Schinner, 1993; Gadd, 2007a, b). Although fungi need a source of carbon and aeration, they can solubilize metals at higher pH values than thiobacilli and could perhaps become important where leaching with such bacteria is not possible and in bioreactors. Leaching of metals with fungi can be effective although a high level of organic acid production may be necessary. Other possible applications of fungal metal

solubilization are the removal of unwanted phosphates, and metal recovery from scrap electronic and computer materials (Brandl, 2001).

The ability of fungi, along with bacteria, to transform metalloids has been utilized successfully in the bioremediation of contaminated land and water. Selenium methylation results in volatilization, a process which has been used to remove selenium from the San Joaquin Valley and Kesterson Reservoir, California, using evaporation pond management and primary pond operation (Thomson-Eagle and Frankenberger, 1992).

Metal immobilization

Fungal biomass provides a metal sink, either by metal biosorption to biomass (cell walls, pigments and extracellular polysaccharides), intracellular accumulation and sequestration, or precipitation of metal compounds onto and/or around hyphae (Gadd, 1993a, 2000b, c, 2001a, b, c, 2007a; Baldrian, 2003; Fomina et al., 2007b, c). Fungi are effective biosorbents for a variety of metals including Ni, Zn, Ag, Cu, Cd, and Pb, and this can be an important passive process in both living and dead biomass (Gadd, 1990, 1993a; Sterflinger, 2000). The presence of chitin, and pigments like melanin, strongly influences the ability of fungi to act as sorbents. In a biotechnological context, fungi and their by-products have received considerable attention as biosorbent materials for metals and radionuclides (de Rome and Gadd, 1987; Gadd and White, 1989, 1990, 1992; White et al., 1995). Fungi can also precipitate several inorganic and organic compounds, e.g., oxalates, oxides, and carbonates, and this can lead to formation of biogenic minerals (mycogenic precipitates) as discussed previously (Gadd, 2007a).

Halide transformations

Fungi have the ability to produce a variety of atmospheric methyl halides. This ability is widespread in both free-living and symbiotic fungi, and is dependent on substrate concentrations and community composition (Redeker et al., 2004). The production of chloromethane (CH₃Cl) by wood-rotting fungi, e.g., *Phellinus* spp., may be particularly significant with one estimate of annual global input to the atmosphere from this source being 160,000t of which 75% is released from tropical and subtropical forests (Watling and Harper, 1998). Filamentous fungi may also contribute to the global circulation of stable iodine and also the long-lived radioiodine, ¹²⁹I (half-life: 1.6 × 10⁷ years), released from nuclear facilities into the environment (Ban-nai et al., 2006).

Fungal symbioses in mineral transformations

One of the most remarkable adaptations of fungi for exploitation of the terrestrial environment is their ability to form mutualistic partnerships with plants (mycorrhizas) and algae or cyanobacteria (lichens). Symbiotic fungi are provided with carbon by the photosynthetic partners

(photobionts), while the fungi may protect the symbiosis from harsh environmental conditions (e.g., desiccation, metal toxicity), increase the absorptive area, and provide increased access to mineral nutrients.

Lichens

Lichens are fungi that exist in facultative or obligate symbioses with one or more photosynthesizing partners, and play an important role in many biogeochemical processes. The lichen symbiosis formed between the fungal partner (mycobiont) and the photosynthesizing partner (algal or cyanobacterial photobiont) enables lichens to grow in practically all surface terrestrial environments: an estimated 6% of the Earth's land surface is covered by lichen-dominated vegetation (Haas and Purvis, 2006). Lichens are pioneer colonizers of fresh rock outcrops. Globally, lichens play an important role in the retention and distribution of nutrient (e.g., C, N) and trace elements, in soil formation, and in rock weathering. Alteration of bedrock minerals and synthesis of biominerals in the proximity of lichens gives rise to different chemical microenvironments and emphasizes their participation in mineral nutrient cycling (Banfield et al., 1999). Lichens can accumulate metals such as lead (Pb), copper (Cu), and many other elements of environmental concern, including radionuclides, to high levels. They can also form a variety of metal-organic biominerals, especially during growth on metal-rich substrates. For example, on copper-sulfide bearing rocks, precipitation of copper oxalate (moolooite) can occur within the lichen thallus (Purvis, 1996; Purvis and Halls, 1996).

Mycorrhizas

Nearly all land plants depend on symbiotic mycorrhizal fungi (Smith and Read, 1997). Two main types of mycorrhizas include endomycorrhizas where the fungus colonizes the interior of host plant root cells (e.g., ericoid and arbuscular mycorrhizas) and ectomycorrhizas where the fungus is located outside plant root cells. Mycorrhizal fungi are involved in proton-promoted and ligand-promoted metal mobilization from mineral sources, metal immobilization within biomass, and extracellular precipitation of mycogenic metal oxalates (Fomina et al., 2004, 2005b). Biogeochemical activities of mycorrhizal fungi lead to changes in the physicochemical characteristics of the root environment and enhanced weathering of soil minerals resulting in metal release. Ectomycorrhizal mycelia may respond to the presence of different soil silicate and phosphate minerals (apatite, quartz, potassium feldspar) by regulating their growth and activity, e.g., colonization, carbon allocation, and substrate acidification (Rosling et al., 2004a, b).

During growth, mycorrhizal fungi often excrete low molecular weight carboxylic acids (Martino et al., 2003; Fomina et al., 2004). The weathering of hornblendes, feldspars, and granitic bedrock in certain soils has been attributed to oxalic, citric, succinic, formic, and malic acid

excretion by ectomycorrhizal hyphae which can produce micro- to millimolar concentrations of these organic acids. Ectomycorrhizal fungi (*Suillus granulatus* and *Paxillus involutus*) can release elements from apatite and wood ash (K, Ca, Ti, Mn, Pb) and accumulate them in the mycelia (Wallander et al., 2003). Ericoid mycorrhizal and ectomycorrhizal fungi can dissolve a variety of cadmium, copper, zinc, and lead-bearing minerals including metal phosphates (Leyval and Joner, 2001; Martino et al., 2003; Fomina et al., 2004, 2005b). Mobilization of phosphorus is generally regarded as one of the most important functions of mycorrhizal fungi.

Summary

The biogeochemical importance of fungi is significant in several key areas. These include organic and inorganic transformations, nutrient and element cycling, rock and mineral transformations, bioweathering, mycogenic mineral formation, fungal-clay interactions, and metal-fungal interactions. While such transformations can occur in both aquatic and terrestrial habitats, it is in the terrestrial environment where fungi probably have the greatest influence especially when considering soil, rock, and mineral surfaces, and the plant-root-soil interface. Of special significance are the mutualistic symbioses, lichens and mycorrhizas. Geochemical transformations that take place can influence plant productivity and the mobility and speciation of toxic elements, and are therefore of considerable socioeconomic relevance. Some fungal transformations have beneficial applications in environmental biotechnology, e.g., in metal and radionuclide leaching, recovery and detoxification, and xenobiotic and organic pollutant degradation. They may also result in adverse effects when these processes are associated with the degradation of foodstuffs, natural products, and building materials, including wood, stone, and concrete.

Bibliography

- Adeyemi, A. O., and Gadd, G. M., 2005. Fungal degradation of calcium-, lead- and silicon-bearing minerals. *Biometals*, **18**, 269–281.
- Arnott, H. J., 1995. Calcium oxalate in fungi. In Khan, S. R. (ed.), *Calcium Oxalate in Biological Systems*. Boca Raton: CRC, pp. 73–111.
- Arocena, J. M., Glowa, K. R., Massicotte, H. B., and Lavkulich, L., 1999. Chemical and mineral composition of ectomycorrhizosphere soils of subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) in the AE horizon of a Luvisol. *Canadian Journal of Soil Science*, **79**, 25–35.
- Baldrian, P., 2003. Interactions of heavy metals with white-rot fungi. *Enzyme and Microbial Technology*, **32**, 78–91.
- Banfield, J. P., Barker, W. W., Welch, S. A., and Taunton, A., 1999. Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 3404–3411.
- Ban-nai, T., Muramatsu, Y., and Amachi, S., 2006. Rate of iodine volatilization and accumulation by filamentous fungi through laboratory cultures. *Chemosphere*, **65**, 2216–2222.

- Barker, W. W., and Banfield, J. F., 1998. Zones of chemical and physical interaction at interfaces between microbial communities and minerals: a model. *Geomicrobiology Journal*, **15**, 223–244.
- Boswell, G. P., Jacobs, H., Davidson, F. A., Gadd, G. M., and Ritz, K., 2002. Functional consequences of nutrient translocation in mycelial fungi. *Journal of Theoretical Biology*, **217**, 459–477.
- Boswell, G. P., Jacobs, H., Davidson, F. A., Gadd, G. M., and Ritz, K., 2003. Growth and function of fungal mycelia in heterogeneous environments. *Bulletin of Mathematical Biology*, **65**, 447–477.
- Boswell, G. P., Jacobs, H., Ritz, K., Gadd, G. M., and Davidson, F. A., 2006. The development of fungal networks in complex environments. *Bulletin of Mathematical Biology*, **69**, 605–634.
- Bowen, A. D., Davidson, F. A., Keatch, R., and Gadd, G. M., 2007a. Effect of nutrient availability on hyphal maturation and topographical sensing in *Aspergillus niger*. *Mycoscience*, **48**, 145–151.
- Bowen, A. D., Davidson, F. A., Keatch, R., and Gadd, G. M., 2007b. Induction of contour sensing in *Aspergillus niger* by stress and its relevance to fungal growth mechanics and hyphal tip structure. *Fungal Genetics and Biology*, **44**, 484–491.
- Braissant, O., Cailleau, G., Aragno, M., and Verrecchia, E. P., 2004. Biologically induced mineralization in the tree *Milicia excelsa* (Moraceae): its causes and consequences to the environment. *Geobiology*, **2**, 59–66.
- Brandl, H., 2001. Heterotrophic leaching. In Gadd, G. M. (ed.), *Fungi in Bioremediation*. Cambridge: Cambridge University Press, pp. 383–423.
- Bronick, C. J., and Lal, R., 2005. Soil structure and management: a review. *Geoderma*, **124**, 3–22.
- Burford, E. P., Kierans, M., and Gadd, G. M., 2003a. Geomycology: fungal growth in mineral substrata. *Mycologist*, **17**, 98–107.
- Burford, E. P., Fomina, M., and Gadd, G. M., 2003b. Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine*, **67**, 1127–1155.
- Burford, E. P., Hillier, S., and Gadd, G. M., 2006. Biomineralization of fungal hyphae with calcite (CaCO₃) and calcium oxalate mono- and dihydrate in carboniferous limestone microcosms. *Geomicrobiology Journal*, **23**, 599–611.
- Burgstaller, W., and Schinner, F., 1993. Leaching of metals with fungi. *Journal of Biotechnology*, **27**, 91–116.
- Butterfield, N. J., 2005. Probable proterozoic fungi. *Palaeobiology*, **31**, 165–182.
- Cerniglia, C. E., and Sutherland, J. B., 2001. Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. In Gadd, G. M. (ed.), *Fungi in Bioremediation*. Cambridge: Cambridge University Press, pp. 136–187.
- Cerniglia, C. E., and Sutherland, J. B., 2006. Relative roles of bacteria and fungi in polycyclic aromatic hydrocarbon biodegradation and bioremediation of contaminated soils. In Gadd, G. M. (ed.), *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, pp. 182–211.
- Cotter-Howells, J. D., and Paterson, E., (2000). Minerals and soil development. In Vaughan, D. J., and Wogelius, R. A. (ed.), *Environmental Mineralogy*. Budapest: Eotvos University Press, pp. 91–124.
- de la Torre, M. A., and Gomez-Alarcon, G., 1994. Manganese and iron oxidation by fungi isolated from building stone. *Microbial Ecology*, **27**, 177–188.
- de Rome, L., and Gadd, G. M., 1987. Copper adsorption by *Rhizopus arrhizus*, *Cladosporium resinae* and *Penicillium italicum*. *Applied Microbiology and Biotechnology*, **26**, 84–90.
- Easton, R. M., 1997. Lichen-rock-mineral interactions: an overview. In McIntosh, J. M., and Groat, L. A. (eds.), *Biological-Mineralogical Interactions* [Short Course Series, Vol. 21]. Ottawa: Mineralogical Association of Canada, pp. 209–239.
- Ehrlich, H. L., 1998. Geomicrobiology: its significance for geology. *Earth-Science Reviews*, **45**, 45–60.
- Ehrlich, H. L., 2002. *Geomicrobiology*. New York: Marcel Dekker.
- Fomina, M., and Gadd, G. M., 2002. Metal sorption by biomass of melanin-producing fungi grown in clay-containing medium. *Journal of Chemical Technology and Biotechnology*, **78**, 23–34.
- Fomina, M., and Gadd, G. M., 2007. Metal and mineral transformations: a mycoremediation perspective. In Robson, G. D., van West, P., and Gadd, G. M. (eds.), *Exploitation of Fungi*. Cambridge: Cambridge University Press, pp. 236–254.
- Fomina, M. A., Alexander, I. J., Hillier, S., and Gadd, G. M., 2004. Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. *Geomicrobiology Journal*, **21**, 351–366.
- Fomina, M., Hillier, S., Charnock, J. M., Melville, K., Alexander, I. J., and Gadd, G. M., 2005a. Role of oxalic acid over-excretion in toxic metal mineral transformations by *Beauveria caledonica*. *Applied and Environmental Microbiology*, **71**, 371–381.
- Fomina, M., Burford, E. P., and Gadd, G. M., 2005b. Toxic metals and fungal communities. In Dighton, J., White, J. F., and Oudemans, P. (eds.), *The Fungal Community: Its Organization and Role in the Ecosystem*, 3rd edn. Boca Raton: CRC, pp. 733–758.
- Fomina, M. A., Alexander, I. J., Colpaert, J. V., and Gadd, G. M., 2005c. Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biology and Biochemistry*, **37**, 851–866.
- Fomina, M., Burford, E. P., and Gadd, G. M., 2006a. Fungal dissolution and transformation of minerals: significance for nutrient and metal mobility. In Gadd, G. M. (ed.), *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, pp. 236–266.
- Fomina, M., Charnock, J. M., Hillier, S., Alexander, I. J., and Gadd, G. M., 2006b. Zinc phosphate transformations by the *Paxillus involutus*/pine ectomycorrhizal association. *Microbial Ecology*, **52**, 526–534.
- Fomina, M., Podgorsky, V. S., Olishvetska, S. V., Kadoshnikov, V. M., Pisanska, I. R., Hillier, S., and Gadd, G. M., 2007a. Fungal deterioration of barrier concrete used in nuclear waste disposal. *Geomicrobiology Journal*, **24**, 643–653.
- Fomina, M., Charnock, J. M., Hillier, S., Alvarez, R., and Gadd, G. M., 2007b. Fungal transformations of uranium oxides. *Environmental Microbiology*, **9**, 1696–1710.
- Fomina, M., Charnock, J., Bowen, A. D., and Gadd, G. M., 2007c. X-ray absorption spectroscopy (XAS) of toxic metal mineral transformations by fungi. *Environmental Microbiology*, **9**, 308–321.
- Gadd, G. M., 1990. Fungi and yeasts for metal accumulation. In Ehrlich, H. L., and Brierley, C. (eds.), *Microbial Mineral Recovery*. New York: McGraw-Hill, pp. 249–275.
- Gadd, G. M., 1992. Metals and microorganisms: a problem of definition. *FEMS Microbiology Letters*, **100**, 197–204.
- Gadd, G. M., 1993a. Interactions of fungi with toxic metals. *New Phytologist*, **124**, 25–60.
- Gadd, G. M., 1993b. Microbial formation and transformation of organometallic and organometalloid compounds. *FEMS Microbiology Reviews*, **11**, 297–316.
- Gadd, G. M., 1999. Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Advances in Microbial Physiology*, **41**, 47–92.
- Gadd, G. M., 2000a. Microbial interactions with tributyltin compounds: detoxification, accumulation, and environmental fate. *Science of the Total Environment*, **258**, 119–127.
- Gadd, G. M., 2000b. Bioremediation potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology*, **11**, 271–279.

- Gadd, G. M., 2000c. Heavy metal pollutants: environmental and biotechnological aspects. In Lederberg, J. (ed.), *Encyclopedia of Microbiology*. New York: Academic, pp. 607–617.
- Gadd, G. M., (ed.), 2001a. *Fungi in Bioremediation*. Cambridge: Cambridge University Press.
- Gadd, G. M., 2001b. Metal transformations. In Gadd, G. M. (ed.) *Fungi in Bioremediation*. Cambridge: Cambridge University Press, pp. 359–382.
- Gadd, G. M., 2001c. Accumulation and transformation of metals by microorganisms. In Rehm, H.-J., Reed, G., Puhler, A., and Stadler, P. (eds.), *Biotechnology, a Multi-Volume Comprehensive Treatise. Vol. 10. Special Processes*. Weinheim: Wiley-VCH, pp. 225–264.
- Gadd, G. M., 2004a. Mycotransformation of organic and inorganic substrates. *Mycologist*, **18**, 60–70.
- Gadd, G. M., 2004b. Microbial influence on metal mobility and application for bioremediation. *Geoderma*, **122**, 109–119.
- Gadd, G. M., 2005. Microorganisms in toxic metal polluted soils. In Buscot, F., and Varma, A. (eds.), *Microorganisms in Soils: Roles in Genesis and Functions*. Berlin: Springer, pp. 325–356.
- Gadd, G. M., (ed.), 2006. *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press.
- Gadd, G. M., 2007a. Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research*, **111**, 3–49.
- Gadd, G. M., 2007b. Fungi and industrial pollutants. In Kubicek, C. P., and Druzhinina, I. S. (eds.), *The Mycota. Vol. IV. Environmental and Microbial Relationships*. Berlin: Springer, pp. 69–84.
- Gadd, G. M., 2008. Transformation and mobilization of metals by microorganisms. In Violante, A., Huang, P. M., and Gadd, G. M. (eds.), *Biophysico-Chemical Processes of Heavy Metals and Metalloids in Soil Environments*. Chichester: Wiley, pp. 53–96.
- Gadd, G. M., and Griffiths, A. J., 1978. Microorganisms and heavy metal toxicity. *Microbial Ecology*, **4**, 303–317.
- Gadd, G. M., and Griffiths, A. J., 1980. Effect of copper on morphology of *Aureobasidium pullulans*. *Transactions of the British Mycological Society*, **74**, 387–392.
- Gadd, G. M., and White, C., 1989. The removal of thorium from simulated acid process streams by fungal biomass. *Biotechnology and Bioengineering*, **33**, 592–597.
- Gadd, G. M., and White, C., 1990. Biosorption of radionuclides by yeast and fungal biomass. *Journal of Chemical Technology and Biotechnology*, **49**, 331–343.
- Gadd, G. M., and White, C., 1992. Removal of thorium from simulated acid process streams by fungal biomass: potential for thorium desorption and reuse of biomass and desorbent. *Journal of Chemical Technology and Biotechnology*, **55**, 39–44.
- Gadd, G. M., Chudek, J. A., Foster, R., and Reed, R. H., 1984. The osmotic responses of *Penicillium ochro-chloron*: changes in internal solute levels in response to copper and salt stress. *Journal of General Microbiology*, **130**, 1969–1975.
- Gadd, G. M., Fomina, M., and Burford, E. P., 2005. Fungal roles and function in rock, mineral and soil transformations. In Gadd, G. M., Semple, K. T., and Lappin-Scott, H. M. (eds.), *Microorganisms in Earth Systems: Advances in Geomicrobiology*. Cambridge: Cambridge University Press, pp. 201–231.
- Gadd, G. M., Burford, E. P., Fomina, M., and Melville, K., 2007. Mineral transformations and biogeochemical cycles: a geomycological perspective. In Gadd, G. M., Watkinson, S. C., and Dyer, P. S. (eds.), *Fungi in the Environment*. Cambridge: Cambridge University Press, pp. 77–111.
- Gharieb, M. M., Wilkinson, S. C., and Gadd, G. M., 1995. Reduction of selenium oxyanions by unicellular, polymorphic and filamentous fungi: cellular location of reduced selenium and implications for tolerance. *Journal of Industrial Microbiology*, **14**, 300–311.
- Gharieb, M. M., Sayer, J. A., and Gadd, G. M., 1998. Solubilization of natural gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and the formation of calcium oxalate by *Aspergillus niger* and *Serpula himantoides*. *Mycological Research*, **102**, 825–830.
- Gharieb, M. M., Kierans, M., and Gadd, G. M., 1999. Transformation and tolerance of tellurite by filamentous fungi: accumulation, reduction and volatilization. *Mycological Research*, **103**, 299–305.
- Gorbushina, A. A., 2007. Life on the rocks, *Environmental Microbiology*, **9**, 1613–1631.
- Gorbushina, A. A., Krumbein, W. E., Hamann, R., Panina, L., Soucharjevsky, S., and Wollenzien, U., 1993. On the role of black fungi in colour change and biodeterioration of antique marbles. *Geomicrobiology Journal*, **11**, 205–221.
- Gorbushina, A. A., Krumbein, W. E., and Volkmann, M., 2002a. Rock surfaces as life indicators: new ways to demonstrate life and traces of former life. *Astrobiology*, **2**, 203–213.
- Gorbushina, A. A., Lyalikova, N. N., Vlasov, D. Y., and Khizhnyak, T. V., 2002b. Microbial communities on the monuments of Moscow and St. Petersburg: biodiversity and trophic relations. *Microbiology*, **71**, 350–356.
- Gorbushina, A. A., Whitehead, K., Dornieden, T., Niesse, A., Schulte, A., and Hedges, J. I., 2003. Black fungal colonies as units of survival: hyphal mycosporines synthesized by rock-dwelling microcolonial fungi. *Canadian Journal of Botany*, **81**, 131–138.
- Grote, G., and Krumbein, W. E., 1992. Microbial precipitation of manganese by bacteria and fungi from desert rock and rock varnish. *Geomicrobiology Journal*, **10**, 49–57.
- Gu, J. D., Ford, T. E., Berke, N. S., and Mitchell, R., 1998. Biodeterioration of concrete by the fungus *Fusarium*. *International Biodegradation and Biodegradation*, **41**, 101–109.
- Haas, J. R., and Purvis, O. W., 2006. Lichen biogeochemistry. In Gadd, G. M. (ed.), *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, pp. 344–276.
- Harvey, R. G., 1997. *Polycyclic Aromatic Hydrocarbons*. Hoboken: Wiley.
- Hawksworth, D. L., 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, **105**, 1422–1432.
- Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., and Hedges, S. B., 2001. Molecular evidence for the early colonisation of land by fungi and plants. *Science*, **293**, 1129–1133.
- Hochella, M. F., 2002. Sustaining Earth: thoughts on the present and future roles in mineralogy in environmental science. *Mineralogical Magazine*, **66**, 627–652.
- Jacobs, H., Boswell, G. P., Ritz, K., Davidson, F. A., and Gadd, G. M., 2002. Solubilization of calcium phosphate as a consequence of carbon translocation by *Rhizoctonia solani*. *FEMS Microbiology Ecology*, **40**, 65–71.
- Jacobs, H., Boswell, G. P., Scrimgeour, C. M., Davidson, F. A., Gadd, G. M., and Ritz, K., 2004. Translocation of carbon by *Rhizoctonia solani* in nutritionally heterogeneous environments. *Mycological Research*, **108**, 453–462.
- Jarosz-Wilkolazka, A., and Gadd, G. M., 2003. Oxalate production by wood-rotting fungi growing in toxic metal-amended medium. *Chemosphere*, **52**, 541–547.
- Kierans, M., Staines, A. M., Bennett, H., and Gadd, G. M., 1991. Silver tolerance and accumulation in yeasts. *Biology of Metals*, **4**, 100–106.

- Kraemer, S. M., Cheah, S. F., Zapf, R., Xu, J. D., Raymond, K. N., and Sposito, G., 1999. Effect of hydroxamate siderophores on Fe release and Pb(II) adsorption by goethite. *Geochimica et Cosmochimica Acta*, **63**, 3003–3008.
- Kumar, R., and Kumar, A. V., 1999. *Biodeterioration of Stone in Tropical Environments: An Overview*. Santa Monica: The Getty Conservation Institute.
- Leyval, C., and Joner, E. J., 2001. Bioavailability of heavy metals in the mycorrhizosphere. In Gobran, G. R., Wenzel, W. W., and Lombi, E. (eds.), *Trace Elements in the Rhizosphere*. Boca Raton: CRC, pp. 165–185.
- Martino, E., Perotto, S., Parsons, R., and Gadd, G. M., 2003. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biology and Biochemistry*, **35**, 133–141.
- Morley, G. F., and Gadd, G. M., 1995. Sorption of toxic metals by fungi and clay minerals. *Mycological Research*, **99**, 1429–1438.
- Nica, D., Davis, J. L., Kirby, L., Zuo, G., and Roberts, D. J., 2000. Isolation and characterization of microorganisms involved in the biodeterioration of concrete in sewers. *International Biodeterioration and Biodegradation*, **46**, 61–68.
- Purvis, O. W., 1996. Interactions of lichens with metals. *Science Progress*, **79**, 283–309.
- Purvis, O. W., and Halls, C., 1996. A review of lichens in metal-enriched environments. *Lichenologist*, **28**, 571–601.
- Redeker, K. R., Treseder, K. K., and Allen, M. F., 2004. Ectomycorrhizal fungi: a new source of atmospheric methyl halides? *Global Change Biology*, **10**, 1009–1016.
- Ritz, K., and Young, I. M., 2004. Interaction between soil structure and fungi. *Mycologist*, **18**, 52–59.
- Rosling, A., Lindahl, B. D., Taylor, A. F. S., and Finlay, R. D., 2004a. Mycelial growth and substrate acidification of ectomycorrhizal fungi in response to different minerals. *FEMS Microbiology Ecology*, **47**, 31–37.
- Rosling, A., Lindahl, B. D., and Finlay, R. D., 2004b. Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. *New Phytologist*, **162**, 795–802.
- Sayer, J. A., and Gadd, G. M., 1997. Solubilization and transformation of insoluble metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycological Research*, **101**, 653–661.
- Sayer, J. A., Cotter-Howells, J. D., Watson, C., Hillier, S., and Gadd, G. M., 1999. Lead mineral transformation by fungi. *Current Biology*, **9**, 691–694.
- Singleton, I., 2001. Fungal remediation of soils contaminated with persistent organic pollutants. In Gadd, G. M. (ed.), *Fungi in Bioremediation*. Cambridge: Cambridge University Press, pp. 79–96.
- Smith, S. E., and Read, D. J., 1997. *Mycorrhizal Symbiosis*, 2nd edn. San Diego: Academic.
- Sterflinger, K., 2000. Fungi as geologic agents. *Geomicrobiology Journal*, **17**, 97–124.
- Stotzky, G., 1966. Influence of clay minerals on microorganisms-II. Effect of various clay species, homoionic clays, and other particles on bacteria. *Canadian Journal of Microbiology*, **12**, 831–848.
- Strasser, H., Burgstaller, W., and Schinner, F., 1994. High yield production of oxalic acid for metal leaching purposes by *Aspergillus niger*. *FEMS Microbiology Letters*, **119**, 365–370.
- Sutherland, J. B., 2004. Degradation of hydrocarbons by yeasts and filamentous fungi. In Arora, D. K. (ed.), *Fungal Biotechnology in Agricultural, Food, and Environmental Applications*. New York: Marcel Dekker, pp. 443–455.
- Taylor, T. N., and Osborn, J. M., 1996. The importance of fungi in shaping the palaeoecosystem. *Review of Palaeobotany and Palynology*, **90**, 249–262.
- Taylor, T. N., Remy, W., and Hass, H., 1994. *Allomyces* in the Devonian. *Nature*, **367**, 601.
- Taylor, T. N., Hass, H., and Kerp, H., 1997. A cyanolichen from the Lower Devonian Rhynie chert. *American Journal of Botany*, **84**, 992–1004.
- Taylor, T. N., Hass, H., Krings, M., and Hanlin, R. T., 2005. Perithecial ascomycetes from the 400 million year old Rhynie Chert: an example of an ancestral polymorphism. *Mycologia*, **97**, 269–285.
- Thomson-Eagle, E. T., and Frankenberger, W. T., 1992. Bioremediation of soils contaminated with selenium. In Lal, R., and Stewart, B. A. (eds.), *Advances in Soil Science*. New York: Springer, pp. 261–309.
- Vaughan, D. J., Patrick, R. A. D., and Wogelius, R. A., 2002. Minerals, metals and molecules: ore and environmental mineralogy in the new millennium. *Mineralogical Magazine*, **66**, 653–676.
- Verrecchia, E. P., 2000. Fungi and sediments. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 69–75.
- Verrecchia, E. P., Braissant, O., and Cailleau, G., 2006. The oxalate-carbonate pathway in soil carbon storage: the role of fungi and oxalotrophic bacteria. In Gadd, G. M. (ed.), *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press, pp. 289–310.
- Wallander, H., Mahmood, S., Hagerberg, D., Johansson, L., and Pallon, J., 2003. Elemental composition of ectomycorrhizal mycelia identified by PCR-RFLP analysis and grown in contact with apatite or wood ash in forest soil. *FEMS Microbiology Ecology*, **44**, 57–65.
- Watling, R., and Harper, D. B., 1998. Chloromethane production by wood-rotting fungi and an estimate of the global flux to the atmosphere. *Mycological Research*, **102**, 769–787.
- Wengel, M., Kothe, E., Schmidt, C. M., Heide, K., and Gleixner, G., 2006. Degradation of organic matter from black shales and charcoal by the wood-rotting fungus *Schizophyllum commune* and release of DOC and heavy metals in the aqueous phase. *Science of the Total Environment*, **367**, 383–393.
- White, C., Wilkinson, S. C., and Gadd, G. M., 1995. The role of microorganisms in biosorption of toxic metals and radionuclides. *International Biodeterioration and Biodegradation*, **35**, 17–40.
- Zhdanova, N. N., Zakharchenko, V. A., Vember, V. V., and Nakonechnaya, L. T., 2000. Fungi from Chernobyl: mycobiota of the inner regions of the containment structures of the damaged nuclear reactor. *Mycological Research*, **104**, 1421–1426.

Cross-references

[Bioerosion](#)
[Calcite Precipitation, Microbially Induced](#)
[Calcium-Biogeochemistry](#)
[Carbon \(Organic Degradation\)](#)
[Carbon Cycle](#)
[Desert Varnish](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Fungi and Lichens](#)
[Isotopes \(Metal\)](#)
[Metalloenzymes](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Selenium](#)
[Siderophores](#)
[Soils](#)

H

HABITAT

The physical location or dwelling place of a particular organism, where an individual ecotype can be found or isolated from. See entry “*Microbial Communities, Structure, and Function*” for further reading.

HALOBACTERIA – HALOPHILES

Helga Stan-Lotter
University of Salzburg, Salzburg, Austria

Definition

Halobacteria (plural), *halobacterium* (singular). Colloquial for halophilic archaeobacteria (or archaea), which are single-celled life forms. Synonyms: Haloarchaea (plural), haloarchaeon (singular).

Halobacterium. The name of a genus of the archaeal family *Halobacteriaceae* (Grant, 2001).

Halophilic. Requires a salt-rich environment for growth and survival.

Introduction

Saline waters dominate the earth, with the oceans holding 97% of the planet's water (a total of 1.338×10^9 km³; <http://ga.water.usgs.gov/edu/earthwherewater.html>). Saline inland seas, groundwater, and saltwater lakes hold another 0.9–0.94%, which exceeds the volume of the world's available freshwater ($\sim 1.1 \times 10^7$ km³). The definition of a hypersaline environment is one that possesses a salt concentration greater than that of seawater (>3.5% w/v). Water-containing environments are usually described as thalassohaline or athalassohaline (see

Chapters “*Saline Lakes*,” “*Soda Lakes*”). Thalassohaline waters are marine-derived and therefore contain, at least initially, the seawater composition; with increasing evaporation, the concentration of various salts is changing. Athalassohaline waters may also receive influx of seawater; however, the chemical composition is mainly determined by geological, geographical, and topographical parameters.

Interactions between halophilic microorganisms and geological materials have been recognized early in the history of mankind and continue to impact human endeavors, including the exploration of energy sources, the storage of waste, the search for extraterrestrial life (see Chapter “*Deep Biosphere of Salt Deposits*”). For example, the evaporation of seawater for the purpose of making salt and the concomitant development of red color of brines, which is due to pigmented microorganisms, have been described already 2,700 years ago (Oren, 2002). It was also observed early that the presence of microorganisms in solar salterns enhances the precipitation and the yield of salt (Oren, 2002). Halite, the mineral of sodium chloride, primarily forms from the evaporation of seawater. Subsurface deposits of halite occur in many areas and are far more abundant than was previously recognized. The advent of widespread oil drilling revealed a frequent association of ancient salt deposits and the presence of the biogenic energy sources, oil and methane (Sassen et al., 1994). The potential impact of viable halophilic microorganisms in salt deposits (see Chapter “*Deep Biosphere of Salt Deposits*”), some of which are being used as storage sites for long-lived radioactive isotopes such as transuranic wastes, has been the focus of much research. The striking discovery of extraterrestrial halite in meteorites (see Chapter “*Deep Biosphere of Salt Deposits*”) and recently, in salt pools on Mars (Osterloo et al., 2008) make a search for halophilic microorganisms on other planets or moons plausible.

Saline environments and their inhabitants

Organisms occur within a range of salinities, from distilled water to saturated salt solutions, and may be loosely classified as non-, slightly, moderately, or extremely halophilic, requiring about 0.2 M, 0.2–0.85 M, 0.85–2.5 M, and 2.5–5.2 M NaCl, respectively, for growth. The largest saline environment, seawater, contains 31–38 g of total dissolved salts per liter, nearly 80% of which is NaCl. Hypersaline environments are characterized by higher salinities than seawater. The oceans are inhabited by a great diversity of organisms; when seawater is evaporating, the biological diversity decreases with increasing concentration of salt. Macroscopic organisms such as salt-tolerant fish (e.g., *Cyprinodon variegatus* and *Menidia beryllina*) are no longer found at concentrations of more than about 11% salt (1.88 M NaCl); copepods (fleas), turbellarian worms, and some rotifers tolerate 15–17% salt. Interestingly, several invertebrates such as brine shrimp (*Artemia salina*) or brine flies (*Ephydra cinerea*) are capable of survival at NaCl concentrations of 30–33% (~5 M; Javor, 1989). However, the dominating organisms in saturated brines are extremely halophilic microorganisms which grow optimally in the presence of 2.5–5.2 M NaCl and are generally unable to grow in less than 1.7 M NaCl. They can occur at such high cell densities (up to 10^8 colony forming units per milliliter) that they cause brines to turn bright red or purplish. Figure 1 shows a commercial salt-producing facility where seawater is evaporating in a series of ponds. The colors of the pools are caused by carotenoid pigments, which are contained in the membranes of haloarchaea, or by the chlorophylls of halophilic algae or cyanobacteria. Typical haloarchaea in such communities are species of the genera *Halobacterium*, *Halorubrum*, and *Halococcus*, halophilic bacteria such as *Ectothiorhodospira halochloris*, *Salinibacter ruber*, cyanobacteria (e.g., *Aphanothece halophytica*, *Phormidium* sp. and *Schizothrix arenaria*), and green algae (e.g., *Dunaliella salina* and *Asteromonas*

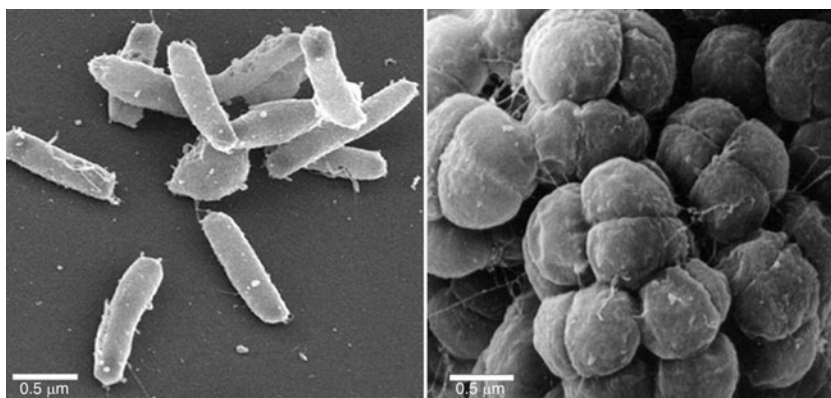
gracilis). Figure 2 shows two characteristic haloarchaeal morphologies – *Halobacterium salinarum* NRC-1 grows as single rod-shaped cells; *Halococcus salifodinae* cells are roundish and tend to aggregate into large clusters.

Crude solar salt contains large numbers of viable bacteria and archaea (about 10^5 – 10^6 colony forming units per milliliter; Oren, 2002). When using this salt for the preservation of fish, meat, and hides, damage in the form of red discoloration of the goods was noted under conditions of moisture and elevated temperatures. These observations started the early research on halophilic microorganisms in the 1920s–1930s. Only much later – in the 1980s – was it recognized that most halophiles from such sources belonged to the archaea. The haloarchaea are not pathogenic; in fact, some are used in traditionally fermented foods in Asia for human consumption (Oren, 2002).

There exist other hypersaline environments which are not directly derived from seawater and are called athalassohaline (see above and Chapter “Soda Lakes”); often they are remnants of prehistoric inland lakes, such as the Dead Sea and the Great Salt Lake. Additional larger lakes include Mono Lake in California, soda lakes in Egypt and China, Lake Magadi in Kenya, and Lake Eyre in Australia. The chemical composition varies depending on the geology of the surroundings, but overall salinity is usually between 200 and 340 g/l of dissolved salts. Soda lakes are an example of naturally occurring alkaline environments which may also be hypersaline, and are characterized by the presence of large amounts of sodium carbonate and correspondingly minute concentrations of Ca^{2+} and Mg^{2+} . The pH of alkaline lakes is usually in the range of 10–11; in some evaporation ponds, it can rise up to 12 (Oren, 2002). Microbial isolates from alkaline hypersaline lakes include the archaea *Natronomonas* sp., *Natronococcus* sp., *Natrialba* sp., and bacteria of the genera *Halomonas*, *Chromohalobacter*, *Spirulina* (the latter is known as nutrient for humans and animals) and others still to be characterized (Javor, 1989; Oren, 2002).



Halobacteria – Halophiles, Figure 1 Lagoons of a commercial salt-producing facility in Namibia, close to the ocean. Red-colored lagoons contain mainly haloarchaea; green color is due to halotolerant algae or cyanobacteria.



Halobacteria – Halophiles, Figure 2 Scanning electron micrograph of cells of *Halobacterium salinarum* NRC-1 (left) and *Halococcus salifodinae* DSM8989T (right). Bars, 0.5 μm. (Photographs were taken by C. Frethem (left) and G. Wanner (right).)

From the Deep Lake in the Vestfold Hills of Antarctica, a psychrophilic archaeon, *Haloarubrum lacusprofundi*, was isolated. Deep Lake has a salinity that is ten times higher than that of seawater (3.6–4.8 M). The lake remains ice-free throughout the year, with water temperatures fluctuating between +10 and –15°C and remaining below 0°C for ~8 months of the year. The microbial diversity in Deep Lake is extremely low and is dominated by haloarchaea, with apparently low numbers of *Dunaliella* sp. and bacteria (of the beta- and gamma-proteobacteria) present in the sediment (Cavicchioli, 2006).

Less explored and unusual hypersaline habitats are represented by anoxic brines near the bottom of the sea, for example in the Mediterranean deeps, which contain saline brines almost saturated with magnesium chloride, and where a wide diversity of novel archaea and bacteria was detected (van der Wielen et al., 2005).

Another environment for halophilic microorganisms are ancient salt deposits; recent reports have described the presence of haloarchaea in Triassic and Permian salts (summarized in McGenity et al., 2000; Stan-Lotter et al., 2004). These viable microorganisms were consistently isolated from surface-sterilized crystals obtained from freshly blasted rock salt or deep drilling bore cores, suggesting that these were survivors from the original depositional event (see Chapter “*Deep Biosphere of Salt Deposits*”).

Finally, halophilic viruses have been detected, which were released from bacteria and archaea; in addition, free virus particles were found by electron microscopical examination of the Dead Sea and in Spanish saltern ponds (Oren, 2002). The viruses (also called bacteriophages) were equally dependent as their hosts on high concentrations of salt for their structural stability. There is little information on the role of viruses in natural environments; suggestions which were made involve ecological functions in controlling the sizes of microbial communities (Oren, 2002). A website with pictures of haloviruses from

the laboratory of M. Dyll-Smith is available: <http://www.microbiol.unimelb.edu.au/people/dyallsmith/research/haloviruses/>

Classification and genomics

Halophilic means salt-loving and this term, often as the prefix hal- or halo-, has been in use since the 1930s. Therefore, members of the bacteria as well as the separate prokaryotic group archaea, which was identified only in the 1970s, may contain such prefixes in their names. Some confusion may arise from this nomenclatural convention, since, for example, the genus with the name *Halobacterium* belongs to the halophilic archaea (or haloarchaea), not to the bacteria.

The number of recognized haloarchaeal genera has increased to 26, according to the International Committee on Systematics of Prokaryotes (<http://www.the-icsp.org>) and the number of validated species at this time is 86. Main taxonomic criteria for the identification and recognition of haloarchaea are the sequence of the 16S rRNA genes and the composition of their characteristic membrane polar lipids, which are unique ether-linked phosphoglycerides (see Fendrihan et al., 2006, for a recent overview). The complete list of required and recommended criteria for the formal identification and recognition of haloarchaeal species was proposed by Oren et al. (1997). For halophilic bacteria belonging to the large family *Halomonadaceae*, a similar list of specific characteristics, which has been revised recently, can be consulted (Arahal et al., 2007).

Five genomes of haloarchaea have been completely sequenced, that of *Halobacterium salinarum* NRC-1, the related strain *Halobacterium salinarum* R1, *Haloarcula marismortui*, *Natronomonas pharaonis*, and *Haloquadratum walsbyi*, and one genome of a halophilic bacterium (*Salinibacter ruber*). The website <http://www.ncbi.nlm.nih.gov/sites/entrez> lists all genome projects, whether complete or still in progress, including references

and detailed descriptions. Comparisons of genomes continue to provide insights about similarity and differences between archaea and bacteria and about the early evolution of prokaryotes. They have, for example, proven that the adaptation mechanism of keeping a high internal salt concentration (see next paragraph) is not unique to archaea, as was thought before, but is used also by *Salinibacter ruber*; this common property as well as some other characteristics suggests the possibility of early lateral gene transfers between prokaryotes (Velieux et al., 2007).

Adaptations to high salinity

Most halophiles are unable to survive outside their high-salt native environments. Some species of the haloarchaea are so fragile that when placed in distilled water they lyse within minutes from the change in osmotic conditions (Grant, 2001). This reflects a general adaptation of the cells' entire intracellular machinery to high concentrations of cations. Halophilic and halotolerant organisms have to expend energy to exclude salt from their cytoplasm to avoid protein aggregation ("salting out"). Two different strategies are known to be used to prevent desiccation through osmotic movement of water out of their cytoplasm, and both work by increasing the internal osmolarity of the cell. In the first, organic compounds, termed "compatible solutes," are accumulated in the cytoplasm; this strategy has been found in many halophilic bacteria and also in some archaea, yeasts, algae, and fungi (Galinski, 1995). Compatible solutes are neutral or zwitterionic and include amino acids, sugars, polyols, betaines, and ectoines, as well as derivatives of some of these compounds; they can be synthesized de novo or taken up from the environment (Galinski, 1995; Grant, 2004). The second adaptation involves the selective import of K^+ ions into the cytoplasm, which can reach internal concentrations of 5 M. This adaptation is restricted to the moderately halophilic bacterial order *Halanaerobiales*, the haloarchaeal family *Halobacteriaceae*, and the extremely halophilic bacterium *Salinibacter ruber* (Oren, 2002). Most proteins of haloarchaea and also of *Salinibacter* contain a large excess of acidic amino acids (glutamate and aspartate) and a low content of basic amino acids (lysine and arginine; Lanyi, 1974; Oren, 2002); these features are thought to represent a specific adaptation to high levels of salt (Mevarech et al., 2000; Oren, 2002). In contrast, the action of compatible solutes is much less specific, with little or no adjustment of intracellular macromolecules required, which is in line with the observation that these compounds act as general stress protectants against heating, freezing, and drying (see Oren, 2002, and references therein).

Conclusions

Halophilic organisms occur in all major groups on the tree of life (see Oren, 2007) and even halophilic viral representatives are known. Halophilic microorganisms in particular

may be found on earth in almost every hypersaline environment – tropical to polar, terrestrial to submarine, acidic to alkaline, or aerobic to anaerobic (Javor, 1989). Initially a little-studied group of microorganisms, halophilic bacteria and archaea have proven to be of interest for life sciences and earth sciences as well; their investigation has resulted in major insights for molecular biology, genomics, biogeochemistry, and – most recently – astrobiology.

Bibliography

- Arahal, D. R., Vreeland, R. H., Litchfield, C. D., Mormile, M. R., Tindall, B. J., Oren, A., Bejar, V., Quesada, E., and Ventosa, A., 2007. Recommended minimal standards for describing new taxa of the family Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2436–2446.
- Cavicchioli, R., 2006. Cold-adapted archaea. *Nature Reviews. Microbiology*, **4**, 331–343.
- Fendrihan, S., Legat, A., Gruber, C., Pfaffenhuemer, M., Weidler, G., Gerbl, F., and Stan-Lotter, H., 2006. Extremely halophilic archaea and the issue of long term microbial survival. *Reviews in Environmental Science and Bio/technology*, **5**, 1569–1605.
- Galinski, E., 1995. Osmoadaptation in bacteria. *Advances in Microbial Physiology*, **37**, 272–328.
- Grant, W. D., 2001. Genus I. *Halobacterium* Elazari-Volcani 1957, 207, ^{AL} emend. Larsen and Grant 1989, 2222. In Boone, D. R., Castenholz, R. W., and Garrity, G. M. (eds.), *Bergey's Manual of Systematic Bacteriology*, 2nd edn. New York: Springer, Vol. I, pp. 301–305.
- Grant, W. D., 2004. Life at low water activity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 1249–1266.
- Javor, B. J., 1989. *Hypersaline Environments: Microbiology and Biogeochemistry*. Berlin: Springer.
- Lanyi, J. K., 1974. Salt-dependent properties of proteins from extremely halophilic bacteria. *Bacteriological Reviews*, **38**, 272–290.
- McGenity, T. J., Gemmell, R. T., Grant, W. D., and Stan-Lotter, H., 2000. Origins of halophilic microorganisms in ancient salt deposits (MiniReview). *Environmental Microbiology*, **2**, 243–250.
- Mevarech, M., Frolov, F., and Gross, I. M., 2000. Halophilic enzymes: proteins with a grain of salt. *Biophysical Chemistry*, **86**, 155–164.
- Oren, A., 2002. *Halophilic Microorganisms and Their Environments*. Dordrecht, The Netherlands: Kluwer.
- Oren, A., 2007. Biodiversity in highly saline environments. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 223–231.
- Oren, A., Ventosa, A., and Grant, W. D., 1997. Proposed minimal standards for description of new taxa in the order Halobacteriales. *International Journal of Systematic Bacteriology*, **47**, 233–236.
- Osterloo, M. M., Hamilton, V. E., Bandfield, J. L., Glotch, T. D., Baldrige, A. M., Christensen, P. R., Tornabene, L. L., and Anderson, F. S., 2008. Chloride-bearing materials in the southern highlands of Mars. *Science*, **319**, 1651–1654.
- Sassen, R., Cole, G. A., Drozd, R., and Roberts, H. H., 1994. Oligocene to holocene hydrocarbon migration and salt dome carbonates, Northern Gulf of Mexico. *Marine and Petroleum Geology*, **11**, 55–65.
- Stan-Lotter, H., Radax, C., McGenity, T. J., Legat, A., Pfaffenhuemer, M., Wieland, H., Gruber, C., and Denner, E. B. M., 2004. From intraterrestrials to extraterrestrials – viable haloarchaea in ancient salt deposits. In Ventosa, A. (ed.), *Halophilic Microorganisms*. Berlin: Springer, pp. 89–102.

van der Wielen, P. W., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., D'Auria, G., de Lange, G. J., Huebner, A., Varnavas, S. P., Thomson, J., Tamburini, C., Marty, D., McGenity, T. J., and Timmis, K. N., and BioDeep Scientific Party, 2005. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, **307**, 121–123.

Velieux, F., Madern, D., Zaccai, G., and Ebel, C., 2007. Molecular adaptation to high salt. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 240–253.

Cross-references

Archaea
Astrobiology
Deep Biosphere of Salt Deposits
Extreme Environments
Hypersaline Environments, Terrestrial
Saline Lakes
Soda Lakes

HAPTOPHYTES

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “*Symbiosis*”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinctive lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “*Biomarkers (Molecular Fossils)*”). For further details, please see entry “*Algae (Eukaryotic)*.”

HEAVY METALS

Please refer to entries “*Acid Rock Drainage*,” “*Arsenic*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Copper*,” “*Geomycology*,” “*Metalloenzymes*,” “*Metals, Acquisition by Marine Bacteria*,” “*Ores, Microbial Precipitation and Oxidation*,” “*Zinc*.”

HISTOLOGY

Michael Gudo¹, Gerta Fleissner², Guenther Fleissner²

¹Morphisto Evolutionsforschung und Anwendung GmbH, Institut für Evolutionswissenschaften, Frankfurt am Main, Germany

²Goethe-University Frankfurt, Frankfurt a. M., Germany

Definition

Histology is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms.

Biopsy is the sample of an organism or part of a tissue that is processed for histological investigations.

Fixation serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

Mineralization is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

Staining is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

Introduction

The term histology was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intra- and extracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kiernan, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are

processed by various histochemical and biophysical techniques according to the aim of the study.

Geobiological samples usually do not consist of organic soft material, alone, like the above mentioned biological materials, but they are often composites of hard inorganic and mineralized organic material, with nearly all biological components converted to inorganic matter. Some geobiological samples are compositions of both, organic and inorganic materials, such as bio films growing on hard substrates, or sessile organisms that adhere to hard substrates like corals, sponges, lichens, or fungi. These samples therefore can seldom be histologically processed like biological material via paraffin embedding and sectioning with traditional microtomes. Special procedures and mechanical devices (like saw microtomes, annular saws or cutting/grindings systems) are necessary to produce sections of geobiological samples (e.g., Hoffmann et al., 2003). By means of special polishing machines, like the ultramill or lapping machines, sections or slices of these sections can be studied by distinct microscopical methods, for example under fluorescent light, X-ray-mappings and topographic element analyses (Janssens et al., 2000) can identify the outline of the biological compounds and their environment, and they may also serve as a biomarker; harsh biochemical treatment can isolate the organic from the inorganic components, but often destroy the original context. Histological analyses may provide tools to identify the biological components even if their former structural context has decayed (Wrede et al., 2008), or help to analyze particular processes or compositions (Neuweiler et al., 2007). Principally these results must be “calibrated” by means of histological investigations of recent tissues in order to understand organ structure and its function (Young and Heath, 2000).

This entry reviews the state of the histological methods and offers the modifications necessary to topographically investigate geobiological samples. *For clarity, this latter information is given in italics.*

Methods

Different from biochemical investigations in the broadest sense, histological analyses are concerned with samples, where the structures and their topographical context are preserved or known before biopsies are taken and only in a controlled way modified by procedures according to the specific aim of the study.

For histological processing of tissues or any other organic compounds, all vital functions have to be stopped by fixation, in order to preserve a distinct structure within or more often isolated from its natural environment. *In geobiology this process often is “history,” but must be known to understand the given results of further analyses.* Usually, the biological samples are embedded into a special medium which provides particular mechanical properties that allow sectioning in the appropriate thickness. The sections are then placed on glass slides or cover

slips, afterwards stained by special chemicals and dyes that have affinities to particular tissues or histological structures. The staining allows the differentiation of cell structures, various types of cell surface and skeleton assemblies, filaments or fibrillar components (e.g., collagenous fibers, elastic fibers, or reticular fibers), tissues, and even complete organs or pathological and genetic modifications of the various structures. Under the microscope the results can be observed and – most often after a thorough comparison to reference samples – evaluated and interpreted.

In geobiology, the selective staining of minerals prevails, although protein staining has been successful in various studies. The major problem in geobiology is that mineralized samples are too hard for conventional section procedures and therefore special preparation methods are necessary (see also below, paragraph “Embedding”). Accordingly, often only the surface of sectioned samples is accessible to the staining which limits the differentiations of components. Structural analyses of biological compounds have to refer to similar structures or organs histologically known from recent specimen. Therefore, histology in geobiology has to refer to a biological histology (Robenek, 1995). Specialized methods are available which either demineralize the sample for showing the biological contents or dissolve the organic leftovers to investigate their “casts” in the inorganic surroundings. Often the resulting histologically processed specimens can be biophysically investigated, and topographic X-ray element mappings (Janssens et al., 2000) are often the method of choice to serve as biomarkers.

Fixation and infiltration

The process of fixation and infiltration of biological specimens is most crucial for the results and aims to stop any autolytic and bacterial decomposition. The most commonly used fixative is formalin; however, other fixatives (e.g., Bouin, Carnoy, Paraformaldehyde, potassium dichromate, chromic acid, or mercury-fixation) are necessary depending on the planned subsequent protocol. The choice of the fixative determines the results, since every fixative has assets and drawbacks for the preservation of particular histological structures and especially the binding sites for differential staining, e.g., in immunohistology. For biophysical analyses, the metal-free fixatives and also metal-free pre- and post-processing protocols are essential.

The fixation process, which is a chemical reaction, has to be controlled and stopped after a particular time that depends on type and size of the tissue, but also on environmental factors like temperature and air pressure. Afterwards the fixative must be removed following a special protocol, which takes care not to destroy structural characteristics (Böck, 1989).

Prior to embedding, the sample has to be slowly transferred to the final substance via several intermediate steps mixable with the respective embedding medium, e.g.,

for the paraffin/wax embedding dehydrants (ethanol, isopropanol, and methanol) and clearants (xylene, toluene, and xylene-replacing clearants).

In case of aerated tissues, it may be necessary to perfuse the sample with a prefix and process fixation and embedding continuously in a vacuum with fluid movement for a proper impregnation of all layers of the specimen.

In geobiology, fixation and conservation also plays a crucial role, since these chemical processes have to be balanced between the necessities for the mineralized and organic components of a geobiological sample (biofilms or water containing fossils; e.g., Desrochers et al., 2007). Every fixative generates chemical reactions, which sometimes may degrade or destroy either the organic or the mineralized components. Accordingly, empirical studies have to be performed before crucial samples are processed in order to ascertain whether the fixative may cause any problems for either the soft or hard tissues of a particular type of samples.

Embedding

Embedding of histological material is performed in order to provide a mechanical stable and hard entity, which can be sectioned to various coherent thin sections. The sections should enable a spatial reconstruction of the original sample after differentiating analyses. The composition of the specimen and the histological program are decisive for the embedding methods of choice. The most common material for tissue embedding is paraffin (wax-embedding), also embedding in celloidin and resin. If the tissues under study are more or less soft (which means without any calcareous or other mineralized structures), the paraffin embedding is the best choice. Celloidin embedding takes much time and is useful for very sophisticated questions (e.g., embedding of complete brains or very large organs). Plastic embeddings are the best choice if the biopsies contain mineralized structures, such as calcium carbonate, calcium phosphate, and silicate, or if organismic components adhere to rocks or other hard substrates, like for example biofilms.

Therefore, these latter embedding methods lend themselves for geobiological samples, which then can be processed like biological material. For resin embedding (Howat et al., 2007) one can choose between epoxy-embedding, methyl-methacrylate-embedding, or glycol-methacrylate-embedding. Various products are commercially available (Obenauf et al., 2007), all of them have well-documented assets and drawbacks. The most drawbacks of plastic embeddings can be seen in their high costs of time and money. On the other hand, resin embedded samples can be sectioned, sawed, milled, or polished by use of various microtomes, saws, milling, or polishing machines.

Sectioning

Whole mount samples can only be investigated from their outer surface. Analyses inside the samples need dissection

with preferably minor destruction of the structural context. In general, penetration of staining chemicals is enhanced afterwards, and microscopic and biophysical inspection is improved as the spatial resolution corresponds to the thickness of the section. Several problems may arise from inappropriate section procedures:

- The samples may be too brittle and break. This effect is reduced by pre-treatment procedures like decalcification or demineralization, also choosing another type of embedding medium may help possibly combined with different section techniques, e.g., a vibrating knife or sample cooling by a special refrigerant device.
- The sections are heavily distorted. A proper selection of the microtome, cutting angle, and cutting velocity may improve the result. The sections may stretch, for example, after floating on a warm water bath about 10°C below the melting point of the embedding wax.
- The requested thickness requires different blades, which cannot be used “universally,” even if section series of varying thickness are necessary for the protocol. For example, thick sections need a hard metal blade, while semithin- and ultrathin sections can be produced only by means of glass or diamond knives.

Biological samples, which are embedded in paraffin or resin, can generally be sectioned by a special device, a microtome. Some microtomes are equipped with a motor-driven support, both for controlling the section thickness as well as the force of the blade. Several types of microtomes are available, each optimized for variably embedded samples: Paraffin embedded samples can be sectioned by sled microtomes or rotary microtomes equipped with metal knives. Resin embedded samples need microtomes which have a special retraction mechanism, to avoid mechanical damage of the knife and the sample. The blades are either manufactured of especially hardened steel for sections between 5 and 30 µm, for semi thin sections (2–5 µm) and ultra thin sections (about 40–100 nm) glass or diamond knives must be used.

Hard materials, like mineralized geobiological probes, cannot be sectioned by conventional microtomes unless the samples have been pre-treated: decalcification or demineralization by different chemicals. While these procedures may cause uncontrolled damage, when applied to the unsectioned samples, preferably control material should be preserved to evaluate the original structural context. Therefore, careful special section devices like diamond milling, saw microtomes, annular saws, or section/grinding-systems especially optimized for this purpose should be applied. About 30 µm thick sections of rather large samples can be achieved by these methods. The sections or grinded pieces can then be further processed, and also partly preserved for subsequent evaluation.

Another method, available since several years, is the laser microtome which may contact free isolate minute samples out of the otherwise untreated entity. The samples must neither be pre-treated nor embedded. Section thickness ranges between 10 and 100 µm. Special

devices allow even for the isolation of minute tissue pieces like a single cellular nucleus.

A satisfying section quality requires a long time of experience especially to decide for the most appropriate procedure. While continuously new embedding products are commercialized, only little has changed concerning the basic techniques of standard microtomy, which is taught as a component part of the education of laboratory and technical assistants.

Mounting

For further histological processing, the sections must be mounted, most often on glass slides or on grids coated with a special synthetic film.

The major difficulty is the choice of the best possible sticking procedures in order to prevent both, a floating resp. a loss of the sections during the staining protocol and undesired background staining by the glue. While before often unreliable “home-made” coating procedures, e.g., by chromic sulphate or polylysine, were used, now various types of pretreated glass slides are commercially available, they have been optimized for selected histological procedures. Thick sections, e.g., sections that are produced by saw microtomes or cutting/grinding-systems, have to be glued to object slides by special sticking resins that, e.g., harden with ultraviolet light; in this aspect, histological sample preparation is quite similar to the preparation of geological thin ground-sections.

Biophysical analyses of geobiological samples requires mounting according to the planned technique (see list of Handbooks of the suppliers). In, for example, X-ray investigations (micro fluorescence analyses), the mounting must be free of auto fluorescence; the sometimes long lasting measuring intervals need a stable non-vibrating mechanical stability.

Staining

Multiple histological staining methods allow identifying cellular structures, tissue components, and organ systems, also enzymatic processes and involved macromolecules. It is even possible to clearly differentiate between organic and inorganic material as well as decaying from living material by selective marking of minerals and molecules.

Stainings can be performed by use of natural stains and dyes or by chemicals often designed for certain projects. New fields of histological analyses have been launched by immunohistochemical stainings combined with fluorescent markers which are very selectively binding to particular proteins. Differential and double stainings, also section series with alternatively stained sections, have revealed important insight into basic cellular processes, information processing, and also pathological resp. genetic changes. The standard protocols are well documented including their modifications for the application to tissues of various origins.

For any staining it is necessary that the structures that should be stained are free of any embedding media. This means that for standard-histology the paraffin has to be removed by special procedures so that the staining chemicals can react with the tissues. If samples are embedded in resins, this process is more crucial and difficult; several resins cannot be removed and accordingly only the surface of the section provides reactive structures for staining.

The staining of cellular structures and tissues is based on chemical affinities of the stains with particular tissues resp. tissue components. In many cases, a particular pretreatment is necessary for adequate results. Pretreatments may be, for example, mordants, which modify the stain so that it reacts with the tissue or modifies the tissues so that the stain can react with the tissue. In some cases mordants generate a reaction that transformed the water soluble stain into a water- and alcohol-stable lacquer. This chemical reaction can, for example, be induced by metal ions like tungsten or molybdenum or by alkali ions like lithium, sodium, or barium (Böck, 1989; Burck, 1988).

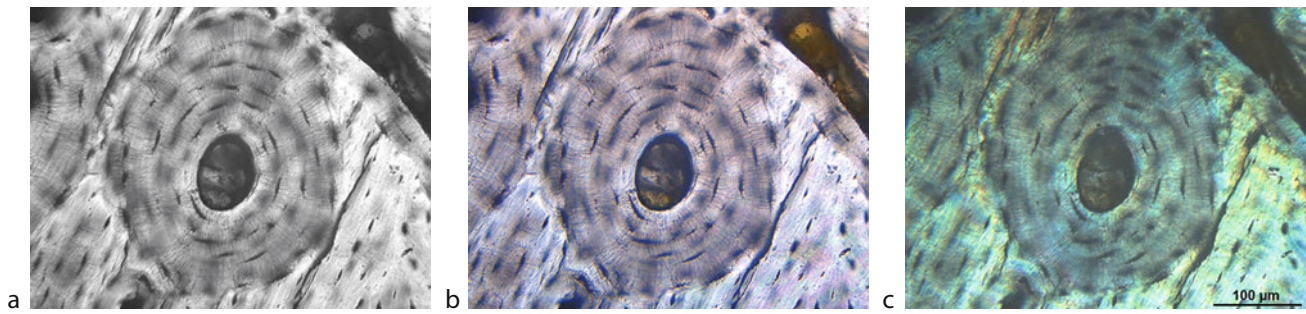
Stainings are usually composed of three steps: in the first step cellular structures like nuclei, etc., are stained. In the second step cell plasma and some extracellular structures are stained, and in the third step the surrounding matrix is stained.

As a result of the staining, the nuclei, cells, various fibers, and other structures or chemical components in a tissue have particular colors that allow differentiation and several diagnostics. Some stainings also provide particular optical characteristics to tissues; they may for example provide polarizing effects to the tissue. Other procedures provide fluorescence-effects or particular ultra-violet-effects of the polished surface and allow further investigations (Figures 1 and 2).

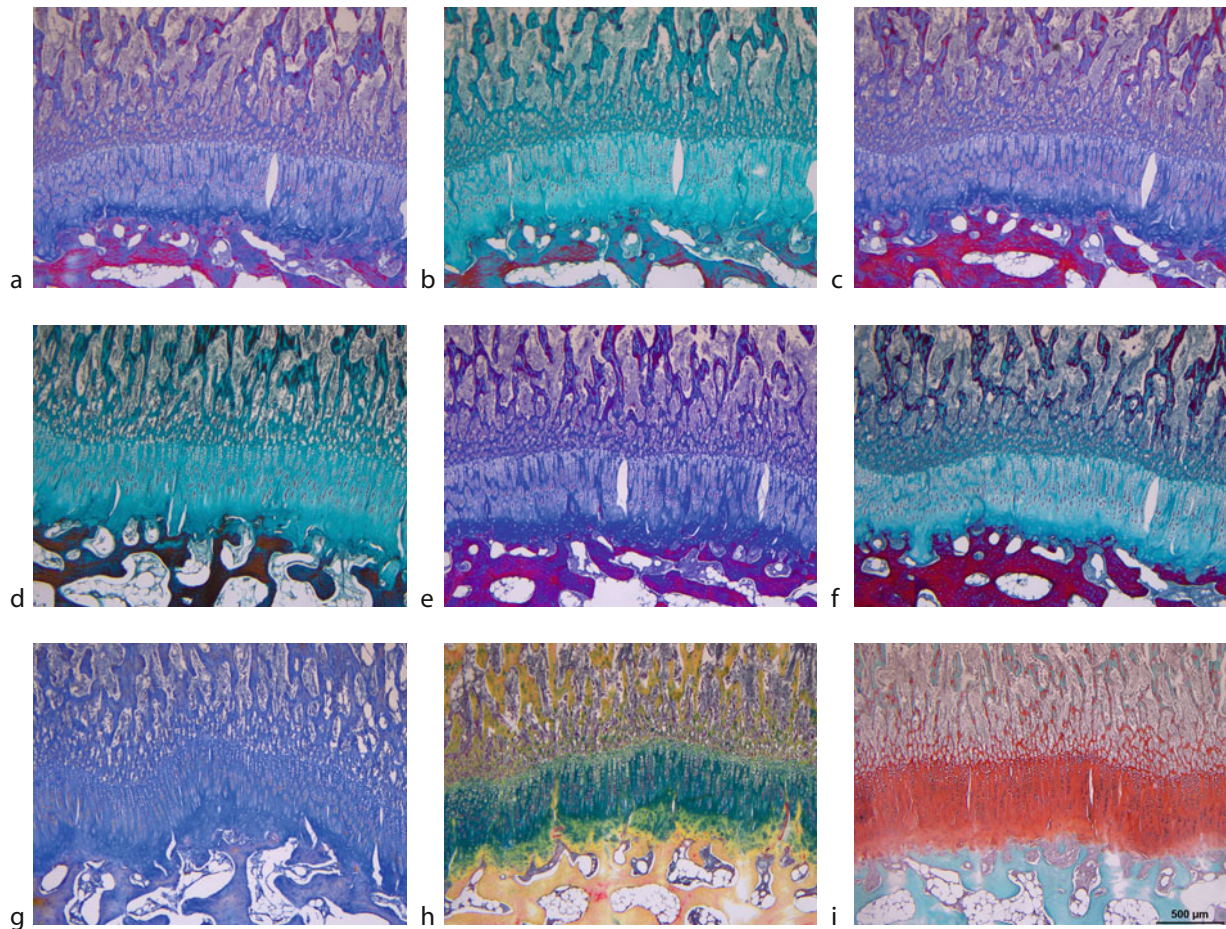
While traditional stainings induce a chemical reaction within tissues and may stain in an unselective way (Horobin and Kiernan, 2002), immunohistology (Polak and Noorden, 2003) selectively marks specific epitopes, proteins, and molecules bound to membrane or subcellular structures. Traditional and immunohistochemical analyses under the light microscope meet the limits of resolution at magnifications of about 1,200×; therefore, immunohistological methods have been upgraded for analyses under the TEM, either by pre-embedding or post-embedding staining protocols.

While traditional stainings are used for topographic and micro anatomical investigations, immunohistological stainings are used to understand the functional context of molecular processes and to allocate the regions in which these processes take place in an organism or in a tissue.

These refined immunohistological staining methods have also been applied to sections of geobiological samples. The analyses could demonstrate biological compounds, thus serving as biomarkers, and in some cases even disclose the genetic origin of the fossilized organisms.



Histology, Figure 1 Cross section of a human long bone under different microscopic optics (thin-ground section of a non-demineralized sample). (a) Under through light with minimal color saturation; (b) same illumination with maximum color saturation; (c) differential interference contrast (After Nomarski). Already without staining procedures characteristic structural details and varying densities can be identified. (a–c same magnification.)



Histology, Figure 2 Section series through a chondral ossification zone of a demineralized porcine long bone after various differential histological staining procedures (Protocols according to Böck et al., 1989; Young and Heath, 2000). (a) Masson Goldner A; (b) Masson Goldner L; (c) Masson Goldner ResF A; (d) Masson Goldner ResF L; (e) Crossmon A; (f) Crossmon L; (g) Azan after Geidis; (h) Movat Pentachrome; (i) Safranin Fastgreen. The layers of young bone (upper area), cartilage (middle area), and secondary ossification area (below) can be analyzed concerning their different structural details (a–i same magnification).

Conclusion

Geobiology is defined as field of science which analyses the interaction of the environment with living matter. Mainly fossilized samples are investigated concerning their systematic relationship and – even more – the origin of this specific form of life. Research results can find their application not only in a better understanding of our life on earth, but also in exploring putative extraterrestrial biology. Another aspect is the transfer of basic sciences to various aspects of biomedical research like the synthesis or degradation of bones and teeth or the control of biogenic mineralization processes. All these research topics cannot be planned and performed or adequately evaluated without a thorough knowledge of the biological structures mainly based on the comparison of living and fossilized specimen. Histology delivers the methods to topographically analyze organisms, organs, tissues, and even molecules. Their modifications by environmental or biogenic processes can be recognized by the investigation of their complex interaction.

Perspectives

Histological investigations provide a huge field of research for biology and geobiology. Both fields have to be combined, since geobiological samples often can only be interpreted with reference to known biological samples. Some examples are histological sections of dinosaur bones or teeth that have to be compared to bones and teeth of recent organisms, others are the discoveries at special fossil-sites, like the Messel pit, the Burgess-Shale, the rocky Hunsrück region, etc., which may provide interesting information about fossilized microscopic structures. And even the investigation of recent samples like biofilms or coatings is of major relevance to understand their composition and emergence in order to discriminate between biogenic and abiotic origin.

Summary

Histology provides methods to topographically investigate the structure of cells, tissues, and organs down to the molecular level, especially in geobiology as reference to recognize, for example, the phylogenetic origin and age and also as a basis to analyze biological in comparison to bio geological turnover processes, which induce structural changes via environmental and climatic conditions.

Bibliography

- Bancroft, J. D., and Gamble, M. (eds.), 2007. *Theory & Practice of Histological Techniques*, 6th edn. Edinburgh: Churchill Livingstone.
- Böck, P. (ed.), 1989. *Romeis Mikroskopische Technik*. München/Wien/Baltimore: Urban & Schwarzenberg.
- Bozzola, J. J., and Russell, L. D., 1999. *Electron Microscopy*, 2nd edn. Boston: Johns and Bartlett.

- Burck, H. C., 1988. *Histologische Technik*, 6th edn. Stuttgart: Thieme.
- Desrochers, A., Bourque, P.-A., and Neuweiler, F., 2007. Diagenetic versus biotic accretionary mechanisms of bryozoan-sponge buildups (Lower Silurian, Anticosti Island, Canada). *Journal of Sedimentary Research*, **77**, 564–571.
- Gerlach, D. (ed.), 1998. *Die Anfänge der histologischen Färbung und der Mikrophotographie – Josef von Gerlach als Wegbegleiter*. Thun und Frankfurt am Main: Verlag Harri Deutsch.
- Hoffmann, F., Janussen, D., Dröse, W., Arp, G., and Reitner, J., 2003. Histological investigation of organisms with hard skeletons: a case study of siliceous sponges. *Biotechnic and Histochemistry*, **78**(3–4), 191–199.
- Horobin, R. W., and Kiernan, J. A., 2002. *Conn's Biological Stains*, 10th edn. Oxford: Bios Scientific.
- Howat, W. J., Warford, A., Mitchell, J. N., Clarke, K. F., Conquer, J. S., and McCafferty, J., 2007. Resin tissue microarrays: a universal format for immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **55**, 21–24.
- Janssens, K. H. A., Adams, F. C. V., and Rindby, A. (eds.), 2000. *Microscopic X-ray Fluorescence Analysis*. Chichester: Wiley.
- Mayer, C., 1819. *Ueber Histologie*. Bonn. Cited in: Gräfe, C. F., Hufeland, C. W., Link, H. F., Rudolphi, K. A., Siebold, E. v. (eds.), 1828. *Encyclopädisches Wörterbuch der medicinischen Wissenschaften*, Band 2. Berlin: J.W. Boike.
- Mulisch, M., and Welsch, U. (eds.), 2010. *Romeis Mikroskopische Technik*. Heidelberg: Spektrum Akademischer Verlag.
- Neuweiler, F., Daoust, I., Bourque, P. A., and Burdige, D. J., 2007. Degradative calcification of a modern siliceous sponge from the Great Bahama Bank, The Bahamas: a guide for interpretation of ancient sponge-bearing limestones. *Journal of Sedimentary Research*, **77**, 552–563.
- Obenauf, R. H., Martin, J., Bostwick, R., et al. (eds.), 2007. *Handbook of Sample Preparation and Handling*, 10th edn. Metuchen: SPEX Certiprep.
- Palm, L. C., and Snelders, H. A. M. (eds.), 1983. *Antoni van Leeuwenhoek 1632–1723*. Studies on the life and work of the Delft scientist commemorating the 350th anniversary of his birthday. Amsterdam: Rodopi.
- Polak, J. M., and Van Noorden, S., 2003. *Introduction to Immunocytochemistry*, 3rd edn. Oxford: Bios Scientific.
- Robenek, H. (ed.), 1995. *Mikroskopie in Forschung und Praxis*. Darmstadt: Git Verlag.
- Ruzin, S. E., 1999. *Plant Microtechnique and Microscopy*. Oxford: Oxford University Press.
- Schüler, D. (ed.), 2006. *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold seeps. *Journal of Microbiological Methods*, **73**, 85–91.
- Young, B., and Heath, J. W., 2000. *Weather's Functional Histology – A Text and Colour Atlas*. Edinburgh: Churchill Livingstone.

Cross-references

[Animal Skeletons, Advent](#)
[Animal Biocalcification, Evolution](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Calcification](#)
[Calcified Cyanobacteria](#)

Carbonate Environments
 Fluorescence-In-Situ-Hybridization (FISH)
 Immunolocalization
 Metalloenzymes
 Metals, Acquisition by Marine Bacteria
 Microbial Biomineralization
 Microbial Communities, Structure, and Function
 Microbial Mats
 Organomineralization
 Scanning Probe Microscopy (Includes Atomic Force Microscopy)
 Sponges (Porifera) and Sponge Microbes

HOT SPRINGS AND GEYSERS

Brian Jones¹, Robin W. Renaut²

¹University of Alberta, Edmonton, Alberta, Canada

²University of Saskatchewan, Saskatoon, SK, Canada

Definition

A *hot spring* is a discharge of hot (>35 – 40°C) water from a vent at the Earth's surface.

A *geyser* is a hot spring characterized by intermittent, turbulent discharges of boiling water and steam.

A *sublacustrine hot spring* is a hot spring that discharges from the floor of a lake.

Introduction

A hot spring is characterized by discharge of hot water from a vent. There is, however, no universally accepted definition of “hot” and the temperature for distinguishing a “warm spring” from a “hot spring” remains contentious (Pentecost et al., 2003). In general usage, a hot spring is one with vent water temperature between about 40°C and boiling point (Renaut and Jones, 2000). It must be remembered, however, that boiling temperature changes with altitude; thus, boiling in Yellowstone National Park occurs at $\sim 92^{\circ}\text{C}$, whereas in New Zealand geothermal areas, which lie closer to sea level, it is at $\sim 100^{\circ}\text{C}$.

The term “geyser” is derived from “Geysir,” located in southwest Iceland. First mentioned in historical records from 1294, the Icelandic word “geysir” means “gusher” or “one who rages.” Accordingly, a geyser is regarded as hot spring that is characterized by intermittent, turbulent discharges of boiling water and steam (Bryan, 2005, 2008).

Geysers and hot springs are end members of a continuous spectrum that includes “intermittent springs” that experience periodic overflows but never erupt, and “pulsating springs,” “perpetual spouters,” or “ebullient springs” that are characterized by continual eruptions. Some hot springs discharge on the floors of lakes and in caves.

Distribution

Hot springs and geysers are found on every continent except mainland Antarctica (Waring, 1965). Many hot springs and geysers, known from historical records, have ceased activity because of natural and anthropogenically induced changes to their plumbing systems. Yellowstone National Park, USA., El Tatio located high in the Andes of northern Chile, the Taupo Volcanic Zone on the North Island of New Zealand, Kronotski National Park in Kamchatka in eastern Russia, and Iceland are the largest known geothermal fields with numerous hot springs and geysers. Bryan (2008), for example, estimated that more than 50% (~ 500) of known geysers are located in Yellowstone National Park, with another 385 in the other four major geothermal areas. Low-enthalpy hot springs that discharge at maximum temperatures of about 35 – 60°C are much more common.

The plumbing system

The hot water discharged by a hot spring or geyser is the final, surface expression of a complex system that involves water, a heat source, and an intricate underground plumbing system. The water is derived from meteoric waters that have seeped deep into the Earth's crust, supplemented in some regions by connate, magmatic, or metamorphic waters. Irrespective of its origin, some spring or geyser water may come from depths of ~ 4 km where it has been magmatically heated to temperatures of $>225^{\circ}\text{C}$. In other settings, however, meteoric waters may only penetrate to shallow depths before being expelled from a low-enthalpy spring vent. The plumbing system of a hot spring is unrestricted and allows continuous movement of water to the surface. In contrast, a geyser has a restriction within its plumbing system, commonly a sinter caprock that precludes continuous vertical flow. The configurations of these plumbing systems remain unknown because of the difficulties in accessing them. Nevertheless, schematic depictions of vast underground, water-filled chambers are probably incorrect. Instead, it seems that the plumbing systems are formed of complex networks of faults, fractures, small cavities, and strata with variable permeability. Geysers are less common than hot springs because of the special plumbing system requirements.

Water trapped below a restriction in a geyser plumbing system will be heated by steam that is moving upwards from deeper parts of the system. The reservoir will then empty as the trapped superheated water is violently discharged. Such an eruption will continue until the reservoir is emptied. The magnitude, frequency, and duration of the eruption depend on reservoir size, the rate of heat transfer, and the rate at which water refills the system once emptied by the eruption. By necessity, therefore, geysers can only exist if the bedrock can withstand the pressures created by the steam and if the bedrock is permeable enough to allow rapid reservoir replenishment.

Water acidity and composition

Most springs and geysers discharge alkaline water with a pH ≥ 7 . Most high-enthalpy geothermal areas (e.g., Taupo Volcanic Zone of New Zealand), however, include springs that discharge acidic waters with a pH ≤ 7 and commonly < 3 . The acidic spring waters usually form through oxidation of H_2S and (or) SO_2 that move upward with steam released upon subsurface boiling and are then absorbed by shallow groundwaters. The pH of the spring water is important because it controls the type of biota living on the discharge apron and the minerals that are precipitated in and around the spring vent.

The composition of the spring water is mainly a function of reactions that take place between the bedrock and the water as it circulates through its plumbing system. In this context, the composition of the bedrock is critical because that will determine the elements that can be transferred to the groundwater.

Precipitates

The type of precipitate that forms in association with a hot spring or geyser is fundamentally controlled by water composition. Thus, waters containing SiO_2 may produce opal-A ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Walter, 1976; Cady and Farmer, 1996; Jones et al., 1997), waters containing CaCO_3 may give rise to calcite and (or) aragonite (Renaut and Jones, 1997), and acidic waters containing Fe may produce jarosite [$\text{KFe}_3(\text{SO}_4)_2(\text{OH})_6$], lepidocrocite ($\gamma\text{-FeOOH}$), and (or) hydrous ferric oxide (mainly goethite: $\alpha\text{-FeOOH}$) (Jones and Renaut, 2007).

Silica

Water discharged from hot springs and geysers commonly has a high content of dissolved SiO_2 (e.g., Geysir with ~ 500 ppm SiO_2). With cooling and evaporation, the waters become supersaturated with respect to amorphous silica, and non-crystalline opal-A containing 1.5–15 wt% H_2O (as molecular water and hydroxyl) is precipitated. Siliceous sinters precipitated around hot spring and geyser vents have been called “geyserite.” Morphologically similar to stromatolites because of their thin laminae and botryoidal structures, geyserite was considered abiogenic because it lacked evidence of permineralized microbes and grew in hot waters that were presumed sterile (Walter, 1976). Scanning electron microscope imaging of “geyserite” collected from the vent areas of many springs and geysers on the North Island of New Zealand, Yellowstone, and elsewhere, however, revealed the presence of well-preserved microbes (Cady and Farmer, 1996; Jones et al., 1997, 2001) and thereby demonstrated that some “geyserite” was not abiogenic.

Sinter precipitated in hot springs and geysers commonly contains significant quantities of other elements, including Fe, Al, Au, Ag, and As. Orange siliceous sinters on the submerged shelf around Champagne Pool, Waiotapu (Figure 1b), for example, are rich in As, Sb, Tl, and Hg, and contain ~ 100 ppm Au and ~ 330 ppm

Ag. Such trace element concentrations commonly appear to be related to microbial adsorption and complexation.

Calcite/aragonite

Folk (1994) suggested that calcite as opposed to aragonite precipitation in hot spring and geyser systems is controlled primarily by water temperature (calcite $< 45^\circ\text{C}$ > aragonite) and (or) Mg:Ca ratio (calcite $< 1:1$ > aragonite). Nevertheless, both calcite and aragonite are found around some hot springs and geyser vents where water temperatures are $> 45^\circ\text{C}$. Spring waters discharged with high PCO_2 may, through rapid CO_2 degassing, quickly attain very high levels of supersaturation with respect to CaCO_3 . This commonly seems to be responsible for calcite and aragonite precipitation even if the waters are $> 75^\circ\text{C}$ and have a Mg:Ca ratio of $< 1:1$. Bizarre and unusual crystal morphologies also seem to be consequence of the high saturation levels.

Fe-rich precipitates

Non-crystalline hydrous ferric oxides (HFO), lepidocrocite, alunite, jarosite, pyrite, and clay minerals (kaolinite) are commonly precipitated in acidic, hot spring systems (Figure 1e). Some non-crystalline HFO contains high concentrations of other elements, including As, Mn, Cu, Zn, Cd, Pb (Jones and Renaut, 2007). Precipitation of the different Fe-rich minerals appears to be related to subtle changes in the pH and sulfate (SO_4^{2-}) of the spring waters.

Biota

Hyperthermophilic microbes, which thrive in waters with a temperature $> 75\text{--}80^\circ\text{C}$, include Bacteria and Archaea (Sheehan et al., 2005). Most Archaea are in the form of cocci, rods, or discs (Stetter, 1996, his Table 3). These non-distinctive morphologies coupled with the problems of laboratory culturing due to their poorly known growth requirements and nutritional needs have made species identification difficult. 16S rRNA sequencing, however, has shown that a diverse array of hyperthermophilic Bacteria, and Archaea thrive at high temperatures and extremes of pH, redox state, and salinity (Stetter, 1996; Kvist et al., 2005). Among the Bacteria, *Aquifex pyrophilus* and *Thermotoga maritima* thrive up to 95°C and 90°C , respectively. Among the Archaea, members of *Pyrobaculum*, *Pyrodictum*, *Pyrococcus*, and *Methanopyrus* survive in temperatures up to 100°C . The thermoacidophile *Sulfolobus*, for example, thrives in sulfur-rich hot springs at a pH of 2–3 and temperatures up to 90°C .

An easy way of collecting microbes that live in the dangerous environs of hot springs/geysers is to place sterile glass slides in the water for 24–72 h. Microbes quickly colonize such substrates and remain attached once the slides are extracted from the hydrothermal waters.

Microbe preservation

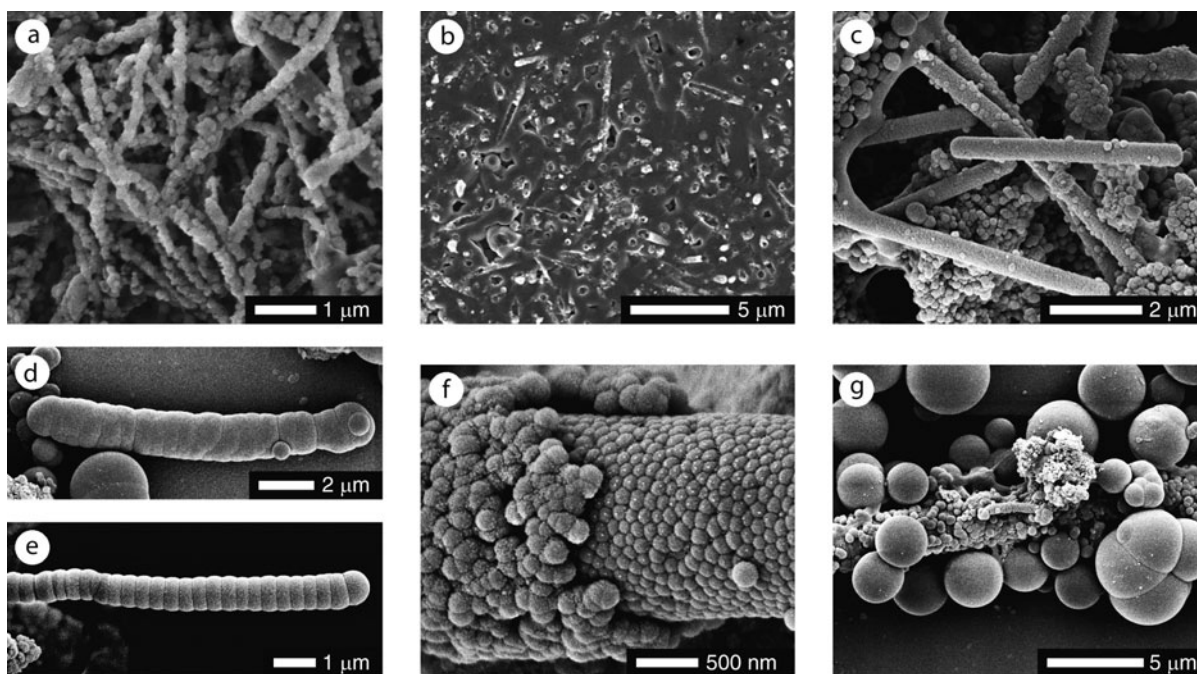
Mineralized microbes are commonly found in precipitates that form in hot spring and geyser systems (Figure 2).



Hot Springs and Geysers, Figure 1 Hot springs and geysers in the Taupo Volcanic Zone on the North Island of New Zealand (a, b, e, f) and El Tatio, northern Chile (c, d). (a) Prince of Wales Feathers and Pohutu during eruptive phase. Note silica precipitates on surrounding Geyser Flats. Whakarewarewa, Rotorua. (b) Margin of Champagne Pool showing exposed siliceous sinters (right) and submerged shelf covered with orange, gold- and silver-rich siliceous sinter. Water has a constant temperature of 75°C and pH of 5.4. Waiotapu geothermal area. (c) Siliceous sinter forming around margin of hot-spring pool, El Tatio. (d) Streamers of filamentous microbes in outflow channel from hot-spring pool, El Tatio. (e) General view of spring in northern part of Waiotapu geothermal area, water temperature of ~80°C and pH of 2.1–2.4. Submerged reddish-brown precipitates formed largely of noncrystalline As-rich hydrous ferric oxide, poorly crystalline lepidocrocite, and crystalline jarosite. (f) Black coating around small hot spring ($T = 100^{\circ}\text{C}$, $\text{pH} = 2.0$) in Kerosene Creek area, north part of Waiotapu geothermal area formed of bitumen generated by the spring system.

Such mineralization, which may involve replacement of the organic tissues and (or) encrustation around the microbes, must take place before the microbes are lost through decay. Microbe preservation is common in non-crystalline precipitates (e.g., opal-A, HFO) but rare in crystalline precipitates (e.g., calcite, aragonite, jarosite), possibly because the former generally precipitate at a faster rate than the latter and organic materials are less prone to destruction by crystal growth.

The microbes can play an active role (i.e., they mediate precipitation through modification of their microenvironment) or a passive role (i.e., they act as templates for precipitation by having nucleation sites on their surfaces) in their own mineralization. Experimental work and interpretation of naturally mineralized microbes indicate that microbes are probably passive during their own mineralization. There is, however, some evidence that some microbes may exert some influence over the initial pattern of opal-A precipitation (Jones et al., 2004).



Hot Springs and Geysers, Figure 2 SEM photomicrographs of siliceous microbes found in Champagne Pool (a, b; $T = 75^{\circ}\text{C}$, $\text{pH} = 5.2$) and Iodine Pool (c–g; $T = >80^{\circ}\text{C}$, $\text{pH} = 8.2\text{--}9.0$), North Island of New Zealand. (a) Silicified filamentous microbes in flocs that circulate in waters of Champagne Pool; (b) Silicified microbes in orange siliceous sinters from shelf around Champagne Pool (see Figure 1b); (c) Silicified rod-shaped microbes; (d and e) Rod-shaped microbes encrusted with different styles of opal-A spheres; (f) Filamentous microbes encrusted with layer of very small opal-A spheres and outer layer of larger opal-A spheres; (g) Silicified filamentous microbes encrusted with opal-A spheres of various sizes.

SEM imaging commonly reveals microbes that appear to be superbly preserved (Figure 2). It is, however, very difficult to identify such microbes in terms of extant taxa because most of the taxonomically important features are lost and DNA is generally not preserved. Thus, identifications must rely solely on morphological features such as general configuration, length, width, and the presence or absence of septa. The problem is compounded by the fact that most extant taxa are defined by DNA sequencing and may not have been imaged to show their morphology.

Conclusion

Hot springs and geysers are spectacular geological features that encompass a wide range of environments that are commonly characterized by extreme conditions (high T , low pH). Although opal-A, calcite/aragonite, and various Fe-rich deposits are the most common minerals precipitated from hot spring and geyser waters, these spring deposits commonly contain significant amounts of other elements, including Au, Ag, As, S, and many others.

Many hot spring and geyser systems are inhabited by a diverse array of microbes (archaea, bacteria, cyanobacteria, fungi, diatoms) that flourish in the warm discharge waters. Such microbes are frequently preserved, typically in opal-A, and thereby provide some insights into the nature of the microbial communities that inhabit these complex environs. Such fossils have received

considerable attention, as they are commonly considered analogous to silicified microbes that are found in much older deposits, especially those in the Precambrian.

Bibliography

- Bryan, T. S., 2005. *Geysers. What They Are and How They Work*, 2nd edn. Missoula, MT: Mountain Press.
- Bryan, T. S., 2008. *The Geysers of Yellowstone*. 4th Edition, University Press of Colorado.
- Folk, R. L., 1994. Interaction between bacteria, nannobacteria, and mineral precipitation in hot springs of central Italy. *Géographie physique et Quaternaire*, **48**, 233–246.
- Cady, S. L., and Farmer, J. D., 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In Block, G. R., and Goode, J. A. (eds), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*. Ciba Foundation Symposium. Chichester, UK: Wiley, pp. 150–173.
- Jones, B., and Renaut, R. W., 1996. Morphology and growth of aragonite crystals in hot-spring travertines at Lake Bogoria, Kenya Rift Valley. *Sedimentology*, **43**, 323–340.
- Jones, B., and Renaut, R. W., 2007. Selective mineralization of microbes in Fe-rich precipitates (jarosite, hydrous ferric oxides) from acid hot springs in the Waiotapu geothermal area, North Island, New Zealand. *Sedimentary Geology*, **194**, 77–98.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1997. Biogenicity of silica precipitation around geysers and hot-spring vents, North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2001. Biogenicity of gold- and silver-bearing siliceous sinters forming in hot (75°C)

- anaerobic spring-waters of Champagne Pool, North Island, New Zealand. *Journal of the Geological Society of London*, **158**, 895–911.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Pentecost, A., Jones, B., and Renaut, R.W., 2003. What is a hot spring? *Canadian Journal of Earth Sciences*, **40**, 1443–1446.
- Kvist, T., Mengewein, A., Manzei, S., Ahring, B. K., and Westermann, P., 2005. Diversity of thermophilic and non-thermophilic crenarchaeota at 80°C. *FEMS Microbiology Ecology*, **244**, 61–68.
- Renaut, R. W., and Jones, B., 1997. Controls on aragonite and calcite precipitation in hot spring travertines at Chemurkeu, Lake Bogoria, Kenya. *Canadian Journal of Earth Sciences*, **34**, 801–814.
- Renaut, R. W., and Jones, B., 2000. Microbial precipitates around continental hot springs and geysers. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 187–195.
- Sheehan, K. B., Patterson, D. J., Dicks, B. L., and Henson, J. M., 2005. *Seen and Unseen. Discovering the Microbes of Yellowstone*. Guilford, CT: The Globe Pequot Press.
- Stetter, K. O., 1996. Hyperthermophilic prokaryotes. *FEMS Microbiology Reviews*, **18**, 149–158.
- Walter, M. R., 1976. Geysers of Yellowstone National Park: an example of abiogenic stromatolites. In Walter, M. R. (ed.), *Stromatolites: Developments in Sedimentology*. Amsterdam, Elsevier, pp. 87–112.
- Waring, G. A., 1965. Thermal springs of the United States and other countries of the world – a summary. *United States Geological Survey Professional Paper*, **492**, 1–383.

Cross-references

[Extreme Environments](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Biomineralization](#)
[Microbial Silicification – Bacteria \(or Passive\)](#)
[Sinter](#)

HYDROGEN

Tori M. Hoehler
 NASA-Ames Research Center, Moffett Field, CA, USA

Synonyms

Dihydrogen; H₂

Definition

A diatomic molecule consisting of two atoms of hydrogen or its heavier isotopes.

Introduction

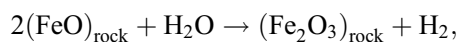
Hydrogen, the most abundant element in the universe by mass and number, exists on Earth principally in combination with oxygen (in H₂O) or carbon (in hydrocarbon and

organic compounds). The diatomic form, H₂ – although present in only trace abundance in the modern oceans and atmosphere – has played a central and evolving role in prebiotic and origin of life chemistry, in linking geochemical and biological cycles, and in shaping the structure, function, and interaction of microbial populations.

Geochemistry of H₂

Geosphere

H₂ is produced by the interaction of water and rocks through a variety of mechanisms (see review in Hoehler, 2005). Among processes that occur in widespread crustal rocks, perhaps the best known and most quantitatively important involves the reduction of water to H₂ by the ferrous iron components of rocks, which can be generalized as



with (FeO)_{rock} representing the ferrous iron component of igneous rock and (Fe₂O₃)_{rock} representing the ferric iron component of alteration minerals (e.g., magnetite). The capacity for H₂ generation via this mechanism is greatest in minerals with high ferrous iron contents, such as the ultramafic minerals olivine and pyroxene, where the process is called serpentinization. Basalts, with a lower ferrous iron content, have a smaller capacity to produce H₂, and granites (where ferrous iron-bearing phases are typically present as minor components) smaller still. The rates of hydrogen generation by water–rock reaction depend significantly on both temperature and the exposure of fresh surface area to water.

H₂ may also be generated via the radiolytic cleavage of water by α, β, or γ radiation produced during decay of isotopes in the host rocks (Spinks and Woods, 1964). Although oxidative species generated simultaneously during the process have potential to recombine with the hydrogen, radiolysis is nonetheless believed responsible for elevated H₂ concentrations (up to millimolar levels) in fluid emanations from a number of continental rock units. This mechanism is generally most important for granites, in which the content of the principal radiogenic elements U, Th, and K is generally highest among major crustal rock types.

Fracturing of silicate rocks may also serve to liberate H₂ that is formed either in solid-state reactions involving trace water impurities in the mineral lattice (Freund et al., 2002) or by direct reduction of water at the fractured surface (Kita et al., 1982).

Among these processes, serpentinization offers the greatest potential capacity for H₂ generation (per unit volume of rock), by orders of magnitude. However, multiple factors – including mineral composition, fluid flow and chemistry, temperature regime, and mechanical processing – ultimately determine which mode of lithogenic H₂ production may prevail in a given environment.

Atmosphere

H₂ is a trace component of the modern atmosphere, with an abundance of about 0.5 ppm (Levine, 1985) and a residence time of less than 1 year (Atkinson et al., 1979), but likely higher levels in the early atmosphere would have had important geochemical and biological implications. Atmospheric H₂ abundance reflects a balance of sources and sinks. Major source terms (excluding anthropogenic sources) include geological efflux (e.g., volcanic and hydrothermal vent emissions) and biological production. Sinks include oxidative atmospheric reactions, biological consumption, and escape to space. The low abundance of H₂ in the modern atmosphere reflects the dominance of oxidative reactions and biological consumption. The early, prebiotic Earth largely lacked such sinks and had a geological source term that was expectedly larger as a result of higher rates of geothermal emanation and a potentially more reducing character in the early mantle and crust (leading to higher H₂ concentrations in geothermal emanations). Estimates of atmospheric H₂ abundance at this time depend principally on theories regarding the oxidation state of the early mantle and crust, and consideration of the factors constraining H₂ escape to space. Even at the low end, these estimates generally predict atmospheric H₂ abundance of hundreds to thousands of parts per million, 3–4 orders of magnitude higher than in the present day.

H₂ itself is considered photochemically inert in the lower atmosphere (Levine, 1985), but it participates in a variety of reactions involving the radical products of photolysis. By serving as a sink for the oxidizing OH radical (Atkinson et al., 1979), and influencing the abundance of the atmospheric reductants H, HCO, and H₂CO (Canuto et al., 1983), H₂ directly affects the oxidation state of the atmosphere. As such, its relative abundance in the early atmosphere may have significantly affected redox speciation in the volatile nitrogen, carbon, and sulfur systems. In turn, this balance (e.g., the CH₄/CO₂ ratio, in the carbon system) affects the atmospheric radiation budget (through differing efficiencies of IR absorption), the potential for formation of photochemical hazes (Sagan and Chyba, 1997), and the formation and stability of simple organics in atmospheric chemical processes.

Above and beyond its role in buffering the oxidation state of the atmosphere through chemical interactions, escape of hydrogen to space can contribute to the irreversible oxidation of the planet, by carrying electrons out of the system (Walker, 1977). This process occurs at low rates in the modern, oxidized atmosphere, where the low abundance of H₂ results in low escape rates. However, H₂ escape from an early atmosphere containing orders of magnitude higher H₂ concentrations may have contributed substantially to the overall oxidation of the planet.

H₂ in prebiotic and origin of life chemistry

Prebiotic chemistry

The emergence of life must depend significantly on development of an inventory of abiotically generated compounds

that can serve as building blocks for biochemistry. Two of the most potentially important endogenous sources for such compounds – synthesis in hydrothermal vent settings and in atmospheric chemical processes – are known or inferred to depend strongly on H₂ for the yield and nature of compounds formed. In vent settings, H₂ from water–rock reactions is implicated as the reductant in Fischer–Tropsch type synthesis of methane, higher hydrocarbons, and simple organic acids, and is predicted to support the synthesis of amino acids and other prebiotic compounds. Miller–Urey type spark discharge experiments that simulate atmospheric chemical processes have a similar dependence that is demonstrated experimentally. For example, the yield of amino acids from simpler precursors in such experiments depends directly on the abundance of H₂, regardless of whether reduced or neutral starting materials (i.e., CH₄/NH₃ (Miller and Urey, 1959), or CO₂/N₂ (Schlesinger and Miller, 1983)) are used. Given its significance in prebiotic synthesis and atmospheric redox and photochemistry on the early Earth (see above), H₂ likely played an important overall role in establishing the environmental and chemical context from which life emerged.

Origin of life

Although the specific chemistry leading to the origin of life is not known, H₂ is proposed in some scenarios to have been a key component of the earliest metabolism (Wächtershauser, 1993). The oxidation of H₂ serves a potentially important dual purpose with respect to such metabolism, in yielding both reducing power and H⁺. Reducing power is necessary for generating organic biomolecules when the carbon source is CO₂. The establishment of H⁺ gradients (specifically, across membranes) can be used to store energy for subsequent coupling to biochemistry. Such dual-purpose chemistry underpins the metabolism of a wide range of modern organisms, including those thought to represent Earth's earliest life. The widespread availability of H₂ in geothermal emanations (e.g., Zobell, 1947; Elderfield and Schulz, 1996) and, presumably, in the prebiotic oceans and atmosphere would have made it a ubiquitous and reliable substrate on which to base the first metabolic chemistry.

H₂ and biology

The reversible oxidation of molecular hydrogen, $\text{H}_2 \rightleftharpoons \text{H}^+ + 2\text{e}^-$, is among the most widely utilized reactions in microbial metabolism. Hydrogenases, the enzymes which catalyze this transformation, are present in all three phylogenetic domains of life (Fenchel and Finlay, 1995; Vignais et al., 2001; Schwarz and Friedrich, 2003). They are particularly widely represented in the Bacteria and Archaea, including the most deeply branching and least derived lineages (Vignais et al., 2001; Schwarz and Friedrich, 2003), suggesting that, exclusive of a possible role in origin of life chemistry, H₂ metabolism has been an important contributor to biochemistry since the very early stages of evolution.

Since its emergence in metabolism, H_2 -based redox chemistry has come to be associated with an extremely broad range of microbial metabolic strategies, including oxygenic and anoxygenic phototrophy, anaerobic chemolitho- and chemoheterotrophy, and aerobic chemolithotrophy (Schwarz and Friedrich, 2003). H_2/H^+ can serve as electron donor, acceptor, or both, in these processes. This broad metabolic utility and versatility casts H_2 in a highly central role in the ecology of microbial communities, and in the geochemistry they mediate.

Lithotrophic communities

Organisms capable of H_2 metabolism can potentially be supported directly by geochemical production of H_2 (by, e.g., water–rock reaction or radiolysis). As such, they have potential to exist independently of energy input from light or the direct products of photosynthesis, oxygen, and organic matter. Such communities are therefore frequently envisioned as important contributors to Earth's earliest biosphere, to the rock-hosted subsurface biosphere, and, potentially, to subsurface life on other worlds. H_2 -based, photosynthesis-independent communities have been documented or inferred for a range of systems and H_2 -producing mechanisms, including vent fluids sourced in serpentinizing systems, basalt-hosted aquifers, and continental groundwaters bearing a signal of radiolytic H_2 production.

Heterotrophic communities

Systems driven by anaerobic decomposition of organic matter typically host a great diversity of H_2 -cycling organisms. Under anaerobic conditions, the complete degradation of complex organics cannot be accomplished by a single organism. Instead, the overall process is mediated, in a series of individual steps, by communities of microorganisms having different metabolic capabilities (Schink, 1988). The individual steps are linked through the transfer of electrons from one group of microbes to another, with H_2 serving as one of the most commonly employed molecular carriers of electrons. The pool of H_2 in such systems is typically characterized by low concentrations (subnanomolar to micromolar) and rapid turnover, with resultant residence times frequently in the range of seconds (Hoehler et al., 2002). Combined with a strong influence on the thermodynamics of reactions that produce or consume it (resulting from its typically high stoichiometric coefficient in such reactions), this effect casts H_2 as a key regulator of degradation pathways, community structure, and carbon and energy flow in such environments. Specifically, fluctuations in H_2 concentration are documented, in a process known as “interspecies H_2 transfer,” to affect the H_2 -cycling reactions by inhibition or stimulation, alteration of products, or even reversal (see review in Hoehler, 2005).

H_2 thus occupies a central role in anoxic ecosystems. Anoxia prevailed globally prior to about 2.4 billion years ago and continues to prevail in most organic-bearing

aqueous environments (including ocean, lake, and wetland sediments and animal digestive tracts). In the past and present, such systems represent a major control on the redox chemistry of the oceans and atmosphere, and the ultimate biological filter on material passing into the rock record.

Phototrophic communities

Most of the known photosynthetic microorganisms, representing both anoxygenic and oxygenic photosynthesis, possess a capability to engage in H_2 metabolism (Schwarz and Friedrich, 2003; Vignais et al., 1985). Most anoxygenic photosynthesizers, including representatives of the purple sulfur, purple non-sulfur, green sulfur, and green non-sulfur bacteria, can utilize H_2 as an electron donor for photosynthetic carbon fixation, and several are also capable of utilizing H_2 as an electron donor for the chemical reduction of inorganic oxidants. Some representatives of the cyanobacteria (oxygenic photosynthesizers) are similarly capable when operating in an anoxygenic photosynthetic mode of metabolism. H_2 is produced obligately during the conduct of nitrogen fixation and can also be photo-produced by the nitrogenase enzyme complex independently of nitrogen fixation. These capabilities, which occur widely among the cyanobacteria and anoxygenic photosynthesizers, are among the principal mechanisms being explored for biological production of H_2 as an alternative energy source. H_2 is also generated as a product of fermentation, a capability demonstrated in some cyanobacteria and purple sulfur bacteria. The fermentative mode of H_2 production appears to dominate the H_2 cycle in a variety of cyanobacterial “mats,” resulting in significant fluxes of H_2 out of the system (Hoehler et al., 2001; Hoehler et al., 2002). During the 1–2 billion years of Earth's history in which cyanobacterial mats dominated biological productivity on Earth, such fluxes may have contributed significantly to atmospheric H_2 levels and, by enhanced rates of H_2 escape to space, the overall oxidation of the planet.

Eukarya

Although not as widespread as in the *Bacteria* and *Archaea*, H_2 metabolism is also found in the *Eukarya*. In a variety of deep-branching anaerobic Eukaryotes, metabolism is tied to, or dependent on, an H_2 -based symbiosis with Bacterial or Archaeal partners (see review in Fenchel and Finlay, 1995). The driver of most of these symbioses is an H_2 -generating organelle, the hydrogenosome, which appears in some cases to be derived from a mitochondrion. These hydrogenosomes carry out fermentation, rather than oxidative phosphorylation, of pyruvate – and thereby produce H_2 . Microbial symbionts (which may include methanogens, sulfate reducers, and (in one case) photosynthetic purple non-sulfur bacteria) increase the energetic yield of this fermentation by consuming the end product H_2 . The symbiosis significantly enhances the growth of the host, and is, in some cases, so evolved that methanogen symbionts have lost their cell walls, and exhibit synchronization of cell

division with the host. Among photosynthetic Eukarya, green algae exhibit an active H_2 metabolism, and are considered a promising potential source of “biohydrogen” for alternative energy systems.

Summary

Hydrogen has played an important role in Earth's geochemistry and biology since the earliest stages of the planet's history. H_2 is produced by a variety of geochemical processes and is postulated to have been a significant component of the early, prebiotic atmosphere, where it had direct or indirect effects on atmospheric redox chemistry and radiation budget, and contributed to planetary oxidation by escape to space. Prebiotic and origin of life chemistry may have significantly involved H_2 , and its prominent use among deeply branching organisms in the tree of life reflects, at very least, a key role in metabolism since the biosphere's early stages. In the modern biosphere, H_2 biochemistry is associated with a broad variety of metabolic strategies, in all three domains of life. Although it now represents a trace component of the oceans and atmosphere, the significance of H_2 for planetary chemistry persists through its central role in the microbial world.

Bibliography

- Atkinson, R., Darnall, K. R., Lloyd, A. C., Winer, A. M., and Pitts, J. N. Jr., 1979. Kinetics and mechanisms of the reaction of the hydroxyl radical with organic compounds in the gas phase. *Advances in Photochemistry*, **11**, 375–488.
- Canuto, V. M., Levine, J. S., Augustsson, T. R., Imhoff, C. L., and Giampapa, M. S., 1983. The young sun and the atmosphere and photochemistry of the early Earth. *Nature*, **305**, 281–286.
- Elderfield, H., and Schulz, A., 1996. Mid-ocean ridge hydrothermal fluxes and the chemical composition of the ocean. *Annual Review of Earth Planetary Science*, **24**, 191–224.
- Fenchel, T., and Finlay, B. J., 1995. *Ecology and Evolution in Anoxic Worlds*. Oxford: Oxford University Press.
- Freund, F., Dickinson, J. T., and Cash, M., 2002. Hydrogen in rocks: an energy source for deep microbial communities. *Astrobiology*, **2**, 83–92.
- Hoehler, T. M., 2005. Biogeochemistry of dihydrogen. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Biogeochemical Cycles of Elements*. New York: Marcel Dekker, Vol. 43, pp. 9–48.
- Hoehler, T. M., Bebout, B. M., and DesMarais, D. J., 2001. The role of microbial mats in the production of reduced gases on the early Earth. *Nature*, **412**, 324–327.
- Hoehler, T. M., Albert, D. B., Alperin, M. J., Bebout, B. M., Martens, C. S., and DesMarais, D. J., 2002. Comparative ecology of H_2 cycling in sedimentary and phototrophic ecosystems. *Antonie Van Leeuwenhoek*, **81**, 575–585.
- Kita, I., Masuo, S., and Wakita, A., 1982. H_2 generation by reaction between H_2O and crushed rock: an experimental study of H_2 degassing from the active fault zone. *Journal of Geophysical Research*, **87**, 10789–10795.
- Levine, J. S., 1985. The photochemistry of the early atmosphere. In Levine, J. S. (ed.), *The Photochemistry of Atmospheres*. San Diego, CA: Academic Press, p. 518.
- Miller, S. L., and Urey, H., 1959. Organic compound synthesis on the primitive Earth. *Science*, **130**, 245–251.
- Sagan, C., and Chyba, C., 1997. The early faint sun paradox: organic shielding of ultraviolet-labile greenhouse gases. *Science*, **276**, 1217–1221.
- Schink, B., 1988. Principles and limits of anaerobic degradation. In Zehnder, A. J. B. (ed.), *Biology of Anaerobic Microorganisms*. New York: Wiley-Interscience, pp. 771–846.
- Schlesinger, G., and Miller, S. L., 1983. Prebiotic synthesis in atmospheres containing CH_4 , CO , and CO_2 : II. Hydrogen cyanide, formaldehyde, and ammonia. *Journal of Molecular Evolution*, **19**, 383–390.
- Schwarz, E., and Friedrich, B., 2003. The H_2 -metabolizing prokaryotes. In Dworkin, M., et al. (eds.), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. New York: Springer.
- Spinks, J. W. T., and Woods, R. J., 1964. *An Introduction to Radiation Chemistry*. New York: Wiley.
- Vignais, P., Colbeau, A., Willison, J. C., and Jouanneau, Y., 1985. Hydrogenase, nitrogenase, and hydrogen metabolism in the photosynthetic bacteria. In Rose, A. H., and Tempest, D. W. (eds.), *Advances in Microbial Physiology*. New York: Academic Press, Vol. 26, pp. 155–234.
- Vignais, P. M., Billoud, B., and Meyer, J., 2001. Classification and phylogeny of hydrogenases. *FEMS Microbiology Reviews*, **25**, 455–501.
- Wächtershäuser, G., 1993. The cradle chemistry of life: on the origin of natural products in a pyrite-pulled chemo-autotrophic origin of life. *Pure and Applied Chemistry*, **65**(6), 1343.
- Walker, J. C. G., 1977. *Evolution of the Atmosphere*. New York: Macmillan.
- Zobell, C. E., 1947. Microbial transformation of molecular hydrogen in marine sediments, with particular reference to petroleum. *Bulletin of the American Association of Petroleum Geochemists*, **31**(10), 1709–1751.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Astrobiology](#)
[Chemolithotrophy](#)
[Early Earth](#)
[Fermentation](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)
[Photosynthesis](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

HYDROTHERMAL ENVIRONMENTS, FOSSIL

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Synonyms

Black and White Smoker communities; Volcanogenic massive sulfide deposits – VMS

Definition

“Black smokers” represent extreme hydrothermal activities that are commonly located in deep sea environments

related with mid-ocean ridges and/or oceanic crust fault systems. Hydrothermal vent systems result from hydrothermal circulation of fluids controlling the transfer of energy and various matter from the interior to the surface of the oceanic crust, respectively the sediment surface. This hydrothermal circulation is a fundamental geological process and has been permanently influencing the chemistry of the oceans, and in consequence, also the composition of the entire earth crust. Seafloor hydrothermal activity has a major impact on the chemistry and life processes of the oceans (Parson et al., 1995; Humphries et al., 1995; van Dover, 2000). This entry focuses on volcanogenic massive sulfide deposits (VMS) and not on sedimentary exhalative deposits (SEDEX) – however, the boundary between both environments is not very strict. Massive sulfide deposits, hydrothermally altered rocks, and special communities of organisms are an important record of this process. Ancient seafloor hydrothermal activity is recorded in massive sulfides from the 3.5 GY-old Pilbara region of Western Australia (Barley, 1992). The new findings of hydrothermal vents with microbialites from 1.43 GY-old Precambrian rocks in northern China (Li and Kusky, 2007) are also of great interest. Within the Phanerozoic, roughly 20 sites are known with fossil hydrothermal vent communities (Little et al., 1998).

Geobiological notes

Hydrothermal vents have become one of the most fascinating fields of research in marine sciences. The recognition of complex vent communities consisting of organisms dependent on chemolithotrophy and extreme conditions of very high temperature, pressure, and chemistry at the ocean ridges was a great discovery (Humphris et al., 1995). There are many reasons to believe that life originated at hydrothermal vent environments in the late Hadean. However, only few publications are dealing with the fossil record of hydrothermal environments connected with preserved organism communities.

There are about 20 known Phanerozoic VMS sites with preserved hydrothermal vent communities, ranging from the Silurian to the Eocene. Most of these sites are found in the Ural Mountains and on Cyprus, some of them in Ireland, California, Philippines, and New Caledonia. All occurrences are linked to remains of oceanic crust (ophiolites). The investigation of vent systems is still a challenge, because the sites are normally small and most of them were destroyed during subduction of oceanic crust.

The vent communities contain assemblages of inarticulate and rhynchonellid brachiopods, gastropods, bivalves, and also monoplacophorans (Little et al., 1998; Kiel and Little, 2006). Remains of worm tubes are very characteristic and may be related to alvinellid polychaetes and pogonophorid-vestimentiferan chemotrophic worms. Most of the fossil taxa of the vent sites are endemic. However, the diagenetic loss of the original shell material makes it very difficult to classify the worm tubes in a modern

taxonomic scheme. Surprisingly, no arthropods were found at the fossil sites, whereas arthropods are very common in modern vent environments. There is an ongoing discussion on the origin of the vent fauna. New groups of organisms may have immigrated from the ambient environment and adapted to the extreme conditions of the vent sites and vice versa, some of them have left this environment. The origin of the vent faunas is thoroughly discussed in Kiel and Little (2006) and Little and Vrijenhoek (2003). Kiel and Little (2006) suggested that the mollusk seep faunas (hydrothermal vents and cold seeps) have a significantly longer evolutionary history than normal marine mollusks and show a tight taxonomic relationship with deep-sea mollusk faunas. The oldest known Phanerozoic hydrothermal vent is of Silurian age (Little et al., 1998). The discovery of the Mesoproterozoic (1.43 GY) hydrothermal vents in northern China was of great importance due to very well-preserved filamentous microbial communities forming massive sulfidic microbialites (Li and Kusky, 2007).

Conclusion

Fossil hydrothermal vent systems are known since the early Archaean and represent important geobiological environments. These environments have a significant key role in biological evolutionary processes. However, only few (ca. 20) sites are known worldwide in the Phanerozoic with preserved vents faunas. The oldest known fossil hydrothermal vent system with a metazoan community is known from the Silurian of the Ural Mountains (Little et al., 1998). Many of the vent organisms probably have a significantly longer evolutionary history than normal marine taxa (Kiel and Little, 2006).

Bibliography

- Barley, M. E., 1992. A review of Archaean volcanic-hosted massive sulphide and sulphate mineralisation in Western Australia. *Economic Geology and the Bulletin of the Society of Economic Geologists*, **87**, 855–872.
- Humphris, S. E., Zierenberg, R. A., Mullineaux, L. S., and Thomson, R. E., 1995. Seafloor hydrothermal systems. *Geophysical Monograph (AGU)*, **91**, 1–466.
- Kiel, S., and Little, C. T. S., 2006. Cold seep mollusks are older than the general marine mollusk fauna. *Science*, **313**, 1429–1431.
- Li, J., and Kusky, T. M., 2007. World's largest known Precambrian fossil black smoker chimneys and associated microbial vent communities, North China: implications for early life. *Gondwana Research*, **12**, 84–100.
- Little, C. T. S., and Vrijenhoek, R. C., 2003. Are hydrothermal vent animals living fossils? *Trends in Ecology and Evolution*, **18**, 582–588.
- Little, C. T. S., Herrington, R. J., Maslennikov, V. V., and Zaykov, V. V., 1998. The fossil record of hydrothermal vent communities. In Mills, R. and Harrison, K. (eds.), *Modern Ocean Floor Processes and the Geological Record*. Geological Society London Special Publications, 148, pp. 259–270.
- Parson, L. M., Walker, C. L., and Dixon, D. R., 1995. Hydrothermal vents and processes. *Geological Society London Special Publication*, **87**, 1–411.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton, NJ: Princeton University Press, 424 p.

Cross-references

Cold Seeps
 Deep Biosphere of the Ocean Deep Sea
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Origin of Life

HYDROTHERMAL ENVIRONMENTS, MARINE

Gilberto E. Flores, Anna-Louise Reysenbach
 Portland State University, Portland, OR, USA

Definition

Marine hydrothermal environments are one of the most extreme environments on Earth, yet they support highly productive biological communities over a wide range of physical and chemical environments. The following review is a general discussion of the types of microorganisms found in these different environments preceded by brief descriptions on hydrothermal fluid generation, mineral deposit formation, and microbial metabolism.

Introduction

The discovery of deep-sea hydrothermal vents and the lush biological communities associated with them in the late 1970s ushered in a new era for marine biologists. Prior to this discovery, life in the deep-sea was thought to be dependent on the settling of detrital material from the productive, overlying surface waters. While this is true for much of the ocean basins, the level of production observed at these newly discovered vent sites was too great to be supported by these mechanisms alone. It soon became evident that life in these environments was supported by chemosynthetic primary production in which microorganisms harnessed the abundant geochemical energy available in the hydrothermal fluids. This challenged one of the basic ecological premises that all ecosystems on Earth were dependent on light energy and driven by photosynthetic primary production.

Since those initial revelations, research efforts have focused on assessing the microbial diversity and attempting to understand the intimate associations between microbial productivity, geology, and geochemistry in marine hydrothermal environments. This article is a general discussion of the trends that are beginning to emerge from these efforts. Included in the discussion are overviews of the geologic setting of marine hydrothermal systems, the generation of hydrothermal fluids from different geologic settings, and the types of structures formed.

For additional in-depth reviews, the Geophysical Monographs published by the American Geophysical Union (AGU) are valuable resources (Humphris et al.,

1995; German et al., 2004; Wilcock et al., 2004; Christie et al., 2006).

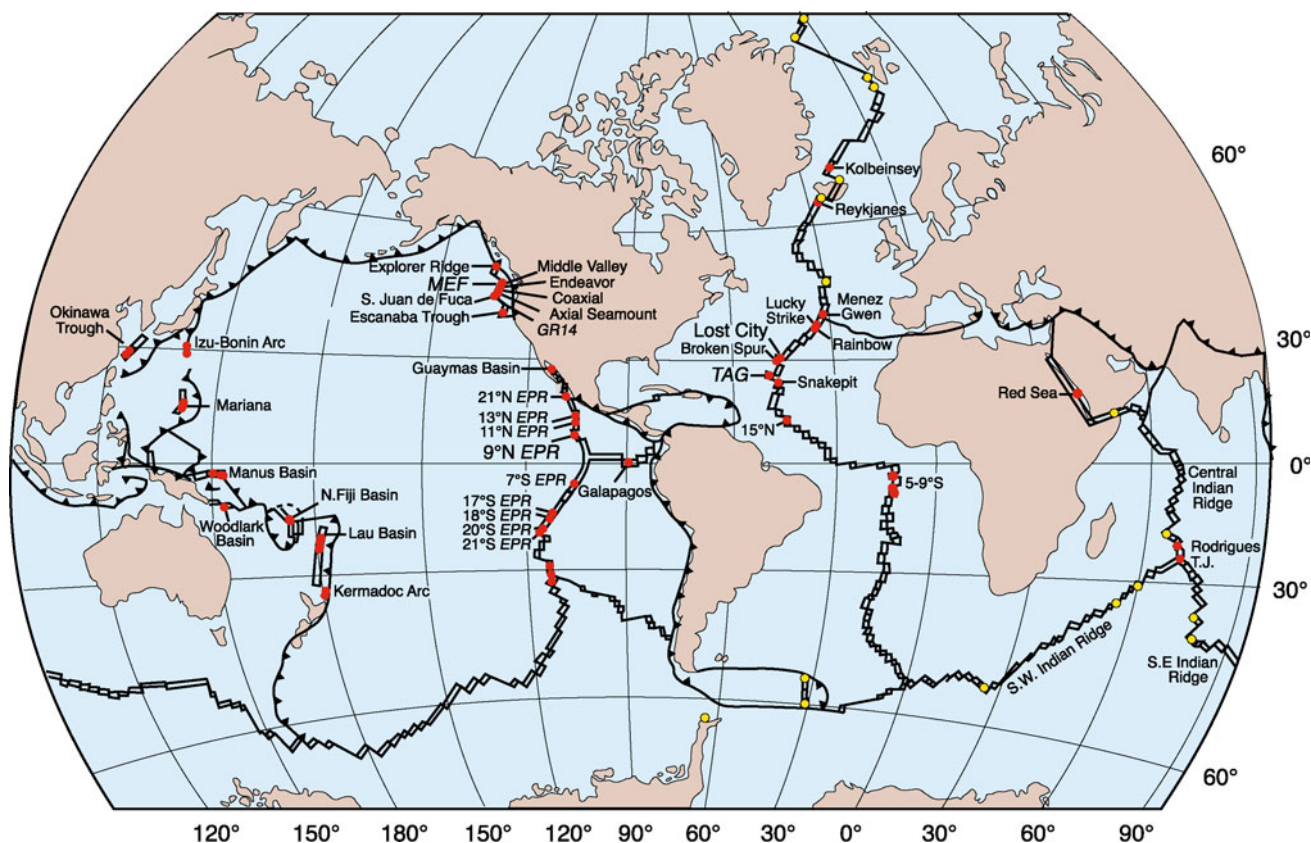
Geological setting

Most seafloor hydrothermal vent systems are associated with extensional tectonic activity and heated by magmatic heat as it is convected into the crust (Seyfried and Mottl, 1995). The most well-studied areas of hydrothermal circulation are along divergent plate boundaries where basaltic seafloor is made and mid-ocean ridges (MOR) arise (Figure 1). Hydrothermal circulation along MORs results from the active heating of seawater that percolates through newly formed basaltic crust. Hydrothermal venting is also commonly found along convergent plate margins where an oceanic plate is subducted beneath a continental plate forming island arcs and back-arc basins (Christie and Fisher, 2006). Vents along back-arc basins are actively heated in the same way as those along MOR, although the fluids tend to be more heterogeneous due to the variability in magma composition and additional inputs from the subducting plate (GAOM et al., 2006). A third location where seafloor hydrothermal systems can be found is at intraplate volcanic hot spots. Volcanic hot spot systems are not directly associated with tectonic plate margins, but are actively heated as plumes of molten magma push up through the mantle and crust (Seyfried and Mottl, 1995).

The recently discovered Lost City vent field more than 15 km off axis of the Mid-Atlantic Ridge (MAR) represents a new type of seafloor hydrothermal system as it appears to be at least partially heated by exothermic reactions between mantle derived peridotite and seawater (Kelley et al., 2001). These reactions have a dramatic impact on the composition of the hydrothermal fluids, which in turn affects the microbial community as discussed in the following sections. Although Lost City represents the first vent type of its kind discovered, similar systems are believed to exist along margins of slow- and ultraslow-spreading ridges where uplifting of ultramafic massifs are common.

Hydrothermal fluids

Seawater heated in contact with subsurface rocks dramatically alters the chemical composition of the end member fluids. The degree of alteration is influenced by several factors including the initial composition of the seawater, the type and structure of the host rock, the presence of sediment overlaying the host rock, and the type, depth, and size of the heat source (reviewed in Tivey, 2007). Understanding these interactions and how they affect the composition of hydrothermal fluids is important as the fluids set the physiological parameters and provide the metabolic menu for colonizing microbes. For comparison, representative compositions of hydrothermal fluids from different settings are presented in Table 1.



Hydrothermal Environments, Marine, Figure 1 Global distribution of known (red) hydrothermal venting along mid-ocean ridges (MOR), back-arc basins, rifted arcs, and at submerged island-arc volcanoes. Yellow circles indicate areas where mid-water column chemical anomalies have been detected suggesting hydrothermal activity. EPR East Pacific Rise; TAG Trans Atlantic Geotraverse; MEF Main Endeavor Field; GR-14 Sea Cliff hydrothermal field on the northern Gorda Ridge. Figure 1 from Tivey (2007).

Basalt hosted hydrothermal fluids

Water–rock reactions begin as cold seawater infiltrates shallow basalt in the recharge zones (Figure 2). Initial low temperature reactions ($\leq 60^\circ\text{C}$) result in the removal of alkali metals from seawater and the oxidation of basaltic minerals (i.e., olivine and plagioclase), with the concomitant leaching of silica (Si), sulfur (S), and sometimes magnesium (Mg) from the minerals (reviewed in Alt, 1995). Magnesium is eventually completely removed from the fluids as Mg-rich clays precipitate at temperatures above 150°C . Anhydrite (CaSO_4) precipitation and seawater sulfate (SO_4^{2-}) reduction also occur in the recharge zone at temperatures above 150°C , resulting in the complete removal of SO_4^{2-} from the fluids. As the fluid continues through the crust, reactions with iron-bearing minerals such as olivine and pyroxene result in highly reducing fluids with elevated concentrations of hydrogen gas (H_2) (Tivey, 2007). Upon reaching the reaction zone where fluids are heated upward of 400°C , the fluid obtains its chemical signature by leaching S and metals (copper-Cu, iron-Fe, manganese-Mn, zinc-Zn) from the surrounding rocks (Alt, 1995). Phase separation

may also occur in the reaction zone if temperature and pressure are greater than 407°C and 298 bars, respectively (Von Damm, 1995). Phase separation can have a significant impact on the composition of end member fluids as volatiles such as hydrogen-sulfide (H_2S) tend to partition in the vapor phase while metals such as Fe become enriched in the liquid (brine) phase. Gases such as helium (He), methane (CH_4), and carbon dioxide (CO_2) may also be added to the hydrothermal fluids as volatiles from the underlying magma (Alt, 1995). In total, these reactions result in end member fluids that are acidic (pH 2.8–4.5), highly reduced, rich in H_2S and metals, and heated up to 400°C . The fluid is also extremely buoyant relative to seawater and is forced back to the seafloor through the discharge zone and may emerge as diffuse or focused flow depending on the degree of subsurface mixing during the ascent (Figure 2).

Other factors, such as differences in host rock and sediment cover, can contribute to the chemical composition of end member fluids. The impact that different host rocks have on fluid chemistry is best illustrated by the ultramafic hosted Rainbow vent field along the MAR

Hydrothermal Environments, Marine, Table 1 Representative chemical compositions of hydrothermal fluids from different geologic settings

Characteristic	Deep seawater	Mid-ocean ridge	Back-arc basin	Sediment hosted	Rainbow vent field	Lost City
T (°C)	2	≤405	278–334	100–315	365	≤91
pH (25°C)	8	2.8–4.5	<1–5.0	5.1–5.9	2.8	10–11
SO ₄ , mmol/kg	28	0	0	0	0	1–4
Mg, mmol/kg	53	0	0	0	0	<1
Cl, mmol/kg	545	30.5–1,245	255–790	412–668	750	548
Na, mmol/kg	464	10.6–983	210–590	315–560	553	479–485
Ca, mmol/kg	10.2	4.02–109	6.5–89	160–257	67	<30
K, mmol/kg	10.1	1.17–58.7	10.5–79	13.5–49.2	20	–
H ₂ S, mmol/kg	–	0–19.5	1.3–13.1	1.10–5.98	1	<0.064
H ₂ , mmol/kg	–	0.0005–38	0.035–0.5	–	13	<1–15
CO ₂ , mmol/kg	2.36	3.56–39.9	14.4–200	–	N/A	bdl
CH ₄ , mmol/kg	–	0.007–2.58	0.005–0.6	–	0.13–2.2	1–2
NH ₃ , mmol/kg	–	<0.65	–	5.6–15.6	–	–
Fe, μmol/kg	–	7–18,700	13–2,500	0–180	24,000	–
Mn, μmol/kg	–	59–3,300	12–7,100	10–236	2,250	–
Cu, μmol/kg	–	0–150	0.003–34	<0.02–1.1	140	–
Zn, μmol/kg	–	0–780	7.6–3,000	0.1–40	160	–
Pb, μmol/kg	–	0.183–0.1630	0.036–3.9	<0.02–0.652	0.148	–

Modified after Tivey (2007). *bdl* below detection limit

(Douville et al., 2002). Fluids from Rainbow vent field are lower in pH, have much higher Fe concentrations, are enriched in H₂ and CH₄, and are depleted in H₂S relative to fluids from most MOR systems (reviewed in McCollom, 2007). These differences in composition are related to the same serpentinization reactions that fuel the Lost City vent field (see below) although the heat sources differ. Sediments overlying the host rock can also affect the composition of end member fluids (German and Von Damm, 2004). In general, sediment-hosted systems generate fluids that are higher in pH (5.1–5.9) and lower in metal contents than those from unsedimented systems. Chemical differences largely depend on the amount and type of organic matter present in the sediments (Tivey, 2007).

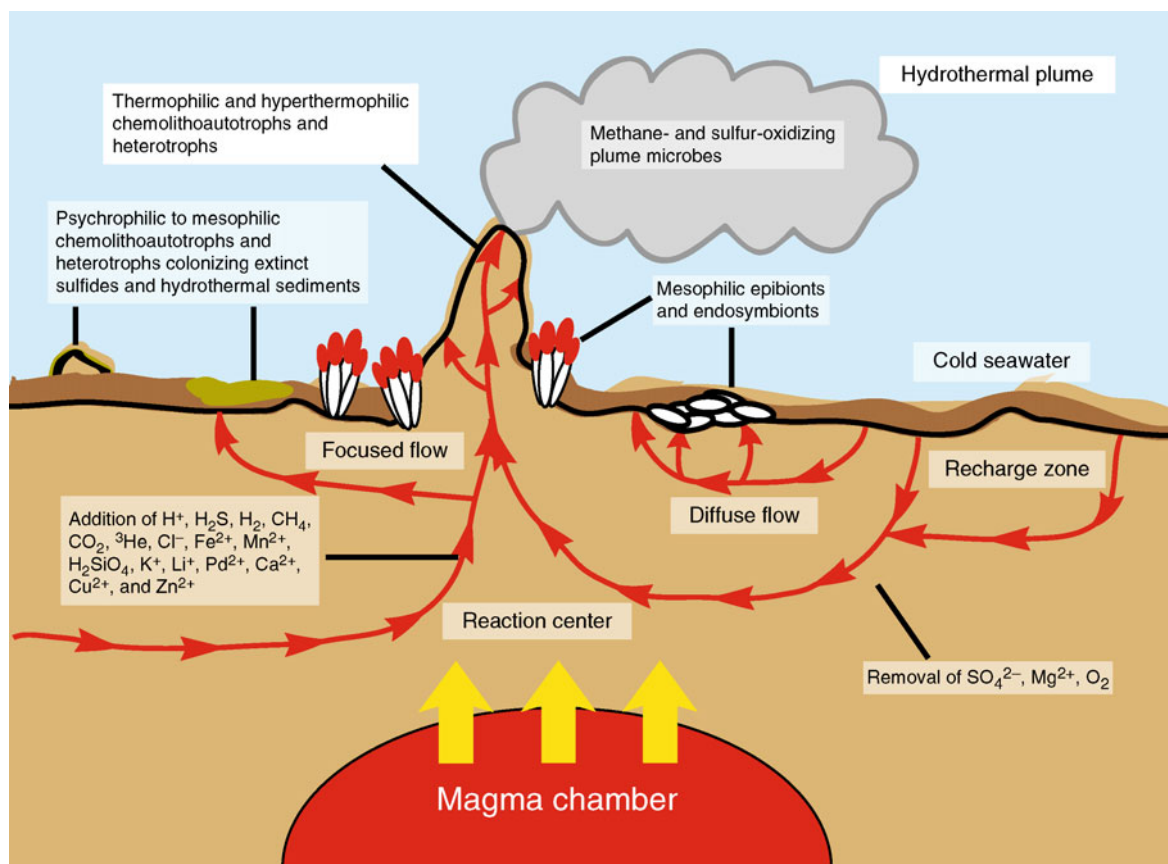
Lost City hydrothermal fluids

Hydrothermal fluid generation at Lost City begins much in the same way as fluid generation at MOR systems, with the infiltration of seawater into rock. However, the rock is not basalt but rather mantle-derived peridotite which reacts with seawater to form serpentinite (Kelley et al., 2001). Serpentinization reactions result in the hydration of minerals such as olivine and produces large amounts of heat, H₂, and CH₄. This heat is believed to at least partially drive hydrothermal circulation at Lost City although other heat sources have been suggested (Kelley et al., 2001; Allen and Seyfried, 2004). Regardless of the heat source, the end member fluid chemistry is dramatically different than MOR vent systems and is indicative of seawater–rock reactions below 150°C. The most striking differences are the alkaline pH (≈10–11), the lower temperatures (up to 93°C), the elevated calcium (Ca) content, and the low concentrations of metals and H₂S found

in the fluids (Kelley et al., 2001, 2005). Methane and H₂ concentrations are also elevated relative to basalt-hosted MOR systems (Table 1).

Hydrothermal structures MOR sulfide deposits

When acidic hydrothermal fluids rich in reduced chemical species interact with cold, oxygenated seawater, minerals rapidly precipitate out of solution forming large deposits (reviewed in Juniper and Tebo, 1995). Mineral phases found in these deposits include sulfides, sulfates, oxides, hydroxides, silicates, and carbonates. Deposit formation can occur in the subsurface as the fluids ascend through oceanic basalt and mix with infiltrating seawater or directly on the seafloor as high temperature fluids erupt into bottom seawater. Subsurface precipitation of minerals generates stockwork deposits in the shallow crust and results in low-temperature venting on the seafloor. Low-temperature fluids are generally low in metal sulfides and as a result, precipitate oxides upon reaching the seafloor. In contrast, fluids that have not been diluted and cooled in the subsurface rapidly precipitate metal sulfides such as pyrite (FeS₂), chalcopyrite (CuFeS₂), and sphalerite (ZnS) upon mixing with seawater. Precipitation of metal sulfides results in the appearance of “black smoke” and ultimately forms massive sulfide deposits (>60% sulfide minerals). Several types of sulfide features are found in vent fields, with the most spectacular being the black smoker chimney (Figure 3a). Other sulfide structures generated at marine hydrothermal vents include diffuse flow beehives, flanges, and complex flow sulfide mounds (Van Dover, 2000). In all cases, active sulfide features provide extreme physical and geochemical gradients that



Hydrothermal Environments, Marine, Figure 2 Hydrothermal circulation along MOR depicting compositional changes of seawater and microbial habitats supported by hydrothermal fluids. *Small arrows* indicate fluid flow while *large arrows* indicate heat transfer from magmatic source. Image is not to scale. Adapted from Reysenbach (2001).

sustain diverse microbial populations even after fluid flow has ceased.

Lost City carbonate deposits

In contrast to the sulfide structures found along basalt-hosted MOR systems, hydrothermal fluids venting at Lost City produce large white chimneys and flanges composed primarily of aragonite (CaCO_3) and brucite ($\text{Mg}(\text{OH})_2$) (Figure 3b) (Kelley et al., 2001). Active structures are highly porous and emit fluids from complex networks of centimeter sized channels (Kelley et al., 2005). Extinct structures are less porous resulting from the replacement of aragonite with calcite (CaCO_3) and the dissolution of brucite. The largest structure at Lost City, Poseidon, is over 60 m tall, making it the tallest seafloor hydrothermal feature yet discovered (Kelley et al., 2001).

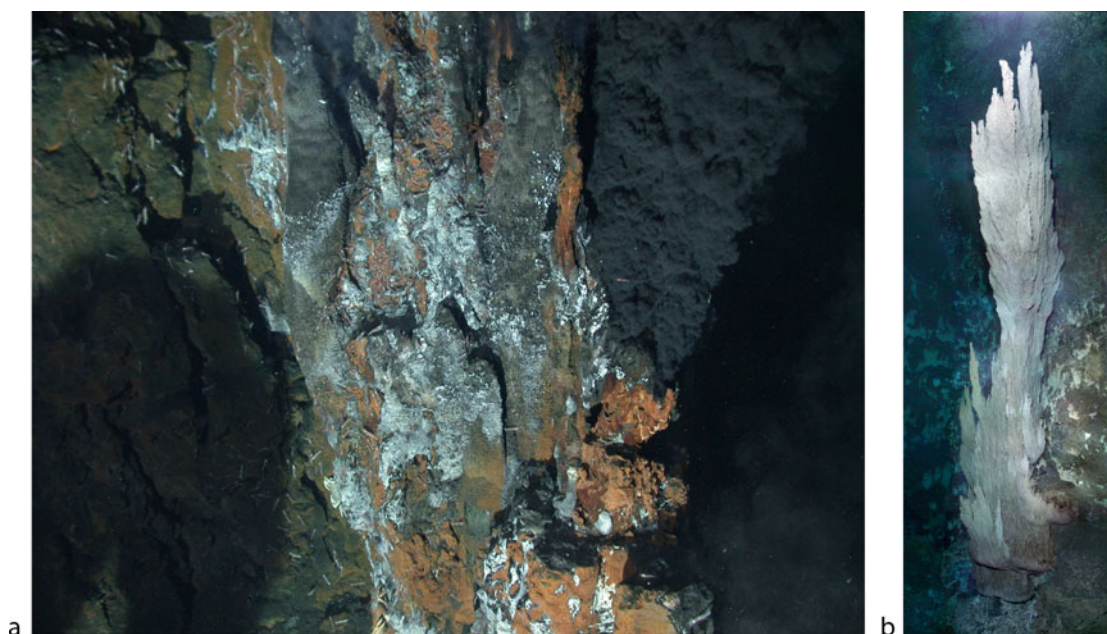
Microbial metabolism

The hydrothermal fluids provide the energy and nutrients for a thriving microbial ecosystem. Additionally, the porous sulfide deposits are excellent habitats for the metabolic diversity of microorganisms.

Terminology

Energy sources for microbial metabolism include light-dependent reactions (less important at deep-sea vents) and light-independent chemical reactions and are described by the prefixes photo- and chemo-, respectively. Energy sources are further classified depending on whether the source of electrons is inorganic (-litho-) or organic (-organo-). For carbon sources, the suffix -autotroph describes an organism that obtains carbon from an inorganic source (CO_2), while the term -heterotroph describes an organism that obtains carbon from an organic source. For example, a microorganism that is able to gain energy and electrons from H_2 and use this energy to fix CO_2 is referred to as a chemolithoautotroph, while humans who require organic carbon and energy sources are described as chemoorganoheterotrophs, as are many microorganisms. Some microorganisms are not obligatory autotrophs and will assimilate organic carbon substances if they are available. The term mixotroph is used to describe these organisms which appear to be common in most environments.

Microorganisms also have varying requirements for oxygen (O_2) as an oxidant. Some organisms have an



Hydrothermal Environments, Marine, Figure 3 Photographs of active chimney structures from different hydrothermal environments. (a) Portion of a massive sulfide chimney from the Eastern Lau Spreading Center emanating “black smoke” from multiple orifices. Note the orange Fe-oxide crust on the outer portions of the structure. (b) Photo mosaic of a 10 m carbonate chimney growing on the side of a serpentine cliff from the Lost City Vent Field. Lost City photo courtesy of D.S. Kelley, University of Washington and the Lost City Team.

obligate need (aerobes) while others cannot tolerate O_2 at all (anaerobes). Furthermore, some organisms require low levels of O_2 (microaerophile) while others can switch between aerobic and anaerobic metabolisms (facultative anaerobe).

At deep-sea vents, the heat and pH preference provides an additional habitat selection. All microbes have a preferred temperature range at which optimal growth is achieved. Psychrophiles have optimal growth $\leq 15^\circ\text{C}$, mesophiles grow optimally between 25 and 40°C , thermophiles and hyperthermophiles grow optimally between 45 and 80°C , and $\geq 80^\circ\text{C}$, respectively.

The pH gradients at deep-sea vents can vary from as low as pH 2.5 to over pH 8.0, as the acidic hydrothermal fluid mixes with the cold seawater. Thus, there are habitats of low pH where acidophiles thrive; however, by far the majority of habitats at vents available for microbial colonization are circumneutral (pH $>6 <8$), and these organisms are therefore referred to as neutrophiles.

Energy sources

Seafloor hydrothermal ecosystems are supported almost entirely by chemosynthetic primary production fueled by the chemical disequilibrium between the oxygenated seawater and the reduced hydrothermal fluids. Microorganisms capture this energy by way of reduction/oxidation (redox) reactions in which seawater typically provides the oxidant (O_2 , SO_4^{2-} , NO_3^- , CO_2) and the hydrothermal fluids provide the reductant (H_2 , H_2S , HS^- , S^0 ,

$S_2O_2^{3-}$, Fe^{2+}). Redox reactions generate energy that is used to fix CO_2 into cellular material which then provides organic carbon for various heterotrophic microbes and animals that make up the rest of the community. Some of the most thermodynamically favorable reactions found in marine hydrothermal environments are presented in Table 2. Reactions are expressed in terms of the change in free energy (ΔG), where the more negative the value the more energy an organism will gain by catalyzing the reaction. Calculated values suggest that aerobic oxidation of reduced sulfur compounds (HS^- , S^0 , $S_2O_2^{3-}$) are the most energetically favorable and potentially the most significant reactions in terms of biomass production in marine hydrothermal environments. It is important to note, however, that thermodynamic calculations can be misleading as they do not consider the bioavailability of certain reactants and potential kinetic inhibition under different environmental conditions (Takai et al., 2006). Several past studies have attempted to model the overall energetics in a variety of marine hydrothermal systems considering such factors (McCollom and Shock, 1997; McCollom, 2000; McCollom, 2007). An interesting conclusion from a theoretical study is that four times as much energy per kg of hydrothermal fluid is available to H_2 oxidizers in ultramafic hosted systems than to sulfur oxidizers because of the increased concentrations of H_2 (McCollom, 2007). This suggests that H_2 oxidizers may be more important than sulfur oxidizers in ultramafic hosted systems (McCollom, 2007). A recent report lends support to

Hydrothermal Environments, Marine, Table 2 Examples of thermodynamically favorable redox reactions utilized by microorganisms from marine hydrothermal environments

Conditions	Electron donor	Electron acceptor	Free energy ΔG^a (kJ/mol)	Metabolic process
Aerobic	H ₂	O ₂	−237	Hydrogen oxidation
	HS [−]	O ₂	−797	Sulfide oxidation
	S ⁰	O ₂	−585	Sulfur oxidation
	S ₂ O ₂ ^{3−}	O ₂	−952	Thiosulfate oxidation
	Fe ²⁺	O ₂	−44.3	Iron oxidation
	NH ₄ ⁺	O ₂	−275	Nitrification
	CH ₄ + other C-1 compounds	O ₂	−810	Methane oxidation
	Organics	O ₂	−477	Heterotrophic metabolism
	Organics	O ₂	−477	Heterotrophic metabolism
Anaerobic	H ₂	NO ₃ [−]	−239	Denitrification
	H ₂	S ⁰	−98.3	Sulfur reduction
	H ₂	SO ₄ ^{2−}	−38.1	Sulfate reduction
	H ₂	CO ₂ ^{2−}	−34.7	Methanogenesis
	Organics	SO ₄ ^{2−}	−40.6	Heterotrophic sulfate reduction
	Organics	S ⁰	−25.1	Heterotrophic sulfur reduction
	Organics	Organics	−38.5	Fermentation ^b
	Organics	Organics	−38.5	Fermentation ^b

Compiled and adapted from Jannasch (1995) and Karl (1995b).

^aValues calculated using standard conditions (pH 7, 25°C, 1 atm)

^bExample reaction calculated from acetate + ethanol → butyrate + water

this hypothesis, as the diversity of H₂-oxidizing bacteria was found to increase as the concentration of H₂ gas increased in vents sampled from the ultramafic hosted Logatchev vent field along the MAR (Perner et al., 2007). These studies speak to the tight coupling of geochemistry and microbial primary production in marine hydrothermal environments.

Microbial habitats and diversity

Seafloor hydrothermal systems provide several habitats over a wide range of physical environments that sustain distinct microbial communities. Such environments include active and inactive vent structures, biotic and abiotic surfaces receiving hydrothermal fluid inputs, inside vent animals (endosymbionts), and hydrothermal vent plumes (Figure 2) (reviewed in Karl, 1995a; Van Dover, 2000; Kelley et al., 2002; Takai et al., 2006). Many of these environments are exposed to varying inputs of hydrothermal fluids, resulting in fluctuating temperatures and chemistry. Below is a brief discussion of each of these habitats and their associated microbial biodiversity. These biodiversity assessments are based on growing and characterizing isolates under laboratory conditions (culturing techniques) and through molecular phylogenetic approaches that are culture-independent. This latter approach is based on extracting genomic DNA from environmental samples, and then amplifying a diagnostic phylogenetic marker for Bacteria and Archaea, the small subunit rRNA gene (16S rRNA), using the polymerase chain reaction.

Active sulfide structures

Using culture-dependent and culture-independent techniques, a rich diversity of Bacteria and Archaea have been found associated with active sulfide structures (reviewed

in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Free-living microorganisms supported by active sulfide structures include mesophilic, thermophilic, and hyperthermophilic chemolithoautotrophs, heterotrophs, and mixotrophs. Organisms can be anaerobes, facultative anaerobes, microaerophiles, and obligate aerobes.

In general, Bacteria are the most numerically abundant microorganisms associated with active sulfide structures (reviewed in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Their abundance can range anywhere from 35 to 99% of the microbial population, depending on the method used to measure abundance, the type of vent feature, and where within a chimney structure one examines (Harmsen et al., 1997; Takai and Horikoshi, 1999; Takai et al., 2001; Hoek et al., 2003; Schrenk et al., 2003; Nakagawa et al., 2006).

Within the Bacteria, members of the epsilon-Proteobacteria have consistently been found to dominate microbial communities in deep-sea hydrothermal environments (Reysenbach et al., 2000; Campbell et al., 2001; Hoek et al., 2003; Nakagawa et al., 2005). These free-living deep-sea epsilon-Proteobacteria are mesophilic to moderately thermophilic. Recently, several representatives have been obtained in pure culture that are chemolithoautotrophs capable of oxidizing H₂ and sulfur compounds while using O₂, NO₃[−], or sulfur compounds as terminal electron acceptors (reviewed in Campbell et al., 2006). Like in cave environments, the epsilon-Proteobacteria play an essential role in sulfur cycle at deep-sea vents.

Other commonly identified but less abundant bacterial members of the free-living microbial communities include the thermophilic *Aquificales* and *Thermotogales* (reviewed in Miroshnichenko and Bonch-Osmolovskaya, 2006). The *Aquificales* are globally distributed in marine

hydrothermal environments but are restricted to active sulfide structures and hydrothermal fluids due to their thermophilic lifestyle. They are generally chemolithoautotrophs and chemoorganotrophs capable of oxidizing H_2 , S^0 , and $S_2O_3^{2-}$ either aerobically or anaerobically (NO_3^- or sulfur compounds) (Reysenbach, 2001a). The *Thermotogales*, on the other hand, are strictly anaerobic, thermophilic heterotrophic fermenters (Reysenbach, 2001b).

As stated above, Archaea are less abundant in active sulfide structures, yet they play a key biogeochemical role especially at the higher temperatures. Some of the most exhaustive studies on microbial diversity at deep-sea vents have focused solely on the Archaea revealing a high phylogenetic and metabolic diversity within this domain (Takai and Horikoshi, 1999; Takai et al., 2001; Nercissian et al., 2003; McCliment et al., 2006). Most of the Archaea found here are thermophilic to hyperthermophilic and are thus restricted to areas most influenced by hydrothermal fluids. Within the Archaea, the two main phyla, the Crenarchaeota and Euryarchaeota, are well represented, while the enigmatic "Korarchaeota" and "Nanoarchaeota" are rarely detected (reviewed in Takai et al., 2006).

Crenarchaeota commonly detected at active sulfide structures include cultivated members of the Desulfurococcales (e.g., *Pyrodictium* and *Pyrolobus*) and several uncultivated groups such as the deep-sea hydrothermal vent crenarchaeotic group (DHVC1), and the marine group I (Takai and Horikoshi, 1999; Takai et al., 2006). *Pyrodictium* is an example of a hyperthermophilic mixotrophic organism with the ability to use either H_2 or complex organics to reduce elemental sulfur (reviewed in Reysenbach et al., 2002). *Pyrolobus* is also a hyperthermophile but, unlike *Pyrodictium*, *Pyrolobus* is an obligate chemolithoautotroph that can only use H_2 to reduce nitrate (NO_3^-), thiosulfate, ($S_2O_3^{2-}$) or O_2 .

Euryarchaeota commonly found at marine hydrothermal systems include the hyperthermophilic methanogens (*Methanocaldococcus* spp.), the hyperthermophilic SO_4^{2-} reducing *Archaeoglobales*, and a variety of thermophilic fermentative *Thermococcus* species (Takai and Horikoshi, 1999; Takai et al., 2006). While these are readily grown in the laboratory, their relative abundance in the environment has been shown to vary from one site to another (Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercissian et al., 2003; McCliment et al., 2006). Several lineages often detected at vent sites around the globe have been classified as the Deep-sea Hydrothermal Vent Euryarchaeotal Groups (DHVEG) (Takai and Horikoshi, 1999; Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercissian et al., 2003). Several subdivisions exist within this group but at least one lineage, the DHVEG-2, appears to be endemic to deep-sea vents. Little was known about this lineage until Moussard et al. (2006) showed optimal activity and thermostability of a DNA polymerase belonging to this lineage which suggested a thermophilic lifestyle. Thermophily was confirmed when the first isolate

was obtained in pure culture (Reysenbach et al., 2006). The organism, *Aciduliprofundum boonei*, is a heterotrophic thermoacidophile capable of fermenting peptides and reducing Fe and S. Isolation of this organism was significant because it is the first obligate acidophile isolated from a deep-sea vent and the first member of the DHVEG-2 lineage to be grown. Prior to this discovery, the absence of acidophiles from a deep-sea vent had always puzzled microbiologists as hydrothermal fluids are acidic, and geochemists had predicted the presence of acidic microhabitats within sulfide chimneys (Von Damm, 1995; Tivey, 2004).

Inactive sulfide structures

Hydrothermal fluid flow through individual sulfide structures is temporary as subsurface flow paths constantly change and chimneys collapse. Inactive sulfides are subjected to low-temperature oxidative weathering in which the reduced sulfur moiety is converted to soluble SO_4^{2-} and the metals, mostly Fe, are converted to insoluble oxyhydroxide crusts (Juniper and Tebo, 1995). Oxidation occurs abiotically as oxygenated seawater penetrates the rock, but can also be mediated by S- and Fe-oxidizing bacteria (reviewed in Edwards, 2004; Edwards et al., 2005). While microbial mediated sulfide weathering in terrestrial systems has been studied for decades, this area of research is in its infancy in the deep sea (Edwards et al., 2003a, b; Edwards, 2004; Rogers et al., 2003; Suzuki et al., 2004). Culturing studies have resulted in the isolation of several psychrophilic, neutrophilic Fe-oxidizing bacteria belonging to the alpha- and gamma-divisions of the Proteobacteria from sulfide rocks collected along the Juan de Fuca Ridge (Edwards et al., 2003a). A follow-up study with one of the gamma-Proteobacteria isolates showed that the organism promoted the dissolution of native sulfide minerals under anaerobic conditions (Edwards, 2004). Although the mechanism of dissolution remains unclear, these studies illustrate that Fe-oxidizing bacteria are associated with extinct sulfide minerals on the seafloor and can actively promote the dissolution of these minerals at least in the laboratory.

Carbonate structures of Lost City

Microbial communities found in active carbonate structures at the Lost City vent field differ from those found in active sulfide structures due to the contrasting physical and geochemical conditions. Inner portions of carbonate chimneys in contact with hydrothermal fluids tend to be covered by thick microbial biofilms dominated by a single Archaeal phylotype belonging to the Methanosarcinales (Schrenk et al., 2004; Brazelton et al., 2006). The Methanosarcinales are the most metabolically diverse of all the methanogens, with various representatives known to generate CH_4 from acetate and H_2/CO_2 or by dismuting methyl compounds (Boone et al., 2001). Members of the Methanosarcinales also participate in a nonobligatory syntrophic relationship

with SO_4^{2-} reducing bacteria that results in the anaerobic oxidation of methane (AOM) in oceanic cold seep environments (Orphan et al., 2001). A second archaeal phylotype belonging to the closely related ANME-1 was identified in weakly flowing and extinct carbonate chimneys (Brazelton et al., 2006). Like the Methanosarcinales, the ANME-1 are involved in AOM but appear to mediate this process without a SO_4^{2-} reducing partner and at lower temperatures (Orphan et al., 2002). As the Methanosarcinales and the ANME-1 found at Lost City were only identified by molecular techniques, it is difficult to speculate on whether they are producers or consumers of CH_4 .

In contrast to the Archaea, Bacteria appear to be more diverse in active and inactive carbonate chimneys (Brazelton, et al., 2006). The most diverse bacterial group at Lost City is the gamma-Proteobacteria which includes close relatives of known chemolithoautotrophic S- and HS^- -oxidizers (e.g., *Thiomicrospira* spp.) and several methylophilic (CH_4 -oxidizing) genera (e.g., *Methylobacter*, *Methylomonas* and *Methylophaga*). Other chemolithoautotrophic S-oxidizing bacteria belonging to the epsilon-Proteobacteria are also found at Lost City.

Perhaps the most interesting trend in bacterial diversity observed at Lost City is not what is present but what has not yet been detected. Specifically, neither any known thermophilic H_2 -oxidizing Bacteria (i.e., *Aquificales*; common in most deep-sea and terrestrial hot spring environments) nor members of the delta-Proteobacteria (the SO_4^{2-} -reducing syntrophs found in other AOM environments), were identified in carbonate chimneys (Brazelton et al., 2006). The lack of bacterial H_2 oxidizers is unexpected as geochemical modeling suggests that H_2 oxidation provides four times as much energy per kg of hydrothermal fluid than S oxidation in ultramafic hosted systems (McCollom, 2007). This suggests that available energy may not be the main constraint driving which microbial metabolisms can be supported in particular environments. It is important to note, however, that relatively few studies have been conducted at Lost City and that these observed trends may be due to the lack of adequate sampling or incorrect inferences based solely on 16S rRNA gene similarity.

Biotic and abiotic surfaces

Microbes are opportunistic organisms and will colonize any surface where growth substrates are provided. In areas influenced by hydrothermal fluids, this includes the sulfide deposits as described above and surfaces of invertebrate animals (reviewed in Van Dover, 2000). Some of these relationships are commensal as the host does not appear to gain an advantage by being colonized while others truly are symbiotic (episymbiosis), with both the host and microbes benefiting. An example of a commensal relationship is found between the giant tube worm *Riftia pachyptila* and the diverse microbial communities that colonize the outside surfaces of its chitinous

tube (López-García et al., 2002). Most of the microbes identified on the outside of the tube are members of the ubiquitous epsilon-Proteobacteria and are likely oxidizing the reduced sulfur compounds in the hydrothermal fluids. An episymbiotic relationship is found on the polychaete worm *Alvinella pompejana* (Van Dover, 2000). *Alvinella pompejana* lives in tubes on the sides of black smoker chimneys where water temperatures can exceed 70°C (reviewed in Desbruyeres et al., 1998). It has a fully functional digestive system and appears to feed upon microbial mats inside of its tube as well on sulfide surfaces. The worm also houses diverse microbial communities on its dorsal surfaces including microaerophilic H_2S -oxidizing members of the epsilon-Proteobacteria. Although the exact nature of this relationship is unknown, it is thought that the bacteria may detoxify H_2S or provide nutritional supplement to the worm.

In addition to surfaces of vent animals, microbes often colonize rocks and sediments surrounding hydrothermal vents. These environments are primarily sustained by hydrothermal fluids flowing diffusely through the underlying porous sediments. The most well-studied and possibly most diverse microbial mat system found in marine hydrothermal environments is in the sediment-hosted Guaymas Basin. Microbes found here include thermophilic/mesophilic methanogens and methanotrophs (Dhillon et al., 2005), SO_4^{2-} reducing Bacteria (Kallmeyer and Boetius, 2004), H_2S -oxidizing gamma-Proteobacteria (Nelson et al., 1989), and even spore forming Mn-oxidizers (Dick et al., 2006). Much of this diversity is fueled by the presence of overlying sediments which provides different combinations of organic/inorganic substrates typically not found in non-sediment-hosted systems.

Endosymbionts

Several invertebrate animals form obligate symbiotic relationships in which the bacteria are hosted inside the animal cells. The most well-studied example is in the giant tube worm *Riftia pachyptila* (reviewed in Minic and Hervé, 2004). As adults, *Riftia* completely lack a mouth, the digestive system and anus and are sustained by chemoautotrophic H_2S -oxidizing Bacteria belonging to the gamma-Proteobacteria (Cavanaugh et al., 1981; Felbeck, 1981; Jones, 1981; Minic and Hervé, 2004). In this relationship, *Riftia* provide the bacteria with inorganic carbon, O_2 , H_2S , and NO_3^- and, in turn are provided with organic carbon. Other invertebrate organisms such as the giant clam *Calypotgena magnifica* and the mytilid mussels *Bathymodiolus* spp. also house H_2S -oxidizing endosymbionts in their gill tissues (Van Dover, 2000). The symbiotic relationship between mussels and bacteria, however, is not obligate as they retain some of their filter feeding capabilities. In addition, certain species of *Bathymodiolus* have methanotrophic (CH_4 -oxidizing) endosymbionts and even dual symbionts with both sulfur- and CH_4 -oxidizing bacteria.

Hydrothermal plumes

Upon reaching neutral buoyancy, hydrothermal vent plumes carrying elevated concentrations of S, metals (Fe and Mn), H₂, and CH₄ extend hundreds of meters laterally into the water column. Not surprising, elevated biomass has been detected in plumes, suggesting that these environments are areas of high productivity (reviewed in Winn et al., 1995). Methane oxidation has been measured in plume waters collected along the Juan de Fuca Ridge, further supporting microbial activity in vent plumes (DeAngelis et al., 1993). More recently, Sunamura et al. (2004) used culture-independent techniques to assess the diversity of microbes in plumes over the Suiyo Seamount. Members of the gamma- and epsilon-Proteobacteria were dominant in the plume waters and are likely using reduced sulfur compounds because of their close phylogenetic relationship with known sulfur-oxidizers (Sunamura et al., 2004).

The first aerobic anoxygenic photosynthetic bacteria from the deep sea were isolated from plumes (Yurkov and Beatty, 1998). These organisms belong to the alpha-Proteobacteria and appear to supplement heterotrophic growth with photosynthesis under laboratory conditions. Their presence in deep-sea hydrothermal environments seems puzzling because of the lack of sunlight, but high-temperature fluids ($\approx 350^{\circ}\text{C}$) have been detected to emit photons capable of supporting facultative photosynthetic bacteria (Van Dover et al., 1996). Their role and significance in the environment, however, remain unknown.

Conclusions

Although we know significantly more about the diversity of microorganisms from deep-sea vents since their discovery in the late 1970s, we still have a rudimentary understanding of their ecology and geobiology. Recently, the discovery of acidophiles (Reysenbach et al., 2006), high-temperature nitrogen fixation (Mehta and Baross, 2006), and high-temperature Fe reduction (Kashefi and Lovley, 2003) provided additional insights into the roles microbes play in biogeochemical cycling at vents. Furthermore, studies are now being undertaken to explore the temporal changes that occur in the microbial communities as the available carbon and energy sources change (e.g., Pagé et al., 2008). Whether these changes are due to the in situ biological activity or due to the geochemical constraints of the hydrothermal fluids and mixing and subsurface reaction zones is still unknown. It is clear that deep-sea vents represent one of the few places on Earth where the “bio” is so tightly coupled to the “geo.”

Bibliography

- Allen, D. A., and Seyfried, W. E., 2004. Serpentinization and heat generation: constraints from Lost City and Rainbow hydrothermal systems. *Geochimica et Cosmochimica Acta*, **68**, 1347–1354.
- Alt, J. C., 1995. Subseafloor processes in mid-ocean ridge hydrothermal systems. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and*

- Geological Interactions*. Washington, DC: American Geophysical Union, pp. 85–114.
- Boone, D. R., Whitman, W. B., and Koga, Y., 2001. Order III Methanosarcinales. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 268–293.
- Brazelton, W. J., Schrenk, M. O., Kelley, D. S., and Baross, J. A., 2006. Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Applied and Environmental Microbiology*, **72**, 6257–6270.
- Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K., 2006. The versatile ϵ -proteobacteria: key players in sulphidic habitats. *Nature Reviews in Microbiology*, **4**, 458–468.
- Campbell, B. J., Jeannot, C., Kostka, J. E., Luther, G. W. III, and Cary, S. C., 2001. Growth and phylogenetic properties of novel bacteria belonging to the epsilon subdivision of the Proteobacteria enriched from *Alvinella pompejana* and deep-sea hydrothermal vents. *Applied and Environmental Microbiology*, **67**, 4566–4572.
- Cary, S. C., Campbell, B. J., and DeLong, E. F., 2004. Studying the deep subsurface biosphere: emerging technologies and applications. In Wilcock, W. S. D., et al. (eds.), *The Subseafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union, pp. 383–399.
- Cavanaugh, C. M., Gardiner, S. L., Jones, M. L., Jannasch, H. W., and Waterbury, J. B., 1981. Prokaryotic cells in hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts. *Science*, **213**, 340–342.
- Christie, D. M., and Fisher, C. R., 2006. Geological, biological, chemical, and physical interactions in back-arc spreading systems – an introduction. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 1–4.
- Christie, D. M., Fisher, C. R., Lee, S.-M., and Givens, S. (eds.), 2006. *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union.
- DeAngelis, M. A., Lilley, M. D., Olsen, E. J., and Baross, J. A., 1993. Methane oxidation in deep-sea hydrothermal plumes of the Endeavor Segment of the Juan de Fuca Ridge. *Deep-Sea Research I*, **40**, 1169–1186.
- Desbruyères, D., Chevillon, P., Alayse, A.-M., Jollivet, D., Lallier, F. H., Jouin-Toulmond, C., Zal, F., Sarradin, P.-M., Cosson, R., Caprais, J.-C., Arndt, C., O'Brien, J., Guezennec, J., Hourdez, S., Riso, R., Gaill, F., Laubier, L., and Toulmond, A., 1998. Biology and ecology of the “Pompeii worm” (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. *Deep-Sea Research II*, **45**, 383–422.
- Dhillon, A., Lever, M., Lloyd, K. G., Albert, D. B., Sogin, M. L., and Teske, A., 2005. Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (*mcrA*) genes in hydrothermal sediments of the Guaymas Basin. *Applied and Environmental Microbiology*, **71**, 4592–4601.
- Dick, G. J., Lee, Y. E., and Tebo, B. M., 2006. Manganese(II)-oxidizing *Bacillus* spores in Guaymas hydrothermal sediments and plumes. *Applied and Environmental Microbiology*, **72**, 3184–3190.
- Douville, E., Charlou, J. L., Oelkers, E. H., Bienvu, P., Colon, C. F. J., Donval, J. P., Rouquet, Y., Prieur, D., and Appriou, P., 2002. The Rainbow vent fluids (36° 14' N, MAR): the influence of ultramafic rocks and phase separation on trace metal content in Mid-Atlantic Ridge hydrothermal fluids. *Chemical Geology*, **184**, 37–48.

- Edwards, K. J., 2004. Formation and degradation of seafloor hydrothermal sulfide deposits. In Amend, J. P., et al. (eds.), *Sulfur Biogeochemistry: Past and Present*. Boulder, CO: The Geological Society of America, pp.83–96.
- Edwards, K. J., Bach, W., and McCollom, T. M., 2005. Geomicrobiology in oceanography: microbe-mineral interactions at and below the seafloor. *Trends in Microbiology*, **13**, 449–456.
- Edwards, K. J., Rogers, D. R., Wirsén, C. O., and McCollom, T. M., 2003a. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing, chemolithoautotrophic α - and γ -*Proteobacteria* from the deep sea. *Applied and Environmental Microbiology*, **69**, 2906–2913.
- Edwards, K. J., McCollom, T. M., Konishi, H., and Buseck, P. R., 2003b. Seafloor bioalteration of sulfide minerals: results from in situ incubation studies. *Geochimica et Cosmochimica Acta*, **67**, 2843–2856.
- Felbeck, H., 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). *Science*, **213**, 336–338.
- Gamo, T., Ishibashi, J., Tsunogai, U., Okamura, K., and Chiba, H., 2006. Unique geochemistry of submarine hydrothermal fluids from arc-back-arc settings of the western Pacific. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 147–161.
- German, C. R., Lin, J., and Parson, L. M. (eds.), 2004. *Mid-Ocean Ridges: Hydrothermal Interactions Between the Lithosphere and Oceans*. Washington, DC: American Geophysical Union.
- German, C. R., and Von Damm, K. L., 2004. Hydrothermal processes. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry, Volume 6: The Oceans and Marine Geochemistry*. London: Elsevier.
- Harmsen, H. J. M., Prieur, D., and Jeanthon, C., 1997. Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture of thermophilic subpopulations. *Applied and Environmental Microbiology*, **63**, 2876–2883.
- Hoek, J., Banta, A., Hubler, F., and Reysenbach, A.-L., 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. *Geobiology*, **1**, 119–127.
- Huber, J. A., Mark Welch, D. B., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A., and Sogin, M. L., 2007. Microbial population structures in the deep marine biosphere. *Science*, **318**, 97–100.
- Humphris, S. E., Zierenberg, R. A., Mullineaux, L. S., and Thomson, R. E. (eds.), 1995. *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union.
- Jannasch, H. W., 1995. Microbial interactions with hydrothermal fluids. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union, pp. 273–296.
- Jones, M. L., 1981. *Riftia pachyptila* Jones: observations on the vestimentiferan worm from the Galapagos Rift. *Science*, **213**, 333–336.
- Juniper, S. K., and Tebo, B. M., 1995. Microbe-metal interactions and mineral deposition at hydrothermal vents. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 219–253.
- Kallmeyer, J., and Boetius, A., 2004. Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Applied and Environmental Microbiology*, **70**, 1231–1233.
- Karl, D. M., 1995a. *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press.
- Karl, D. M., 1995b. Ecology of free-living, hydrothermal vent microbial communities. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 35–124.
- Kashefi, K., and Lovley, D. R., 2003. Extending the upper temperature limit for life. *Science*, **301**, 934.
- Kelley, D. S., Karson, J. A., Blackman, D. K., Früh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Schrenk, M. O., Roe, K. K., Lebon, G. T., Rivizzigno, P., and the AT3-60 Shipboard Party., 2001. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30°N. *Nature*, **412**, 145–149.
- Kelley, D. S., Baross, J. A., and Delaney, J. R., 2002. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annual Reviews in Earth and Planetary Sciences*, **30**, 385–491.
- Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Simmons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- López-García, P., Gaill, F., and Moreira, D., 2002. Wide bacterial diversity associated with tubes of the vent worm *Riftia pachyptila*. *Environmental Microbiology*, **4**, 204–215.
- McCliment, E. A., Voglesonger, K. M., O'Day, P. A., Dunn, E. E., Holloway, J. R., and Cary, S. C., 2006. Colonization of nascent, deep-sea hydrothermal vents by a novel archaeal and nanoarchaeal assemblage. *Environmental Microbiology*, **8**, 114–125.
- McCollom, T. M., 2000. Geochemical constraints on primary productivity in submarine hydrothermal vent plumes. *Deep-Sea Research I*, **47**, 85–101.
- McCollom, T. M., 2007. Geochemical constraints on sources of metabolic energy for chemolithoautotrophy in ultramafic-hosted deep-sea hydrothermal systems. *Astrobiology*, **7**, 933–950.
- McCollom, T. M., and Shock, E. L., 1997. Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta*, **61**, 4375–4391.
- Mehta, M. P., and Baross, J. A., 2006. Nitrogen fixation at 92 degrees C by a hydrothermal vent archaeon. *Science*, **314**, 1783–1786.
- Minic, Z., and Hervé, G., 2004. Biochemical and enzymological aspects of the symbiosis between the deep-sea tubeworm *Riftia pachyptila* and its bacterial endosymbiont. *European Journal of Biochemistry*, **271**, 3093–3102.
- Miroshnichenko, M. L., and Bonch-Osmolovskaya, E. A., 2006. Recent developments in the thermophilic microbiology of deep-sea hydrothermal vent. *Extremophiles*, **10**, 85–96.
- Moussard, H., Henneke, G., Moreira, D., Jouffe, V., Lopez-Garcia, P., and Jeanthon, C., 2006. Thermophilic lifestyle for an uncultured archaeon from hydrothermal vents: evidence from environmental genomics. *Applied and Environmental Microbiology*, **72**, 2268–2271.
- Nakagawa, S., Takai, K., Inagaki, F., Hirayama, H., Nunoura, T., Horikoshi, K., and Sako, Y., 2005. Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field. *Environmental Microbiology*, **7**, 1619–1632.
- Nakagawa, T., Takai, K., Suzuki, Y., Hirayama, H., Konno, U., Tsunogai, U., and Horikoshi, K., 2006. Geomicrobiological exploration and characterization of a novel deep-sea hydrothermal system at the TOTO caldera in the Mariana Volcanic Arc. *Environmental Microbiology*, **8**, 37–49.
- Nelson, D. C., Wirsén, C. O., and Jannasch, H. W., 1989. Characterization of large, autotrophic *Beggiatoa* spp. abundant at

- hydrothermal vents of the Guaymas Basin. *Applied and Environmental Microbiology*, **55**, 2909–2917.
- Nercessian, O., Reysenbach, A.-L., Prieur, D., and Jeanthon, C., 2003. Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13°N). *Environmental Microbiology*, **5**, 492–502.
- Nercessian, O., Prokofeva, M., Lebedinski, A. I., L'Haridon, S., Cary, C., Prieur, D., and Jeanthon, C., 2004. Design of 16S rRNA-targeted oligonucleotide probes for detecting cultured and uncultured archaeal lineages in high-temperature environments. *Environmental Microbiology*, **6**, 170–182.
- Orphan, V. J., House, C. H., Hinrichs, K.-W., McKeegan, K. D., and DeLong, E. F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., House, C. H., Hinrichs, K.-W., McKeegan, K. D., and DeLong, E. F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences*, **99**, 7663–7668.
- Pagé, A., Tivey, M. K., Stakes, D. S., and Reysenbach, A.-L., 2008. Temporal and spatial archaeal colonization of hydrothermal vent deposits. *Environmental Microbiology*, **10**, 874–884.
- Perner, M., Kuever, J., Seifert, R., Pape, T., Koschinsky, A., Schmidt, K., Strauss, H., and Imhoff, J. F., 2007. The influence of ultramafic rocks on microbial communities at the Logatchev hydrothermal field, located 15°N on the Mid-Atlantic Ridge. *FEMS Microbiology Ecology*, **61**, 97–109.
- Reysenbach, A.-L., 2001a. Phylum Aquificae. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 359–367.
- Reysenbach, A.-L., 2001b. Phylum Thermotogae. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 369–387.
- Reysenbach, A.-L., 2001. Deep-sea ridges, microbiology. In Steele, J. H., Thorpe, S. A., and Turekian, K. K. (eds.), *Encyclopedia of Ocean Sciences*. London, UK: Academic Press, Vol. 2, pp. 693–700.
- Reysenbach, A.-L., Longnecker, K., and Kirshtein, J., 2000. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. *Applied and Environmental Microbiology*, **66**, 3798–3806.
- Reysenbach, A.-L., Götz, D., and Yernool, D., 2002. Microbial diversity of marine and terrestrial thermal springs. In Staley, J. T., and Reysenbach, A.-L. (eds.), *Biodiversity of Microbial Life*. New York: Wiley-Liss, Inc., pp. 345–421.
- Reysenbach, A.-L., Liu, Y., Banta, A. B., Beveridge, T. J., Kirshtein, J. D., Schouten, S., Tivey, M. K., Von Damm, K. L., and Voytek, M. A., 2006. A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature*, **442**, 444–447.
- Rogers, D. R., Santelli, C. M., and Edwards, K. J., 2003. Geomicrobiology of deep-sea deposits: estimating community diversity from low-temperature seafloor rocks and minerals. *Geobiology*, **1**, 109–117.
- Schmeisser, C., Steele, H., and Streit, W. R., 2007. Metagenomics, biotechnology with non-culturable microbes. *Applied Microbiology and Technology*, **75**, 955–962.
- Schrenk, M. O., Kelley, D. S., Delaney, J. R., and Baross, J. A., 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology*, **69**, 3580–3592.
- Schrenk, M. O., Kelley, D. S., Bolton, S. A., and Baross, J. A., 2004. Low archaeal diversity linked to seafloor geochemical processes at the Lost City hydrothermal field, Mid-Atlantic Ridge. *Environmental Microbiology*, **6**, 1086–1095.
- Seyfried, W. E. Jr., and Mottl, M. J., 1995. Geologic setting and chemistry of deep-sea hydrothermal vents. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 1–34.
- Sunamura, M., Higashi, Y., Miyako, C., Ishibashi, J., and Maruyama, A., 2004. Two *Bacteria* are predominant in the Suiyo Seamount hydrothermal plume. *Applied and Environmental Microbiology*, **70**, 1190–1198.
- Suzuki, Y., Inagaki, F., Takai, K., Nealson, K. H., and Horikoshi, K., 2004. Microbial diversity in inactive chimney structures from deep-sea hydrothermal systems. *Microbial Ecology*, **47**, 186–196.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Takai, K., Komatsu, T., Inagaki, F., and Horikoshi, K., 2001. Distribution of archaea in a black smoker chimney structure. *Applied and Environmental Microbiology*, **67**, 3618–3629.
- Takai, K., Nakagawa, S., Reysenbach, A.-L., and Hoek, J., 2006. Microbial ecology of mid-ocean ridges and back-arc basins. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 185–213.
- Tivey, M. K., 2004. Environmental conditions within active seafloor vent structures: sensitivity to vent fluid composition and fluid flow. In Wilcock, W. S. D., et al. (eds.), *The Seafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union, pp. 137–152.
- Tivey, M. K., 2007. Generation of seafloor hydrothermal vent fluids and associated mineral deposits. *Oceanography*, **20**, 50–65.
- Von Damm, K. L., 1995. Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union, pp. 222–247.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton, NJ: Princeton University Press.
- Van Dover, C. L., Reynolds, G. T., Chave, A. D., and Tyson, J. A., 1996. Light at deep-sea hydrothermal vents. *Geophysical Research Letters*, **23**, 2049–2052.
- Wilcock, W. S. D., DeLong, E. F., Kelley, D. S., Baross, J. A., and Cary, S. C. (eds.), 2004. *The Seafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union.
- Winn, C. D., Cowen, J. P., and Karl, D. M., 1995. Microbes in deep-sea hydrothermal plumes. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 255–273.
- Yurkov, V., and Beatty, J. T., 1998. Isolation of aerobic anoxygenic photosynthetic bacteria from black smoker plume waters of the Juan de Fuca Ridge in the Pacific Ocean. *Applied and Environmental Microbiology*, **64**, 337–341.

Cross-references

[Acidophiles](#)
[Archaea](#)
[Bacteria](#)
[Beggiatoa](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Extreme Environments](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(II\)-Reducing Prokaryotes](#)
[Hot Springs and Geysers](#)

[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Terrestrial](#)
[Iron Sulfide Formation](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Methanogens](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Origin of Life](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

HYDROTHERMAL ENVIRONMENTS, TERRESTRIAL

Robin W. Renaut¹, Brian Jones²

¹University of Saskatchewan, Saskatoon, SK, Canada

²University of Alberta, Edmonton, Alberta, Canada

Definition

Hydrothermal Environments, Terrestrial. Areas on the Earth's surface that are under the influence of geothermal waters, steam, and associated gases discharged from hot springs, geysers, and fumaroles.

Introduction

Terrestrial hydrothermal environments are those settings where fluids discharge either at or close to the land surface at a temperature that is significantly above the local ambient air temperature. The hydrothermal processes transfer heat and dissolved matter to the surface in a liquid or vapor (gas) phase. Those fluids originate at variable depths below the Earth's surface and have a wide range of temperature and chemical composition. Visible features of terrestrial hydrothermal environments include hot springs, geysers, fumaroles, and steam vents. Travertine (calcite and aragonite: CaCO_3) and sinter (mainly opal-A: $\text{SiO}_2 \cdot n\text{H}_2\text{O}$), precipitated from thermal water, commonly form mounds and terraces around many spring and geyser vents. In contrast, chemical reactions between acidic hydrothermal fluids and the host bedrock and soils usually produce extensive hydrothermal alteration that generates a very different type of landscape.

Since the pioneering studies of life in hydrothermal environments, begun in the 1960s by Brock (1978) and colleagues, interest in these environments has increased significantly for many reasons (Bock and Goode, 1996; Renaut and Jones, 2000; Jones and Renaut, 2003a). The extremophiles that inhabit modern high-temperature settings, which include thermophiles and thermoacidophiles, have implications for understanding the origin of life on the Earth and potentially on other planets. Many of the rocks precipitated around hot springs have stromatolitic fabrics and contain numerous fossilized microbes. Many recent studies have tried to determine if such microbes

directly influence mineral precipitation, or are simply trapped in essentially abiotic mineral deposits. From a geological perspective, surface hydrothermal environments can provide clues to subsurface processes including the potential for epithermal ore mineralization and for geothermal energy development (Pirajno, 1992; Bogie et al., 2005).

Geothermal fields

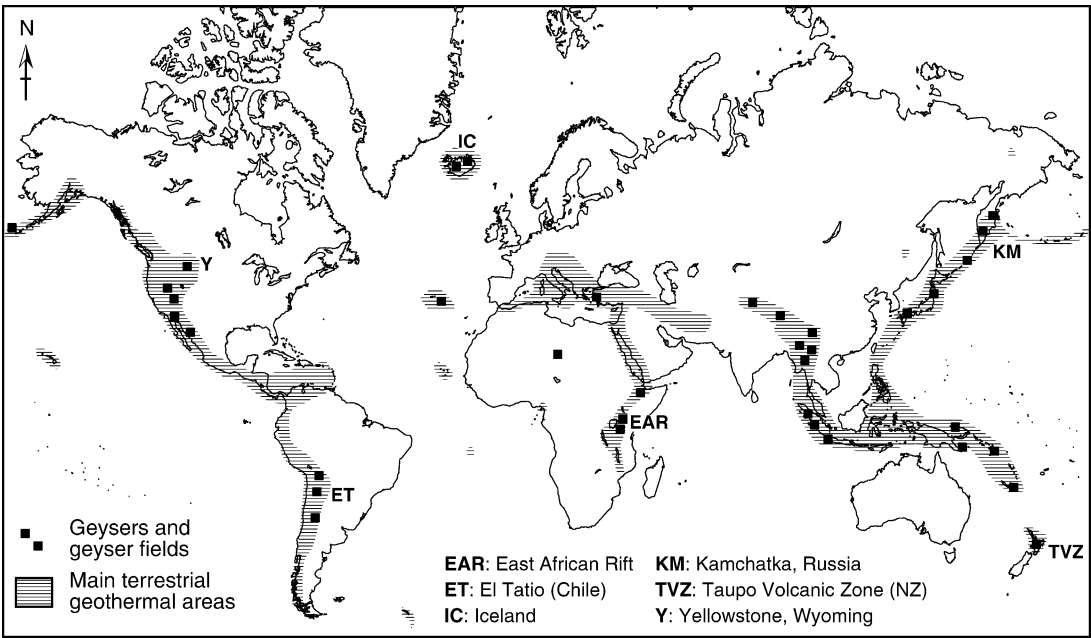
The geothermal gradient, a measure of the increase in temperature with depth in the Earth's crust, has an average value of $25\text{--}30^\circ\text{C km}^{-1}$, but this may rise to several hundred $^\circ\text{C km}^{-1}$ in regions of volcanic activity. Geothermal systems are found in regions with normal and slightly elevated geothermal gradients, but high-temperature systems are most common in regions of active tectonics and volcanism near plate margins (Figure 1). Although geothermal fields can extend over several thousand square kilometers, the individual surface areas of hydrothermal discharge tend to be localized and of limited extent within those domains.

Using stable isotopic evidence, Craig (1963) confirmed that most hydrothermal fluids are meteoric in origin. Some hydrothermal systems are partly fed by connate (formation) waters that were buried along with the host sediments; other hydrothermal fluids may derive from juvenile (magmatic) and metamorphic waters, but these are usually minor components if present (Nicholson, 1993; Giggenbach, 1994).

The fluids that feed high-temperature (high-enthalpy) liquid-dominated hydrothermal systems derive mainly from cool meteoric water that has circulated to a few kilometers depth (up to ~ 5 km) via faults, fractures, and permeable horizons. The heated fluids react with host rocks, acquire solutes, and typically evolve to become saline ($3,000\text{--}5,000 \text{ mg kg}^{-1}$) and chloride-rich, forming geothermal reservoirs with temperatures of up to $\sim 400^\circ\text{C}$. Those fluids then rise convectively and may boil with the decreasing pressure during ascent to create a two-phase (steam + liquid) zone below the land surface. The liquid phase may discharge at the surface as hot springs, whereas the vapors released on boiling (steam separation) may discharge at the surface as fumaroles. Rising hot fluids may mix with shallow groundwaters before surface discharge, which may modify their composition and temperature. Not all geothermal systems are of high-temperature type. In low-temperature (low-enthalpy) systems, the reservoir temperature may be $<150^\circ\text{C}$, and the fluids may not boil before being discharged as springs.

Thermal fluids

Geothermal fluids are commonly classified according to their dominant anion, into chloride waters, sulfate waters, and bicarbonate waters (Table 1) (Henley and Ellis, 1983; Nicholson, 1993). Waters of mixed anion composition are also found. The composition of the discharged fluids



Hydrothermal Environments, Terrestrial, Figure 1 Distribution of high-temperature (high-enthalpy) terrestrial geothermal environments. Modified from Kühn (2004) and Bryan (2005).

Hydrothermal Environments, Terrestrial, Table 1 Selected chemical analyses of hot spring and geyser waters

Water type	Source	pH	T°C	Na	K	Ca	Mg	B	Cl	SO ₄	HCO ₃	SiO ₂
<i>Chloride waters</i>												
Ohaaki Pool, NZ	A	7.1	95	860	82	2.5	0.1	32	1,060	100	679	338
El Tatio, Chile	B	7.4	85	4,600	520	280	0.3	187	8,220	38	39	256
<i>Sulfate waters</i>												
Waiotapu, NZ	A	2.2	99	32	6.6	4.0	0.8	2	6	338	<1	308
Roaring Mt., Yellowstone	C	2.4	92	48	42	3.8	0	—	43	435	—	335
<i>Bicarbonate waters</i>												
L. Bogoria, Kenya	D	9.02	99	1,710	38	0.2	0.05	—	340	65	3,750	135
Mammoth, Yellowstone	E	7.2	69	130	54	323	67	4.1	163	563	755	54
<i>Mixed anion waters</i>												
Rotokawa, NZ	A	6.6	90	380	32	30	0.8	16.3	477	103	173	182
North Waiotapu, NZ	F	3.1	99	153	28	12.9	2.1	—	182	338	0	363

Sources of data: *A* Nicholson (1993, Table 2.1), expressed in mg kg⁻¹. *B* Cusicanqui et al. (1986), expressed in mg kg⁻¹. *C* Brock (1978, Table 2.3), expressed in mg l⁻¹. *D* Renaut and Jones (1997), expressed in mg l⁻¹. *E* Bargar (1978), average of 15 analyses, expressed in ppm. *F* Jones et al. (2000), expressed in ppm

profoundly affects the characteristics of the terrestrial surface environment, including any mineral deposits and hydrothermal alteration, and the biota that inhabit the areas of hydrothermal discharge.

Chloride waters (also termed “alkali-chloride” or “neutral chloride” waters)

These saline waters typify the deep geothermal fluids in most high-temperature geothermal systems and where discharged at the surface, they commonly overlie permeable zones of major thermal upflow (Nicholson, 1993).

They have near neutral pH and are chloride-rich with variable SO₄²⁻ and HCO₃⁻ concentrations. Chloride waters are typically rich in dissolved silica and have Na⁺ and K⁺ as the dominant cations. Carbon dioxide is usually the main dissolved gas with much lower amounts of H₂S.

At the land surface, chloride fluids commonly discharge from hot and boiling springs, often with clear bluish-green waters, and from most geysers (Figure 2a). Such fluids characteristically precipitate large quantities of silica sinter (see Chapter Sinter). If the fluids are rich in HCO₃⁻, they may boil at greater depths and may

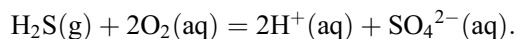
precipitate both sinter and travertine upon CO_2 degassing at the land surface.

Spectacular examples of geysers and springs that eject chloride waters are found in most of the major geothermal sites, including those in Yellowstone National Park (Bryan, 2008), the Taupo Volcanic Zone of North Island, New Zealand (Ellis and Mahon, 1977; Jones and Renaut, 2003b), El Tatio in northern Chile (Cusicanqui et al., 1976; Fernandez-Turiel et al., 2005), and Iceland (Torfason, 1985). The sinter that precipitates around many geyser and spring vents and on their discharge aprons as mounds and terraces is commonly colonized by diverse, varicolored, microbial communities.

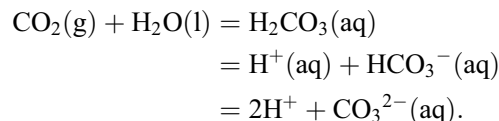
Sulfate waters ("acid sulfate waters")

Sulfate-rich waters form through condensation of geothermal gases in shallow oxygenated groundwater. Steam, gases, and other volatiles (e.g., NH_3 , As, B) that were originally dissolved in deep geothermal fluids can separate from chloride waters when they undergo boiling at depth, and rise toward the surface. The steam-heated acidic waters commonly boil at the water table because the separated steam contains high enthalpy from the deeper geothermal fluid.

Sulfate, the dominant anion, is formed by oxidation of condensed hydrogen sulfide (Nicholson, 1993):

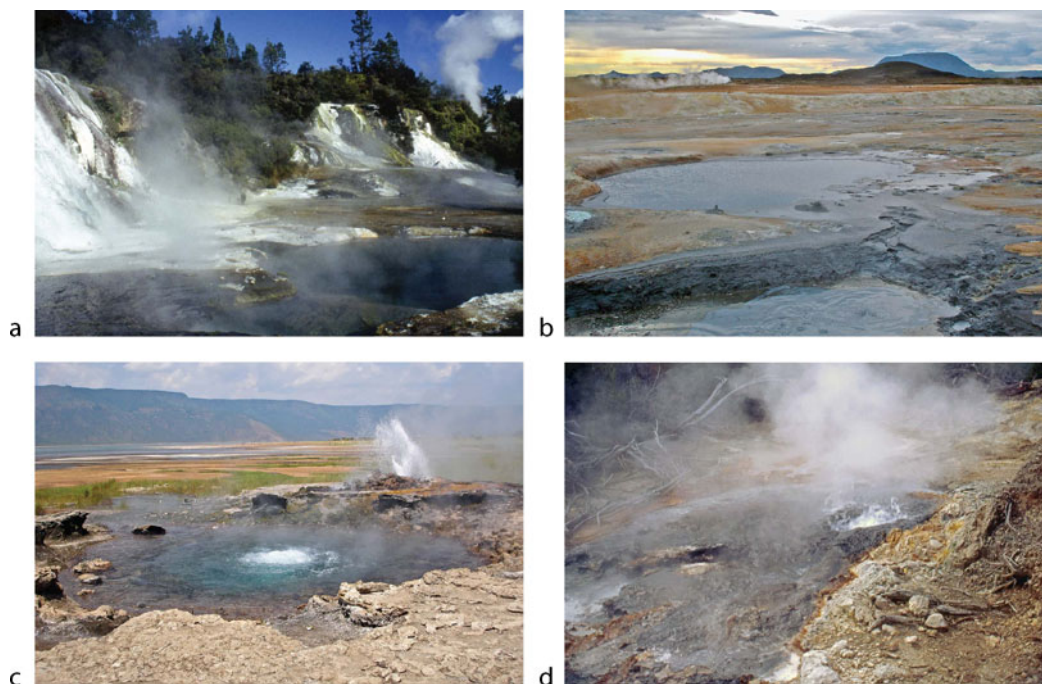


The protons produced may be supplemented by condensation of CO_2 to create acidic waters:



The pH is typically between 2.8 and ~ 5 . Chloride is usually present in minor amounts. Bicarbonate is usually absent because it is lost from solution as CO_2 gas. These acidic fluids, typically present above the water table close to the land surface (<100 m depth), are usually found around the margins of geothermal fields at sites distant (few hundred meters to several kilometers) from the main chloride-rich upflow.

Acidic waters form distinctive surface environments characterized by hot springs, turbid boiling mud pools, or steaming ground commonly with extensive pits and collapse features resulting from hydrothermal alteration (Figure 2b). The acidic waters leach the host rocks



Hydrothermal Environments, Terrestrial, Figure 2 Variations in modern hydrothermal environments according to water chemistry. (a) Hot springs, geysers, and extensive silica sinter coating a fault scarp at Orakeikorako, New Zealand. The fluids are alkaline chloride waters. (b) Muddy hot springs and fumaroles at Myvatn, northern Iceland. The fluids are acid sulfate in composition. Hydrothermal alteration of young volcanic rocks has produced extensive clays and Fe oxides at the land surface. (c) Boiling hot spring and perpetually spouting hot spring (*right*) rimmed by calcite travertine on the shoreline of Lake Bogoria, Kenya. The spring water is of sodium bicarbonate composition. (d) Small geyser discharging mixed acid chloride-sulfate waters (pH: 2.1–2.6) near Hakereteke Stream, north Waiotapu, New Zealand. Thin Fe-rich sinter covers the substrate.

producing extensive clays (mainly kaolinite), commonly with alunite, alunogen, jarosite, Fe-oxyhydroxides, pyrite, cristobalite, and native sulfur. Little sinter forms in acidic waters, but alteration of volcanic rocks by sulfuric acid can leave behind abundant opaline silica in place, termed “silica residue” (Rodgers et al., 2002). Well-developed examples of acid sulfate water discharge are found at Tikitere (New Zealand), Myvatn (northern Iceland), and Norris Geyser Basin (Yellowstone National Park).

Bicarbonate waters

Bicarbonate-rich hot waters are formed mainly when steam and gases (mainly CO_2) condense in shallow groundwaters. Although most common around the margins of geothermal fields, they may also form in sites distant from volcanic activity. They often form where geothermal fluids are in contact with limestone or volcanic bedrock. Such fluids normally have a near-neutral pH when discharged. The initial acidity of the rising CO_2 -rich fluids is neutralized by hydrolysis reactions with bedrock that consume protons (H^+) leaving waters dominated by HCO_3^- and Na^+ . Sulfate and chloride have variable concentrations. At the land surface, these fluids discharge from springs that range from warm to boiling and most have clear water. Travertine deposits, composed of calcite and (or) aragonite, commonly form around the vent and downslope of these springs (Figure 2c) where sufficient Ca^{2+} is available. Sinter is generally absent, but minor amounts can form from very hot and boiling bicarbonate waters. Hydrothermal alteration at the surface is generally very minor, though more extensive at depth.

Carbonate deposits associated with such springs may be localized around their vents or form vast discharge aprons. Examples include the large terrace mounds, fissure ridges, and related features at Pamukkale in Turkey, Mammoth Hot Springs in Wyoming, and at many sites in central Italy (Bargar, 1978; Chafetz and Folk, 1984; Pentecost, 2005). The travertines precipitate from bicarbonate waters over a wide temperature range, from ambient to boiling. Most carbonate precipitation has been attributed to rapid loss of CO_2 upon discharge of the spring waters at the land surface (Renaut and Jones, 1997; Pentecost, 2005). Microorganisms, principally cyanobacteria, may play a role in some carbonate precipitation through photosynthetic removal of CO_2 , but at high temperatures most precipitation is probably driven by abiotic degassing of carbon dioxide.

Mixed waters

Waters that contain two anions with similar concentrations form by several different processes. *Sulfate-chloride waters* typically have a pH of ~ 2 –5, but can have a pH as low as 0. They originate through subsurface mixing of sulfate and chloride waters, oxidation of H_2S in chloride waters, near-surface condensation of volcanic gases in meteoric waters, or dissolution of sulfate-bearing bedrock by migrating thermal chloride waters (Nicholson, 1993).

Such waters discharge as warm to hot springs, commonly with thin sinter deposits and sulfate-bearing minerals around the vent. Native sulfur may also be present.

Examples of acidic sulfate-chloride springs are found in the northern part of the Waiotapu geothermal area of North Island, New Zealand (Figure 2d) (Jones and Renaut, 2006). These springs discharge waters a pH of 2.1–2.6 at temperatures $>90^\circ\text{C}$. Spicular sinters containing silicified microbes have precipitated around these springs together with kaolinite, jarosite, and gypsum.

Dilute *chloride-bicarbonate* waters form where chloride waters have been diluted by groundwater or bicarbonate water (Nicholson, 1993). They typically are found in springs of variable temperature around the margins of thermal upflow zones, but waters of this composition also discharge in low-temperature systems. The fluids are typically Cl-dominated, have variable HCO_3^- concentrations, and a neutral pH of 6–8. Minor travertine and (or) sinter may form around the vents, but many of these springs lack surface deposits.

Types of geothermal systems

The environmental characteristics of terrestrial hydrothermal sites are strongly controlled by the type of geothermal system that supplies their fluids. Many classifications of geothermal systems have been proposed (e.g., Heiken, 1982; Henley and Ellis, 1983; Pirajno, 1992; Nicholson, 1993). Kühn (2004) recognized two major groups. In *static systems*, heat may be transferred to the surface by conduction without convective fluid movement. In *dynamic systems*, heat is transferred to the surface by moving fluids undergoing convective circulation.

Static (conductive) geothermal systems

Static geothermal systems depend on above-average conductive heat flow in the crust. In static magmatic systems, fluids can be heated by granitic plutons. Other conductive systems are hosted by porous sedimentary rocks in deep sedimentary basins. Static systems generally have little impact at the surface. However, trapped formation waters, which are typically saline, chloride-rich and have temperatures of ~ 70 – 150°C , can be released by tectonic movements and discharge at the surface as thermal springs with variable temperature and salinity, depending on the degree of mixing with shallow groundwaters. Many examples are known from Europe, especially Germany (Kühn, 2004).

Dynamic (convective) geothermal systems

Most high-temperature geothermal systems are linked to magmatic intrusions (volcano-plutonic and volcanic), which provide the heat that drives the convective circulation. In such systems, reservoir temperatures are commonly $>150^\circ\text{C}$ at depths of a few kilometers. Such systems are most common in sites of geologically young volcanism and in tectonically active regions such as areas of faulting, rifting, and caldera collapse, and at plate

margins in general. As in other hydrothermal systems, the waters are predominantly meteoric, and the ascending fluids commonly boil before or during surface discharge. The hydrothermal systems vary with tectonic location and type of volcanism (Pirajno, 1992). Many are associated with silica-rich and andesitic volcanism, whereas others are associated with basaltic volcanism.

Silica-andesitic systems

High-temperature hydrothermal systems associated with silica-rich volcanism are highly variable. Some systems are liquid dominated, producing springs and geysers, whereas others are vapor dominated, generating mainly steaming ground and fumaroles at the land surface.

In regions of low relief (Figure 3a), magmatic systems supply heat and gases to meteoric groundwater and give rise to convective columns that can allow deep chloride-rich waters to flow directly to the surface (e.g., Taupo Volcanic Zone, New Zealand). The deep fluids may discharge as springs and geysers above the main upflow or may move laterally some distance away from upflow column. Steam zones commonly develop in sites marginal to the main upflow. Oxidation of H_2S and other gases in groundwater produces acid sulfate waters, generating fumaroles and steaming ground. Condensation of CO_2 in groundwater may produce neutral to alkaline bicarbonate springs around the margins of the field from which travertine may be formed. These types of system commonly have fumaroles, steaming ground, and both acid and alkaline springs located near each other because of the low topographic relief and limited potential for lateral flow of fluids near the land surface.

In areas of high relief (Figure 3b), such as andesitic stratovolcanoes, a thick two-phase zone (liquid–steam–gas) commonly develops below the land surface. At high elevations, fumaroles, steam vents, and acid sulfate springs fed by condensate are common features. Deep chloride-rich waters normally do not reach the surface but may flow laterally and mix with groundwater or cooled, descending sulfate waters before discharging as hot springs on the volcano flanks or other areas of relatively low relief. Numerous examples are found in Indonesia, the Philippines, and Japan.

Some silicic systems are vapor dominated (e.g., Lardarello, Italy; The Geysers, California). The deep thermal reservoir contains steam, probably fed by saline boiling water, most of which is contained in convective cells capped by low-permeability rocks. Fluids escaping the reservoir produce steaming ground and fumaroles at the surface and some thermal springs similar to those in high-relief regions. Surface fluids can be of acid sulfate or bicarbonate type.

Basaltic systems

High-temperature basaltic systems occur at mid-oceanic ridges (see Chapter *Hydrothermal Environments, Marine*) and in continental rifts. Many tectonic and hydrogeological settings are possible (Pirajno, 1992), but

in most examples hydrothermal fluids rise along permeable faults discharging at hot springs and geysers on the rift basin floor (Figure 2c), including subaqueous discharge on the floors of lakes. Fluid compositions are variable and depend partly on the bedrock composition, which may be volcanic, basement rocks, or valley-fill sediments.

Low temperature systems

Low-temperature geothermal systems are common and of diverse origins. Most are characterized by dilute waters that are discharged as springs with maximum temperatures of about 30–60°C. They form in regions of both normal and above-average heat flow. They are common where meteoric fluids return to the surface having been heated to temperatures above ambient surface temperature. These springs commonly occur in regions of deep faulting and folding, in regions of tectonic uplift where hot rocks have been elevated to higher levels in the crust, and from the residual heat of cooling plutons (Nicholson, 1993). Meteoric fluids circulate at depth through faults, fractures, and permeable rocks. The chemical composition of the fluids reflects such factors as the bedrock mineralogical composition, depth and temperature of fluid circulation, the rates of recharge, and degree of mixing with near-surface waters before discharge. Such low-enthalpy systems are common in western Canada, Europe, Australia, and on most continents.

The surface environment in hydrothermal areas

Areas of hydrothermal discharge are distinctive features of the landscape. Where they are extensive, they can influence the local ecology at all scales, from large mammals to microbes. The impact of hydrothermal discharge at the land surface ranges from negligible, where dilute warm springs flow quietly from small vents at temperatures little above ambient, to major, where high-temperature and acidic fluids destroy vegetation, precipitate mineral deposits, or flood the landscape. In general, areas of neutral to alkaline hydrothermal discharge are mainly constructive with new rocks (sinter, travertine, and others) precipitated from hydrothermal fluids upon an existing landscape (Figure 2a, c). In contrast, where the fluids are mainly acidic, the net impact on the surface environment is often destructive and the landscape commonly becomes nearly barren and pock-marked by craters that result from collapse due to hydrothermal alteration of the host bedrock or hydrothermal explosions (Figure 2b, d). The main hydrothermal surface features are hot springs and geysers, fumaroles, steam vents, and mudpots. Some hydrothermal springs also discharge on the floors of lakes.

Hot springs

Hot springs are sites where geothermally heated groundwater discharges at the land surface (see Chapter *Hot Springs and Geysers*). There is no universally accepted definition of a hot spring (Pentecost et al., 2003). Some definitions place the lower temperature limit at an

arbitrary value (e.g., human body temperature: $\sim 37^{\circ}\text{C}$; or some fixed value: 20° , 35° , 50°C , etc.), whereas others define a hot spring by its temperature relationship to local ambient surface water temperature, mean air temperature, or a similar environmental factor. As generally used, the term hot spring is given to those springs that discharge at between $\sim 35^{\circ}\text{C}$ and the local boiling point.

Individual hot springs vary greatly in size (<10 cm to ~ 80 m in vent diameter), depth, shape, and discharge. The most common forms are circular and subcircular pools where water flows from poorly consolidated sediments or hydrothermally altered ground (Figure 4a). Very large hot springs include Grand Prismatic Spring at Yellowstone National Park and Champagne Pool at Waiotapu in New Zealand. Some large springs are composite with multiple vents discharging into a single basin (e.g., Frying Pan Lake, Waimangu, New Zealand). In contrast, shape and size can be highly irregular where flow emanates from a resistant substrate such as volcanic bedrock or from faults and fractures (Figure 4b, c). In the latter case, for example, the vents may be linear fissures without a pool. The vent morphology may change through time as pools coalesce or contract with changes, for example, in surface discharge and earthquake activity.

Geysers (Figure 4d) are hot springs characterized by intermittent turbulent discharge of water that is induced by a vapor phase of gases (mainly steam) that results from boiling. Geysers are rare (Figure 1), with more than 50% of all known geysers in Yellowstone National Park, Wyoming, USA (Bryan, 2005). Well-known geysers include Geysir in Iceland (eponym for geyser) (Torfason, 1985), Old Faithful in Yellowstone National Park (Bryan, 2008), and Pohutu at Whakarewarewa in New Zealand (Jones and Renaut, 2003b). Intermittent springs flow periodically but discharge quietly and lack the forceful ejection of a true geyser. Some springs, termed perpetual spouters (Figure 4e), erupt jets of water and steam constantly, but lack the periodicity of geysers.

Springs also change in morphology as minerals are precipitated in and around their vents (see Chapter Sinter). Mineral precipitation commonly begins around pool margins with build up of a pool-rim dam (Figure 4a), which, depending on hydrostatic pressure, may raise the water level. Water overflowing the dam may then precipitate minerals to form terraces (rimstone dams and pools), oncoids, pisoids, and a range of other spring deposits (Jones and Renaut, 2003a; Pentecost, 2005). At some springs, including many geysers, vents may grow upward forming a range of conical mound deposits (Figure 4d). Microorganisms are commonly fossilized in the spring deposits, and some have been implicated in mineral precipitation.

Fumaroles (steam vents) and mudpots

Fumaroles are vents or openings in the ground that discharge hot water vapor (steam) and (or) volcanic gases. Fumaroles are common features of active volcanoes,

where they can attain temperatures of $>900^{\circ}\text{C}$, but much cooler ($<150^{\circ}\text{C}$) vents are also present at dormant and recently extinct volcanoes and at some high-temperature spring sites, mainly in areas where the water table is relatively low. Fumaroles emit mainly steam (typically $>90\%$), carbon dioxide, and lesser amounts of sulfur (especially H_2S) and chlorine compounds. Minor carbon monoxide, ammonia, boron and fluorine compounds are present in some gases. The predominant steam is mainly superheated groundwater, whereas much of other gas is magmatically sourced. *Solfataras* are areas where fumaroles discharge large volumes of sulfur gases; *mofettes* discharge carbon dioxide, sometimes methane, from fumaroles at temperatures below boiling point of water but above those of ambient air. Mofette gases trapped in valleys and depressions can cause asphyxiation.

The impact of fumaroles on the landscape and environment reflects their abundance, gas composition, temperature, and location. Fumaroles can form on steep and low-angled slopes. On steep slopes, they typically emerge from cracks in the substrate, cm to m in scale, which may be linear, irregular, or funnel-shaped openings where gases have hydrothermally altered the bedrock. On low ground, fumaroles may pockmark the landscape with series of pits (Figure 4f), some meters in diameter. Minerals (*fumarolites*) are commonly precipitated around the vents as rock coatings, many of which are sublimates from the fumarole gases. This diverse array of minerals includes opaline silica, native sulfur, jarosite, Fe-oxyhydroxides, and many salts and rare minerals, some incorporating volatiles.

Rising steam and gases may heat the ground and condense near the land surface or may heat groundwater at shallow depth. The condensed steam and heated water may boil at the water table to produce areas of steaming ground. If H_2S is present in the vapor phase, the resulting sulfuric acid commonly attacks the bedrock and soils and may generate abundant clay minerals, especially kaolinite, producing mudpots (Figure 4g). Forceful eruption of viscous mud can produce mud volcanoes (Figure 4h). The mineralogy of mudpots has received little attention, but includes a similar suite of minerals to other acid sulfate springs.

Steam (hydrothermal) explosions

The surface environment at high-temperature hydrothermal sites occasionally undergoes major disturbances due to hydrothermal eruptions. Such eruptions have several causes but are typically related to the inability of the plumbing system to withstand high fluid pressures generated by boiling. Most eruptions result from the rapid generation of steam due to a sudden pressure reduction (Browne and Lawless, 2001). The explosive release of vapor may lead to break up of sinter or other rocks near the land surface. Blocks become strewn across the landscape, new vents may be formed, and old vents may become inactive because of the changes in the subsurface plumbing system.



Hydrothermal Environments, Terrestrial, Figure 4 Surface features in terrestrial hydrothermal environments. (a) Hot spring with sinter pool-rim dam. Oyster Pool (pH 4.5; T: 65°C), Waiotapu, New Zealand. (b) Hot spring (pH 8.8; 99°C) emerging from fractured trachyphonolite bedrock at Chemurkeu, Lake Bogoria, Kenya. (c) Low-enthalpy, dilute bicarbonate hot spring (pH: 6.6; T: 36°C) at Lobo, Lake Bogoria, Kenya. (d) Clepsydra Geyser, Yellowstone National Park. (e) Perpetually spouting hot spring (pH: 8.7; T: 98.5°C) with mound of subfossil travertine, Lake Bogoria, Kenya. (f) Large (3 m wide) fumarole vent at Sol de Mañana geothermal field, southern Bolivia. (g) Boiling mudpool at Sol de Mañana geothermal field, southern Bolivia. (h) Mud volcano, Tikitere, near Rotorua, New Zealand.

The Waimangu geothermal area on North Island, New Zealand, experienced major hydrothermal explosions on April 1, 1917 that created Frying Pan Lake, with further eruptions in 1924 and 1973 (Lloyd and Keam, 1974). The 1973 eruption, for example, distributed ejecta, which consisted of hydrothermally-altered angular blocks of country rock (up to 30 cm diameter) and fine debris, over an area of $\sim 3 \text{ km}^2$.

Sublacustrine hydrothermal discharge

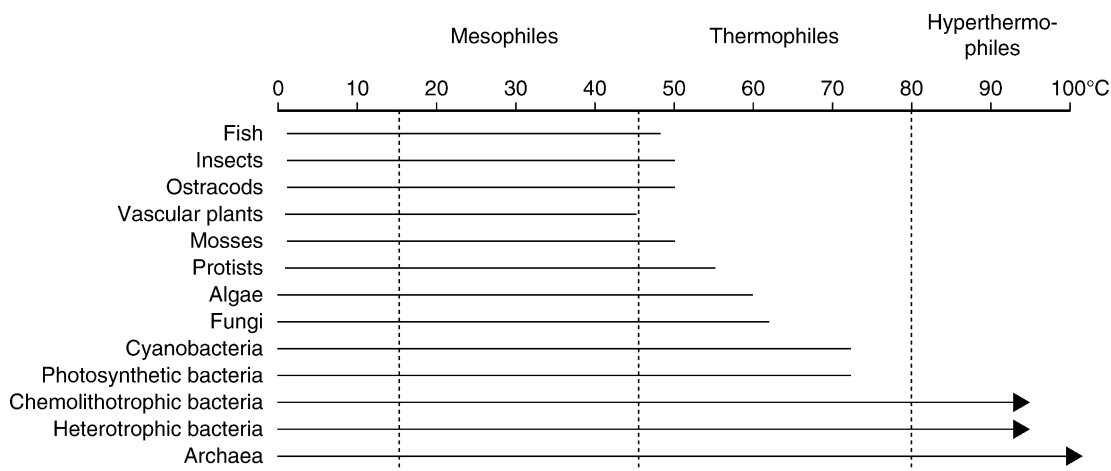
Thermal fluids can discharge directly from lake floors either as underwater springs or as steam and gas vents. Diffuse upward-moving steam and gases may also condense in lake water through permeable sediments without point-sourced vents. Sublacustrine springs are most common on the floors of caldera (crater) lakes, continental rift, and other tectonic lakes, and in lakes produced by subsidence, collapse, or hydrothermal eruptions in areas of acidic hydrothermal discharge. Their impact on life in the lake depends on the respective chemistry and temperatures of the lake water and thermal fluids, the rate and volume of thermal fluid discharge, and lake size, morphology, and climate, which can control seasonal stratification and hydrological closure. Like submarine springs, sublacustrine hot springs may host local communities of thermophilic organisms, and biofilms or microbial mats commonly cover the vent surfaces. Sublacustrine sinter, travertine (tufa), and other mineral deposits (e.g., metal sulfides) can precipitate around vents at sites where thermal fluids mix with lake water, undergo cooling, or exsolve noncondensable gases. Spring deposits include spires, chimneys, mounds, crusts, and various microbialites. Sublacustrine spring deposits commonly incorporate lacustrine organisms, especially planktonic diatoms and freshwater sponges, which can help distinguish them from morphologically similar subaerial spring deposits when examining ancient deposits.

Well-known examples of sublacustrine spring systems include those in Crater Lake (Oregon), Yellowstone Lake (Wyoming), Lakes Tanganyika, Kivu and Bogoria in the East African Rift, and Lake Taupo (North Island, New Zealand). Hot springs on the floor of Lake Taupo at depths of $\sim 140 \text{ m}$, for example, have produced small chimneys formed of opal-A and various Mn-, Fe-, and Hg-precipitates (de Ronde et al., 2002). Crater lakes in active and dormant volcanoes commonly receive discharge from acidic springs and fumaroles that produces acid lake waters.

Life in terrestrial hydrothermal environments

Terrestrial hydrothermal environments host many organisms, but some, notably thermophiles ($\sim 45^\circ\text{--}80^\circ\text{C}$) and hyperthermophiles ($>80^\circ\text{C}$), are unique to hydrothermal settings (see Chapter *Extreme Environments*). Although the ecology of hydrothermal environments has been studied extensively (e.g., Brock, 1978; Reysenbach et al., 2001; Robb et al., 2007; Wiegand, 2007), new discoveries continue to be made regularly. The distribution of microorganisms at hot springs and geysers is controlled mainly by temperature, pH, and water chemistry. Brock (1978) first established the main temperature tolerance ranges for the main groups of organisms in hot-spring systems (Figure 5).

Proximal areas under the influence of near-boiling water ejected from hot springs and geysers were once regarded as being sterile. The ornate and banded silica deposits commonly found around geyser vents were named “geyserite” because they were thought to have formed abiotically (Walter, 1976). It has since been shown that a diverse community of microorganisms inhabit these very hot waters (Blank et al., 2002) and that geyserite commonly contains abundant silicified microbes (Jones et al., 1997). Identifying these microbes is a challenge because many are difficult to culture and their



Hydrothermal Environments, Terrestrial, Figure 5 Upper temperature tolerance limits of selected organisms in hydrothermal environments (modified from Brock, 1978).

morphological diversity is low. DNA analyses, however, show that these extremophiles are biologically diverse (Barns et al., 2004). The hyperthermophiles are dominantly archaea, and some are also thermoacidophiles. In the hottest (anoxic) waters, the archaea are mainly chemolithoautotrophs and chemoorganotrophs, and include coccoid, rod-shaped, and filamentous forms (e.g., *Pyrolobus*, *Pyrococcus*, *Methanothermus*). Hyperthermophilic bacteria (e.g., *Aquifex*, *Geothermobacterium*), some of which are sulfide and hydrogen oxidizing species, also thrive in waters up to boiling point. Viruses also inhabit these high-temperature settings. Thin microbial films locally coat immersed rock and soil surfaces around the edge of the vents (Figure 6a), and filamentous stringers of hyperthermophiles are sometimes seen attached to the substrate in proximal outflow channels.

As water temperature decreases downstream from the spring and geyser vents, the species diversity usually increases. At temperatures between 75 and $\sim 40^{\circ}\text{C}$, microbial mats are commonly well developed in neutral and alkaline waters (Figure 6b). Varicoloured (green, orange, brown) microbial mats built by photosynthetic cyanobacteria often cover the substrate in sites that remain almost constantly moist from thermal outflow, and are commonly several cm thick (Figure 6c). Small coniform stromatolites form in some low-energy pools (Figure 6d, e) (Jones et al., 2002). Common genera include *Oscillatoria*, *Synechococcus*, *Phormidium*, *Anabaena*, and *Spirulina*. *Chloroflexus*, a green sulfur bacterium and anoxygenic photoautotroph, is commonly associated with these mats. Extensive extracellular polymeric gels are produced by some of these communities. Heterotrophic bacterial communities normally underlie the surface layers of these mats (Brock, 1978). Organisms common in waters cooler than $\sim 45^{\circ}\text{C}$ include the cyanobacteria *Calothrix* and *Pleurocapsa*, which often build nodular mats, eukaryotic algae, diatoms, fungi, and protists, some of which become important components of microbial mats.

Acid thermal areas, including areas around some fumaroles (and solfataras), usually have distinctive acidophilic communities. In acidic hot springs where the temperature is $<60^{\circ}\text{C}$, green mats built by the unicellular red alga *Cyanidium caldarium* are often present (Figure 6f) (Brock, 1978), together with a range of bacteria (e.g., *Thiobacillus*). Below pH 5, cyanobacteria are less abundant and mats composed of diatoms and fungi become common. Stromatolitic sinters built by diatoms, for example, are present at Frying Pan Flats at Waiotapu, New Zealand, where the pH is <3 (Figure 6g) (Jones et al., 2000).

Invertebrates, such as mat-grazing ostracods, are common in some hot spring waters that are $<45^{\circ}\text{C}$. Insects are common at some hot springs and include spiders, beetles, and flies. Adult and larval *Ephydrya* spp., for example, feed on microbial mats in hot spring effluent (Figure 6h). Few vertebrates are restricted to thermal aquatic environments, but fish (*Alcolapia grahami*) inhabit hot spring pools with temperatures up to 46°C at Lake Magadi, Kenya (Figure 6i).

Environmental impact of terrestrial hydrothermal activity

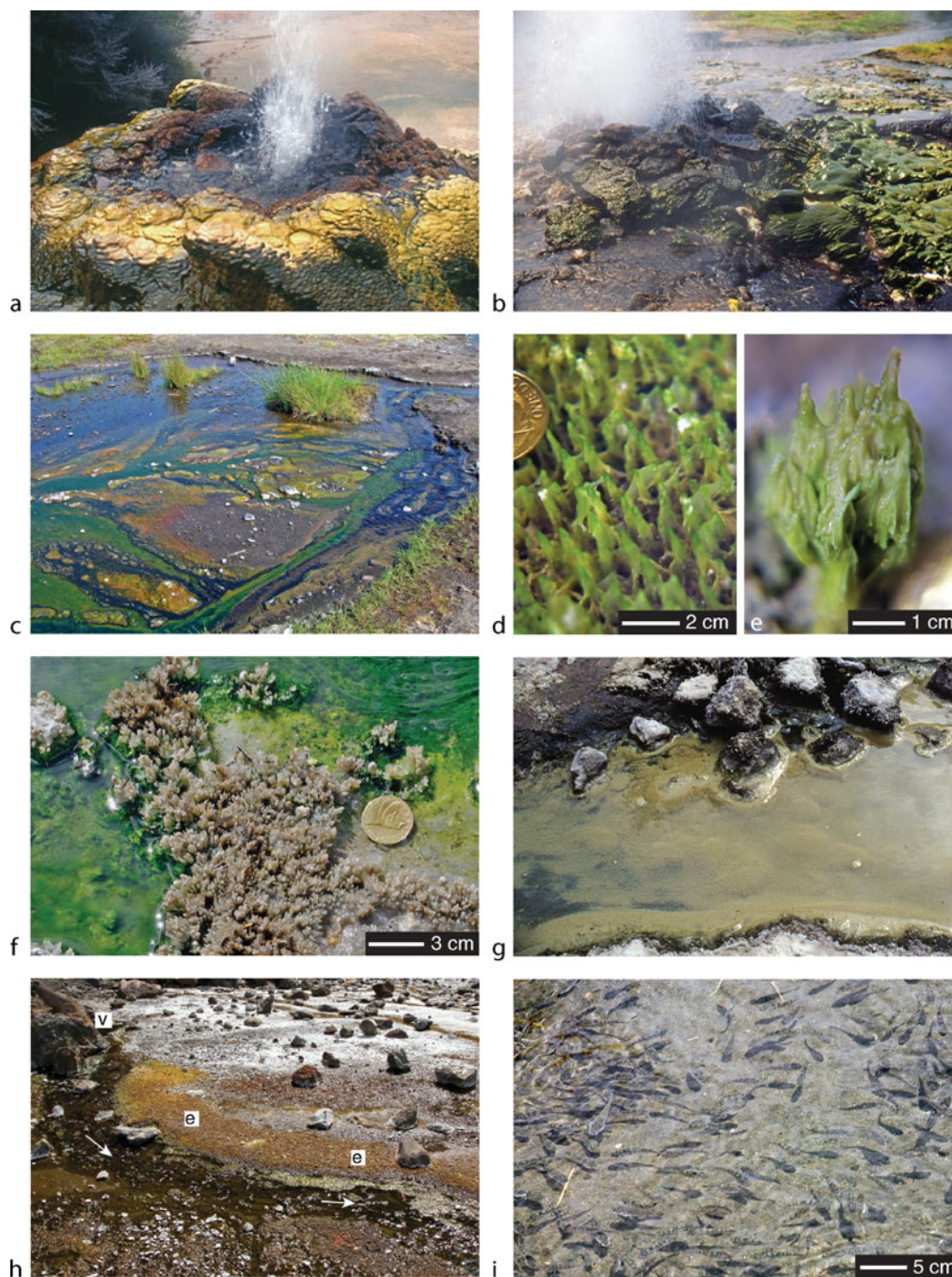
The environmental consequences of terrestrial hydrothermal activity are highly diverse, as a few examples can illustrate. High concentrations of metals such as As that are present in effluent can be absorbed by vegetation in thermal areas with variable effects (Koch et al., 1999). High concentrations of fluoride in dilute hot-spring outflow are a problem (fluorosis) for animals and humans in parts of China, Kenya, Tanzania, and elsewhere (e.g., Xu et al., 1995). Toxins in thermophilic cyanobacterial mats have been blamed for deaths (e.g., Krienitz et al., 2003) of birds. Fumarole gases are commonly toxic and have killed animals and vegetation in many locations (e.g., Baxter et al., 1999). Effluent from geothermal sites can contaminate freshwater aquifers and surface waters. In many cases, the impact of hydrothermal activity is negative from a human perspective, but this is partly balanced by the strong appeal of hot springs, geysers, and fumaroles as tourist attractions.

Temporal and anthropogenic changes in hot spring and geyser activity

Hydrothermal features at the land surface are typically ephemeral, with activity lasting from months up to several hundred thousand years (see Chapter *Hydrothermal Environments, Fossil*), but renewed activity may recur at the same location following volcanic and tectonic events (e.g., faulting) or climate change. Hot springs, geysers, and fumaroles may continue to function provided the basic requirements of a heat source, reliable fluid source, and permeable fluid pathway to the surface are met. Variations in heat source typically occur over long timescales (e.g., magmatic cooling), but water supply and fluid pathways (permeability) to the land surface can vary over much shorter timescales and induce frequent and shorter-term changes in hydrothermal activity.

The fluid recharge is especially sensitive to climate. Sturchio et al. (1993), for example, showed that periods of sinter formation in the arid northern Kenya Rift during the Pleistocene could be correlated with phases of humid climate. Springs that discharged chloride waters and precipitated sinter when water tables were regionally high during wetter periods are now acidic fumaroles or have become extinct. Even very short-term climate changes can have an impact. The activity of geysers and hot springs on the shoreline of Lake Bogoria in Kenya, for example, responds rapidly to minor changes in lake level (<30 cm) that are induced by El Niño rains (Renaut et al., 2005).

Anthropogenic activity can also cause substantial changes to the level of the water table over periods of a few years, as examples from New Zealand demonstrate. Development of the Wairakei geothermal power plant in the late 1950s led to a large fall in the water table, subsidence, and the loss of at least 100 geysers (Glover, 1965; Bryan, 2008). Decreasing geyser activity and other



Hydrothermal Environments, Terrestrial, Figure 6 Life in terrestrial hydrothermal environments. (a) Vent of small geyser (former well bore) at Tokaanu, New Zealand. Despite the boiling water spray and overflow, a diverse microbial community inhabits the area at the vent and their remains are fossilized in the sinter. (b) Thick (up to 10 cm) microbial mats near the vent of a perpetually spouting, Na-HCO₃ hot spring at Lake Bogoria, Kenya (shown in Figure 2c). The mats (*Chloroflexus* and several cyanobacterial species) receive spray from the spring but are absent where water temperature is constantly >70°C. (c) Microbial mats (cyanobacteria) in shallow outflow (pH: 8.8; T: 40–85°C) from a Na-HCO₃ hot spring at Lake Bogoria, Kenya. (d), (e) Small coniform stromatolites, built by *Phormidium* growing in Kirihero spring pool (pH: 7.5; T: 38°C), Tokaanu, New Zealand. (f) Green subaqueous *Cyanidium* mats adjacent to glassy spicular sinter in an acidic (pH 2.6; T: 48°C), shallow spring pool near Hakereteke Stream, north Waiotapu, New Zealand. (g) Soft mats built mainly by diatoms and fungi in acidic outflow channel (pH: 2.6; T: 41°C) from Frying Pan Flat, Waiotapu, New Zealand. (h) Thick accumulation of pupae of *Ephydra* sp. (e) along the edge of a small alkaline hot spring (v) (pH: 8.6; T: 47°C), Lake Magadi, Kenya. The ephydrids feed on the mats on the discharge apron. (i) *Alcolapia grahmi* swimming in the vent pool (pH: 9.9; T: 46°C) of an alkaline hot spring, Lake Magadi, Kenya.

changes in the Whakarewarewa geothermal area over several decades have been attributed to natural changes in the subterranean plumbing system and to increased water extraction in nearby Rotorua.

The history of Geysir in Iceland is known from 1,294 AD because its behavior has been recorded in Icelandic literature. Historical sources show that the geyser activity has varied considerably, with periods of regular discharge alternating with periods of quiescence with eruptions. At Geysir, earthquakes rather than climate variability often seem to cause changes in its patterns of eruption (Torfasen, 1985; Jones et al., 2007).

In some instances, changes in the subsurface aquifer and plumbing systems may trigger renewed activity after years of inactivity. The sudden, renewed activity of hot springs in residential areas can, for example, have a radical impact. This has happened recently (2000–2001) in Rotorua, New Zealand, where house owners awoke to find hot springs and mudpools in what used to be their back gardens, and a hydrothermal eruption (February 2001) sent mud 100 m into the air and created a pool 10 m in diameter in a local park.

Conclusions

Terrestrial hydrothermal environments occupy a small percentage of the Earth's surface but they provide a wealth of information on both geological and biological processes. These environments cover almost the entire spectrum of pH (0 to >10 in some alkaline hot springs) and water temperature range, and it is commonly possible to examine alkaline and highly acidic environments within a few kilometers of each other. For the geologists, they provide clues to mineral–water reactions occurring below the Earth's surface and the origin of some ore minerals. For the biologists, they provide opportunities to study a wide range of extremophiles in natural environments that, with care and permission, are generally accessible. With the discovery of possible siliceous hydrothermal deposits and highly acidic environments on Mars, the scientific interest in these environments is currently high and is likely to continue.

Bibliography

- Bargar, K. E., 1978. Geology and thermal history of Mammoth Hot Springs, Yellowstone National Park, Wyoming. *United States Geological Survey Bulletin*, **1444**.
- Barns, S. M., Fundyga, R. E., Jeffries, M. W., and Pace, N. R., 2004. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 1609–1613.
- Baxter, P. J., Baubron, J. C., and Coutinho, R., 1999. Health hazards and disaster potential of ground gas emissions at Furnas volcano, São Miguel, Azores. *Journal of Volcanology and Geothermal Research*, **92**, 95–106.
- Blank, C. E., Cady, S. L., and Pace, N. R., 2002. Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park. *Applied and Environmental Microbiology*, **68**, 5123–5135.
- Bock, G. R., and Goode, J. A. (eds.), 1996. *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)* (CIBA Foundation Symposium 202). Chichester: Wiley.
- Bogie, I., Lawless, J. V., Rychagov, S., and Belousov, V., 2005. Magmatic-related hydrothermal systems: classification of the types of geothermal systems and their ore mineralization. In Rychagov, S. (ed.), *Geothermal and Mineral Resources of Modern Volcanism Areas (Proceedings of the International Kuril-Kamchatka field workshop, July 16–August 6, 2005)*. Moscow: Óotiskó, pp. 51–73.
- Brock, T. D., 1978. *Thermophilic Organisms and Life and High Temperature*. New York: Springer.
- Browne, P. R. L., and Lawless, J. V., 2001. Characteristics of hydrothermal eruptions, with examples from New Zealand and elsewhere. *Earth-Science Reviews*, **52**, 299–331.
- Bryan, T. S., 2005. *Geysers. What They Are and How They Work*, 2nd edn. Missoula, MT: Mountain Press Publishing Co.
- Bryan, T. S., 2008. *The Geysers of Yellowstone*, 4th edn. Boulder, CO: University Press of Colorado.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Research*, **54**, 289–316.
- Craig, H., 1963. The isotopic geochemistry of water and carbon in geothermal areas. In Tongiorgi, E. (ed.), *Nuclear Geology in Geothermal Areas*. Pisa: Consiglio Nazionale delle Ricerche, Laboratorio di Geologia Nucleare, pp. 17–53.
- Cusicanqui, H., Mahon, W. A. J., and Ellis, A. J., 1976. The geochemistry of the El Tatio Geothermal Field, Northern Chile. In *Proceedings of the 2nd United Nations Symposium on Geothermal Fields*. Berkeley, CA, pp. 703–711.
- De Ronde, C. E. J., Stoffers, P., Garbe-Schöberg, D., Christenson, B. W., Jones, B., Manconi, R., Browne, P. R. L., Hissmann, K., Botz, R., Davy, B. W., Schmitt, M., and Battershill, C. N., 2002. Discovery of active hydrothermal venting in Lake Taupo, New Zealand. *Journal of Volcanology and Geothermal Research*, **115**, 257–275.
- Ellis, A. J., and Mahon, W. A. J., 1977. *Chemistry and Geothermal Systems*. New York: Academic Press.
- Fernandez-Turiel, J. L., Garcia-Valles, M., Gimeno-Torrente, D., Saavedra-Alonso, J., and Martinez-Anent, S., 2005. The hot-springs and geyser sinters of El Tatio, Northern Chile. *Sedimentary Geology*, **180**, 125–147.
- Giggenbach, W. F., 1994. Magma degassing and mineral deposition in hydrothermal systems along convergent plate boundaries. *Economic Geology*, **94**, 1193–1212.
- Glover, R. B., 1965. *Changes in the Wairakei Geyser Valley Springs 1962–1965*. Open File Report, Department of Scientific and Industrial Research, New Zealand.
- Heiken, G., 1982. Geology of geothermal systems. In Edwards, L. M., Chilingar, G. V., Rieke, H. H., and Fertl, W. H. (eds.), *Handbook of Geothermal Energy*. Houston, TX: Gulf Publishing, pp. 177–217.
- Henley, R. W., and Ellis, A. J., 1983. Geothermal systems, ancient and modern: a geochemical review. *Earth-Science Reviews*, **19**, 1–50.
- Jones, B., and Renaut, R. W. (eds.), 2003a. Sedimentology of hot spring systems. *Canadian Journal of Earth Sciences*, **40** (Special Issue), 1439–1738.
- Jones, B., and Renaut, R. W., 2003b. Hot spring and geyser sinters: the integrated product of precipitation, replacement, and deposition. *Canadian Journal of Earth Sciences*, **40**, 1549–1569.
- Jones, B., and Renaut, R. W., 2006. Selective mineralization of microbes in Fe-rich precipitates (jarosite, hydrous ferric oxides) from acid hot springs in the Waitotapu geothermal area, North Island, New Zealand. *Sedimentary Geology*, **194**, 77–98.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1997. Biogenicity of silica precipitation around geysers and hot-spring vents,

- North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2000. Stromatolites forming in acidic hot-spring waters, North Island, New Zealand. *Palaos*, **15**, 450–475.
- Jones, B., Renaut, R. W., Rosen, M. R., and Ansdell, K. M., 2002. Coniform stromatolites from geothermal systems, North Island, New Zealand. *Palaos*, **17**, 84–103.
- Jones, B., Renaut, R. W., Torfason, H., and Owen, R. B., 2007. The historical development of Geysir, Iceland. *Journal of the Geological Society (London)*, **164**, 1241–1252.
- Koch, I., Feldmann, J., Wang, L., Andrewes, P., Reimer, K. J., and Cullen, W. R., 1999. Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. *The Science of the Total Environment*, **236**, 101–117.
- Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J. S., Codd, G. A., and Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology*, **43**, 141–148.
- Kühn, M., 2004. *Reactive Flow Modeling of Hydrothermal Systems*. Berlin: Springer.
- Lloyd, E. F., and Keam, R. F., 1974. Trinity terrace hydrothermal eruption, Waimangu, New Zealand. *New Zealand Journal of Science*, **17**, 511–528.
- Nicholson, K., 1993. *Geothermal Fluids: Chemistry and Exploration Techniques*. Berlin: Springer.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer.
- Pentecost, A., Jones, B., and Renaut, R. W., 2003. What is a hot spring? *Canadian Journal of Earth Sciences*, **40**, 1443–1446.
- Pirajno, F., 1992. *Hydrothermal Mineral Deposits*. Berlin: Springer.
- Renaut, R. W., and Jones, B., 1997. Controls on aragonite and calcite precipitation in hot spring travertines at Chemurkeu, Lake Bogoria, Kenya. *Canadian Journal of Earth Sciences*, **34**, 801–814.
- Renaut, R. W., and Jones, B., 2000. Microbial precipitates around continental hot springs and geysers. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 187–195.
- Renaut, R. W., and Owen, R. B., 2005. The geysers of Lake Bogoria, Kenya Rift Valley, Africa. *GOSA Transactions*, **9**, 4–18.
- Reysenbach, A. L., Voytek, M., and Mancinelli, R. (eds.), 2001. *Thermophiles: Biodiversity, Ecology and Evolution*. New York: Springer.
- Robb, F., Antranikian, G., Grogan, D., and Driessen, A. (eds.), 2007. *Thermophiles: Biology and Technology at High Temperatures*. London: Taylor and Francis.
- Rodgers, K. A., Cook, K. L., Browne, P. R. L., and Campbell, K. A., 2002. The mineralogy, texture and significance of silica derived from alteration by steam condensate in three New Zealand geothermal fields. *Clay Minerals*, **37**, 299–322.
- Sturchio, N. C., Dunkley, P. N., and Smith, M., 1993. Climate-driven variations in geothermal activity in the northern Kenya Rift Valley. *Nature*, **362**, 233–234.
- Torfason, H., 1985. *The Great Geysir*. Reykjavík: Geysir Conservation Committee.
- Walter, M. R., 1976. Geysers of Yellowstone National Park: an example of abiogenic “stromatolites”. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 87–112.
- Wiegel, J., 2007. *Thermophiles: The Keys to Molecular Evolution and the Origin of Life?* London: Taylor and Francis.
- Xu, R. H., Yuan, H. H., and Fan, A., 1995. Endemic fluorosis in China from ingestion of food immersed in hot spring water. *Bulletin of Environmental Contamination and Toxicology*, **54**, 337–341.

Cross-references

[Astrobiology](#)
[Extreme Environments](#)
[Hot Springs and Geysers](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Marine](#)
[Sinter](#)

HYPERHALINE ENVIRONMENTS

Please refer to “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Salinity History of the Earth’s Ocean](#),” “[Saline Lakes](#),” “[Soda Lakes](#).”

GEYSERITE

A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some hot springs. See entries “*Sinter*” and “*Hot Springs and Geysers*” for further reading.

GLASS

See entry “*Basalt (Glass, Endoliths)*.”

GLAUCOPHYTES

Glaucoephytes (glaucoecystophytes or glaucoecystids) are colorless freshwater protists containing plastids derived from primary endosymbiosis (see also entry *Symbiosis*) that is, they are still surrounded by a peptidoglycane wall, which may be viewed as reminiscent of their cyanobacterial origin. Previously, these primitive plastids were also called “cyanelles.” For details, please refer to entry “*Algae (Eukaryotic)*.”

Cross-references

Algae (Eukaryotic)

GOLD

Veit-Enno Hoffmann
University of Göttingen, Göttingen, Germany

Synonyms

Aurum (Latin)

Definition

Precipitation/Bioaccumulation of gold is the precipitation of fine-grained gold colloids by microbial activities.

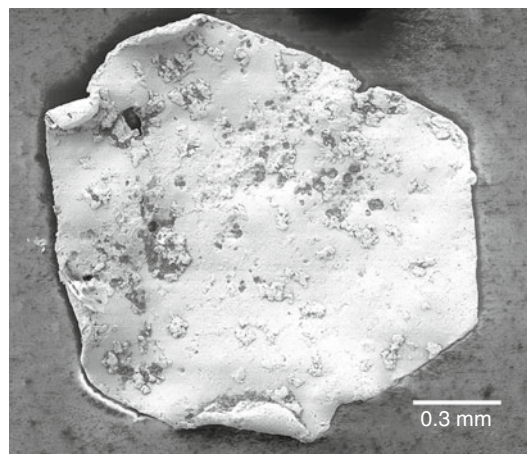
Nugget. A natural gold grain (>5 mm, >200 mg).

The impact of the use of microbial activity for the solution and precipitation of gold was undervalued for a long period, thus affording the opportunity for economic and environmentally friendly gold bio-processing. Furthermore, the current high price of gold has led to intensive research into the precipitation of gold.

Some iron and sulfur oxidizers (e.g., *Acidothiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) contribute to the disintegration of important gold ores (Nordstrom and Southam, 1997). In an environment of gold-bearing rocks, the dissolved gold content is 40 times higher than in comparable non-gold-bearing areas (Benedetti and Boulègue,

1991). It has been shown that the dissolution of gold is dominated by the gold thiosulfate complex $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ which is composed of the above-mentioned bacteria and Actinomycetes (e.g., *Streptomyces fradiae*) (Nordstrom and Southam, 1997; Sand et al., 2001). However, $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ is only stable until the sulfur-oxidation is complete (Lengke and Southam, 2005). Subsequently the gold thiosulfate complex is degraded by *A. thiooxidans*. Thereby gold accumulates in the form of fine-grained colloids inside the bacterial cell and on the surface of the cell membrane. Comparable with the results of Southam and Beveridge (1996) with *Bacillus subtilis* 168, hexagonal-octahedral gold crystals are generated out of the colloids after some months (Figure 1).

As a result of the toxicity of an increasing content of ionized gold, most of the microorganisms that can generate gold die on accumulation of gold. However, various microorganisms, such as bacteria (e.g., *Escherichia coli*, *Pseudomonas maltophilia*, *Plectonema boryanum*), Archaea (e.g., *Pyrococcus furiosus*), Actinomycetes (e.g., *Streptomyces cinamonensis*), fungi (e.g., *Neurospora sitophila*, *Fusarium oxysporum*) and yeasts (e.g., *Endomycopsis fibuligera*, *Sporobolomyces salmonicolor*) from different environments (e.g., hydrothermal, supergene), are partially resistant to the toxicity induced by the accumulation of gold (Kashefi et al., 2001; Nakajima, 2003; Lengke et al., 2006). These bioaccumulation processes are related to various microbial activities (e.g., detoxification, energy generation, passive adsorption). In the laboratory, *Pseudomonas aeruginosa* biofilms demonstrate a higher resistance to the toxicity of gold than planktonic *P. aeruginosa* cells and form solid gold at the base of the biofilms (Karthikeyan and Beveridge, 2002). Reith et al. (2006) isolated a 16SrDNA sequence from natural samples. This sequence was only found on gold grains



Gold, Figure 1 A secondary electron (SE) image of a hexagonal gold crystal from the secondary gold deposit Silberkuhle near Korbach, Germany.

GEYSERITE

A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some hot springs. See entries “*Sinter*” and “*Hot Springs and Geysers*” for further reading.

GLASS

See entry “*Basalt (Glass, Endoliths)*.”

GLAUCOPHYTES

Glaucoephytes (glaucoecystophytes or glaucoecystids) are colorless freshwater protists containing plastids derived from primary endosymbiosis (see also entry *Symbiosis*) that is, they are still surrounded by a peptidoglycane wall, which may be viewed as reminiscent of their cyanobacterial origin. Previously, these primitive plastids were also called “cyanelles.” For details, please refer to entry “*Algae (Eukaryotic)*.”

Cross-references

Algae (Eukaryotic)

GOLD

Veit-Enno Hoffmann
University of Göttingen, Göttingen, Germany

Synonyms

Aurum (Latin)

Definition

Precipitation/Bioaccumulation of gold is the precipitation of fine-grained gold colloids by microbial activities.

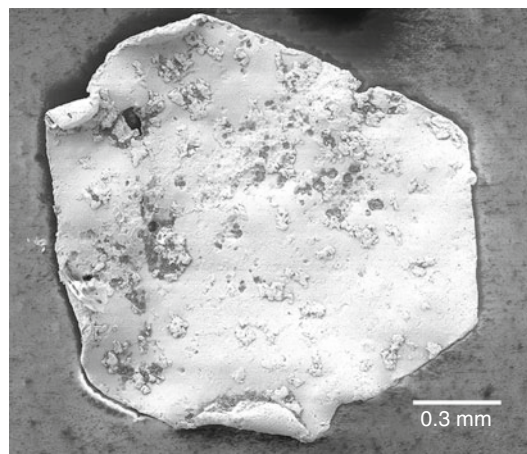
Nugget. A natural gold grain (>5 mm, >200 mg).

The impact of the use of microbial activity for the solution and precipitation of gold was undervalued for a long period, thus affording the opportunity for economic and environmentally friendly gold bio-processing. Furthermore, the current high price of gold has led to intensive research into the precipitation of gold.

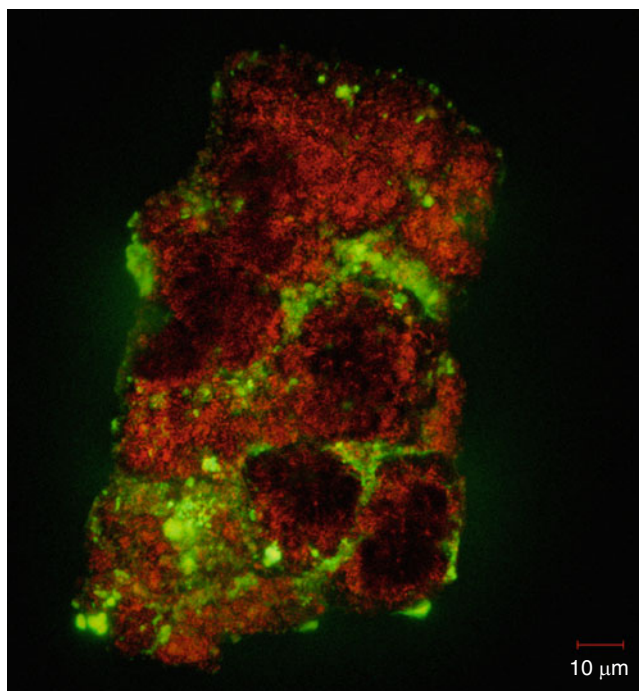
Some iron and sulfur oxidizers (e.g., *Acidothiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) contribute to the disintegration of important gold ores (Nordstrom and Southam, 1997). In an environment of gold-bearing rocks, the dissolved gold content is 40 times higher than in comparable non-gold-bearing areas (Benedetti and Boulègue,

1991). It has been shown that the dissolution of gold is dominated by the gold thiosulfate complex $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ which is composed of the above-mentioned bacteria and Actinomycetes (e.g., *Streptomyces fradiae*) (Nordstrom and Southam, 1997; Sand et al., 2001). However, $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ is only stable until the sulfur-oxidation is complete (Lengke and Southam, 2005). Subsequently the gold thiosulfate complex is degraded by *A. thiooxidans*. Thereby gold accumulates in the form of fine-grained colloids inside the bacterial cell and on the surface of the cell membrane. Comparable with the results of Southam and Beveridge (1996) with *Bacillus subtilis* 168, hexagonal-octahedral gold crystals are generated out of the colloids after some months (Figure 1).

As a result of the toxicity of an increasing content of ionized gold, most of the microorganisms that can generate gold die on accumulation of gold. However, various microorganisms, such as bacteria (e.g., *Escherichia coli*, *Pseudomonas maltophilia*, *Plectonema boryanum*), Archaea (e.g., *Pyrococcus furiosus*), Actinomycetes (e.g., *Streptomyces cinamonensis*), fungi (e.g., *Neurospora sitophila*, *Fusarium oxysporum*) and yeasts (e.g., *Endomycopsis fibuligera*, *Sporobolomyces salmonicolor*) from different environments (e.g., hydrothermal, supergene), are partially resistant to the toxicity induced by the accumulation of gold (Kashefi et al., 2001; Nakajima, 2003; Lengke et al., 2006). These bioaccumulation processes are related to various microbial activities (e.g., detoxification, energy generation, passive adsorption). In the laboratory, *Pseudomonas aeruginosa* biofilms demonstrate a higher resistance to the toxicity of gold than planktonic *P. aeruginosa* cells and form solid gold at the base of the biofilms (Karthikeyan and Beveridge, 2002). Reith et al. (2006) isolated a 16SrDNA sequence from natural samples. This sequence was only found on gold grains



Gold, Figure 1 A secondary electron (SE) image of a hexagonal gold crystal from the secondary gold deposit Silberkuhle near Korbach, Germany.



Gold, Figure 2 Laser scanning micrograph (ex. 488 nm, em. 543 nm) of microorganisms (green) on a gold grain (red) from the Eder River, Germany. Eubacteria labeled by oligonucleotide probe EuB-CY3.

and not in the surrounding sediments. With a similarity of 99%, the sequence correlated to *Cupriavidus (Ralstonia) metallidurans*. Ninety percent of the bacteria in the laboratory experiment died because of the toxicity of AuCl_4^- . The surviving cells adapted themselves to the high concentration of Au(III) ions and precipitated gold. In addition to morphological studies (Bischoff, 1997), this provides evidence for bioaccumulation of secondary gold (e.g., nuggets, gold grains) by bacteria like *C. metallidurans* (Figure 2).

The basal biochemical microbial processes of the biomineralization of gold are not completely understood yet. Precipitation of gold provides the cellular defense of *C. metallidurans* and is controlled by a coupling of efflux, reduction and possibly methylation of gold complexes (Reith et al., 2009). Reith et al. (2007) have published a comprehensive review of the geomicrobiology of gold.

Summary

Different microorganisms are partially resistant to dissolved gold. Various microbial activities precipitate gold. Biofilms of *Cupriavidus metallidurans* accumulate gold naturally and contribute to the formation of secondary gold.

Bibliography

Benedetti, M., and Boulègue, J., 1991. Mechanism of gold transfer and deposition in a supergene environment. *Geochimica et Cosmochimica Acta*, **55**, 1539–1547.

- Bischoff, G. C. O., 1997. The biological origin of bacterioform gold from Australia. *Neues Jahrbuch Geologische Paläontologische Abhandlungen*, **H6**, 329–338.
- Karthikeyan, S., and Beveridge, T. J., 2002. *Pseudomonas aeruginosa* biofilms react with and precipitate toxic soluble gold. *Environmental Microbiology*, **4**(11), 667–675.
- Kashefi, K., Tor, J. M., Nevin, K. P., and Lovley, D. R., 2001. Reductive precipitation of gold by dissimilatory Fe(III) -reducing bacteria and archaea. *Applied and Environmental Microbiology*, **67**(7), 3275–3279.
- Lengke, M. F., and Southam, G., 2005. The effect of thiosulfate-oxidizing bacteria on the stability of the gold–thiosulfate complex. *Geochimica et Cosmochimica Acta*, **69**(15), 3759–3772.
- Lengke, M. F., Ravel, B., Fleet, M. E., Wanger, G., and Southam, G., 2006. Mechanisms of gold bioaccumulation by filamentous cyanobacteria from gold(III)–chloride complex. *Environmental Science & Technology*, **40**(20): 6304–6309.
- Nakajima, A., 2003. Accumulation of gold by microorganisms. *World Journal of Microbiology*, **19**, 369–374.
- Nordstrom, D. K., and Southam G., 1997. Geomicrobiology of sulfide mineral oxidation. *Reviews in Mineralogy and Geochemistry*, **35**, 361–390.
- Reith, F., Rogers, S., McPhail, D. C., and Webb, D., 2006. Biomineralization of gold: biofilms on bacterioform gold. *Science*, **313**, 333–336.
- Reith, F., Lengke, M. F., Falconer, D., Craw, D., and Southam, G., 2007. The geomicrobiology of gold. *ISME Journal*, **1**, 567–584.
- Reith, F., Etschmann, B., Grosse, C., Moors, H., Benotmane, M. A., Monsieurs, P., Grass, G., Doonan, C., Vogt, S., Lai, B., Martinez-Criado, G., George, G. N., Nies, D. H., Mergeay, M., Pring, A., Southam, G., and Brugger, J., 2009. Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(1), 17757–17762.
- Sand, W., Gehrke, T., Jozsa, P.-G., and Schippers, A., 2001. (Bio) chemistry of bacterial leaching – direct vs. indirect bioleaching. *Hydrometallurgy*, **59**, 159–175.
- Southam, G., and Beveridge, T. J., 1996. The occurrence of sulfur and phosphorus within bacterially derived crystalline and pseudocrystalline octahedral gold formed in vitro. *Geochimica et Cosmochimica Acta*, **60**(22), 4369–4376.

Cross-references

[Algae \(Eukaryotic\)](#)
[Archaea](#)
[Bacteria](#)
[Biofilms](#)
[Fungi and Lichens](#)
[Microbial Biomineralization](#)
[Ores, Microbial Precipitation and Oxidation](#)

GONDWANALAND, FORMATION

Joseph G. Meert
 University of Florida, Gainesville, FL, USA

Definition

Gondwanaland or “Gondwana” is the name for the southern half of the Pangaeon supercontinent that existed some 300 million years ago. Gondwanaland is composed of the major continental blocks of South America, Africa,

H

HABITAT

The physical location or dwelling place of a particular organism, where an individual ecotype can be found or isolated from. See entry “*Microbial Communities, Structure, and Function*” for further reading.

HALOBACTERIA – HALOPHILES

Helga Stan-Lotter
University of Salzburg, Salzburg, Austria

Definition

Halobacteria (plural), *halobacterium* (singular). Colloquial for halophilic archaeobacteria (or archaea), which are single-celled life forms. Synonyms: Haloarchaea (plural), haloarchaeon (singular).

Halobacterium. The name of a genus of the archaeal family *Halobacteriaceae* (Grant, 2001).

Halophilic. Requires a salt-rich environment for growth and survival.

Introduction

Saline waters dominate the earth, with the oceans holding 97% of the planet's water (a total of 1.338×10^9 km³; <http://ga.water.usgs.gov/edu/earthwherewater.html>). Saline inland seas, groundwater, and saltwater lakes hold another 0.9–0.94%, which exceeds the volume of the world's available freshwater ($\sim 1.1 \times 10^7$ km³). The definition of a hypersaline environment is one that possesses a salt concentration greater than that of seawater (>3.5% w/v). Water-containing environments are usually described as thalassohaline or athalassohaline (see

Chapters “*Saline Lakes*,” “*Soda Lakes*”). Thalassohaline waters are marine-derived and therefore contain, at least initially, the seawater composition; with increasing evaporation, the concentration of various salts is changing. Athalassohaline waters may also receive influx of seawater; however, the chemical composition is mainly determined by geological, geographical, and topographical parameters.

Interactions between halophilic microorganisms and geological materials have been recognized early in the history of mankind and continue to impact human endeavors, including the exploration of energy sources, the storage of waste, the search for extraterrestrial life (see Chapter “*Deep Biosphere of Salt Deposits*”). For example, the evaporation of seawater for the purpose of making salt and the concomitant development of red color of brines, which is due to pigmented microorganisms, have been described already 2,700 years ago (Oren, 2002). It was also observed early that the presence of microorganisms in solar salterns enhances the precipitation and the yield of salt (Oren, 2002). Halite, the mineral of sodium chloride, primarily forms from the evaporation of seawater. Subsurface deposits of halite occur in many areas and are far more abundant than was previously recognized. The advent of widespread oil drilling revealed a frequent association of ancient salt deposits and the presence of the biogenic energy sources, oil and methane (Sassen et al., 1994). The potential impact of viable halophilic microorganisms in salt deposits (see Chapter “*Deep Biosphere of Salt Deposits*”), some of which are being used as storage sites for long-lived radioactive isotopes such as transuranic wastes, has been the focus of much research. The striking discovery of extraterrestrial halite in meteorites (see Chapter “*Deep Biosphere of Salt Deposits*”) and recently, in salt pools on Mars (Osterloo et al., 2008) make a search for halophilic microorganisms on other planets or moons plausible.

Saline environments and their inhabitants

Organisms occur within a range of salinities, from distilled water to saturated salt solutions, and may be loosely classified as non-, slightly, moderately, or extremely halophilic, requiring about 0.2 M, 0.2–0.85 M, 0.85–2.5 M, and 2.5–5.2 M NaCl, respectively, for growth. The largest saline environment, seawater, contains 31–38 g of total dissolved salts per liter, nearly 80% of which is NaCl. Hypersaline environments are characterized by higher salinities than seawater. The oceans are inhabited by a great diversity of organisms; when seawater is evaporating, the biological diversity decreases with increasing concentration of salt. Macroscopic organisms such as salt-tolerant fish (e.g., *Cyprinodon variegatus* and *Menidia beryllina*) are no longer found at concentrations of more than about 11% salt (1.88 M NaCl); copepods (fleas), turbellarian worms, and some rotifers tolerate 15–17% salt. Interestingly, several invertebrates such as brine shrimp (*Artemia salina*) or brine flies (*Ephydra cinerea*) are capable of survival at NaCl concentrations of 30–33% (~5 M; Javor, 1989). However, the dominating organisms in saturated brines are extremely halophilic microorganisms which grow optimally in the presence of 2.5–5.2 M NaCl and are generally unable to grow in less than 1.7 M NaCl. They can occur at such high cell densities (up to 10^8 colony forming units per milliliter) that they cause brines to turn bright red or purplish. Figure 1 shows a commercial salt-producing facility where seawater is evaporating in a series of ponds. The colors of the pools are caused by carotenoid pigments, which are contained in the membranes of haloarchaea, or by the chlorophylls of halophilic algae or cyanobacteria. Typical haloarchaea in such communities are species of the genera *Halobacterium*, *Halorubrum*, and *Halococcus*, halophilic bacteria such as *Ectothiorhodospira halochloris*, *Salinibacter ruber*, cyanobacteria (e.g., *Aphanothece halophytica*, *Phormidium* sp. and *Schizothrix arenaria*), and green algae (e.g., *Dunaliella salina* and *Asteromonas*

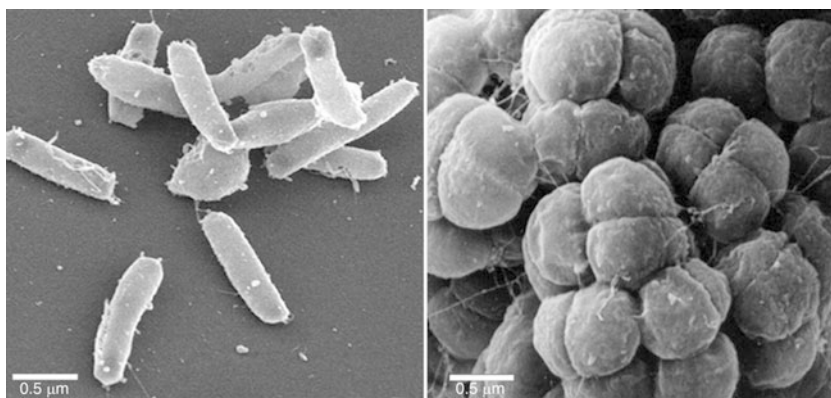
gracilis). Figure 2 shows two characteristic haloarchaeal morphologies – *Halobacterium salinarum* NRC-1 grows as single rod-shaped cells; *Halococcus salifodinae* cells are roundish and tend to aggregate into large clusters.

Crude solar salt contains large numbers of viable bacteria and archaea (about 10^5 – 10^6 colony forming units per milliliter; Oren, 2002). When using this salt for the preservation of fish, meat, and hides, damage in the form of red discoloration of the goods was noted under conditions of moisture and elevated temperatures. These observations started the early research on halophilic microorganisms in the 1920s–1930s. Only much later – in the 1980s – was it recognized that most halophiles from such sources belonged to the archaea. The haloarchaea are not pathogenic; in fact, some are used in traditionally fermented foods in Asia for human consumption (Oren, 2002).

There exist other hypersaline environments which are not directly derived from seawater and are called athalassohaline (see above and Chapter “Soda Lakes”); often they are remnants of prehistoric inland lakes, such as the Dead Sea and the Great Salt Lake. Additional larger lakes include Mono Lake in California, soda lakes in Egypt and China, Lake Magadi in Kenya, and Lake Eyre in Australia. The chemical composition varies depending on the geology of the surroundings, but overall salinity is usually between 200 and 340 g/l of dissolved salts. Soda lakes are an example of naturally occurring alkaline environments which may also be hypersaline, and are characterized by the presence of large amounts of sodium carbonate and correspondingly minute concentrations of Ca^{2+} and Mg^{2+} . The pH of alkaline lakes is usually in the range of 10–11; in some evaporation ponds, it can rise up to 12 (Oren, 2002). Microbial isolates from alkaline hypersaline lakes include the archaea *Natronomonas* sp., *Natronococcus* sp., *Natrialba* sp., and bacteria of the genera *Halomonas*, *Chromohalobacter*, *Spirulina* (the latter is known as nutrient for humans and animals) and others still to be characterized (Javor, 1989; Oren, 2002).



Halobacteria – Halophiles, Figure 1 Lagoons of a commercial salt-producing facility in Namibia, close to the ocean. Red-colored lagoons contain mainly haloarchaea; green color is due to halotolerant algae or cyanobacteria.



Halobacteria – Halophiles, Figure 2 Scanning electron micrograph of cells of *Halobacterium salinarum* NRC-1 (left) and *Halococcus salifodinae* DSM8989T (right). Bars, 0.5 μm. (Photographs were taken by C. Frethem (left) and G. Wanner (right).)

From the Deep Lake in the Vestfold Hills of Antarctica, a psychrophilic archaeon, *Halorubrum lacusprofundi*, was isolated. Deep Lake has a salinity that is ten times higher than that of seawater (3.6–4.8 M). The lake remains ice-free throughout the year, with water temperatures fluctuating between +10 and –15°C and remaining below 0°C for ~8 months of the year. The microbial diversity in Deep Lake is extremely low and is dominated by haloarchaea, with apparently low numbers of *Dunaliella* sp. and bacteria (of the beta- and gamma-proteobacteria) present in the sediment (Cavicchioli, 2006).

Less explored and unusual hypersaline habitats are represented by anoxic brines near the bottom of the sea, for example in the Mediterranean deeps, which contain saline brines almost saturated with magnesium chloride, and where a wide diversity of novel archaea and bacteria was detected (van der Wielen et al., 2005).

Another environment for halophilic microorganisms are ancient salt deposits; recent reports have described the presence of haloarchaea in Triassic and Permian salts (summarized in McGenity et al., 2000; Stan-Lotter et al., 2004). These viable microorganisms were consistently isolated from surface-sterilized crystals obtained from freshly blasted rock salt or deep drilling bore cores, suggesting that these were survivors from the original depositional event (see Chapter “*Deep Biosphere of Salt Deposits*”).

Finally, halophilic viruses have been detected, which were released from bacteria and archaea; in addition, free virus particles were found by electron microscopical examination of the Dead Sea and in Spanish saltern ponds (Oren, 2002). The viruses (also called bacteriophages) were equally dependent as their hosts on high concentrations of salt for their structural stability. There is little information on the role of viruses in natural environments; suggestions which were made involve ecological functions in controlling the sizes of microbial communities (Oren, 2002). A website with pictures of haloviruses from

the laboratory of M. Dyll-Smith is available: <http://www.microbiol.unimelb.edu.au/people/dyallsmith/research/haloviruses/>

Classification and genomics

Halophilic means salt-loving and this term, often as the prefix hal- or halo-, has been in use since the 1930s. Therefore, members of the bacteria as well as the separate prokaryotic group archaea, which was identified only in the 1970s, may contain such prefixes in their names. Some confusion may arise from this nomenclatural convention, since, for example, the genus with the name *Halobacterium* belongs to the halophilic archaea (or haloarchaea), not to the bacteria.

The number of recognized haloarchaeal genera has increased to 26, according to the International Committee on Systematics of Prokaryotes (<http://www.the-icsp.org>) and the number of validated species at this time is 86. Main taxonomic criteria for the identification and recognition of haloarchaea are the sequence of the 16S rRNA genes and the composition of their characteristic membrane polar lipids, which are unique ether-linked phosphoglycerides (see Fendrihan et al., 2006, for a recent overview). The complete list of required and recommended criteria for the formal identification and recognition of haloarchaeal species was proposed by Oren et al. (1997). For halophilic bacteria belonging to the large family *Halomonadaceae*, a similar list of specific characteristics, which has been revised recently, can be consulted (Arahal et al., 2007).

Five genomes of haloarchaea have been completely sequenced, that of *Halobacterium salinarum* NRC-1, the related strain *Halobacterium salinarum* R1, *Haloarcula marismortui*, *Natronomonas pharaonis*, and *Haloquadratum walsbyi*, and one genome of a halophilic bacterium (*Salinibacter ruber*). The website <http://www.ncbi.nlm.nih.gov/sites/entrez> lists all genome projects, whether complete or still in progress, including references

and detailed descriptions. Comparisons of genomes continue to provide insights about similarity and differences between archaea and bacteria and about the early evolution of prokaryotes. They have, for example, proven that the adaptation mechanism of keeping a high internal salt concentration (see next paragraph) is not unique to archaea, as was thought before, but is used also by *Salinibacter ruber*; this common property as well as some other characteristics suggests the possibility of early lateral gene transfers between prokaryotes (Velieux et al., 2007).

Adaptations to high salinity

Most halophiles are unable to survive outside their high-salt native environments. Some species of the haloarchaea are so fragile that when placed in distilled water they lyse within minutes from the change in osmotic conditions (Grant, 2001). This reflects a general adaptation of the cells' entire intracellular machinery to high concentrations of cations. Halophilic and halotolerant organisms have to expend energy to exclude salt from their cytoplasm to avoid protein aggregation ("salting out"). Two different strategies are known to be used to prevent desiccation through osmotic movement of water out of their cytoplasm, and both work by increasing the internal osmolarity of the cell. In the first, organic compounds, termed "compatible solutes," are accumulated in the cytoplasm; this strategy has been found in many halophilic bacteria and also in some archaea, yeasts, algae, and fungi (Galinski, 1995). Compatible solutes are neutral or zwitterionic and include amino acids, sugars, polyols, betaines, and ectoines, as well as derivatives of some of these compounds; they can be synthesized de novo or taken up from the environment (Galinski, 1995; Grant, 2004). The second adaptation involves the selective import of K^+ ions into the cytoplasm, which can reach internal concentrations of 5 M. This adaptation is restricted to the moderately halophilic bacterial order *Halanaerobiales*, the haloarchaeal family *Halobacteriaceae*, and the extremely halophilic bacterium *Salinibacter ruber* (Oren, 2002). Most proteins of haloarchaea and also of *Salinibacter* contain a large excess of acidic amino acids (glutamate and aspartate) and a low content of basic amino acids (lysine and arginine; Lanyi, 1974; Oren, 2002); these features are thought to represent a specific adaptation to high levels of salt (Mevarech et al., 2000; Oren, 2002). In contrast, the action of compatible solutes is much less specific, with little or no adjustment of intracellular macromolecules required, which is in line with the observation that these compounds act as general stress protectants against heating, freezing, and drying (see Oren, 2002, and references therein).

Conclusions

Halophilic organisms occur in all major groups on the tree of life (see Oren, 2007) and even halophilic viral representatives are known. Halophilic microorganisms in particular

may be found on earth in almost every hypersaline environment – tropical to polar, terrestrial to submarine, acidic to alkaline, or aerobic to anaerobic (Javor, 1989). Initially a little-studied group of microorganisms, halophilic bacteria and archaea have proven to be of interest for life sciences and earth sciences as well; their investigation has resulted in major insights for molecular biology, genomics, biogeochemistry, and – most recently – astrobiology.

Bibliography

- Arahal, D. R., Vreeland, R. H., Litchfield, C. D., Mormile, M. R., Tindall, B. J., Oren, A., Bejar, V., Quesada, E., and Ventosa, A., 2007. Recommended minimal standards for describing new taxa of the family Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2436–2446.
- Cavicchioli, R., 2006. Cold-adapted archaea. *Nature Reviews. Microbiology*, **4**, 331–343.
- Fendrihan, S., Legat, A., Gruber, C., Pfaffenhuemer, M., Weidler, G., Gerbl, F., and Stan-Lotter, H., 2006. Extremely halophilic archaea and the issue of long term microbial survival. *Reviews in Environmental Science and Bio/technology*, **5**, 1569–1605.
- Galinski, E., 1995. Osmoadaptation in bacteria. *Advances in Microbial Physiology*, **37**, 272–328.
- Grant, W. D., 2001. Genus I. *Halobacterium* Elazari-Volcani 1957, 207, ^{AL} emend. Larsen and Grant 1989, 2222. In Boone, D. R., Castenholz, R. W., and Garrity, G. M. (eds.), *Bergey's Manual of Systematic Bacteriology*, 2nd edn. New York: Springer, Vol. I, pp. 301–305.
- Grant, W. D., 2004. Life at low water activity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **359**, 1249–1266.
- Javor, B. J., 1989. *Hypersaline Environments: Microbiology and Biogeochemistry*. Berlin: Springer.
- Lanyi, J. K., 1974. Salt-dependent properties of proteins from extremely halophilic bacteria. *Bacteriological Reviews*, **38**, 272–290.
- McGenity, T. J., Gemmell, R. T., Grant, W. D., and Stan-Lotter, H., 2000. Origins of halophilic microorganisms in ancient salt deposits (MiniReview). *Environmental Microbiology*, **2**, 243–250.
- Mevarech, M., Frolov, F., and Gross, I. M., 2000. Halophilic enzymes: proteins with a grain of salt. *Biophysical Chemistry*, **86**, 155–164.
- Oren, A., 2002. *Halophilic Microorganisms and Their Environments*. Dordrecht, The Netherlands: Kluwer.
- Oren, A., 2007. Biodiversity in highly saline environments. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 223–231.
- Oren, A., Ventosa, A., and Grant, W. D., 1997. Proposed minimal standards for description of new taxa in the order Halobacteriales. *International Journal of Systematic Bacteriology*, **47**, 233–236.
- Osterloo, M. M., Hamilton, V. E., Bandfield, J. L., Glotch, T. D., Baldrige, A. M., Christensen, P. R., Tornabene, L. L., and Anderson, F. S., 2008. Chloride-bearing materials in the southern highlands of Mars. *Science*, **319**, 1651–1654.
- Sassen, R., Cole, G. A., Drozd, R., and Roberts, H. H., 1994. Oligocene to holocene hydrocarbon migration and salt dome carbonates, Northern Gulf of Mexico. *Marine and Petroleum Geology*, **11**, 55–65.
- Stan-Lotter, H., Radax, C., McGenity, T. J., Legat, A., Pfaffenhuemer, M., Wieland, H., Gruber, C., and Denner, E. B. M., 2004. From intraterrestrials to extraterrestrials – viable haloarchaea in ancient salt deposits. In Ventosa, A. (ed.), *Halophilic Microorganisms*. Berlin: Springer, pp. 89–102.

van der Wielen, P. W., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., D'Auria, G., de Lange, G. J., Huebner, A., Varnavas, S. P., Thomson, J., Tamburini, C., Marty, D., McGenity, T. J., and Timmis, K. N., and BioDeep Scientific Party, 2005. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, **307**, 121–123.

Velieux, F., Madern, D., Zaccai, G., and Ebel, C., 2007. Molecular adaptation to high salt. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 240–253.

Cross-references

Archaea
Astrobiology
Deep Biosphere of Salt Deposits
Extreme Environments
Hypersaline Environments, Terrestrial
Saline Lakes
Soda Lakes

HAPTOPHYTES

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “*Symbiosis*”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinctive lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “*Biomarkers (Molecular Fossils)*”). For further details, please see entry “*Algae (Eukaryotic)*.”

HEAVY METALS

Please refer to entries “*Acid Rock Drainage*,” “*Arsenic*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Copper*,” “*Geomycology*,” “*Metalloenzymes*,” “*Metals, Acquisition by Marine Bacteria*,” “*Ores, Microbial Precipitation and Oxidation*,” “*Zinc*.”

HISTOLOGY

Michael Gudo¹, Gerta Fleissner², Guenther Fleissner²

¹Morphisto Evolutionsforschung und Anwendung GmbH, Institut für Evolutionswissenschaften, Frankfurt am Main, Germany

²Goethe-University Frankfurt, Frankfurt a. M., Germany

Definition

Histology is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms.

Biopsy is the sample of an organism or part of a tissue that is processed for histological investigations.

Fixation serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

Mineralization is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

Staining is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

Introduction

The term histology was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intra- and extracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kiernan, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are

van der Wielen, P. W., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., D'Auria, G., de Lange, G. J., Huebner, A., Varnavas, S. P., Thomson, J., Tamburini, C., Marty, D., McGenity, T. J., and Timmis, K. N., and BioDeep Scientific Party, 2005. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, **307**, 121–123.

Velieux, F., Madern, D., Zaccari, G., and Ebel, C., 2007. Molecular adaptation to high salt. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 240–253.

Cross-references

Archaea
Astrobiology
Deep Biosphere of Salt Deposits
Extreme Environments
Hypersaline Environments, Terrestrial
Saline Lakes
Soda Lakes

HAPTOPHYTES

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “*Symbiosis*”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinctive lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “*Biomarkers (Molecular Fossils)*”). For further details, please see entry “*Algae (Eukaryotic)*.”

HEAVY METALS

Please refer to entries “*Acid Rock Drainage*,” “*Arsenic*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Copper*,” “*Geomycology*,” “*Metalloenzymes*,” “*Metals, Acquisition by Marine Bacteria*,” “*Ores, Microbial Precipitation and Oxidation*,” “*Zinc*.”

HISTOLOGY

Michael Gudo¹, Gerta Fleissner², Guenther Fleissner²

¹Morphisto Evolutionsforschung und Anwendung GmbH, Institut für Evolutionswissenschaften, Frankfurt am Main, Germany

²Goethe-University Frankfurt, Frankfurt a. M., Germany

Definition

Histology is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms.

Biopsy is the sample of an organism or part of a tissue that is processed for histological investigations.

Fixation serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

Mineralization is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

Staining is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

Introduction

The term histology was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intra- and extracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kiernan, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are

van der Wielen, P. W., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., D'Auria, G., de Lange, G. J., Huebner, A., Varnavas, S. P., Thomson, J., Tamburini, C., Marty, D., McGenity, T. J., and Timmis, K. N., and BioDeep Scientific Party, 2005. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, **307**, 121–123.

Velieux, F., Madern, D., Zaccari, G., and Ebel, C., 2007. Molecular adaptation to high salt. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 240–253.

Cross-references

Archaea
Astrobiology
Deep Biosphere of Salt Deposits
Extreme Environments
Hypersaline Environments, Terrestrial
Saline Lakes
Soda Lakes

HAPTOPHYTES

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “*Symbiosis*”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinctive lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “*Biomarkers (Molecular Fossils)*”). For further details, please see entry “*Algae (Eukaryotic)*.”

HEAVY METALS

Please refer to entries “*Acid Rock Drainage*,” “*Arsenic*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Copper*,” “*Geomycology*,” “*Metalloenzymes*,” “*Metals, Acquisition by Marine Bacteria*,” “*Ores, Microbial Precipitation and Oxidation*,” “*Zinc*.”

HISTOLOGY

Michael Gudo¹, Gerta Fleissner², Guenther Fleissner²

¹Morphisto Evolutionsforschung und Anwendung GmbH, Institut für Evolutionswissenschaften, Frankfurt am Main, Germany

²Goethe-University Frankfurt, Frankfurt a. M., Germany

Definition

Histology is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms.

Biopsy is the sample of an organism or part of a tissue that is processed for histological investigations.

Fixation serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

Mineralization is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

Staining is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

Introduction

The term histology was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intra- and extracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kiernan, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are

processed by various histochemical and biophysical techniques according to the aim of the study.

Geobiological samples usually do not consist of organic soft material, alone, like the above mentioned biological materials, but they are often composites of hard inorganic and mineralized organic material, with nearly all biological components converted to inorganic matter. Some geobiological samples are compositions of both, organic and inorganic materials, such as bio films growing on hard substrates, or sessile organisms that adhere to hard substrates like corals, sponges, lichens, or fungi. These samples therefore can seldom be histologically processed like biological material via paraffin embedding and sectioning with traditional microtomes. Special procedures and mechanical devices (like saw microtomes, annular saws or cutting/grindings systems) are necessary to produce sections of geobiological samples (e.g., Hoffmann et al., 2003). By means of special polishing machines, like the ultramill or lapping machines, sections or slices of these sections can be studied by distinct microscopical methods, for example under fluorescent light, X-ray-mappings and topographic element analyses (Janssens et al., 2000) can identify the outline of the biological compounds and their environment, and they may also serve as a biomarker; harsh biochemical treatment can isolate the organic from the inorganic components, but often destroy the original context. Histological analyses may provide tools to identify the biological components even if their former structural context has decayed (Wrede et al., 2008), or help to analyze particular processes or compositions (Neuweiler et al., 2007). Principally these results must be “calibrated” by means of histological investigations of recent tissues in order to understand organ structure and its function (Young and Heath, 2000).

This entry reviews the state of the histological methods and offers the modifications necessary to topographically investigate geobiological samples. *For clarity, this latter information is given in italics.*

Methods

Different from biochemical investigations in the broadest sense, histological analyses are concerned with samples, where the structures and their topographical context are preserved or known before biopsies are taken and only in a controlled way modified by procedures according to the specific aim of the study.

For histological processing of tissues or any other organic compounds, all vital functions have to be stopped by fixation, in order to preserve a distinct structure within or more often isolated from its natural environment. *In geobiology this process often is “history,” but must be known to understand the given results of further analyses.* Usually, the biological samples are embedded into a special medium which provides particular mechanical properties that allow sectioning in the appropriate thickness. The sections are then placed on glass slides or cover

slips, afterwards stained by special chemicals and dyes that have affinities to particular tissues or histological structures. The staining allows the differentiation of cell structures, various types of cell surface and skeleton assemblies, filaments or fibrillar components (e.g., collagenous fibers, elastic fibers, or reticular fibers), tissues, and even complete organs or pathological and genetic modifications of the various structures. Under the microscope the results can be observed and – most often after a thorough comparison to reference samples – evaluated and interpreted.

In geobiology, the selective staining of minerals prevails, although protein staining has been successful in various studies. The major problem in geobiology is that mineralized samples are too hard for conventional section procedures and therefore special preparation methods are necessary (see also below, paragraph “Embedding”). Accordingly, often only the surface of sectioned samples is accessible to the staining which limits the differentiations of components. Structural analyses of biological compounds have to refer to similar structures or organs histologically known from recent specimen. Therefore, histology in geobiology has to refer to a biological histology (Robenek, 1995). Specialized methods are available which either demineralize the sample for showing the biological contents or dissolve the organic leftovers to investigate their “casts” in the inorganic surroundings. Often the resulting histologically processed specimens can be biophysically investigated, and topographic X-ray element mappings (Janssens et al., 2000) are often the method of choice to serve as biomarkers.

Fixation and infiltration

The process of fixation and infiltration of biological specimens is most crucial for the results and aims to stop any autolytic and bacterial decomposition. The most commonly used fixative is formalin; however, other fixatives (e.g., Bouin, Carnoy, Paraformaldehyde, potassium dichromate, chromic acid, or mercury-fixation) are necessary depending on the planned subsequent protocol. The choice of the fixative determines the results, since every fixative has assets and drawbacks for the preservation of particular histological structures and especially the binding sites for differential staining, e.g., in immunohistology. For biophysical analyses, the metal-free fixatives and also metal-free pre- and post-processing protocols are essential.

The fixation process, which is a chemical reaction, has to be controlled and stopped after a particular time that depends on type and size of the tissue, but also on environmental factors like temperature and air pressure. Afterwards the fixative must be removed following a special protocol, which takes care not to destroy structural characteristics (Böck, 1989).

Prior to embedding, the sample has to be slowly transferred to the final substance via several intermediate steps mixable with the respective embedding medium, e.g.,

for the paraffin/wax embedding dehydrants (ethanol, isopropanol, and methanol) and clearants (xylene, toluene, and xylene-replacing clearants).

In case of aerated tissues, it may be necessary to perfuse the sample with a prefix and process fixation and embedding continuously in a vacuum with fluid movement for a proper impregnation of all layers of the specimen.

In geobiology, fixation and conservation also plays a crucial role, since these chemical processes have to be balanced between the necessities for the mineralized and organic components of a geobiological sample (biofilms or water containing fossils; e.g., Desrochers et al., 2007). Every fixative generates chemical reactions, which sometimes may degrade or destroy either the organic or the mineralized components. Accordingly, empirical studies have to be performed before crucial samples are processed in order to ascertain whether the fixative may cause any problems for either the soft or hard tissues of a particular type of samples.

Embedding

Embedding of histological material is performed in order to provide a mechanical stable and hard entity, which can be sectioned to various coherent thin sections. The sections should enable a spatial reconstruction of the original sample after differentiating analyses. The composition of the specimen and the histological program are decisive for the embedding methods of choice. The most common material for tissue embedding is paraffin (wax-embedding), also embedding in celloidin and resin. If the tissues under study are more or less soft (which means without any calcareous or other mineralized structures), the paraffin embedding is the best choice. Celloidin embedding takes much time and is useful for very sophisticated questions (e.g., embedding of complete brains or very large organs). Plastic embeddings are the best choice if the biopsies contain mineralized structures, such as calcium carbonate, calcium phosphate, and silicate, or if organismic components adhere to rocks or other hard substrates, like for example biofilms.

Therefore, these latter embedding methods lend themselves for geobiological samples, which then can be processed like biological material. For resin embedding (Howat et al., 2007) one can choose between epoxy-embedding, methyl-methacrylate-embedding, or glycol-methacrylate-embedding. Various products are commercially available (Obenauf et al., 2007), all of them have well-documented assets and drawbacks. The most drawbacks of plastic embeddings can be seen in their high costs of time and money. On the other hand, resin embedded samples can be sectioned, sawed, milled, or polished by use of various microtomes, saws, milling, or polishing machines.

Sectioning

Whole mount samples can only be investigated from their outer surface. Analyses inside the samples need dissection

with preferably minor destruction of the structural context. In general, penetration of staining chemicals is enhanced afterwards, and microscopic and biophysical inspection is improved as the spatial resolution corresponds to the thickness of the section. Several problems may arise from inappropriate section procedures:

- The samples may be too brittle and break. This effect is reduced by pre-treatment procedures like decalcification or demineralization, also choosing another type of embedding medium may help possibly combined with different section techniques, e.g., a vibrating knife or sample cooling by a special refrigerant device.
- The sections are heavily distorted. A proper selection of the microtome, cutting angle, and cutting velocity may improve the result. The sections may stretch, for example, after floating on a warm water bath about 10°C below the melting point of the embedding wax.
- The requested thickness requires different blades, which cannot be used “universally,” even if section series of varying thickness are necessary for the protocol. For example, thick sections need a hard metal blade, while semithin- and ultrathin sections can be produced only by means of glass or diamond knives.

Biological samples, which are embedded in paraffin or resin, can generally be sectioned by a special device, a microtome. Some microtomes are equipped with a motor-driven support, both for controlling the section thickness as well as the force of the blade. Several types of microtomes are available, each optimized for variably embedded samples: Paraffin embedded samples can be sectioned by sled microtomes or rotary microtomes equipped with metal knives. Resin embedded samples need microtomes which have a special retraction mechanism, to avoid mechanical damage of the knife and the sample. The blades are either manufactured of especially hardened steel for sections between 5 and 30 µm, for semi thin sections (2–5 µm) and ultra thin sections (about 40–100 nm) glass or diamond knives must be used.

Hard materials, like mineralized geobiological probes, cannot be sectioned by conventional microtomes unless the samples have been pre-treated: decalcification or demineralization by different chemicals. While these procedures may cause uncontrolled damage, when applied to the unsectioned samples, preferably control material should be preserved to evaluate the original structural context. Therefore, careful special section devices like diamond milling, saw microtomes, annular saws, or section/grinding-systems especially optimized for this purpose should be applied. About 30 µm thick sections of rather large samples can be achieved by these methods. The sections or grinded pieces can then be further processed, and also partly preserved for subsequent evaluation.

Another method, available since several years, is the laser microtome which may contact free isolate minute samples out of the otherwise untreated entity. The samples must neither be pre-treated nor embedded. Section thickness ranges between 10 and 100 µm. Special

devices allow even for the isolation of minute tissue pieces like a single cellular nucleus.

A satisfying section quality requires a long time of experience especially to decide for the most appropriate procedure. While continuously new embedding products are commercialized, only little has changed concerning the basic techniques of standard microtomy, which is taught as a component part of the education of laboratory and technical assistants.

Mounting

For further histological processing, the sections must be mounted, most often on glass slides or on grids coated with a special synthetic film.

The major difficulty is the choice of the best possible sticking procedures in order to prevent both, a floating resp. a loss of the sections during the staining protocol and undesired background staining by the glue. While before often unreliable “home-made” coating procedures, e.g., by chromic sulphate or polylysine, were used, now various types of pretreated glass slides are commercially available, they have been optimized for selected histological procedures. Thick sections, e.g., sections that are produced by saw microtomes or cutting/grinding-systems, have to be glued to object slides by special sticking resins that, e.g., harden with ultraviolet light; in this aspect, histological sample preparation is quite similar to the preparation of geological thin ground-sections.

Biophysical analyses of geobiological samples requires mounting according to the planned technique (see list of Handbooks of the suppliers). In, for example, X-ray investigations (micro fluorescence analyses), the mounting must be free of auto fluorescence; the sometimes long lasting measuring intervals need a stable non-vibrating mechanical stability.

Staining

Multiple histological staining methods allow identifying cellular structures, tissue components, and organ systems, also enzymatic processes and involved macromolecules. It is even possible to clearly differentiate between organic and inorganic material as well as decaying from living material by selective marking of minerals and molecules.

Stainings can be performed by use of natural stains and dyes or by chemicals often designed for certain projects. New fields of histological analyses have been launched by immunohistochemical stainings combined with fluorescent markers which are very selectively binding to particular proteins. Differential and double stainings, also section series with alternatively stained sections, have revealed important insight into basic cellular processes, information processing, and also pathological resp. genetic changes. The standard protocols are well documented including their modifications for the application to tissues of various origins.

For any staining it is necessary that the structures that should be stained are free of any embedding media. This means that for standard-histology the paraffin has to be removed by special procedures so that the staining chemicals can react with the tissues. If samples are embedded in resins, this process is more crucial and difficult; several resins cannot be removed and accordingly only the surface of the section provides reactive structures for staining.

The staining of cellular structures and tissues is based on chemical affinities of the stains with particular tissues resp. tissue components. In many cases, a particular pretreatment is necessary for adequate results. Pretreatments may be, for example, mordants, which modify the stain so that it reacts with the tissue or modifies the tissues so that the stain can react with the tissue. In some cases mordants generate a reaction that transformed the water soluble stain into a water- and alcohol-stable lacquer. This chemical reaction can, for example, be induced by metal ions like tungsten or molybdenum or by alkali ions like lithium, sodium, or barium (Böck, 1989; Burck, 1988).

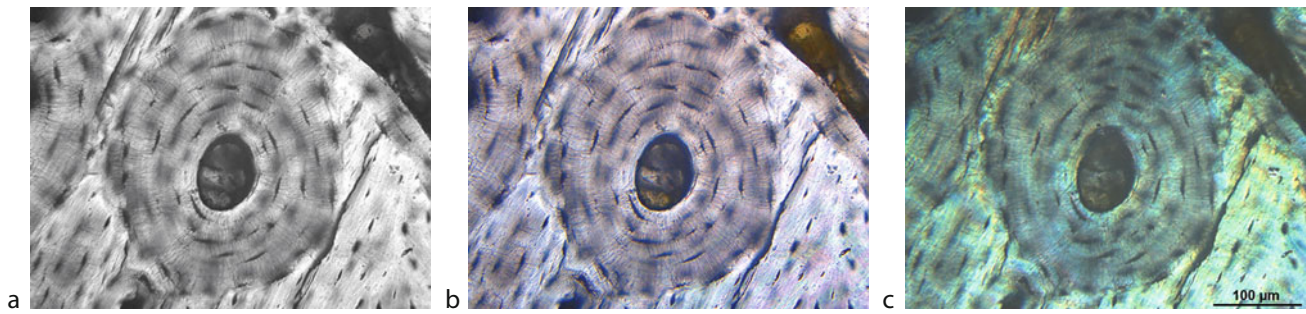
Stainings are usually composed of three steps: in the first step cellular structures like nuclei, etc., are stained. In the second step cell plasma and some extracellular structures are stained, and in the third step the surrounding matrix is stained.

As a result of the staining, the nuclei, cells, various fibers, and other structures or chemical components in a tissue have particular colors that allow differentiation and several diagnostics. Some stainings also provide particular optical characteristics to tissues; they may for example provide polarizing effects to the tissue. Other procedures provide fluorescence-effects or particular ultra-violet-effects of the polished surface and allow further investigations (Figures 1 and 2).

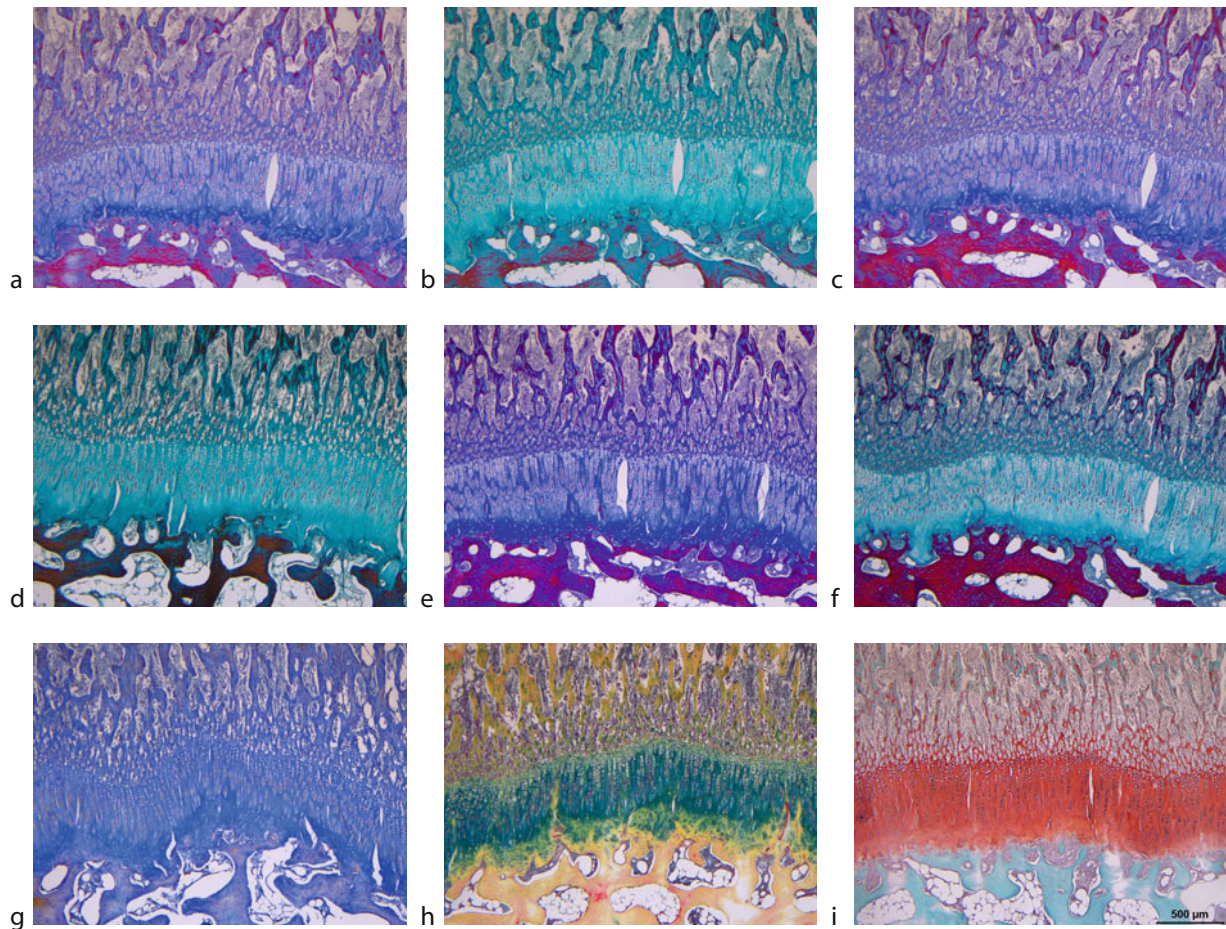
While traditional stainings induce a chemical reaction within tissues and may stain in an unselective way (Horobin and Kiernan, 2002), immunohistology (Polak and Noorden, 2003) selectively marks specific epitopes, proteins, and molecules bound to membrane or subcellular structures. Traditional and immunohistochemical analyses under the light microscope meet the limits of resolution at magnifications of about 1,200 $\times$; therefore, immunohistological methods have been upgraded for analyses under the TEM, either by pre-embedding or post-embedding staining protocols.

While traditional stainings are used for topographic and micro anatomical investigations, immunohistological stainings are used to understand the functional context of molecular processes and to allocate the regions in which these processes take place in an organism or in a tissue.

These refined immunohistological staining methods have also been applied to sections of geobiological samples. The analyses could demonstrate biological compounds, thus serving as biomarkers, and in some cases even disclose the genetic origin of the fossilized organisms.



Histology, Figure 1 Cross section of a human long bone under different microscopic optics (thin-ground section of a non-demineralized sample). (a) Under through light with minimal color saturation; (b) same illumination with maximum color saturation; (c) differential interference contrast (After Nomarski). Already without staining procedures characteristic structural details and varying densities can be identified. (a–c same magnification.)



Histology, Figure 2 Section series through a chondral ossification zone of a demineralized porcine long bone after various differential histological staining procedures (Protocols according to Böck et al., 1989; Young and Heath, 2000). (a) Masson Goldner A; (b) Masson Goldner L; (c) Masson Goldner ResF A; (d) Masson Goldner ResF L; (e) Crossmon A; (f) Crossmon L; (g) Azan after Geidis; (h) Movat Pentachrome; (i) Safranin Fastgreen. The layers of young bone (upper area), cartilage (middle area), and secondary ossification area (below) can be analyzed concerning their different structural details (a–i same magnification).

Conclusion

Geobiology is defined as field of science which analyses the interaction of the environment with living matter. Mainly fossilized samples are investigated concerning their systematic relationship and – even more – the origin of this specific form of life. Research results can find their application not only in a better understanding of our life on earth, but also in exploring putative extraterrestrial biology. Another aspect is the transfer of basic sciences to various aspects of biomedical research like the synthesis or degradation of bones and teeth or the control of biogenic mineralization processes. All these research topics cannot be planned and performed or adequately evaluated without a thorough knowledge of the biological structures mainly based on the comparison of living and fossilized specimen. Histology delivers the methods to topographically analyze organisms, organs, tissues, and even molecules. Their modifications by environmental or biogenic processes can be recognized by the investigation of their complex interaction.

Perspectives

Histological investigations provide a huge field of research for biology and geobiology. Both fields have to be combined, since geobiological samples often can only be interpreted with reference to known biological samples. Some examples are histological sections of dinosaur bones or teeth that have to be compared to bones and teeth of recent organisms, others are the discoveries at special fossil-sites, like the Messel pit, the Burgess-Shale, the rocky Hunsrück region, etc., which may provide interesting information about fossilized microscopic structures. And even the investigation of recent samples like biofilms or coatings is of major relevance to understand their composition and emergence in order to discriminate between biogenic and abiotic origin.

Summary

Histology provides methods to topographically investigate the structure of cells, tissues, and organs down to the molecular level, especially in geobiology as reference to recognize, for example, the phylogenetic origin and age and also as a basis to analyze biological in comparison to bio geological turnover processes, which induce structural changes via environmental and climatic conditions.

Bibliography

- Bancroft, J. D., and Gamble, M. (eds.), 2007. *Theory & Practice of Histological Techniques*, 6th edn. Edinburgh: Churchill Livingstone.
- Böck, P. (ed.), 1989. *Romeis Mikroskopische Technik*. München/Wien/Baltimore: Urban & Schwarzenberg.
- Bozzola, J. J., and Russell, L. D., 1999. *Electron Microscopy*, 2nd edn. Boston: Johns and Bartlett.

- Burck, H. C., 1988. *Histologische Technik*, 6th edn. Stuttgart: Thieme.
- Desrochers, A., Bourque, P.-A., and Neuweiler, F., 2007. Diagenetic versus biotic accretionary mechanisms of bryozoan-sponge buildups (Lower Silurian, Anticosti Island, Canada). *Journal of Sedimentary Research*, **77**, 564–571.
- Gerlach, D. (ed.), 1998. *Die Anfänge der histologischen Färbung und der Mikrophotographie – Josef von Gerlach als Wegbegleiter*. Thun und Frankfurt am Main: Verlag Harri Deutsch.
- Hoffmann, F., Janussen, D., Dröse, W., Arp, G., and Reitner, J., 2003. Histological investigation of organisms with hard skeletons: a case study of siliceous sponges. *Biotechnic and Histochemistry*, **78**(3–4), 191–199.
- Horobin, R. W., and Kiernan, J. A., 2002. *Conn's Biological Stains*, 10th edn. Oxford: Bios Scientific.
- Howat, W. J., Warford, A., Mitchell, J. N., Clarke, K. F., Conquer, J. S., and McCafferty, J., 2007. Resin tissue microarrays: a universal format for immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **55**, 21–24.
- Janssens, K. H. A., Adams, F. C. V., and Rindby, A. (eds.), 2000. *Microscopic X-ray Fluorescence Analysis*. Chichester: Wiley.
- Mayer, C., 1819. *Ueber Histologie*. Bonn. Cited in: Gräfe, C. F., Hufeland, C. W., Link, H. F., Rudolphi, K. A., Siebold, E. v. (eds.), 1828. *Encyclopädisches Wörterbuch der medicinischen Wissenschaften*, Band 2. Berlin: J.W. Boike.
- Mulisch, M., and Welsch, U. (eds.), 2010. *Romeis Mikroskopische Technik*. Heidelberg: Spektrum Akademischer Verlag.
- Neuweiler, F., Daoust, I., Bourque, P. A., and Burdige, D. J., 2007. Degradative calcification of a modern siliceous sponge from the Great Bahama Bank, The Bahamas: a guide for interpretation of ancient sponge-bearing limestones. *Journal of Sedimentary Research*, **77**, 552–563.
- Obenauf, R. H., Martin, J., Bostwick, R., et al. (eds.), 2007. *Handbook of Sample Preparation and Handling*, 10th edn. Metuchen: SPEX Certiprep.
- Palm, L. C., and Snelders, H. A. M. (eds.), 1983. *Antoni van Leeuwenhoek 1632–1723*. Studies on the life and work of the Delft scientist commemorating the 350th anniversary of his birthday. Amsterdam: Rodopi.
- Polak, J. M., and Van Noorden, S., 2003. *Introduction to Immunocytochemistry*, 3rd edn. Oxford: Bios Scientific.
- Robenek, H. (ed.), 1995. *Mikroskopie in Forschung und Praxis*. Darmstadt: Git Verlag.
- Ruzin, S. E., 1999. *Plant Microtechnique and Microscopy*. Oxford: Oxford University Press.
- Schüler, D. (ed.), 2006. *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold seeps. *Journal of Microbiological Methods*, **73**, 85–91.
- Young, B., and Heath, J. W., 2000. *Weather's Functional Histology – A Text and Colour Atlas*. Edinburgh: Churchill Livingstone.

Cross-references

[Animal Skeletons, Advent](#)
[Animal Biocalcification, Evolution](#)
[Biofilms](#)
[Biomarkers \(Molecular Fossils\)](#)
[Calcification](#)
[Calcified Cyanobacteria](#)

Carbonate Environments
 Fluorescence-In-Situ-Hybridization (FISH)
 Immunolocalization
 Metalloenzymes
 Metals, Acquisition by Marine Bacteria
 Microbial Biomineralization
 Microbial Communities, Structure, and Function
 Microbial Mats
 Organomineralization
 Scanning Probe Microscopy (Includes Atomic Force Microscopy)
 Sponges (Porifera) and Sponge Microbes

HOT SPRINGS AND GEYSERS

Brian Jones¹, Robin W. Renaut²

¹University of Alberta, Edmonton, Alberta, Canada

²University of Saskatchewan, Saskatoon, SK, Canada

Definition

A *hot spring* is a discharge of hot (>35 – 40°C) water from a vent at the Earth's surface.

A *geyser* is a hot spring characterized by intermittent, turbulent discharges of boiling water and steam.

A *sublacustrine hot spring* is a hot spring that discharges from the floor of a lake.

Introduction

A hot spring is characterized by discharge of hot water from a vent. There is, however, no universally accepted definition of “hot” and the temperature for distinguishing a “warm spring” from a “hot spring” remains contentious (Pentecost et al., 2003). In general usage, a hot spring is one with vent water temperature between about 40°C and boiling point (Renaut and Jones, 2000). It must be remembered, however, that boiling temperature changes with altitude; thus, boiling in Yellowstone National Park occurs at $\sim 92^{\circ}\text{C}$, whereas in New Zealand geothermal areas, which lie closer to sea level, it is at $\sim 100^{\circ}\text{C}$.

The term “geyser” is derived from “Geysir,” located in southwest Iceland. First mentioned in historical records from 1294, the Icelandic word “geysir” means “gusher” or “one who rages.” Accordingly, a geyser is regarded as hot spring that is characterized by intermittent, turbulent discharges of boiling water and steam (Bryan, 2005, 2008).

Geysers and hot springs are end members of a continuous spectrum that includes “intermittent springs” that experience periodic overflows but never erupt, and “pulsating springs,” “perpetual spouters,” or “ebullient springs” that are characterized by continual eruptions. Some hot springs discharge on the floors of lakes and in caves.

Distribution

Hot springs and geysers are found on every continent except mainland Antarctica (Waring, 1965). Many hot springs and geysers, known from historical records, have ceased activity because of natural and anthropogenically induced changes to their plumbing systems. Yellowstone National Park, USA., El Tatio located high in the Andes of northern Chile, the Taupo Volcanic Zone on the North Island of New Zealand, Kronotski National Park in Kamchatka in eastern Russia, and Iceland are the largest known geothermal fields with numerous hot springs and geysers. Bryan (2008), for example, estimated that more than 50% (~ 500) of known geysers are located in Yellowstone National Park, with another 385 in the other four major geothermal areas. Low-enthalpy hot springs that discharge at maximum temperatures of about 35 – 60°C are much more common.

The plumbing system

The hot water discharged by a hot spring or geyser is the final, surface expression of a complex system that involves water, a heat source, and an intricate underground plumbing system. The water is derived from meteoric waters that have seeped deep into the Earth's crust, supplemented in some regions by connate, magmatic, or metamorphic waters. Irrespective of its origin, some spring or geyser water may come from depths of ~ 4 km where it has been magmatically heated to temperatures of $>225^{\circ}\text{C}$. In other settings, however, meteoric waters may only penetrate to shallow depths before being expelled from a low-enthalpy spring vent. The plumbing system of a hot spring is unrestricted and allows continuous movement of water to the surface. In contrast, a geyser has a restriction within its plumbing system, commonly a sinter caprock that precludes continuous vertical flow. The configurations of these plumbing systems remain unknown because of the difficulties in accessing them. Nevertheless, schematic depictions of vast underground, water-filled chambers are probably incorrect. Instead, it seems that the plumbing systems are formed of complex networks of faults, fractures, small cavities, and strata with variable permeability. Geysers are less common than hot springs because of the special plumbing system requirements.

Water trapped below a restriction in a geyser plumbing system will be heated by steam that is moving upwards from deeper parts of the system. The reservoir will then empty as the trapped superheated water is violently discharged. Such an eruption will continue until the reservoir is emptied. The magnitude, frequency, and duration of the eruption depend on reservoir size, the rate of heat transfer, and the rate at which water refills the system once emptied by the eruption. By necessity, therefore, geysers can only exist if the bedrock can withstand the pressures created by the steam and if the bedrock is permeable enough to allow rapid reservoir replenishment.

- anaerobic spring-waters of Champagne Pool, North Island, New Zealand. *Journal of the Geological Society of London*, **158**, 895–911.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Pentecost, A., Jones, B., and Renaut, R.W., 2003. What is a hot spring? *Canadian Journal of Earth Sciences*, **40**, 1443–1446.
- Kvist, T., Mengewein, A., Manzei, S., Ahring, B. K., and Westermann, P., 2005. Diversity of thermophilic and non-thermophilic crenarchaeota at 80°C. *FEMS Microbiology Ecology*, **244**, 61–68.
- Renaut, R. W., and Jones, B., 1997. Controls on aragonite and calcite precipitation in hot spring travertines at Chemurkeu, Lake Bogoria, Kenya. *Canadian Journal of Earth Sciences*, **34**, 801–814.
- Renaut, R. W., and Jones, B., 2000. Microbial precipitates around continental hot springs and geysers. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 187–195.
- Sheehan, K. B., Patterson, D. J., Dicks, B. L., and Henson, J. M., 2005. *Seen and Unseen. Discovering the Microbes of Yellowstone*. Guilford, CT: The Globe Pequot Press.
- Stetter, K. O., 1996. Hyperthermophilic prokaryotes. *FEMS Microbiology Reviews*, **18**, 149–158.
- Walter, M. R., 1976. Geysers of Yellowstone National Park: an example of abiogenic stromatolites. In Walter, M. R. (ed.), *Stromatolites: Developments in Sedimentology*. Amsterdam, Elsevier, pp. 87–112.
- Waring, G. A., 1965. Thermal springs of the United States and other countries of the world – a summary. *United States Geological Survey Professional Paper*, **492**, 1–383.

Cross-references

[Extreme Environments](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Biomineralization](#)
[Microbial Silicification – Bacteria \(or Passive\)](#)
[Sinter](#)

HYDROGEN

Tori M. Hoehler
 NASA-Ames Research Center, Moffett Field, CA, USA

Synonyms

Dihydrogen; H₂

Definition

A diatomic molecule consisting of two atoms of hydrogen or its heavier isotopes.

Introduction

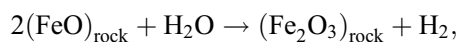
Hydrogen, the most abundant element in the universe by mass and number, exists on Earth principally in combination with oxygen (in H₂O) or carbon (in hydrocarbon and

organic compounds). The diatomic form, H₂ – although present in only trace abundance in the modern oceans and atmosphere – has played a central and evolving role in prebiotic and origin of life chemistry, in linking geochemical and biological cycles, and in shaping the structure, function, and interaction of microbial populations.

Geochemistry of H₂

Geosphere

H₂ is produced by the interaction of water and rocks through a variety of mechanisms (see review in Hoehler, 2005). Among processes that occur in widespread crustal rocks, perhaps the best known and most quantitatively important involves the reduction of water to H₂ by the ferrous iron components of rocks, which can be generalized as



with (FeO)_{rock} representing the ferrous iron component of igneous rock and (Fe₂O₃)_{rock} representing the ferric iron component of alteration minerals (e.g., magnetite). The capacity for H₂ generation via this mechanism is greatest in minerals with high ferrous iron contents, such as the ultramafic minerals olivine and pyroxene, where the process is called serpentinization. Basalts, with a lower ferrous iron content, have a smaller capacity to produce H₂, and granites (where ferrous iron-bearing phases are typically present as minor components) smaller still. The rates of hydrogen generation by water–rock reaction depend significantly on both temperature and the exposure of fresh surface area to water.

H₂ may also be generated via the radiolytic cleavage of water by α, β, or γ radiation produced during decay of isotopes in the host rocks (Spinks and Woods, 1964). Although oxidative species generated simultaneously during the process have potential to recombine with the hydrogen, radiolysis is nonetheless believed responsible for elevated H₂ concentrations (up to millimolar levels) in fluid emanations from a number of continental rock units. This mechanism is generally most important for granites, in which the content of the principal radiogenic elements U, Th, and K is generally highest among major crustal rock types.

Fracturing of silicate rocks may also serve to liberate H₂ that is formed either in solid-state reactions involving trace water impurities in the mineral lattice (Freund et al., 2002) or by direct reduction of water at the fractured surface (Kita et al., 1982).

Among these processes, serpentinization offers the greatest potential capacity for H₂ generation (per unit volume of rock), by orders of magnitude. However, multiple factors – including mineral composition, fluid flow and chemistry, temperature regime, and mechanical processing – ultimately determine which mode of lithogenic H₂ production may prevail in a given environment.

Atmosphere

H₂ is a trace component of the modern atmosphere, with an abundance of about 0.5 ppm (Levine, 1985) and a residence time of less than 1 year (Atkinson et al., 1979), but likely higher levels in the early atmosphere would have had important geochemical and biological implications. Atmospheric H₂ abundance reflects a balance of sources and sinks. Major source terms (excluding anthropogenic sources) include geological efflux (e.g., volcanic and hydrothermal vent emissions) and biological production. Sinks include oxidative atmospheric reactions, biological consumption, and escape to space. The low abundance of H₂ in the modern atmosphere reflects the dominance of oxidative reactions and biological consumption. The early, prebiotic Earth largely lacked such sinks and had a geological source term that was expectedly larger as a result of higher rates of geothermal emanation and a potentially more reducing character in the early mantle and crust (leading to higher H₂ concentrations in geothermal emanations). Estimates of atmospheric H₂ abundance at this time depend principally on theories regarding the oxidation state of the early mantle and crust, and consideration of the factors constraining H₂ escape to space. Even at the low end, these estimates generally predict atmospheric H₂ abundance of hundreds to thousands of parts per million, 3–4 orders of magnitude higher than in the present day.

H₂ itself is considered photochemically inert in the lower atmosphere (Levine, 1985), but it participates in a variety of reactions involving the radical products of photolysis. By serving as a sink for the oxidizing OH radical (Atkinson et al., 1979), and influencing the abundance of the atmospheric reductants H, HCO, and H₂CO (Canuto et al., 1983), H₂ directly affects the oxidation state of the atmosphere. As such, its relative abundance in the early atmosphere may have significantly affected redox speciation in the volatile nitrogen, carbon, and sulfur systems. In turn, this balance (e.g., the CH₄/CO₂ ratio, in the carbon system) affects the atmospheric radiation budget (through differing efficiencies of IR absorption), the potential for formation of photochemical hazes (Sagan and Chyba, 1997), and the formation and stability of simple organics in atmospheric chemical processes.

Above and beyond its role in buffering the oxidation state of the atmosphere through chemical interactions, escape of hydrogen to space can contribute to the irreversible oxidation of the planet, by carrying electrons out of the system (Walker, 1977). This process occurs at low rates in the modern, oxidized atmosphere, where the low abundance of H₂ results in low escape rates. However, H₂ escape from an early atmosphere containing orders of magnitude higher H₂ concentrations may have contributed substantially to the overall oxidation of the planet.

H₂ in prebiotic and origin of life chemistry

Prebiotic chemistry

The emergence of life must depend significantly on development of an inventory of abiotically generated compounds

that can serve as building blocks for biochemistry. Two of the most potentially important endogenous sources for such compounds – synthesis in hydrothermal vent settings and in atmospheric chemical processes – are known or inferred to depend strongly on H₂ for the yield and nature of compounds formed. In vent settings, H₂ from water–rock reactions is implicated as the reductant in Fischer–Tropsch type synthesis of methane, higher hydrocarbons, and simple organic acids, and is predicted to support the synthesis of amino acids and other prebiotic compounds. Miller–Urey type spark discharge experiments that simulate atmospheric chemical processes have a similar dependence that is demonstrated experimentally. For example, the yield of amino acids from simpler precursors in such experiments depends directly on the abundance of H₂, regardless of whether reduced or neutral starting materials (i.e., CH₄/NH₃ (Miller and Urey, 1959), or CO₂/N₂ (Schlesinger and Miller, 1983)) are used. Given its significance in prebiotic synthesis and atmospheric redox and photochemistry on the early Earth (see above), H₂ likely played an important overall role in establishing the environmental and chemical context from which life emerged.

Origin of life

Although the specific chemistry leading to the origin of life is not known, H₂ is proposed in some scenarios to have been a key component of the earliest metabolism (Wächtershauser, 1993). The oxidation of H₂ serves a potentially important dual purpose with respect to such metabolism, in yielding both reducing power and H⁺. Reducing power is necessary for generating organic biomolecules when the carbon source is CO₂. The establishment of H⁺ gradients (specifically, across membranes) can be used to store energy for subsequent coupling to biochemistry. Such dual-purpose chemistry underpins the metabolism of a wide range of modern organisms, including those thought to represent Earth's earliest life. The widespread availability of H₂ in geothermal emanations (e.g., Zobell, 1947; Elderfield and Schulz, 1996) and, presumably, in the prebiotic oceans and atmosphere would have made it a ubiquitous and reliable substrate on which to base the first metabolic chemistry.

H₂ and biology

The reversible oxidation of molecular hydrogen, $\text{H}_2 \rightleftharpoons \text{H}^+ + 2\text{e}^-$, is among the most widely utilized reactions in microbial metabolism. Hydrogenases, the enzymes which catalyze this transformation, are present in all three phylogenetic domains of life (Fenchel and Finlay, 1995; Vignais et al., 2001; Schwarz and Friedrich, 2003). They are particularly widely represented in the Bacteria and Archaea, including the most deeply branching and least derived lineages (Vignais et al., 2001; Schwarz and Friedrich, 2003), suggesting that, exclusive of a possible role in origin of life chemistry, H₂ metabolism has been an important contributor to biochemistry since the very early stages of evolution.

Since its emergence in metabolism, H_2 -based redox chemistry has come to be associated with an extremely broad range of microbial metabolic strategies, including oxygenic and anoxygenic phototrophy, anaerobic chemolitho- and chemoheterotrophy, and aerobic chemolithotrophy (Schwarz and Friedrich, 2003). H_2/H^+ can serve as electron donor, acceptor, or both, in these processes. This broad metabolic utility and versatility casts H_2 in a highly central role in the ecology of microbial communities, and in the geochemistry they mediate.

Lithotrophic communities

Organisms capable of H_2 metabolism can potentially be supported directly by geochemical production of H_2 (by, e.g., water–rock reaction or radiolysis). As such, they have potential to exist independently of energy input from light or the direct products of photosynthesis, oxygen, and organic matter. Such communities are therefore frequently envisioned as important contributors to Earth's earliest biosphere, to the rock-hosted subsurface biosphere, and, potentially, to subsurface life on other worlds. H_2 -based, photosynthesis-independent communities have been documented or inferred for a range of systems and H_2 -producing mechanisms, including vent fluids sourced in serpentinizing systems, basalt-hosted aquifers, and continental groundwaters bearing a signal of radiolytic H_2 production.

Heterotrophic communities

Systems driven by anaerobic decomposition of organic matter typically host a great diversity of H_2 -cycling organisms. Under anaerobic conditions, the complete degradation of complex organics cannot be accomplished by a single organism. Instead, the overall process is mediated, in a series of individual steps, by communities of microorganisms having different metabolic capabilities (Schink, 1988). The individual steps are linked through the transfer of electrons from one group of microbes to another, with H_2 serving as one of the most commonly employed molecular carriers of electrons. The pool of H_2 in such systems is typically characterized by low concentrations (sub-nanomolar to micromolar) and rapid turnover, with resultant residence times frequently in the range of seconds (Hoehler et al., 2002). Combined with a strong influence on the thermodynamics of reactions that produce or consume it (resulting from its typically high stoichiometric coefficient in such reactions), this effect casts H_2 as a key regulator of degradation pathways, community structure, and carbon and energy flow in such environments. Specifically, fluctuations in H_2 concentration are documented, in a process known as “interspecies H_2 transfer,” to affect the H_2 -cycling reactions by inhibition or stimulation, alteration of products, or even reversal (see review in Hoehler, 2005).

H_2 thus occupies a central role in anoxic ecosystems. Anoxia prevailed globally prior to about 2.4 billion years ago and continues to prevail in most organic-bearing

aqueous environments (including ocean, lake, and wet-land sediments and animal digestive tracts). In the past and present, such systems represent a major control on the redox chemistry of the oceans and atmosphere, and the ultimate biological filter on material passing into the rock record.

Phototrophic communities

Most of the known photosynthetic microorganisms, representing both anoxygenic and oxygenic photosynthesis, possess a capability to engage in H_2 metabolism (Schwarz and Friedrich, 2003; Vignais et al., 1985). Most anoxygenic photosynthesizers, including representatives of the purple sulfur, purple non-sulfur, green sulfur, and green non-sulfur bacteria, can utilize H_2 as an electron donor for photosynthetic carbon fixation, and several are also capable of utilizing H_2 as an electron donor for the chemical reduction of inorganic oxidants. Some representatives of the cyanobacteria (oxygenic photosynthesizers) are similarly capable when operating in an anoxygenic photosynthetic mode of metabolism. H_2 is produced obligately during the conduct of nitrogen fixation and can also be photo-produced by the nitrogenase enzyme complex independently of nitrogen fixation. These capabilities, which occur widely among the cyanobacteria and anoxygenic photosynthesizers, are among the principal mechanisms being explored for biological production of H_2 as an alternative energy source. H_2 is also generated as a product of fermentation, a capability demonstrated in some cyanobacteria and purple sulfur bacteria. The fermentative mode of H_2 production appears to dominate the H_2 cycle in a variety of cyanobacterial “mats,” resulting in significant fluxes of H_2 out of the system (Hoehler et al., 2001; Hoehler et al., 2002). During the 1–2 billion years of Earth's history in which cyanobacterial mats dominated biological productivity on Earth, such fluxes may have contributed significantly to atmospheric H_2 levels and, by enhanced rates of H_2 escape to space, the overall oxidation of the planet.

Eukarya

Although not as widespread as in the *Bacteria* and *Archaea*, H_2 metabolism is also found in the *Eukarya*. In a variety of deep-branching anaerobic Eukaryotes, metabolism is tied to, or dependent on, an H_2 -based symbiosis with Bacterial or Archaeal partners (see review in Fenchel and Finlay, 1995). The driver of most of these symbioses is an H_2 -generating organelle, the hydrogenosome, which appears in some cases to be derived from a mitochondrion. These hydrogenosomes carry out fermentation, rather than oxidative phosphorylation, of pyruvate – and thereby produce H_2 . Microbial symbionts (which may include methanogens, sulfate reducers, and (in one case) photosynthetic purple non-sulfur bacteria) increase the energetic yield of this fermentation by consuming the end product H_2 . The symbiosis significantly enhances the growth of the host, and is, in some cases, so evolved that methanogen symbionts have lost their cell walls, and exhibit synchronization of cell

division with the host. Among photosynthetic Eukarya, green algae exhibit an active H_2 metabolism, and are considered a promising potential source of “biohydrogen” for alternative energy systems.

Summary

Hydrogen has played an important role in Earth's geochemistry and biology since the earliest stages of the planet's history. H_2 is produced by a variety of geochemical processes and is postulated to have been a significant component of the early, prebiotic atmosphere, where it had direct or indirect effects on atmospheric redox chemistry and radiation budget, and contributed to planetary oxidation by escape to space. Prebiotic and origin of life chemistry may have significantly involved H_2 , and its prominent use among deeply branching organisms in the tree of life reflects, at very least, a key role in metabolism since the biosphere's early stages. In the modern biosphere, H_2 biochemistry is associated with a broad variety of metabolic strategies, in all three domains of life. Although it now represents a trace component of the oceans and atmosphere, the significance of H_2 for planetary chemistry persists through its central role in the microbial world.

Bibliography

- Atkinson, R., Darnall, K. R., Lloyd, A. C., Winer, A. M., and Pitts, J. N. Jr., 1979. Kinetics and mechanisms of the reaction of the hydroxyl radical with organic compounds in the gas phase. *Advances in Photochemistry*, **11**, 375–488.
- Canuto, V. M., Levine, J. S., Augustsson, T. R., Imhoff, C. L., and Giampapa, M. S., 1983. The young sun and the atmosphere and photochemistry of the early Earth. *Nature*, **305**, 281–286.
- Elderfield, H., and Schulz, A., 1996. Mid-ocean ridge hydrothermal fluxes and the chemical composition of the ocean. *Annual Review of Earth Planetary Science*, **24**, 191–224.
- Fenchel, T., and Finlay, B. J., 1995. *Ecology and Evolution in Anoxic Worlds*. Oxford: Oxford University Press.
- Freund, F., Dickinson, J. T., and Cash, M., 2002. Hydrogen in rocks: an energy source for deep microbial communities. *Astrobiology*, **2**, 83–92.
- Hoehler, T. M., 2005. Biogeochemistry of dihydrogen. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Biogeochemical Cycles of Elements*. New York: Marcel Dekker, Vol. 43, pp. 9–48.
- Hoehler, T. M., Bebout, B. M., and DesMarais, D. J., 2001. The role of microbial mats in the production of reduced gases on the early Earth. *Nature*, **412**, 324–327.
- Hoehler, T. M., Albert, D. B., Alperin, M. J., Bebout, B. M., Martens, C. S., and DesMarais, D. J., 2002. Comparative ecology of H_2 cycling in sedimentary and phototrophic ecosystems. *Antonie Van Leeuwenhoek*, **81**, 575–585.
- Kita, I., Masuo, S., and Wakita, A., 1982. H_2 generation by reaction between H_2O and crushed rock: an experimental study of H_2 degassing from the active fault zone. *Journal of Geophysical Research*, **87**, 10789–10795.
- Levine, J. S., 1985. The photochemistry of the early atmosphere. In Levine, J. S. (ed.), *The Photochemistry of Atmospheres*. San Diego, CA: Academic Press, p. 518.
- Miller, S. L., and Urey, H., 1959. Organic compound synthesis on the primitive Earth. *Science*, **130**, 245–251.
- Sagan, C., and Chyba, C., 1997. The early faint sun paradox: organic shielding of ultraviolet-labile greenhouse gases. *Science*, **276**, 1217–1221.
- Schink, B., 1988. Principles and limits of anaerobic degradation. In Zehnder, A. J. B. (ed.), *Biology of Anaerobic Microorganisms*. New York: Wiley-Interscience, pp. 771–846.
- Schlesinger, G., and Miller, S. L., 1983. Prebiotic synthesis in atmospheres containing CH_4 , CO , and CO_2 : II. Hydrogen cyanide, formaldehyde, and ammonia. *Journal of Molecular Evolution*, **19**, 383–390.
- Schwarz, E., and Friedrich, B., 2003. The H_2 -metabolizing prokaryotes. In Dworkin, M., et al. (eds.), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. New York: Springer.
- Spinks, J. W. T., and Woods, R. J., 1964. *An Introduction to Radiation Chemistry*. New York: Wiley.
- Vignais, P., Colbeau, A., Willison, J. C., and Jouanneau, Y., 1985. Hydrogenase, nitrogenase, and hydrogen metabolism in the photosynthetic bacteria. In Rose, A. H., and Tempest, D. W. (eds.), *Advances in Microbial Physiology*. New York: Academic Press, Vol. 26, pp. 155–234.
- Vignais, P. M., Billoud, B., and Meyer, J., 2001. Classification and phylogeny of hydrogenases. *FEMS Microbiology Reviews*, **25**, 455–501.
- Wächtershäuser, G., 1993. The cradle chemistry of life: on the origin of natural products in a pyrite-pulled chemo-autotrophic origin of life. *Pure and Applied Chemistry*, **65**(6), 1343.
- Walker, J. C. G., 1977. *Evolution of the Atmosphere*. New York: Macmillan.
- Zobell, C. E., 1947. Microbial transformation of molecular hydrogen in marine sediments, with particular reference to petroleum. *Bulletin of the American Association of Petroleum Geochemists*, **31**(10), 1709–1751.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Astrobiology](#)
[Chemolithotrophy](#)
[Early Earth](#)
[Fermentation](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)
[Photosynthesis](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

HYDROTHERMAL ENVIRONMENTS, FOSSIL

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Synonyms

Black and White Smoker communities; Volcanogenic massive sulfide deposits – VMS

Definition

“Black smokers” represent extreme hydrothermal activities that are commonly located in deep sea environments

division with the host. Among photosynthetic Eukarya, green algae exhibit an active H_2 metabolism, and are considered a promising potential source of “biohydrogen” for alternative energy systems.

Summary

Hydrogen has played an important role in Earth's geochemistry and biology since the earliest stages of the planet's history. H_2 is produced by a variety of geochemical processes and is postulated to have been a significant component of the early, prebiotic atmosphere, where it had direct or indirect effects on atmospheric redox chemistry and radiation budget, and contributed to planetary oxidation by escape to space. Prebiotic and origin of life chemistry may have significantly involved H_2 , and its prominent use among deeply branching organisms in the tree of life reflects, at very least, a key role in metabolism since the biosphere's early stages. In the modern biosphere, H_2 biochemistry is associated with a broad variety of metabolic strategies, in all three domains of life. Although it now represents a trace component of the oceans and atmosphere, the significance of H_2 for planetary chemistry persists through its central role in the microbial world.

Bibliography

- Atkinson, R., Darnall, K. R., Lloyd, A. C., Winer, A. M., and Pitts, J. N. Jr., 1979. Kinetics and mechanisms of the reaction of the hydroxyl radical with organic compounds in the gas phase. *Advances in Photochemistry*, **11**, 375–488.
- Canuto, V. M., Levine, J. S., Augustsson, T. R., Imhoff, C. L., and Giampapa, M. S., 1983. The young sun and the atmosphere and photochemistry of the early Earth. *Nature*, **305**, 281–286.
- Elderfield, H., and Schulz, A., 1996. Mid-ocean ridge hydrothermal fluxes and the chemical composition of the ocean. *Annual Review of Earth Planetary Science*, **24**, 191–224.
- Fenchel, T., and Finlay, B. J., 1995. *Ecology and Evolution in Anoxic Worlds*. Oxford: Oxford University Press.
- Freund, F., Dickinson, J. T., and Cash, M., 2002. Hydrogen in rocks: an energy source for deep microbial communities. *Astrobiology*, **2**, 83–92.
- Hoehler, T. M., 2005. Biogeochemistry of dihydrogen. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Biogeochemical Cycles of Elements*. New York: Marcel Dekker, Vol. 43, pp. 9–48.
- Hoehler, T. M., Bebout, B. M., and DesMarais, D. J., 2001. The role of microbial mats in the production of reduced gases on the early Earth. *Nature*, **412**, 324–327.
- Hoehler, T. M., Albert, D. B., Alperin, M. J., Bebout, B. M., Martens, C. S., and DesMarais, D. J., 2002. Comparative ecology of H_2 cycling in sedimentary and phototrophic ecosystems. *Antonie Van Leeuwenhoek*, **81**, 575–585.
- Kita, I., Masuo, S., and Wakita, A., 1982. H_2 generation by reaction between H_2O and crushed rock: an experimental study of H_2 degassing from the active fault zone. *Journal of Geophysical Research*, **87**, 10789–10795.
- Levine, J. S., 1985. The photochemistry of the early atmosphere. In Levine, J. S. (ed.), *The Photochemistry of Atmospheres*. San Diego, CA: Academic Press, p. 518.
- Miller, S. L., and Urey, H., 1959. Organic compound synthesis on the primitive Earth. *Science*, **130**, 245–251.
- Sagan, C., and Chyba, C., 1997. The early faint sun paradox: organic shielding of ultraviolet-labile greenhouse gases. *Science*, **276**, 1217–1221.
- Schink, B., 1988. Principles and limits of anaerobic degradation. In Zehnder, A. J. B. (ed.), *Biology of Anaerobic Microorganisms*. New York: Wiley-Interscience, pp. 771–846.
- Schlesinger, G., and Miller, S. L., 1983. Prebiotic synthesis in atmospheres containing CH_4 , CO , and CO_2 : II. Hydrogen cyanide, formaldehyde, and ammonia. *Journal of Molecular Evolution*, **19**, 383–390.
- Schwarz, E., and Friedrich, B., 2003. The H_2 -metabolizing prokaryotes. In Dworkin, M., et al. (eds.), *The Prokaryotes: An Evolving Electronic Resource for the Microbiological Community*. New York: Springer.
- Spinks, J. W. T., and Woods, R. J., 1964. *An Introduction to Radiation Chemistry*. New York: Wiley.
- Vignais, P., Colbeau, A., Willison, J. C., and Jouanneau, Y., 1985. Hydrogenase, nitrogenase, and hydrogen metabolism in the photosynthetic bacteria. In Rose, A. H., and Tempest, D. W. (eds.), *Advances in Microbial Physiology*. New York: Academic Press, Vol. 26, pp. 155–234.
- Vignais, P. M., Billoud, B., and Meyer, J., 2001. Classification and phylogeny of hydrogenases. *FEMS Microbiology Reviews*, **25**, 455–501.
- Wächtershäuser, G., 1993. The cradle chemistry of life: on the origin of natural products in a pyrite-pulled chemo-autotrophic origin of life. *Pure and Applied Chemistry*, **65**(6), 1343.
- Walker, J. C. G., 1977. *Evolution of the Atmosphere*. New York: Macmillan.
- Zobell, C. E., 1947. Microbial transformation of molecular hydrogen in marine sediments, with particular reference to petroleum. *Bulletin of the American Association of Petroleum Geochemists*, **31**(10), 1709–1751.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Astrobiology](#)
[Chemolithotrophy](#)
[Early Earth](#)
[Fermentation](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Origin of Life](#)
[Photosynthesis](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

HYDROTHERMAL ENVIRONMENTS, FOSSIL

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Synonyms

Black and White Smoker communities; Volcanogenic massive sulfide deposits – VMS

Definition

“Black smokers” represent extreme hydrothermal activities that are commonly located in deep sea environments

related with mid-ocean ridges and/or oceanic crust fault systems. Hydrothermal vent systems result from hydrothermal circulation of fluids controlling the transfer of energy and various matter from the interior to the surface of the oceanic crust, respectively the sediment surface. This hydrothermal circulation is a fundamental geological process and has been permanently influencing the chemistry of the oceans, and in consequence, also the composition of the entire earth crust. Seafloor hydrothermal activity has a major impact on the chemistry and life processes of the oceans (Parson et al., 1995; Humphries et al., 1995; van Dover, 2000). This entry focuses on volcanogenic massive sulfide deposits (VMS) and not on sedimentary exhalative deposits (SEDEX) – however, the boundary between both environments is not very strict. Massive sulfide deposits, hydrothermally altered rocks, and special communities of organisms are an important record of this process. Ancient seafloor hydrothermal activity is recorded in massive sulfides from the 3.5 GY-old Pilbara region of Western Australia (Barley, 1992). The new findings of hydrothermal vents with microbialites from 1.43 GY-old Precambrian rocks in northern China (Li and Kusky, 2007) are also of great interest. Within the Phanerozoic, roughly 20 sites are known with fossil hydrothermal vent communities (Little et al., 1998).

Geobiological notes

Hydrothermal vents have become one of the most fascinating fields of research in marine sciences. The recognition of complex vent communities consisting of organisms dependent on chemolithotrophy and extreme conditions of very high temperature, pressure, and chemistry at the ocean ridges was a great discovery (Humphris et al., 1995). There are many reasons to believe that life originated at hydrothermal vent environments in the late Hadean. However, only few publications are dealing with the fossil record of hydrothermal environments connected with preserved organism communities.

There are about 20 known Phanerozoic VMS sites with preserved hydrothermal vent communities, ranging from the Silurian to the Eocene. Most of these sites are found in the Ural Mountains and on Cyprus, some of them in Ireland, California, Philippines, and New Caledonia. All occurrences are linked to remains of oceanic crust (ophiolites). The investigation of vent systems is still a challenge, because the sites are normally small and most of them were destroyed during subduction of oceanic crust.

The vent communities contain assemblages of inarticulate and rhynchonellid brachiopods, gastropods, bivalves, and also monoplacophorans (Little et al., 1998; Kiel and Little, 2006). Remains of worm tubes are very characteristic and may be related to alvinellid polychaetes and pogonophorid-vestimentiferan chemotrophic worms. Most of the fossil taxa of the vent sites are endemic. However, the diagenetic loss of the original shell material makes it very difficult to classify the worm tubes in a modern

taxonomic scheme. Surprisingly, no arthropods were found at the fossil sites, whereas arthropods are very common in modern vent environments. There is an ongoing discussion on the origin of the vent fauna. New groups of organisms may have immigrated from the ambient environment and adapted to the extreme conditions of the vent sites and vice versa, some of them have left this environment. The origin of the vent faunas is thoroughly discussed in Kiel and Little (2006) and Little and Vrijenhoek (2003). Kiel and Little (2006) suggested that the mollusk seep faunas (hydrothermal vents and cold seeps) have a significantly longer evolutionary history than normal marine mollusks and show a tight taxonomic relationship with deep-sea mollusk faunas. The oldest known Phanerozoic hydrothermal vent is of Silurian age (Little et al., 1998). The discovery of the Mesoproterozoic (1.43 GY) hydrothermal vents in northern China was of great importance due to very well-preserved filamentous microbial communities forming massive sulfidic microbialites (Li and Kusky, 2007).

Conclusion

Fossil hydrothermal vent systems are known since the early Archaean and represent important geobiological environments. These environments have a significant key role in biological evolutionary processes. However, only few (ca. 20) sites are known worldwide in the Phanerozoic with preserved vents faunas. The oldest known fossil hydrothermal vent system with a metazoan community is known from the Silurian of the Ural Mountains (Little et al., 1998). Many of the vent organisms probably have a significantly longer evolutionary history than normal marine taxa (Kiel and Little, 2006).

Bibliography

- Barley, M. E., 1992. A review of Archaean volcanic-hosted massive sulphide and sulphate mineralisation in Western Australia. *Economic Geology and the Bulletin of the Society of Economic Geologists*, **87**, 855–872.
- Humphris, S. E., Zierenberg, R. A., Mullineaux, L. S., and Thomson, R. E., 1995. Seafloor hydrothermal systems. *Geophysical Monograph (AGU)*, **91**, 1–466.
- Kiel, S., and Little, C. T. S., 2006. Cold seep mollusks are older than the general marine mollusk fauna. *Science*, **313**, 1429–1431.
- Li, J., and Kusky, T. M., 2007. World's largest known Precambrian fossil black smoker chimneys and associated microbial vent communities, North China: implications for early life. *Gondwana Research*, **12**, 84–100.
- Little, C. T. S., and Vrijenhoek, R. C., 2003. Are hydrothermal vent animals living fossils? *Trends in Ecology and Evolution*, **18**, 582–588.
- Little, C. T. S., Herrington, R. J., Maslennikov, V. V., and Zaykov, V. V., 1998. The fossil record of hydrothermal vent communities. In Mills, R. and Harrison, K. (eds.), *Modern Ocean Floor Processes and the Geological Record*. Geological Society London Special Publications, 148, pp. 259–270.
- Parson, L. M., Walker, C. L., and Dixon, D. R., 1995. Hydrothermal vents and processes. *Geological Society London Special Publication*, **87**, 1–411.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton, NJ: Princeton University Press, 424 p.

Cross-references

Cold Seeps
 Deep Biosphere of the Ocean Deep Sea
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Origin of Life

HYDROTHERMAL ENVIRONMENTS, MARINE

Gilberto E. Flores, Anna-Louise Reysenbach
 Portland State University, Portland, OR, USA

Definition

Marine hydrothermal environments are one of the most extreme environments on Earth, yet they support highly productive biological communities over a wide range of physical and chemical environments. The following review is a general discussion of the types of microorganisms found in these different environments preceded by brief descriptions on hydrothermal fluid generation, mineral deposit formation, and microbial metabolism.

Introduction

The discovery of deep-sea hydrothermal vents and the lush biological communities associated with them in the late 1970s ushered in a new era for marine biologists. Prior to this discovery, life in the deep-sea was thought to be dependent on the settling of detrital material from the productive, overlying surface waters. While this is true for much of the ocean basins, the level of production observed at these newly discovered vent sites was too great to be supported by these mechanisms alone. It soon became evident that life in these environments was supported by chemosynthetic primary production in which microorganisms harnessed the abundant geochemical energy available in the hydrothermal fluids. This challenged one of the basic ecological premises that all ecosystems on Earth were dependent on light energy and driven by photosynthetic primary production.

Since those initial revelations, research efforts have focused on assessing the microbial diversity and attempting to understand the intimate associations between microbial productivity, geology, and geochemistry in marine hydrothermal environments. This article is a general discussion of the trends that are beginning to emerge from these efforts. Included in the discussion are overviews of the geologic setting of marine hydrothermal systems, the generation of hydrothermal fluids from different geologic settings, and the types of structures formed.

For additional in-depth reviews, the Geophysical Monographs published by the American Geophysical Union (AGU) are valuable resources (Humphris et al.,

1995; German et al., 2004; Wilcock et al., 2004; Christie et al., 2006).

Geological setting

Most seafloor hydrothermal vent systems are associated with extensional tectonic activity and heated by magmatic heat as it is convected into the crust (Seyfried and Mottl, 1995). The most well-studied areas of hydrothermal circulation are along divergent plate boundaries where basaltic seafloor is made and mid-ocean ridges (MOR) arise (Figure 1). Hydrothermal circulation along MORs results from the active heating of seawater that percolates through newly formed basaltic crust. Hydrothermal venting is also commonly found along convergent plate margins where an oceanic plate is subducted beneath a continental plate forming island arcs and back-arc basins (Christie and Fisher, 2006). Vents along back-arc basins are actively heated in the same way as those along MOR, although the fluids tend to be more heterogeneous due to the variability in magma composition and additional inputs from the subducting plate (GAOM et al., 2006). A third location where seafloor hydrothermal systems can be found is at intraplate volcanic hot spots. Volcanic hot spot systems are not directly associated with tectonic plate margins, but are actively heated as plumes of molten magma push up through the mantle and crust (Seyfried and Mottl, 1995).

The recently discovered Lost City vent field more than 15 km off axis of the Mid-Atlantic Ridge (MAR) represents a new type of seafloor hydrothermal system as it appears to be at least partially heated by exothermic reactions between mantle derived peridotite and seawater (Kelley et al., 2001). These reactions have a dramatic impact on the composition of the hydrothermal fluids, which in turn affects the microbial community as discussed in the following sections. Although Lost City represents the first vent type of its kind discovered, similar systems are believed to exist along margins of slow- and ultraslow-spreading ridges where uplifting of ultramafic massifs are common.

Hydrothermal fluids

Seawater heated in contact with subsurface rocks dramatically alters the chemical composition of the end member fluids. The degree of alteration is influenced by several factors including the initial composition of the seawater, the type and structure of the host rock, the presence of sediment overlaying the host rock, and the type, depth, and size of the heat source (reviewed in Tivey, 2007). Understanding these interactions and how they affect the composition of hydrothermal fluids is important as the fluids set the physiological parameters and provide the metabolic menu for colonizing microbes. For comparison, representative compositions of hydrothermal fluids from different settings are presented in Table 1.

Cross-references

Cold Seeps
 Deep Biosphere of the Ocean Deep Sea
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Origin of Life

HYDROTHERMAL ENVIRONMENTS, MARINE

Gilberto E. Flores, Anna-Louise Reysenbach
 Portland State University, Portland, OR, USA

Definition

Marine hydrothermal environments are one of the most extreme environments on Earth, yet they support highly productive biological communities over a wide range of physical and chemical environments. The following review is a general discussion of the types of microorganisms found in these different environments preceded by brief descriptions on hydrothermal fluid generation, mineral deposit formation, and microbial metabolism.

Introduction

The discovery of deep-sea hydrothermal vents and the lush biological communities associated with them in the late 1970s ushered in a new era for marine biologists. Prior to this discovery, life in the deep-sea was thought to be dependent on the settling of detrital material from the productive, overlying surface waters. While this is true for much of the ocean basins, the level of production observed at these newly discovered vent sites was too great to be supported by these mechanisms alone. It soon became evident that life in these environments was supported by chemosynthetic primary production in which microorganisms harnessed the abundant geochemical energy available in the hydrothermal fluids. This challenged one of the basic ecological premises that all ecosystems on Earth were dependent on light energy and driven by photosynthetic primary production.

Since those initial revelations, research efforts have focused on assessing the microbial diversity and attempting to understand the intimate associations between microbial productivity, geology, and geochemistry in marine hydrothermal environments. This article is a general discussion of the trends that are beginning to emerge from these efforts. Included in the discussion are overviews of the geologic setting of marine hydrothermal systems, the generation of hydrothermal fluids from different geologic settings, and the types of structures formed.

For additional in-depth reviews, the Geophysical Monographs published by the American Geophysical Union (AGU) are valuable resources (Humphris et al.,

1995; German et al., 2004; Wilcock et al., 2004; Christie et al., 2006).

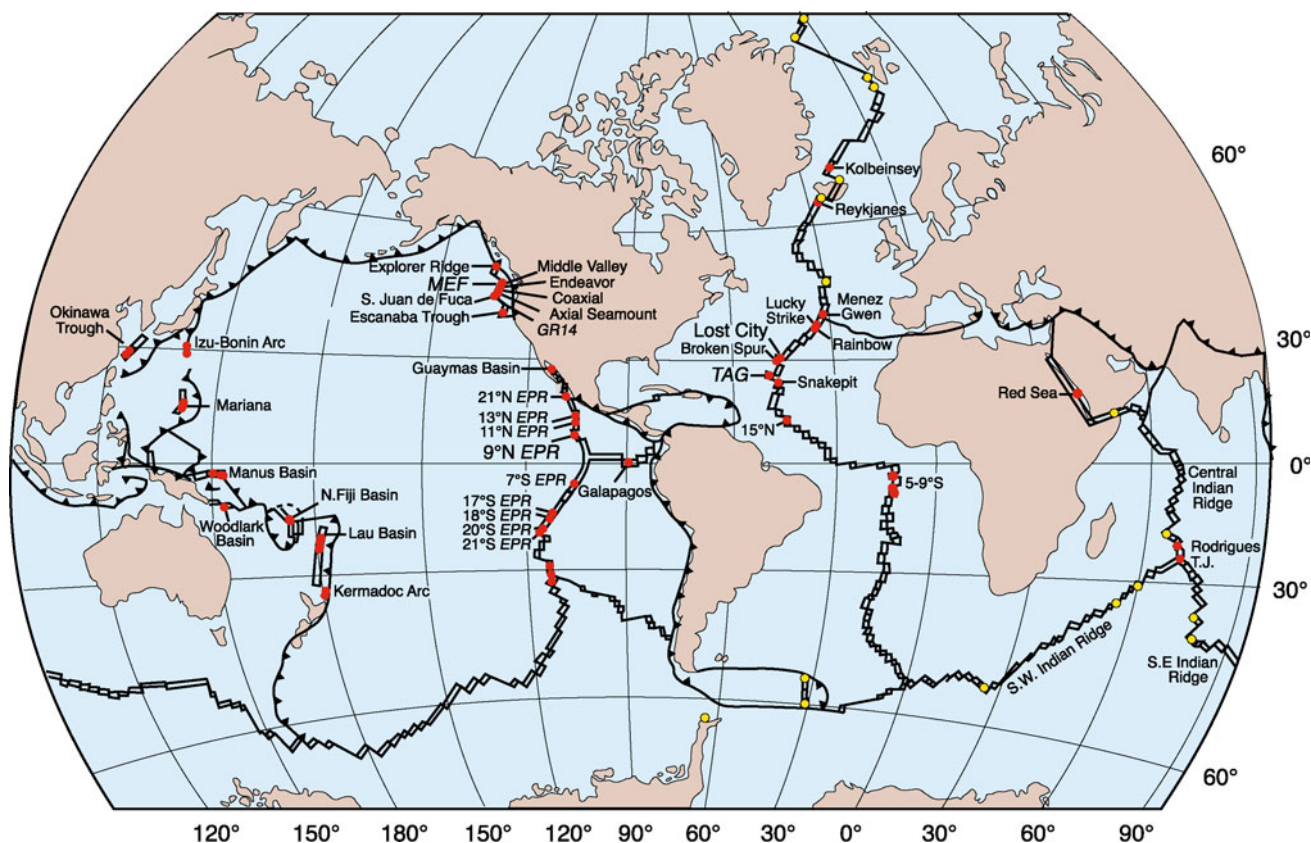
Geological setting

Most seafloor hydrothermal vent systems are associated with extensional tectonic activity and heated by magmatic heat as it is convected into the crust (Seyfried and Mottl, 1995). The most well-studied areas of hydrothermal circulation are along divergent plate boundaries where basaltic seafloor is made and mid-ocean ridges (MOR) arise (Figure 1). Hydrothermal circulation along MORs results from the active heating of seawater that percolates through newly formed basaltic crust. Hydrothermal venting is also commonly found along convergent plate margins where an oceanic plate is subducted beneath a continental plate forming island arcs and back-arc basins (Christie and Fisher, 2006). Vents along back-arc basins are actively heated in the same way as those along MOR, although the fluids tend to be more heterogeneous due to the variability in magma composition and additional inputs from the subducting plate (GAOM et al., 2006). A third location where seafloor hydrothermal systems can be found is at intraplate volcanic hot spots. Volcanic hot spot systems are not directly associated with tectonic plate margins, but are actively heated as plumes of molten magma push up through the mantle and crust (Seyfried and Mottl, 1995).

The recently discovered Lost City vent field more than 15 km off axis of the Mid-Atlantic Ridge (MAR) represents a new type of seafloor hydrothermal system as it appears to be at least partially heated by exothermic reactions between mantle derived peridotite and seawater (Kelley et al., 2001). These reactions have a dramatic impact on the composition of the hydrothermal fluids, which in turn affects the microbial community as discussed in the following sections. Although Lost City represents the first vent type of its kind discovered, similar systems are believed to exist along margins of slow- and ultraslow-spreading ridges where uplifting of ultramafic massifs are common.

Hydrothermal fluids

Seawater heated in contact with subsurface rocks dramatically alters the chemical composition of the end member fluids. The degree of alteration is influenced by several factors including the initial composition of the seawater, the type and structure of the host rock, the presence of sediment overlaying the host rock, and the type, depth, and size of the heat source (reviewed in Tivey, 2007). Understanding these interactions and how they affect the composition of hydrothermal fluids is important as the fluids set the physiological parameters and provide the metabolic menu for colonizing microbes. For comparison, representative compositions of hydrothermal fluids from different settings are presented in Table 1.



Hydrothermal Environments, Marine, Figure 1 Global distribution of known (red) hydrothermal venting along mid-ocean ridges (MOR), back-arc basins, rifted arcs, and at submerged island-arc volcanoes. Yellow circles indicate areas where mid-water column chemical anomalies have been detected suggesting hydrothermal activity. EPR East Pacific Rise; TAG Trans Atlantic Geotraverse; MEF Main Endeavor Field; GR-14 Sea Cliff hydrothermal field on the northern Gorda Ridge. Figure 1 from Tivey (2007).

Basalt hosted hydrothermal fluids

Water–rock reactions begin as cold seawater infiltrates shallow basalt in the recharge zones (Figure 2). Initial low temperature reactions ($\leq 60^\circ\text{C}$) result in the removal of alkali metals from seawater and the oxidation of basaltic minerals (i.e., olivine and plagioclase), with the concomitant leaching of silica (Si), sulfur (S), and sometimes magnesium (Mg) from the minerals (reviewed in Alt, 1995). Magnesium is eventually completely removed from the fluids as Mg-rich clays precipitate at temperatures above 150°C . Anhydrite (CaSO_4) precipitation and seawater sulfate (SO_4^{2-}) reduction also occur in the recharge zone at temperatures above 150°C , resulting in the complete removal of SO_4^{2-} from the fluids. As the fluid continues through the crust, reactions with iron-bearing minerals such as olivine and pyroxene result in highly reducing fluids with elevated concentrations of hydrogen gas (H_2) (Tivey, 2007). Upon reaching the reaction zone where fluids are heated upward of 400°C , the fluid obtains its chemical signature by leaching S and metals (copper-Cu, iron-Fe, manganese-Mn, zinc-Zn) from the surrounding rocks (Alt, 1995). Phase separation

may also occur in the reaction zone if temperature and pressure are greater than 407°C and 298 bars, respectively (Von Damm, 1995). Phase separation can have a significant impact on the composition of end member fluids as volatiles such as hydrogen-sulfide (H_2S) tend to partition in the vapor phase while metals such as Fe become enriched in the liquid (brine) phase. Gases such as helium (He), methane (CH_4), and carbon dioxide (CO_2) may also be added to the hydrothermal fluids as volatiles from the underlying magma (Alt, 1995). In total, these reactions result in end member fluids that are acidic (pH 2.8–4.5), highly reduced, rich in H_2S and metals, and heated up to 400°C . The fluid is also extremely buoyant relative to seawater and is forced back to the seafloor through the discharge zone and may emerge as diffuse or focused flow depending on the degree of subsurface mixing during the ascent (Figure 2).

Other factors, such as differences in host rock and sediment cover, can contribute to the chemical composition of end member fluids. The impact that different host rocks have on fluid chemistry is best illustrated by the ultramafic hosted Rainbow vent field along the MAR

Hydrothermal Environments, Marine, Table 1 Representative chemical compositions of hydrothermal fluids from different geologic settings

Characteristic	Deep seawater	Mid-ocean ridge	Back-arc basin	Sediment hosted	Rainbow vent field	Lost City
T (°C)	2	<405	278–334	100–315	365	≤91
pH (25°C)	8	2.8–4.5	<1–5.0	5.1–5.9	2.8	10–11
SO ₄ , mmol/kg	28	0	0	0	0	1–4
Mg, mmol/kg	53	0	0	0	0	<1
Cl, mmol/kg	545	30.5–1,245	255–790	412–668	750	548
Na, mmol/kg	464	10.6–983	210–590	315–560	553	479–485
Ca, mmol/kg	10.2	4.02–109	6.5–89	160–257	67	<30
K, mmol/kg	10.1	1.17–58.7	10.5–79	13.5–49.2	20	–
H ₂ S, mmol/kg	–	0–19.5	1.3–13.1	1.10–5.98	1	<0.064
H ₂ , mmol/kg	–	0.0005–38	0.035–0.5	–	13	<1–15
CO ₂ , mmol/kg	2.36	3.56–39.9	14.4–200	–	N/A	bdl
CH ₄ , mmol/kg	–	0.007–2.58	0.005–0.6	–	0.13–2.2	1–2
NH ₃ , mmol/kg	–	<0.65	–	5.6–15.6	–	–
Fe, μmol/kg	–	7–18,700	13–2,500	0–180	24,000	–
Mn, μmol/kg	–	59–3,300	12–7,100	10–236	2,250	–
Cu, μmol/kg	–	0–150	0.003–34	<0.02–1.1	140	–
Zn, μmol/kg	–	0–780	7.6–3,000	0.1–40	160	–
Pb, μmol/kg	–	0.183–0.1630	0.036–3.9	<0.02–0.652	0.148	–

Modified after Tivey (2007). *bdl* below detection limit

(Douville et al., 2002). Fluids from Rainbow vent field are lower in pH, have much higher Fe concentrations, are enriched in H₂ and CH₄, and are depleted in H₂S relative to fluids from most MOR systems (reviewed in McCollom, 2007). These differences in composition are related to the same serpentinization reactions that fuel the Lost City vent field (see below) although the heat sources differ. Sediments overlying the host rock can also affect the composition of end member fluids (German and Von Damm, 2004). In general, sediment-hosted systems generate fluids that are higher in pH (5.1–5.9) and lower in metal contents than those from unsedimented systems. Chemical differences largely depend on the amount and type of organic matter present in the sediments (Tivey, 2007).

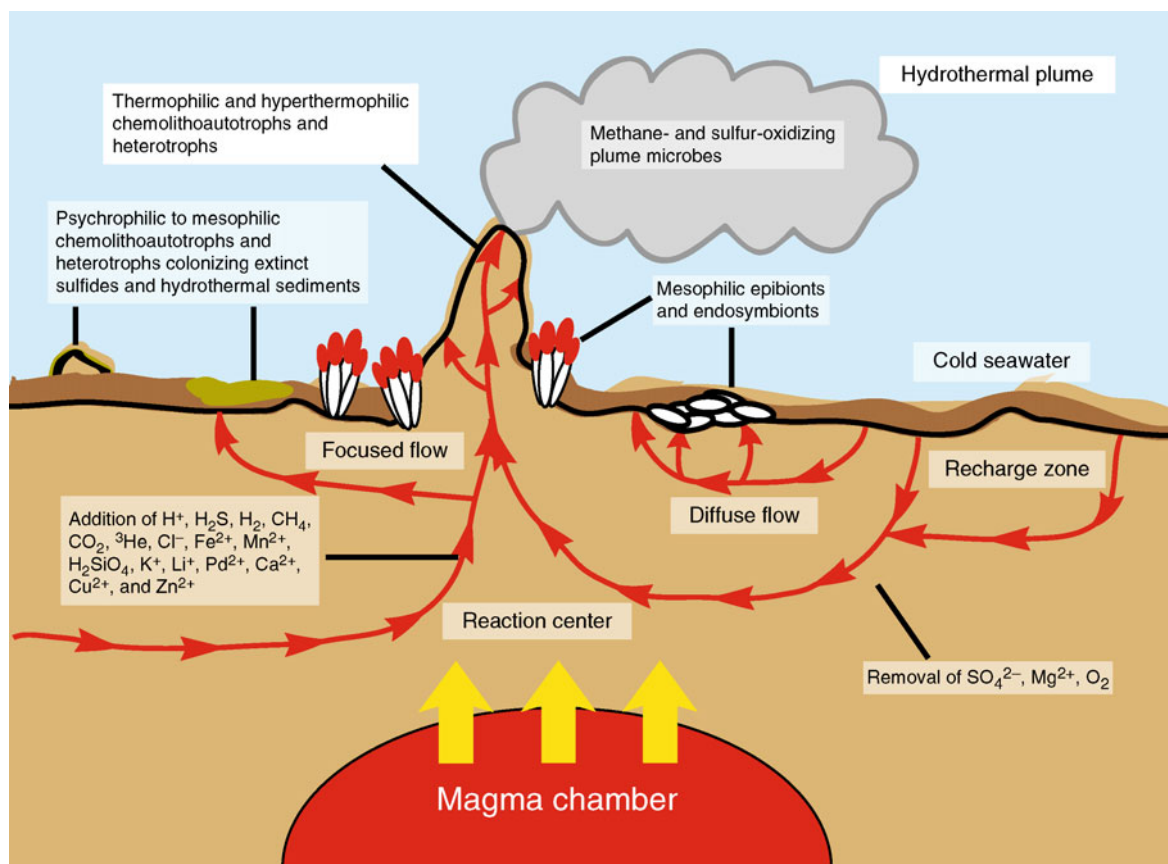
Lost City hydrothermal fluids

Hydrothermal fluid generation at Lost City begins much in the same way as fluid generation at MOR systems, with the infiltration of seawater into rock. However, the rock is not basalt but rather mantle-derived peridotite which reacts with seawater to form serpentinite (Kelley et al., 2001). Serpentinization reactions result in the hydration of minerals such as olivine and produces large amounts of heat, H₂, and CH₄. This heat is believed to at least partially drive hydrothermal circulation at Lost City although other heat sources have been suggested (Kelley et al., 2001; Allen and Seyfried, 2004). Regardless of the heat source, the end member fluid chemistry is dramatically different than MOR vent systems and is indicative of seawater–rock reactions below 150°C. The most striking differences are the alkaline pH (≈10–11), the lower temperatures (up to 93°C), the elevated calcium (Ca) content, and the low concentrations of metals and H₂S found

in the fluids (Kelley et al., 2001, 2005). Methane and H₂ concentrations are also elevated relative to basalt-hosted MOR systems (Table 1).

Hydrothermal structures MOR sulfide deposits

When acidic hydrothermal fluids rich in reduced chemical species interact with cold, oxygenated seawater, minerals rapidly precipitate out of solution forming large deposits (reviewed in Juniper and Tebo, 1995). Mineral phases found in these deposits include sulfides, sulfates, oxides, hydroxides, silicates, and carbonates. Deposit formation can occur in the subsurface as the fluids ascend through oceanic basalt and mix with infiltrating seawater or directly on the seafloor as high temperature fluids erupt into bottom seawater. Subsurface precipitation of minerals generates stockwork deposits in the shallow crust and results in low-temperature venting on the seafloor. Low-temperature fluids are generally low in metal sulfides and as a result, precipitate oxides upon reaching the seafloor. In contrast, fluids that have not been diluted and cooled in the subsurface rapidly precipitate metal sulfides such as pyrite (FeS₂), chalcopyrite (CuFeS₂), and sphalerite (ZnS) upon mixing with seawater. Precipitation of metal sulfides results in the appearance of “black smoke” and ultimately forms massive sulfide deposits (>60% sulfide minerals). Several types of sulfide features are found in vent fields, with the most spectacular being the black smoker chimney (Figure 3a). Other sulfide structures generated at marine hydrothermal vents include diffuse flow beehives, flanges, and complex flow sulfide mounds (Van Dover, 2000). In all cases, active sulfide features provide extreme physical and geochemical gradients that



Hydrothermal Environments, Marine, Figure 2 Hydrothermal circulation along MOR depicting compositional changes of seawater and microbial habitats supported by hydrothermal fluids. *Small arrows* indicate fluid flow while *large arrows* indicate heat transfer from magmatic source. Image is not to scale. Adapted from Reysenbach (2001).

sustain diverse microbial populations even after fluid flow has ceased.

Lost City carbonate deposits

In contrast to the sulfide structures found along basalt-hosted MOR systems, hydrothermal fluids venting at Lost City produce large white chimneys and flanges composed primarily of aragonite ($CaCO_3$) and brucite ($Mg(OH)_2$) (Figure 3b) (Kelley et al., 2001). Active structures are highly porous and emit fluids from complex networks of centimeter sized channels (Kelley et al., 2005). Extinct structures are less porous resulting from the replacement of aragonite with calcite ($CaCO_3$) and the dissolution of brucite. The largest structure at Lost City, Poseidon, is over 60 m tall, making it the tallest seafloor hydrothermal feature yet discovered (Kelley et al., 2001).

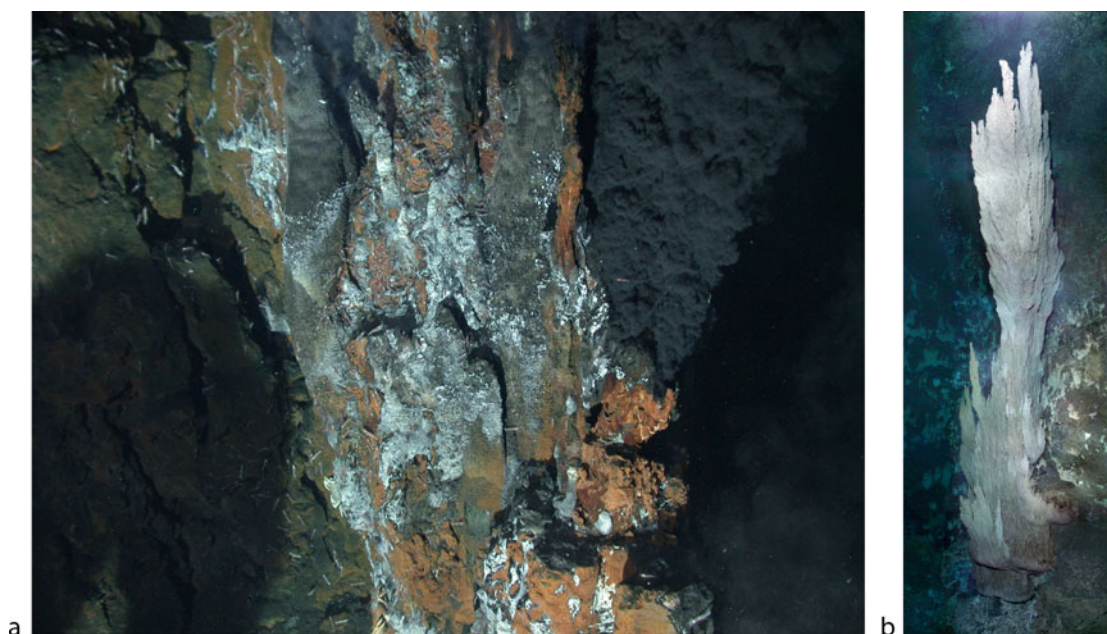
Microbial metabolism

The hydrothermal fluids provide the energy and nutrients for a thriving microbial ecosystem. Additionally, the porous sulfide deposits are excellent habitats for the metabolic diversity of microorganisms.

Terminology

Energy sources for microbial metabolism include light-dependent reactions (less important at deep-sea vents) and light-independent chemical reactions and are described by the prefixes photo- and chemo-, respectively. Energy sources are further classified depending on whether the source of electrons is inorganic (-litho-) or organic (-organo-). For carbon sources, the suffix -autotroph describes an organism that obtains carbon from an inorganic source (CO_2), while the term -heterotroph describes an organism that obtains carbon from an organic source. For example, a microorganism that is able to gain energy and electrons from H_2 and use this energy to fix CO_2 is referred to as a chemolithoautotroph, while humans who require organic carbon and energy sources are described as chemoorganoheterotrophs, as are many microorganisms. Some microorganisms are not obligatory autotrophs and will assimilate organic carbon substances if they are available. The term mixotroph is used to describe these organisms which appear to be common in most environments.

Microorganisms also have varying requirements for oxygen (O_2) as an oxidant. Some organisms have an



Hydrothermal Environments, Marine, Figure 3 Photographs of active chimney structures from different hydrothermal environments. (a) Portion of a massive sulfide chimney from the Eastern Lau Spreading Center emanating “black smoke” from multiple orifices. Note the orange Fe-oxide crust on the outer portions of the structure. (b) Photo mosaic of a 10 m carbonate chimney growing on the side of a serpentine cliff from the Lost City Vent Field. Lost City photo courtesy of D.S. Kelley, University of Washington and the Lost City Team.

obligate need (aerobes) while others cannot tolerate O_2 at all (anaerobes). Furthermore, some organisms require low levels of O_2 (microaerophile) while others can switch between aerobic and anaerobic metabolisms (facultative anaerobe).

At deep-sea vents, the heat and pH preference provides an additional habitat selection. All microbes have a preferred temperature range at which optimal growth is achieved. Psychrophiles have optimal growth $\leq 15^\circ C$, mesophiles grow optimally between 25 and $40^\circ C$, thermophiles and hyperthermophiles grow optimally between 45 and $80^\circ C$, and $\geq 80^\circ C$, respectively.

The pH gradients at deep-sea vents can vary from as low as pH 2.5 to over pH 8.0, as the acidic hydrothermal fluid mixes with the cold seawater. Thus, there are habitats of low pH where acidophiles thrive; however, by far the majority of habitats at vents available for microbial colonization are circumneutral (pH $>6 <8$), and these organisms are therefore referred to as neutrophiles.

Energy sources

Seafloor hydrothermal ecosystems are supported almost entirely by chemosynthetic primary production fueled by the chemical disequilibrium between the oxygenated seawater and the reduced hydrothermal fluids. Microorganisms capture this energy by way of reduction/oxidation (redox) reactions in which seawater typically provides the oxidant (O_2 , SO_4^{2-} , NO_3^- , CO_2) and the hydrothermal fluids provide the reductant (H_2 , H_2S , HS^- , S^0 ,

$S_2O_2^{3-}$, Fe^{2+}). Redox reactions generate energy that is used to fix CO_2 into cellular material which then provides organic carbon for various heterotrophic microbes and animals that make up the rest of the community. Some of the most thermodynamically favorable reactions found in marine hydrothermal environments are presented in Table 2. Reactions are expressed in terms of the change in free energy (ΔG), where the more negative the value the more energy an organism will gain by catalyzing the reaction. Calculated values suggest that aerobic oxidation of reduced sulfur compounds (HS^- , S^0 , $S_2O_2^{3-}$) are the most energetically favorable and potentially the most significant reactions in terms of biomass production in marine hydrothermal environments. It is important to note, however, that thermodynamic calculations can be misleading as they do not consider the bioavailability of certain reactants and potential kinetic inhibition under different environmental conditions (Takai et al., 2006). Several past studies have attempted to model the overall energetics in a variety of marine hydrothermal systems considering such factors (McCollom and Shock, 1997; McCollom, 2000; McCollom, 2007). An interesting conclusion from a theoretical study is that four times as much energy per kg of hydrothermal fluid is available to H_2 oxidizers in ultramafic hosted systems than to sulfur oxidizers because of the increased concentrations of H_2 (McCollom, 2007). This suggests that H_2 oxidizers may be more important than sulfur oxidizers in ultramafic hosted systems (McCollom, 2007). A recent report lends support to

Hydrothermal Environments, Marine, Table 2 Examples of thermodynamically favorable redox reactions utilized by microorganisms from marine hydrothermal environments

Conditions	Electron donor	Electron acceptor	Free energy ΔG^a (kJ/mol)	Metabolic process
Aerobic	H ₂	O ₂	−237	Hydrogen oxidation
	HS [−]	O ₂	−797	Sulfide oxidation
	S ⁰	O ₂	−585	Sulfur oxidation
	S ₂ O ₂ ^{3−}	O ₂	−952	Thiosulfate oxidation
	Fe ²⁺	O ₂	−44.3	Iron oxidation
	NH ₄ ⁺	O ₂	−275	Nitrification
	CH ₄ + other C-1 compounds	O ₂	−810	Methane oxidation
	Organics	O ₂	−477	Heterotrophic metabolism
	Organics	O ₂	−477	Heterotrophic metabolism
Anaerobic	H ₂	NO ₃ [−]	−239	Denitrification
	H ₂	S ⁰	−98.3	Sulfur reduction
	H ₂	SO ₄ ^{2−}	−38.1	Sulfate reduction
	H ₂	CO ₂ ^{2−}	−34.7	Methanogenesis
	Organics	SO ₄ ^{2−}	−40.6	Heterotrophic sulfate reduction
	Organics	S ⁰	−25.1	Heterotrophic sulfur reduction
	Organics	Organics	−38.5	Fermentation ^b
	Organics	Organics	−38.5	Fermentation ^b

Compiled and adapted from Jannasch (1995) and Karl (1995b).

^aValues calculated using standard conditions (pH 7, 25°C, 1 atm)

^bExample reaction calculated from acetate + ethanol → butyrate + water

this hypothesis, as the diversity of H₂-oxidizing bacteria was found to increase as the concentration of H₂ gas increased in vents sampled from the ultramafic hosted Logatchev vent field along the MAR (Perner et al., 2007). These studies speak to the tight coupling of geochemistry and microbial primary production in marine hydrothermal environments.

Microbial habitats and diversity

Seafloor hydrothermal systems provide several habitats over a wide range of physical environments that sustain distinct microbial communities. Such environments include active and inactive vent structures, biotic and abiotic surfaces receiving hydrothermal fluid inputs, inside vent animals (endosymbionts), and hydrothermal vent plumes (Figure 2) (reviewed in Karl, 1995a; Van Dover, 2000; Kelley et al., 2002; Takai et al., 2006). Many of these environments are exposed to varying inputs of hydrothermal fluids, resulting in fluctuating temperatures and chemistry. Below is a brief discussion of each of these habitats and their associated microbial biodiversity. These biodiversity assessments are based on growing and characterizing isolates under laboratory conditions (culturing techniques) and through molecular phylogenetic approaches that are culture-independent. This latter approach is based on extracting genomic DNA from environmental samples, and then amplifying a diagnostic phylogenetic marker for Bacteria and Archaea, the small subunit rRNA gene (16S rRNA), using the polymerase chain reaction.

Active sulfide structures

Using culture-dependent and culture-independent techniques, a rich diversity of Bacteria and Archaea have been found associated with active sulfide structures (reviewed

in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Free-living microorganisms supported by active sulfide structures include mesophilic, thermophilic, and hyperthermophilic chemolithoautotrophs, heterotrophs, and mixotrophs. Organisms can be anaerobes, facultative anaerobes, microaerophiles, and obligate aerobes.

In general, Bacteria are the most numerically abundant microorganisms associated with active sulfide structures (reviewed in Jannasch, 1995; Karl, 1995b; Kelley et al., 2002; Takai et al., 2006). Their abundance can range anywhere from 35 to 99% of the microbial population, depending on the method used to measure abundance, the type of vent feature, and where within a chimney structure one examines (Harmsen et al., 1997; Takai and Horikoshi, 1999; Takai et al., 2001; Hoek et al., 2003; Schrenk et al., 2003; Nakagawa et al., 2006).

Within the Bacteria, members of the epsilon-Proteobacteria have consistently been found to dominate microbial communities in deep-sea hydrothermal environments (Reysenbach et al., 2000; Campbell et al., 2001; Hoek et al., 2003; Nakagawa et al., 2005). These free-living deep-sea epsilon-Proteobacteria are mesophilic to moderately thermophilic. Recently, several representatives have been obtained in pure culture that are chemolithoautotrophs capable of oxidizing H₂ and sulfur compounds while using O₂, NO₃[−], or sulfur compounds as terminal electron acceptors (reviewed in Campbell et al., 2006). Like in cave environments, the epsilon-Proteobacteria play an essential role in sulfur cycle at deep-sea vents.

Other commonly identified but less abundant bacterial members of the free-living microbial communities include the thermophilic *Aquificales* and *Thermotogales* (reviewed in Miroshnichenko and Bonch-Osmolovskaya, 2006). The *Aquificales* are globally distributed in marine

hydrothermal environments but are restricted to active sulfide structures and hydrothermal fluids due to their thermophilic lifestyle. They are generally chemolithoautotrophs and chemoorganotrophs capable of oxidizing H_2 , S^0 , and $S_2O_3^{2-}$ either aerobically or anaerobically (NO_3^- or sulfur compounds) (Reysenbach, 2001a). The *Thermotogales*, on the other hand, are strictly anaerobic, thermophilic heterotrophic fermenters (Reysenbach, 2001b).

As stated above, Archaea are less abundant in active sulfide structures, yet they play a key biogeochemical role especially at the higher temperatures. Some of the most exhaustive studies on microbial diversity at deep-sea vents have focused solely on the Archaea revealing a high phylogenetic and metabolic diversity within this domain (Takai and Horikoshi, 1999; Takai et al., 2001; Nercissian et al., 2003; McCliment et al., 2006). Most of the Archaea found here are thermophilic to hyperthermophilic and are thus restricted to areas most influenced by hydrothermal fluids. Within the Archaea, the two main phyla, the Crenarchaeota and Euryarchaeota, are well represented, while the enigmatic "Korarchaeota" and "Nanoarchaeota" are rarely detected (reviewed in Takai et al., 2006).

Crenarchaeota commonly detected at active sulfide structures include cultivated members of the Desulfurococcales (e.g., *Pyrodictium* and *Pyrolobus*) and several uncultivated groups such as the deep-sea hydrothermal vent crenarchaeotic group (DHVC1), and the marine group I (Takai and Horikoshi, 1999; Takai et al., 2006). *Pyrodictium* is an example of a hyperthermophilic mixotrophic organism with the ability to use either H_2 or complex organics to reduce elemental sulfur (reviewed in Reysenbach et al., 2002). *Pyrolobus* is also a hyperthermophile but, unlike *Pyrodictium*, *Pyrolobus* is an obligate chemolithoautotroph that can only use H_2 to reduce nitrate (NO_3^-), thiosulfate, ($S_2O_3^{2-}$) or O_2 .

Euryarchaeota commonly found at marine hydrothermal systems include the hyperthermophilic methanogens (*Methanocaldococcus* spp.), the hyperthermophilic SO_4^{2-} reducing *Archaeoglobales*, and a variety of thermophilic fermentative *Thermococcus* species (Takai and Horikoshi, 1999; Takai et al., 2006). While these are readily grown in the laboratory, their relative abundance in the environment has been shown to vary from one site to another (Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercissian et al., 2003; McCliment et al., 2006). Several lineages often detected at vent sites around the globe have been classified as the Deep-sea Hydrothermal Vent Euryarchaeotal Groups (DHVEG) (Takai and Horikoshi, 1999; Reysenbach et al., 2000; Takai et al., 2001; Hoek et al., 2003; Nercissian et al., 2003). Several subdivisions exist within this group but at least one lineage, the DHVEG-2, appears to be endemic to deep-sea vents. Little was known about this lineage until Moussard et al. (2006) showed optimal activity and thermostability of a DNA polymerase belonging to this lineage which suggested a thermophilic lifestyle. Thermophily was confirmed when the first isolate

was obtained in pure culture (Reysenbach et al., 2006). The organism, *Aciduliprofundum boonei*, is a heterotrophic thermoacidophile capable of fermenting peptides and reducing Fe and S. Isolation of this organism was significant because it is the first obligate acidophile isolated from a deep-sea vent and the first member of the DHVEG-2 lineage to be grown. Prior to this discovery, the absence of acidophiles from a deep-sea vent had always puzzled microbiologists as hydrothermal fluids are acidic, and geochemists had predicted the presence of acidic microhabitats within sulfide chimneys (Von Damm, 1995; Tivey, 2004).

Inactive sulfide structures

Hydrothermal fluid flow through individual sulfide structures is temporary as subsurface flow paths constantly change and chimneys collapse. Inactive sulfides are subjected to low-temperature oxidative weathering in which the reduced sulfur moiety is converted to soluble SO_4^{2-} and the metals, mostly Fe, are converted to insoluble oxyhydroxide crusts (Juniper and Tebo, 1995). Oxidation occurs abiotically as oxygenated seawater penetrates the rock, but can also be mediated by S- and Fe-oxidizing bacteria (reviewed in Edwards, 2004; Edwards et al., 2005). While microbial mediated sulfide weathering in terrestrial systems has been studied for decades, this area of research is in its infancy in the deep sea (Edwards et al., 2003a, b; Edwards, 2004; Rogers et al., 2003; Suzuki et al., 2004). Culturing studies have resulted in the isolation of several psychrophilic, neutrophilic Fe-oxidizing bacteria belonging to the alpha- and gamma-divisions of the Proteobacteria from sulfide rocks collected along the Juan de Fuca Ridge (Edwards et al., 2003a). A follow-up study with one of the gamma-Proteobacteria isolates showed that the organism promoted the dissolution of native sulfide minerals under anaerobic conditions (Edwards, 2004). Although the mechanism of dissolution remains unclear, these studies illustrate that Fe-oxidizing bacteria are associated with extinct sulfide minerals on the seafloor and can actively promote the dissolution of these minerals at least in the laboratory.

Carbonate structures of Lost City

Microbial communities found in active carbonate structures at the Lost City vent field differ from those found in active sulfide structures due to the contrasting physical and geochemical conditions. Inner portions of carbonate chimneys in contact with hydrothermal fluids tend to be covered by thick microbial biofilms dominated by a single Archaeal phylotype belonging to the Methanosarcinales (Schrenk et al., 2004; Brazelton et al., 2006). The Methanosarcinales are the most metabolically diverse of all the methanogens, with various representatives known to generate CH_4 from acetate and H_2/CO_2 or by dismuting methyl compounds (Boone et al., 2001). Members of the Methanosarcinales also participate in a nonobligatory syntrophic relationship

with SO_4^{2-} reducing bacteria that results in the anaerobic oxidation of methane (AOM) in oceanic cold seep environments (Orphan et al., 2001). A second archaeal phylotype belonging to the closely related ANME-1 was identified in weakly flowing and extinct carbonate chimneys (Brazelton et al., 2006). Like the Methanosarcinales, the ANME-1 are involved in AOM but appear to mediate this process without a SO_4^{2-} reducing partner and at lower temperatures (Orphan et al., 2002). As the Methanosarcinales and the ANME-1 found at Lost City were only identified by molecular techniques, it is difficult to speculate on whether they are producers or consumers of CH_4 .

In contrast to the Archaea, Bacteria appear to be more diverse in active and inactive carbonate chimneys (Brazelton, et al., 2006). The most diverse bacterial group at Lost City is the gamma-Proteobacteria which includes close relatives of known chemolithoautotrophic S- and HS^- -oxidizers (e.g., *Thiomicrospira* spp.) and several methylophilic (CH_4 -oxidizing) genera (e.g., *Methylobacter*, *Methylomonas* and *Methylophaga*). Other chemolithoautotrophic S-oxidizing bacteria belonging to the epsilon-Proteobacteria are also found at Lost City.

Perhaps the most interesting trend in bacterial diversity observed at Lost City is not what is present but what has not yet been detected. Specifically, neither any known thermophilic H_2 -oxidizing Bacteria (i.e., *Aquificales*; common in most deep-sea and terrestrial hot spring environments) nor members of the delta-Proteobacteria (the SO_4^{2-} -reducing syntrophs found in other AOM environments), were identified in carbonate chimneys (Brazelton et al., 2006). The lack of bacterial H_2 oxidizers is unexpected as geochemical modeling suggests that H_2 oxidation provides four times as much energy per kg of hydrothermal fluid than S oxidation in ultramafic hosted systems (McCollom, 2007). This suggests that available energy may not be the main constraint driving which microbial metabolisms can be supported in particular environments. It is important to note, however, that relatively few studies have been conducted at Lost City and that these observed trends may be due to the lack of adequate sampling or incorrect inferences based solely on 16S rRNA gene similarity.

Biotic and abiotic surfaces

Microbes are opportunistic organisms and will colonize any surface where growth substrates are provided. In areas influenced by hydrothermal fluids, this includes the sulfide deposits as described above and surfaces of invertebrate animals (reviewed in Van Dover, 2000). Some of these relationships are commensal as the host does not appear to gain an advantage by being colonized while others truly are symbiotic (episymbiosis), with both the host and microbes benefiting. An example of a commensal relationship is found between the giant tube worm *Riftia pachyptila* and the diverse microbial communities that colonize the outside surfaces of its chitinous

tube (López-García et al., 2002). Most of the microbes identified on the outside of the tube are members of the ubiquitous epsilon-Proteobacteria and are likely oxidizing the reduced sulfur compounds in the hydrothermal fluids. An episymbiotic relationship is found on the polychaete worm *Alvinella pompejana* (Van Dover, 2000). *Alvinella pompejana* lives in tubes on the sides of black smoker chimneys where water temperatures can exceed 70°C (reviewed in Desbruyeres et al., 1998). It has a fully functional digestive system and appears to feed upon microbial mats inside of its tube as well on sulfide surfaces. The worm also houses diverse microbial communities on its dorsal surfaces including microaerophilic H_2S -oxidizing members of the epsilon-Proteobacteria. Although the exact nature of this relationship is unknown, it is thought that the bacteria may detoxify H_2S or provide nutritional supplement to the worm.

In addition to surfaces of vent animals, microbes often colonize rocks and sediments surrounding hydrothermal vents. These environments are primarily sustained by hydrothermal fluids flowing diffusely through the underlying porous sediments. The most well-studied and possibly most diverse microbial mat system found in marine hydrothermal environments is in the sediment-hosted Guaymas Basin. Microbes found here include thermophilic/mesophilic methanogens and methanotrophs (Dhillon et al., 2005), SO_4^{2-} reducing Bacteria (Kallmeyer and Boetius, 2004), H_2S -oxidizing gamma-Proteobacteria (Nelson et al., 1989), and even spore forming Mn-oxidizers (Dick et al., 2006). Much of this diversity is fueled by the presence of overlying sediments which provides different combinations of organic/inorganic substrates typically not found in non-sediment-hosted systems.

Endosymbionts

Several invertebrate animals form obligate symbiotic relationships in which the bacteria are hosted inside the animal cells. The most well-studied example is in the giant tube worm *Riftia pachyptila* (reviewed in Minic and Hervé, 2004). As adults, *Riftia* completely lack a mouth, the digestive system and anus and are sustained by chemoautotrophic H_2S -oxidizing Bacteria belonging to the gamma-Proteobacteria (Cavanaugh et al., 1981; Felbeck, 1981; Jones, 1981; Minic and Hervé, 2004). In this relationship, *Riftia* provide the bacteria with inorganic carbon, O_2 , H_2S , and NO_3^- and, in turn are provided with organic carbon. Other invertebrate organisms such as the giant clam *Calypotgena magnifica* and the mytilid mussels *Bathymodiolus* spp. also house H_2S -oxidizing endosymbionts in their gill tissues (Van Dover, 2000). The symbiotic relationship between mussels and bacteria, however, is not obligate as they retain some of their filter feeding capabilities. In addition, certain species of *Bathymodiolus* have methanotrophic (CH_4 -oxidizing) endosymbionts and even dual symbionts with both sulfur- and CH_4 -oxidizing bacteria.

Hydrothermal plumes

Upon reaching neutral buoyancy, hydrothermal vent plumes carrying elevated concentrations of S, metals (Fe and Mn), H₂, and CH₄ extend hundreds of meters laterally into the water column. Not surprising, elevated biomass has been detected in plumes, suggesting that these environments are areas of high productivity (reviewed in Winn et al., 1995). Methane oxidation has been measured in plume waters collected along the Juan de Fuca Ridge, further supporting microbial activity in vent plumes (DeAngelis et al., 1993). More recently, Sunamura et al. (2004) used culture-independent techniques to assess the diversity of microbes in plumes over the Suiyo Seamount. Members of the gamma- and epsilon-Proteobacteria were dominant in the plume waters and are likely using reduced sulfur compounds because of their close phylogenetic relationship with known sulfur-oxidizers (Sunamura et al., 2004).

The first aerobic anoxygenic photosynthetic bacteria from the deep sea were isolated from plumes (Yurkov and Beatty, 1998). These organisms belong to the alpha-Proteobacteria and appear to supplement heterotrophic growth with photosynthesis under laboratory conditions. Their presence in deep-sea hydrothermal environments seems puzzling because of the lack of sunlight, but high-temperature fluids (≈350°C) have been detected to emit photons capable of supporting facultative photosynthetic bacteria (Van Dover et al., 1996). Their role and significance in the environment, however, remain unknown.

Conclusions

Although we know significantly more about the diversity of microorganisms from deep-sea vents since their discovery in the late 1970s, we still have a rudimentary understanding of their ecology and geobiology. Recently, the discovery of acidophiles (Reysenbach et al., 2006), high-temperature nitrogen fixation (Mehta and Baross, 2006), and high-temperature Fe reduction (Kashefi and Lovley, 2003) provided additional insights into the roles microbes play in biogeochemical cycling at vents. Furthermore, studies are now being undertaken to explore the temporal changes that occur in the microbial communities as the available carbon and energy sources change (e.g., Pagé et al., 2008). Whether these changes are due to the in situ biological activity or due to the geochemical constraints of the hydrothermal fluids and mixing and subsurface reaction zones is still unknown. It is clear that deep-sea vents represent one of the few places on Earth where the “bio” is so tightly coupled to the “geo.”

Bibliography

- Allen, D. A., and Seyfried, W. E., 2004. Serpentinization and heat generation: constraints from Lost City and Rainbow hydrothermal systems. *Geochimica et Cosmochimica Acta*, **68**, 1347–1354.
- Alt, J. C., 1995. Subseafloor processes in mid-ocean ridge hydrothermal systems. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and*

- Geological Interactions*. Washington, DC: American Geophysical Union, pp. 85–114.
- Boone, D. R., Whitman, W. B., and Koga, Y., 2001. Order III Methanosarcinales. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 268–293.
- Brazelton, W. J., Schrenk, M. O., Kelley, D. S., and Baross, J. A., 2006. Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Applied and Environmental Microbiology*, **72**, 6257–6270.
- Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K., 2006. The versatile ε-proteobacteria: key players in sulphidic habitats. *Nature Reviews in Microbiology*, **4**, 458–468.
- Campbell, B. J., Jeannot, C., Kostka, J. E., Luther, G. W. III, and Cary, S. C., 2001. Growth and phylogenetic properties of novel bacteria belonging to the epsilon subdivision of the Proteobacteria enriched from *Alvinella pompejana* and deep-sea hydrothermal vents. *Applied and Environmental Microbiology*, **67**, 4566–4572.
- Cary, S. C., Campbell, B. J., and DeLong, E. F., 2004. Studying the deep subsurface biosphere: emerging technologies and applications. In Wilcock, W. S. D., et al. (eds.), *The Subseafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union, pp. 383–399.
- Cavanaugh, C. M., Gardiner, S. L., Jones, M. L., Jannasch, H. W., and Waterbury, J. B., 1981. Prokaryotic cells in hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts. *Science*, **213**, 340–342.
- Christie, D. M., and Fisher, C. R., 2006. Geological, biological, chemical, and physical interactions in back-arc spreading systems – an introduction. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 1–4.
- Christie, D. M., Fisher, C. R., Lee, S.-M., and Givens, S. (eds.), 2006. *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union.
- DeAngelis, M. A., Lilley, M. D., Olsen, E. J., and Baross, J. A., 1993. Methane oxidation in deep-sea hydrothermal plumes of the Endeavor Segment of the Juan de Fuca Ridge. *Deep-Sea Research I*, **40**, 1169–1186.
- Desbruyères, D., Chevillon, P., Alayse, A.-M., Jollivet, D., Lallier, F. H., Jouin-Toulmond, C., Zal, F., Sarradin, P.-M., Cosson, R., Caprais, J.-C., Arndt, C., O'Brien, J., Guezennec, J., Hourdez, S., Riso, R., Gaill, F., Laubier, L., and Toulmond, A., 1998. Biology and ecology of the “Pompeii worm” (*Alvinella pompejana* Desbruyères and Laubier), a normal dweller of an extreme deep-sea environment: a synthesis of current knowledge and recent developments. *Deep-Sea Research II*, **45**, 383–422.
- Dhillon, A., Lever, M., Lloyd, K. G., Albert, D. B., Sogin, M. L., and Teske, A., 2005. Methanogen diversity evidenced by molecular characterization of methyl coenzyme M reductase A (*mcrA*) genes in hydrothermal sediments of the Guaymas Basin. *Applied and Environmental Microbiology*, **71**, 4592–4601.
- Dick, G. J., Lee, Y. E., and Tebo, B. M., 2006. Manganese(II)-oxidizing *Bacillus* spores in Guaymas hydrothermal sediments and plumes. *Applied and Environmental Microbiology*, **72**, 3184–3190.
- Douville, E., Charlou, J. L., Oelkers, E. H., Bienvu, P., Colon, C. F. J., Donval, J. P., Rouquet, Y., Prieur, D., and Appriou, P., 2002. The Rainbow vent fluids (36° 14' N, MAR): the influence of ultramafic rocks and phase separation on trace metal content in Mid-Atlantic Ridge hydrothermal fluids. *Chemical Geology*, **184**, 37–48.

- Edwards, K. J., 2004. Formation and degradation of seafloor hydrothermal sulfide deposits. In Amend, J. P., et al. (eds.), *Sulfur Biogeochemistry: Past and Present*. Boulder, CO: The Geological Society of America, pp.83–96.
- Edwards, K. J., Bach, W., and McCollom, T. M., 2005. Geomicrobiology in oceanography: microbe-mineral interactions at and below the seafloor. *Trends in Microbiology*, **13**, 449–456.
- Edwards, K. J., Rogers, D. R., Wirsén, C. O., and McCollom, T. M., 2003a. Isolation and characterization of novel psychrophilic, neutrophilic, Fe-oxidizing, chemolithoautotrophic α - and γ -*Proteobacteria* from the deep sea. *Applied and Environmental Microbiology*, **69**, 2906–2913.
- Edwards, K. J., McCollom, T. M., Konishi, H., and Buseck, P. R., 2003b. Seafloor bioalteration of sulfide minerals: results from in situ incubation studies. *Geochimica et Cosmochimica Acta*, **67**, 2843–2856.
- Felbeck, H., 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, *Riftia pachyptila* Jones (Vestimentifera). *Science*, **213**, 336–338.
- Gamo, T., Ishibashi, J., Tsunogai, U., Okamura, K., and Chiba, H., 2006. Unique geochemistry of submarine hydrothermal fluids from arc-back-arc settings of the western Pacific. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 147–161.
- German, C. R., Lin, J., and Parson, L. M. (eds.), 2004. *Mid-Ocean Ridges: Hydrothermal Interactions Between the Lithosphere and Oceans*. Washington, DC: American Geophysical Union.
- German, C. R., and Von Damm, K. L., 2004. Hydrothermal processes. In Holland, H. D., and Turekian, K. K. (eds.), *Treatise on Geochemistry, Volume 6: The Oceans and Marine Geochemistry*. London: Elsevier.
- Harmsen, H. J. M., Prieur, D., and Jeanthon, C., 1997. Distribution of microorganisms in deep-sea hydrothermal vent chimneys investigated by whole-cell hybridization and enrichment culture of thermophilic subpopulations. *Applied and Environmental Microbiology*, **63**, 2876–2883.
- Hoek, J., Banta, A., Hubler, F., and Reysenbach, A.-L., 2003. Microbial diversity of a sulphide spire located in the Edmond deep-sea hydrothermal vent field on the Central Indian Ridge. *Geobiology*, **1**, 119–127.
- Huber, J. A., Mark Welch, D. B., Morrison, H. G., Huse, S. M., Neal, P. R., Butterfield, D. A., and Sogin, M. L., 2007. Microbial population structures in the deep marine biosphere. *Science*, **318**, 97–100.
- Humphris, S. E., Zierenberg, R. A., Mullineaux, L. S., and Thomson, R. E. (eds.), 1995. *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union.
- Jannasch, H. W., 1995. Microbial interactions with hydrothermal fluids. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union, pp. 273–296.
- Jones, M. L., 1981. *Riftia pachyptila* Jones: observations on the vestimentiferan worm from the Galapagos Rift. *Science*, **213**, 333–336.
- Juniper, S. K., and Tebo, B. M., 1995. Microbe-metal interactions and mineral deposition at hydrothermal vents. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 219–253.
- Kallmeyer, J., and Boetius, A., 2004. Effects of temperature and pressure on sulfate reduction and anaerobic oxidation of methane in hydrothermal sediments of Guaymas Basin. *Applied and Environmental Microbiology*, **70**, 1231–1233.
- Karl, D. M., 1995a. *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press.
- Karl, D. M., 1995b. Ecology of free-living, hydrothermal vent microbial communities. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 35–124.
- Kashefi, K., and Lovley, D. R., 2003. Extending the upper temperature limit for life. *Science*, **301**, 934.
- Kelley, D. S., Karson, J. A., Blackman, D. K., Früh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Schrenk, M. O., Roe, K. K., Lebon, G. T., Rivizzigno, P., and the AT3-60 Shipboard Party., 2001. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30°N. *Nature*, **412**, 145–149.
- Kelley, D. S., Baross, J. A., and Delaney, J. R., 2002. Volcanoes, fluids, and life at mid-ocean ridge spreading centers. *Annual Reviews in Earth and Planetary Sciences*, **30**, 385–491.
- Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson, D., Buckman, K., Bradley, A. S., Brazelton, W. J., Roe, K., Elend, M. J., Delacour, A., Bernasconi, S. M., Lilley, M. D., Baross, J. A., Simmons, R. E., and Sylva, S. P., 2005. A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science*, **307**, 1428–1434.
- López-García, P., Gaill, F., and Moreira, D., 2002. Wide bacterial diversity associated with tubes of the vent worm *Riftia pachyptila*. *Environmental Microbiology*, **4**, 204–215.
- McCliment, E. A., Voglesonger, K. M., O'Day, P. A., Dunn, E. E., Holloway, J. R., and Cary, S. C., 2006. Colonization of nascent, deep-sea hydrothermal vents by a novel archaeal and nanoarchaeal assemblage. *Environmental Microbiology*, **8**, 114–125.
- McCollom, T. M., 2000. Geochemical constraints on primary productivity in submarine hydrothermal vent plumes. *Deep-Sea Research I*, **47**, 85–101.
- McCollom, T. M., 2007. Geochemical constraints on sources of metabolic energy for chemolithoautotrophy in ultramafic-hosted deep-sea hydrothermal systems. *Astrobiology*, **7**, 933–950.
- McCollom, T. M., and Shock, E. L., 1997. Geochemical constraints on chemolithoautotrophic metabolism by microorganisms in seafloor hydrothermal systems. *Geochimica et Cosmochimica Acta*, **61**, 4375–4391.
- Mehta, M. P., and Baross, J. A., 2006. Nitrogen fixation at 92 degrees C by a hydrothermal vent archaeon. *Science*, **314**, 1783–1786.
- Minic, Z., and Hervé, G., 2004. Biochemical and enzymological aspects of the symbiosis between the deep-sea tubeworm *Riftia pachyptila* and its bacterial endosymbiont. *European Journal of Biochemistry*, **271**, 3093–3102.
- Miroshnichenko, M. L., and Bonch-Osmolovskaya, E. A., 2006. Recent developments in the thermophilic microbiology of deep-sea hydrothermal vent. *Extremophiles*, **10**, 85–96.
- Moussard, H., Henneke, G., Moreira, D., Jouffe, V., Lopez-Garcia, P., and Jeanthon, C., 2006. Thermophilic lifestyle for an uncultured archaeon from hydrothermal vents: evidence from environmental genomics. *Applied and Environmental Microbiology*, **72**, 2268–2271.
- Nakagawa, S., Takai, K., Inagaki, F., Hirayama, H., Nunoura, T., Horikoshi, K., and Sako, Y., 2005. Distribution, phylogenetic diversity and physiological characteristics of epsilon-Proteobacteria in a deep-sea hydrothermal field. *Environmental Microbiology*, **7**, 1619–1632.
- Nakagawa, T., Takai, K., Suzuki, Y., Hirayama, H., Konno, U., Tsunogai, U., and Horikoshi, K., 2006. Geomicrobiological exploration and characterization of a novel deep-sea hydrothermal system at the TOTO caldera in the Mariana Volcanic Arc. *Environmental Microbiology*, **8**, 37–49.
- Nelson, D. C., Wirsén, C. O., and Jannasch, H. W., 1989. Characterization of large, autotrophic *Beggiatoa* spp. abundant at

- hydrothermal vents of the Guaymas Basin. *Applied and Environmental Microbiology*, **55**, 2909–2917.
- Nercessian, O., Reysenbach, A.-L., Prieur, D., and Jeanthon, C., 2003. Archaeal diversity associated with in situ samplers deployed on hydrothermal vents on the East Pacific Rise (13°N). *Environmental Microbiology*, **5**, 492–502.
- Nercessian, O., Prokofeva, M., Lebedinski, A. I., L'Haridon, S., Cary, C., Prieur, D., and Jeanthon, C., 2004. Design of 16S rRNA-targeted oligonucleotide probes for detecting cultured and uncultured archaeal lineages in high-temperature environments. *Environmental Microbiology*, **6**, 170–182.
- Orphan, V. J., House, C. H., Hinrichs, K.-W., McKeegan, K. D., and DeLong, E. F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Orphan, V. J., House, C. H., Hinrichs, K.-W., McKeegan, K. D., and DeLong, E. F., 2002. Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proceedings of the National Academy of Sciences*, **99**, 7663–7668.
- Pagé, A., Tivey, M. K., Stakes, D. S., and Reysenbach, A.-L., 2008. Temporal and spatial archaeal colonization of hydrothermal vent deposits. *Environmental Microbiology*, **10**, 874–884.
- Perner, M., Kuever, J., Seifert, R., Pape, T., Koschinsky, A., Schmidt, K., Strauss, H., and Imhoff, J. F., 2007. The influence of ultramafic rocks on microbial communities at the Logatchev hydrothermal field, located 15°N on the Mid-Atlantic Ridge. *FEMS Microbiology Ecology*, **61**, 97–109.
- Reysenbach, A.-L., 2001a. Phylum Aquificae. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 359–367.
- Reysenbach, A.-L., 2001b. Phylum Thermotogae. In Boone, D. R., et al. (eds.), *Bergey's Manual of Systematic Bacteriology, The Archaea, Photosynthetic Bacteria, and Deeply Branched Bacteria*, 2nd edn. Berlin: Springer, Vol. 1, pp. 369–387.
- Reysenbach, A.-L., 2001. Deep-sea ridges, microbiology. In Steele, J. H., Thorpe, S. A., and Turekian, K. K. (eds.), *Encyclopedia of Ocean Sciences*. London, UK: Academic Press, Vol. 2, pp. 693–700.
- Reysenbach, A.-L., Longnecker, K., and Kirshtein, J., 2000. Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic Ridge hydrothermal vent. *Applied and Environmental Microbiology*, **66**, 3798–3806.
- Reysenbach, A.-L., Götz, D., and Yernool, D., 2002. Microbial diversity of marine and terrestrial thermal springs. In Staley, J. T., and Reysenbach, A.-L. (eds.), *Biodiversity of Microbial Life*. New York: Wiley-Liss, Inc., pp. 345–421.
- Reysenbach, A.-L., Liu, Y., Banta, A. B., Beveridge, T. J., Kirshtein, J. D., Schouten, S., Tivey, M. K., Von Damm, K. L., and Voytek, M. A., 2006. A ubiquitous thermoacidophilic archaeon from deep-sea hydrothermal vents. *Nature*, **442**, 444–447.
- Rogers, D. R., Santelli, C. M., and Edwards, K. J., 2003. Geomicrobiology of deep-sea deposits: estimating community diversity from low-temperature seafloor rocks and minerals. *Geobiology*, **1**, 109–117.
- Schmeisser, C., Steele, H., and Streit, W. R., 2007. Metagenomics, biotechnology with non-culturable microbes. *Applied Microbiology and Technology*, **75**, 955–962.
- Schrenk, M. O., Kelley, D. S., Delaney, J. R., and Baross, J. A., 2003. Incidence and diversity of microorganisms within the walls of an active deep-sea sulfide chimney. *Applied and Environmental Microbiology*, **69**, 3580–3592.
- Schrenk, M. O., Kelley, D. S., Bolton, S. A., and Baross, J. A., 2004. Low archaeal diversity linked to seafloor geochemical processes at the Lost City hydrothermal field, Mid-Atlantic Ridge. *Environmental Microbiology*, **6**, 1086–1095.
- Seyfried, W. E. Jr., and Mottl, M. J., 1995. Geologic setting and chemistry of deep-sea hydrothermal vents. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 1–34.
- Sunamura, M., Higashi, Y., Miyako, C., Ishibashi, J., and Maruyama, A., 2004. Two *Bacteria* are predominant in the Suiyo Seamount hydrothermal plume. *Applied and Environmental Microbiology*, **70**, 1190–1198.
- Suzuki, Y., Inagaki, F., Takai, K., Nealson, K. H., and Horikoshi, K., 2004. Microbial diversity in inactive chimney structures from deep-sea hydrothermal systems. *Microbial Ecology*, **47**, 186–196.
- Takai, K., and Horikoshi, K., 1999. Genetic diversity of archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Takai, K., Komatsu, T., Inagaki, F., and Horikoshi, K., 2001. Distribution of archaea in a black smoker chimney structure. *Applied and Environmental Microbiology*, **67**, 3618–3629.
- Takai, K., Nakagawa, S., Reysenbach, A.-L., and Hoek, J., 2006. Microbial ecology of mid-ocean ridges and back-arc basins. In Christie, D. M., et al. (eds.), *Back-Arc Spreading Centers: Geological, Biological, Chemical, and Physical Interactions*. Washington, DC: American Geophysical Union, pp. 185–213.
- Tivey, M. K., 2004. Environmental conditions within active seafloor vent structures: sensitivity to vent fluid composition and fluid flow. In Wilcock, W. S. D., et al. (eds.), *The Seafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union, pp. 137–152.
- Tivey, M. K., 2007. Generation of seafloor hydrothermal vent fluids and associated mineral deposits. *Oceanography*, **20**, 50–65.
- Von Damm, K. L., 1995. Controls on the chemistry and temporal variability of seafloor hydrothermal fluids. In Humphris, S. E., et al. (eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions*. Washington, DC: American Geophysical Union, pp. 222–247.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton, NJ: Princeton University Press.
- Van Dover, C. L., Reynolds, G. T., Chave, A. D., and Tyson, J. A., 1996. Light at deep-sea hydrothermal vents. *Geophysical Research Letters*, **23**, 2049–2052.
- Wilcock, W. S. D., DeLong, E. F., Kelley, D. S., Baross, J. A., and Cary, S. C. (eds.), 2004. *The Seafloor Biosphere at Mid-Ocean Ridges*. Washington, DC: American Geophysical Union.
- Winn, C. D., Cowen, J. P., and Karl, D. M., 1995. Microbes in deep-sea hydrothermal plumes. In Karl, D. M. (ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. New York: CRC Press, pp. 255–273.
- Yurkov, V., and Beatty, J. T., 1998. Isolation of aerobic anoxygenic photosynthetic bacteria from black smoker plume waters of the Juan de Fuca Ridge in the Pacific Ocean. *Applied and Environmental Microbiology*, **64**, 337–341.

Cross-references

[Acidophiles](#)
[Archaea](#)
[Bacteria](#)
[Beggiatoa](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Extreme Environments](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(II\)-Reducing Prokaryotes](#)
[Hot Springs and Geysers](#)

[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Terrestrial](#)
[Iron Sulfide Formation](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Methanogens](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Origin of Life](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

HYDROTHERMAL ENVIRONMENTS, TERRESTRIAL

Robin W. Renaut¹, Brian Jones²

¹University of Saskatchewan, Saskatoon, SK, Canada

²University of Alberta, Edmonton, Alberta, Canada

Definition

Hydrothermal Environments, Terrestrial. Areas on the Earth's surface that are under the influence of geothermal waters, steam, and associated gases discharged from hot springs, geysers, and fumaroles.

Introduction

Terrestrial hydrothermal environments are those settings where fluids discharge either at or close to the land surface at a temperature that is significantly above the local ambient air temperature. The hydrothermal processes transfer heat and dissolved matter to the surface in a liquid or vapor (gas) phase. Those fluids originate at variable depths below the Earth's surface and have a wide range of temperature and chemical composition. Visible features of terrestrial hydrothermal environments include hot springs, geysers, fumaroles, and steam vents. Travertine (calcite and aragonite: CaCO_3) and sinter (mainly opal-A: $\text{SiO}_2 \cdot n\text{H}_2\text{O}$), precipitated from thermal water, commonly form mounds and terraces around many spring and geyser vents. In contrast, chemical reactions between acidic hydrothermal fluids and the host bedrock and soils usually produce extensive hydrothermal alteration that generates a very different type of landscape.

Since the pioneering studies of life in hydrothermal environments, begun in the 1960s by Brock (1978) and colleagues, interest in these environments has increased significantly for many reasons (Bock and Goode, 1996; Renaut and Jones, 2000; Jones and Renaut, 2003a). The extremophiles that inhabit modern high-temperature settings, which include thermophiles and thermoacidophiles, have implications for understanding the origin of life on the Earth and potentially on other planets. Many of the rocks precipitated around hot springs have stromatolitic fabrics and contain numerous fossilized microbes. Many recent studies have tried to determine if such microbes

directly influence mineral precipitation, or are simply trapped in essentially abiotic mineral deposits. From a geological perspective, surface hydrothermal environments can provide clues to subsurface processes including the potential for epithermal ore mineralization and for geothermal energy development (Pirajno, 1992; Bogie et al., 2005).

Geothermal fields

The geothermal gradient, a measure of the increase in temperature with depth in the Earth's crust, has an average value of $25\text{--}30^\circ\text{C km}^{-1}$, but this may rise to several hundred $^\circ\text{C km}^{-1}$ in regions of volcanic activity. Geothermal systems are found in regions with normal and slightly elevated geothermal gradients, but high-temperature systems are most common in regions of active tectonics and volcanism near plate margins (Figure 1). Although geothermal fields can extend over several thousand square kilometers, the individual surface areas of hydrothermal discharge tend to be localized and of limited extent within those domains.

Using stable isotopic evidence, Craig (1963) confirmed that most hydrothermal fluids are meteoric in origin. Some hydrothermal systems are partly fed by connate (formation) waters that were buried along with the host sediments; other hydrothermal fluids may derive from juvenile (magmatic) and metamorphic waters, but these are usually minor components if present (Nicholson, 1993; Giggenbach, 1994).

The fluids that feed high-temperature (high-enthalpy) liquid-dominated hydrothermal systems derive mainly from cool meteoric water that has circulated to a few kilometers depth (up to ~ 5 km) via faults, fractures, and permeable horizons. The heated fluids react with host rocks, acquire solutes, and typically evolve to become saline ($3,000\text{--}5,000 \text{ mg kg}^{-1}$) and chloride-rich, forming geothermal reservoirs with temperatures of up to $\sim 400^\circ\text{C}$. Those fluids then rise convectively and may boil with the decreasing pressure during ascent to create a two-phase (steam + liquid) zone below the land surface. The liquid phase may discharge at the surface as hot springs, whereas the vapors released on boiling (steam separation) may discharge at the surface as fumaroles. Rising hot fluids may mix with shallow groundwaters before surface discharge, which may modify their composition and temperature. Not all geothermal systems are of high-temperature type. In low-temperature (low-enthalpy) systems, the reservoir temperature may be $<150^\circ\text{C}$, and the fluids may not boil before being discharged as springs.

Thermal fluids

Geothermal fluids are commonly classified according to their dominant anion, into chloride waters, sulfate waters, and bicarbonate waters (Table 1) (Henley and Ellis, 1983; Nicholson, 1993). Waters of mixed anion composition are also found. The composition of the discharged fluids

[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Terrestrial](#)
[Iron Sulfide Formation](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Methanogens](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Origin of Life](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Thiomargarita](#)
[Thiotrophic Bacteria](#)

HYDROTHERMAL ENVIRONMENTS, TERRESTRIAL

Robin W. Renaut¹, Brian Jones²

¹University of Saskatchewan, Saskatoon, SK, Canada

²University of Alberta, Edmonton, Alberta, Canada

Definition

Hydrothermal Environments, Terrestrial. Areas on the Earth's surface that are under the influence of geothermal waters, steam, and associated gases discharged from hot springs, geysers, and fumaroles.

Introduction

Terrestrial hydrothermal environments are those settings where fluids discharge either at or close to the land surface at a temperature that is significantly above the local ambient air temperature. The hydrothermal processes transfer heat and dissolved matter to the surface in a liquid or vapor (gas) phase. Those fluids originate at variable depths below the Earth's surface and have a wide range of temperature and chemical composition. Visible features of terrestrial hydrothermal environments include hot springs, geysers, fumaroles, and steam vents. Travertine (calcite and aragonite: CaCO_3) and sinter (mainly opal-A: $\text{SiO}_2 \cdot n\text{H}_2\text{O}$), precipitated from thermal water, commonly form mounds and terraces around many spring and geyser vents. In contrast, chemical reactions between acidic hydrothermal fluids and the host bedrock and soils usually produce extensive hydrothermal alteration that generates a very different type of landscape.

Since the pioneering studies of life in hydrothermal environments, begun in the 1960s by Brock (1978) and colleagues, interest in these environments has increased significantly for many reasons (Bock and Goode, 1996; Renaut and Jones, 2000; Jones and Renaut, 2003a). The extremophiles that inhabit modern high-temperature settings, which include thermophiles and thermoacidophiles, have implications for understanding the origin of life on the Earth and potentially on other planets. Many of the rocks precipitated around hot springs have stromatolitic fabrics and contain numerous fossilized microbes. Many recent studies have tried to determine if such microbes

directly influence mineral precipitation, or are simply trapped in essentially abiotic mineral deposits. From a geological perspective, surface hydrothermal environments can provide clues to subsurface processes including the potential for epithermal ore mineralization and for geothermal energy development (Pirajno, 1992; Bogie et al., 2005).

Geothermal fields

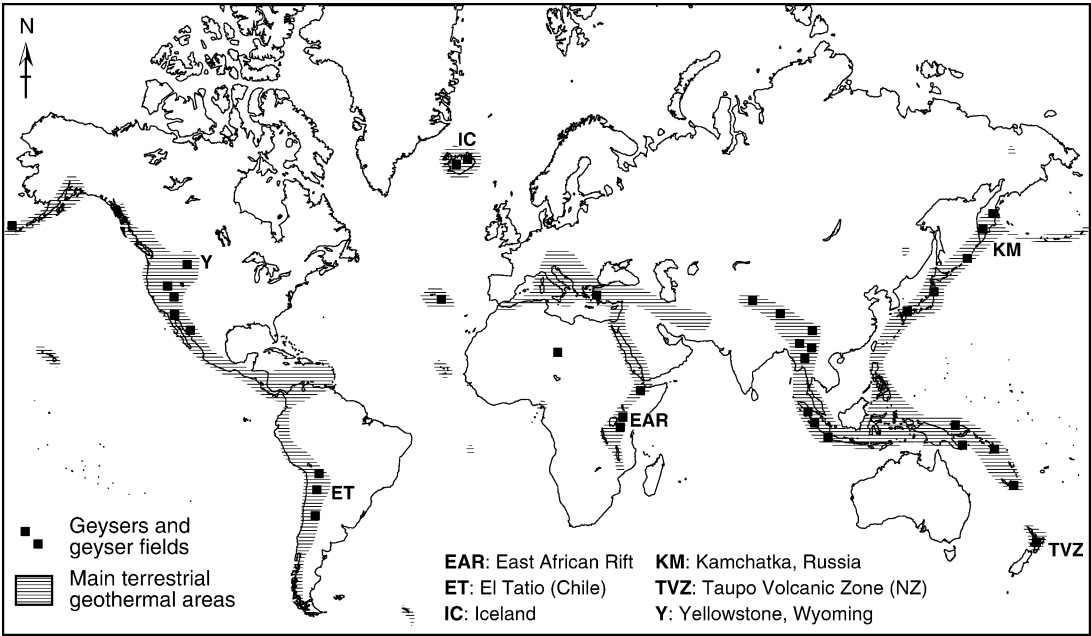
The geothermal gradient, a measure of the increase in temperature with depth in the Earth's crust, has an average value of $25\text{--}30^\circ\text{C km}^{-1}$, but this may rise to several hundred $^\circ\text{C km}^{-1}$ in regions of volcanic activity. Geothermal systems are found in regions with normal and slightly elevated geothermal gradients, but high-temperature systems are most common in regions of active tectonics and volcanism near plate margins (Figure 1). Although geothermal fields can extend over several thousand square kilometers, the individual surface areas of hydrothermal discharge tend to be localized and of limited extent within those domains.

Using stable isotopic evidence, Craig (1963) confirmed that most hydrothermal fluids are meteoric in origin. Some hydrothermal systems are partly fed by connate (formation) waters that were buried along with the host sediments; other hydrothermal fluids may derive from juvenile (magmatic) and metamorphic waters, but these are usually minor components if present (Nicholson, 1993; Giggenbach, 1994).

The fluids that feed high-temperature (high-enthalpy) liquid-dominated hydrothermal systems derive mainly from cool meteoric water that has circulated to a few kilometers depth (up to ~ 5 km) via faults, fractures, and permeable horizons. The heated fluids react with host rocks, acquire solutes, and typically evolve to become saline ($3,000\text{--}5,000 \text{ mg kg}^{-1}$) and chloride-rich, forming geothermal reservoirs with temperatures of up to $\sim 400^\circ\text{C}$. Those fluids then rise convectively and may boil with the decreasing pressure during ascent to create a two-phase (steam + liquid) zone below the land surface. The liquid phase may discharge at the surface as hot springs, whereas the vapors released on boiling (steam separation) may discharge at the surface as fumaroles. Rising hot fluids may mix with shallow groundwaters before surface discharge, which may modify their composition and temperature. Not all geothermal systems are of high-temperature type. In low-temperature (low-enthalpy) systems, the reservoir temperature may be $<150^\circ\text{C}$, and the fluids may not boil before being discharged as springs.

Thermal fluids

Geothermal fluids are commonly classified according to their dominant anion, into chloride waters, sulfate waters, and bicarbonate waters (Table 1) (Henley and Ellis, 1983; Nicholson, 1993). Waters of mixed anion composition are also found. The composition of the discharged fluids



Hydrothermal Environments, Terrestrial, Figure 1 Distribution of high-temperature (high-enthalpy) terrestrial geothermal environments. Modified from Kühn (2004) and Bryan (2005).

Hydrothermal Environments, Terrestrial, Table 1 Selected chemical analyses of hot spring and geyser waters

Water type	Source	pH	T°C	Na	K	Ca	Mg	B	Cl	SO ₄	HCO ₃	SiO ₂
<i>Chloride waters</i>												
Ohaaki Pool, NZ	A	7.1	95	860	82	2.5	0.1	32	1,060	100	679	338
El Tatio, Chile	B	7.4	85	4,600	520	280	0.3	187	8,220	38	39	256
<i>Sulfate waters</i>												
Waiotapu, NZ	A	2.2	99	32	6.6	4.0	0.8	2	6	338	<1	308
Roaring Mt., Yellowstone	C	2.4	92	48	42	3.8	0	—	43	435	—	335
<i>Bicarbonate waters</i>												
L. Bogoria, Kenya	D	9.02	99	1,710	38	0.2	0.05	—	340	65	3,750	135
Mammoth, Yellowstone	E	7.2	69	130	54	323	67	4.1	163	563	755	54
<i>Mixed anion waters</i>												
Rotokawa, NZ	A	6.6	90	380	32	30	0.8	16.3	477	103	173	182
North Waiotapu, NZ	F	3.1	99	153	28	12.9	2.1	—	182	338	0	363

Sources of data: *A* Nicholson (1993, Table 2.1), expressed in mg kg⁻¹. *B* Cusicanqui et al. (1986), expressed in mg kg⁻¹. *C* Brock (1978, Table 2.3), expressed in mg l⁻¹. *D* Renaut and Jones (1997), expressed in mg l⁻¹. *E* Bargar (1978), average of 15 analyses, expressed in ppm. *F* Jones et al. (2000), expressed in ppm

profoundly affects the characteristics of the terrestrial surface environment, including any mineral deposits and hydrothermal alteration, and the biota that inhabit the areas of hydrothermal discharge.

Chloride waters (also termed “alkali-chloride” or “neutral chloride” waters)

These saline waters typify the deep geothermal fluids in most high-temperature geothermal systems and where discharged at the surface, they commonly overlie permeable zones of major thermal upflow (Nicholson, 1993).

They have near neutral pH and are chloride-rich with variable SO₄²⁻ and HCO₃⁻ concentrations. Chloride waters are typically rich in dissolved silica and have Na⁺ and K⁺ as the dominant cations. Carbon dioxide is usually the main dissolved gas with much lower amounts of H₂S.

At the land surface, chloride fluids commonly discharge from hot and boiling springs, often with clear bluish-green waters, and from most geysers (Figure 2a). Such fluids characteristically precipitate large quantities of silica sinter (see Chapter Sinter). If the fluids are rich in HCO₃⁻, they may boil at greater depths and may

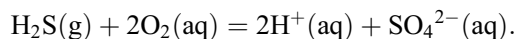
precipitate both sinter and travertine upon CO₂ degassing at the land surface.

Spectacular examples of geysers and springs that eject chloride waters are found in most of the major geothermal sites, including those in Yellowstone National Park (Bryan, 2008), the Taupo Volcanic Zone of North Island, New Zealand (Ellis and Mahon, 1977; Jones and Renaut, 2003b), El Tatio in northern Chile (Cusicanqui et al., 1976; Fernandez-Turiel et al., 2005), and Iceland (Torfason, 1985). The sinter that precipitates around many geyser and spring vents and on their discharge aprons as mounds and terraces is commonly colonized by diverse, varicolored, microbial communities.

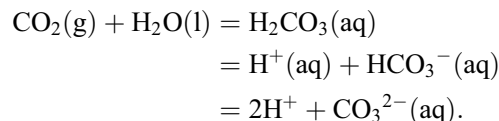
Sulfate waters ("acid sulfate waters")

Sulfate-rich waters form through condensation of geothermal gases in shallow oxygenated groundwater. Steam, gases, and other volatiles (e.g., NH₃, As, B) that were originally dissolved in deep geothermal fluids can separate from chloride waters when they undergo boiling at depth, and rise toward the surface. The steam-heated acidic waters commonly boil at the water table because the separated steam contains high enthalpy from the deeper geothermal fluid.

Sulfate, the dominant anion, is formed by oxidation of condensed hydrogen sulfide (Nicholson, 1993):

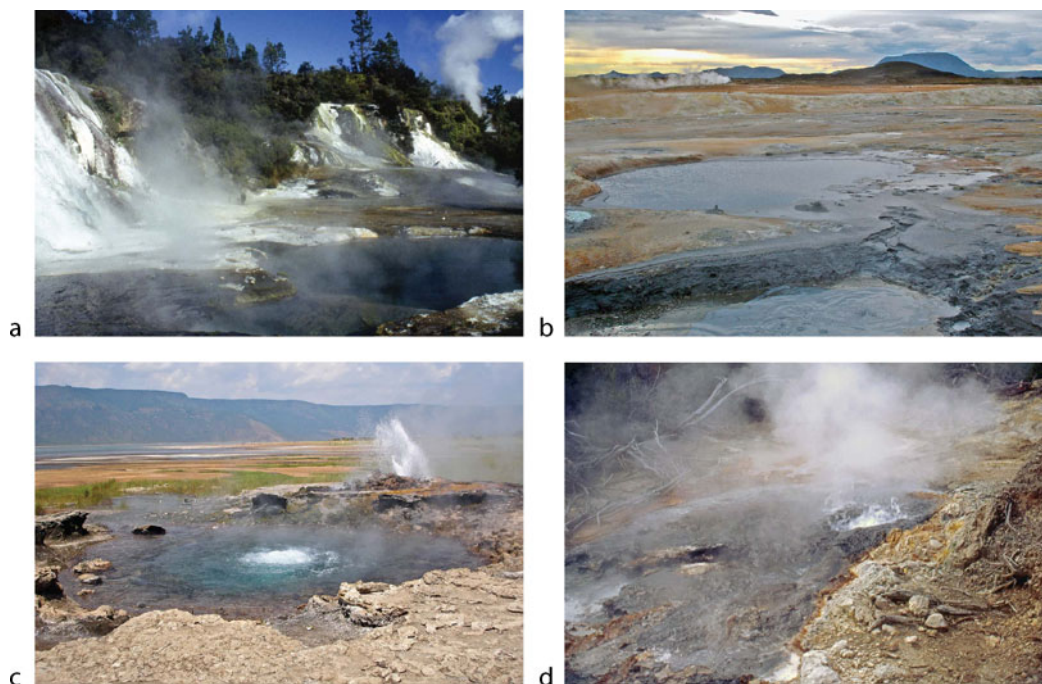


The protons produced may be supplemented by condensation of CO₂ to create acidic waters:



The pH is typically between 2.8 and ~5. Chloride is usually present in minor amounts. Bicarbonate is usually absent because it is lost from solution as CO₂ gas. These acidic fluids, typically present above the water table close to the land surface (<100 m depth), are usually found around the margins of geothermal fields at sites distant (few hundred meters to several kilometers) from the main chloride-rich upflow.

Acidic waters form distinctive surface environments characterized by hot springs, turbid boiling mud pools, or steaming ground commonly with extensive pits and collapse features resulting from hydrothermal alteration (Figure 2b). The acidic waters leach the host rocks



Hydrothermal Environments, Terrestrial, Figure 2 Variations in modern hydrothermal environments according to water chemistry. (a) Hot springs, geysers, and extensive silica sinter coating a fault scarp at Orakeikorako, New Zealand. The fluids are alkaline chloride waters. (b) Muddy hot springs and fumaroles at Myvatn, northern Iceland. The fluids are acid sulfate in composition. Hydrothermal alteration of young volcanic rocks has produced extensive clays and Fe oxides at the land surface. (c) Boiling hot spring and perpetually spouting hot spring (*right*) rimmed by calcite travertine on the shoreline of Lake Bogoria, Kenya. The spring water is of sodium bicarbonate composition. (d) Small geyser discharging mixed acid chloride-sulfate waters (pH: 2.1–2.6) near Hakereteke Stream, north Waiotapu, New Zealand. Thin Fe-rich sinter covers the substrate.

producing extensive clays (mainly kaolinite), commonly with alunite, alunogen, jarosite, Fe-oxyhydroxides, pyrite, cristobalite, and native sulfur. Little sinter forms in acidic waters, but alteration of volcanic rocks by sulfuric acid can leave behind abundant opaline silica in place, termed “silica residue” (Rodgers et al., 2002). Well-developed examples of acid sulfate water discharge are found at Tikitere (New Zealand), Myvatn (northern Iceland), and Norris Geyser Basin (Yellowstone National Park).

Bicarbonate waters

Bicarbonate-rich hot waters are formed mainly when steam and gases (mainly CO_2) condense in shallow groundwaters. Although most common around the margins of geothermal fields, they may also form in sites distant from volcanic activity. They often form where geothermal fluids are in contact with limestone or volcanic bedrock. Such fluids normally have a near-neutral pH when discharged. The initial acidity of the rising CO_2 -rich fluids is neutralized by hydrolysis reactions with bedrock that consume protons (H^+) leaving waters dominated by HCO_3^- and Na^+ . Sulfate and chloride have variable concentrations. At the land surface, these fluids discharge from springs that range from warm to boiling and most have clear water. Travertine deposits, composed of calcite and (or) aragonite, commonly form around the vent and downslope of these springs (Figure 2c) where sufficient Ca^{2+} is available. Sinter is generally absent, but minor amounts can form from very hot and boiling bicarbonate waters. Hydrothermal alteration at the surface is generally very minor, though more extensive at depth.

Carbonate deposits associated with such springs may be localized around their vents or form vast discharge aprons. Examples include the large terrace mounds, fissure ridges, and related features at Pamukkale in Turkey, Mammoth Hot Springs in Wyoming, and at many sites in central Italy (Bargar, 1978; Chafetz and Folk, 1984; Pentecost, 2005). The travertines precipitate from bicarbonate waters over a wide temperature range, from ambient to boiling. Most carbonate precipitation has been attributed to rapid loss of CO_2 upon discharge of the spring waters at the land surface (Renaut and Jones, 1997; Pentecost, 2005). Microorganisms, principally cyanobacteria, may play a role in some carbonate precipitation through photosynthetic removal of CO_2 , but at high temperatures most precipitation is probably driven by abiotic degassing of carbon dioxide.

Mixed waters

Waters that contain two anions with similar concentrations form by several different processes. *Sulfate-chloride waters* typically have a pH of ~ 2 –5, but can have a pH as low as 0. They originate through subsurface mixing of sulfate and chloride waters, oxidation of H_2S in chloride waters, near-surface condensation of volcanic gases in meteoric waters, or dissolution of sulfate-bearing bedrock by migrating thermal chloride waters (Nicholson, 1993).

Such waters discharge as warm to hot springs, commonly with thin sinter deposits and sulfate-bearing minerals around the vent. Native sulfur may also be present.

Examples of acidic sulfate-chloride springs are found in the northern part of the Waiotapu geothermal area of North Island, New Zealand (Figure 2d) (Jones and Renaut, 2006). These springs discharge waters a pH of 2.1–2.6 at temperatures $>90^\circ\text{C}$. Spicular sinters containing silicified microbes have precipitated around these springs together with kaolinite, jarosite, and gypsum.

Dilute *chloride-bicarbonate* waters form where chloride waters have been diluted by groundwater or bicarbonate water (Nicholson, 1993). They typically are found in springs of variable temperature around the margins of thermal upflow zones, but waters of this composition also discharge in low-temperature systems. The fluids are typically Cl-dominated, have variable HCO_3^- concentrations, and a neutral pH of 6–8. Minor travertine and (or) sinter may form around the vents, but many of these springs lack surface deposits.

Types of geothermal systems

The environmental characteristics of terrestrial hydrothermal sites are strongly controlled by the type of geothermal system that supplies their fluids. Many classifications of geothermal systems have been proposed (e.g., Heiken, 1982; Henley and Ellis, 1983; Pirajno, 1992; Nicholson, 1993). Kühn (2004) recognized two major groups. In *static systems*, heat may be transferred to the surface by conduction without convective fluid movement. In *dynamic systems*, heat is transferred to the surface by moving fluids undergoing convective circulation.

Static (conductive) geothermal systems

Static geothermal systems depend on above-average conductive heat flow in the crust. In static magmatic systems, fluids can be heated by granitic plutons. Other conductive systems are hosted by porous sedimentary rocks in deep sedimentary basins. Static systems generally have little impact at the surface. However, trapped formation waters, which are typically saline, chloride-rich and have temperatures of ~ 70 – 150°C , can be released by tectonic movements and discharge at the surface as thermal springs with variable temperature and salinity, depending on the degree of mixing with shallow groundwaters. Many examples are known from Europe, especially Germany (Kühn, 2004).

Dynamic (convective) geothermal systems

Most high-temperature geothermal systems are linked to magmatic intrusions (volcano-plutonic and volcanic), which provide the heat that drives the convective circulation. In such systems, reservoir temperatures are commonly $>150^\circ\text{C}$ at depths of a few kilometers. Such systems are most common in sites of geologically young volcanism and in tectonically active regions such as areas of faulting, rifting, and caldera collapse, and at plate

margins in general. As in other hydrothermal systems, the waters are predominantly meteoric, and the ascending fluids commonly boil before or during surface discharge. The hydrothermal systems vary with tectonic location and type of volcanism (Pirajno, 1992). Many are associated with silica-rich and andesitic volcanism, whereas others are associated with basaltic volcanism.

Silica-andesitic systems

High-temperature hydrothermal systems associated with silica-rich volcanism are highly variable. Some systems are liquid dominated, producing springs and geysers, whereas others are vapor dominated, generating mainly steaming ground and fumaroles at the land surface.

In regions of low relief (Figure 3a), magmatic systems supply heat and gases to meteoric groundwater and give rise to convective columns that can allow deep chloride-rich waters to flow directly to the surface (e.g., Taupo Volcanic Zone, New Zealand). The deep fluids may discharge as springs and geysers above the main upflow or may move laterally some distance away from upflow column. Steam zones commonly develop in sites marginal to the main upflow. Oxidation of H_2S and other gases in groundwater produces acid sulfate waters, generating fumaroles and steaming ground. Condensation of CO_2 in groundwater may produce neutral to alkaline bicarbonate springs around the margins of the field from which travertine may be formed. These types of system commonly have fumaroles, steaming ground, and both acid and alkaline springs located near each other because of the low topographic relief and limited potential for lateral flow of fluids near the land surface.

In areas of high relief (Figure 3b), such as andesitic stratovolcanoes, a thick two-phase zone (liquid–steam–gas) commonly develops below the land surface. At high elevations, fumaroles, steam vents, and acid sulfate springs fed by condensate are common features. Deep chloride-rich waters normally do not reach the surface but may flow laterally and mix with groundwater or cooled, descending sulfate waters before discharging as hot springs on the volcano flanks or other areas of relatively low relief. Numerous examples are found in Indonesia, the Philippines, and Japan.

Some silicic systems are vapor dominated (e.g., Lardarello, Italy; The Geysers, California). The deep thermal reservoir contains steam, probably fed by saline boiling water, most of which is contained in convective cells capped by low-permeability rocks. Fluids escaping the reservoir produce steaming ground and fumaroles at the surface and some thermal springs similar to those in high-relief regions. Surface fluids can be of acid sulfate or bicarbonate type.

Basaltic systems

High-temperature basaltic systems occur at mid-oceanic ridges (see Chapter *Hydrothermal Environments, Marine*) and in continental rifts. Many tectonic and hydrogeological settings are possible (Pirajno, 1992), but

in most examples hydrothermal fluids rise along permeable faults discharging at hot springs and geysers on the rift basin floor (Figure 2c), including subaqueous discharge on the floors of lakes. Fluid compositions are variable and depend partly on the bedrock composition, which may be volcanic, basement rocks, or valley-fill sediments.

Low temperature systems

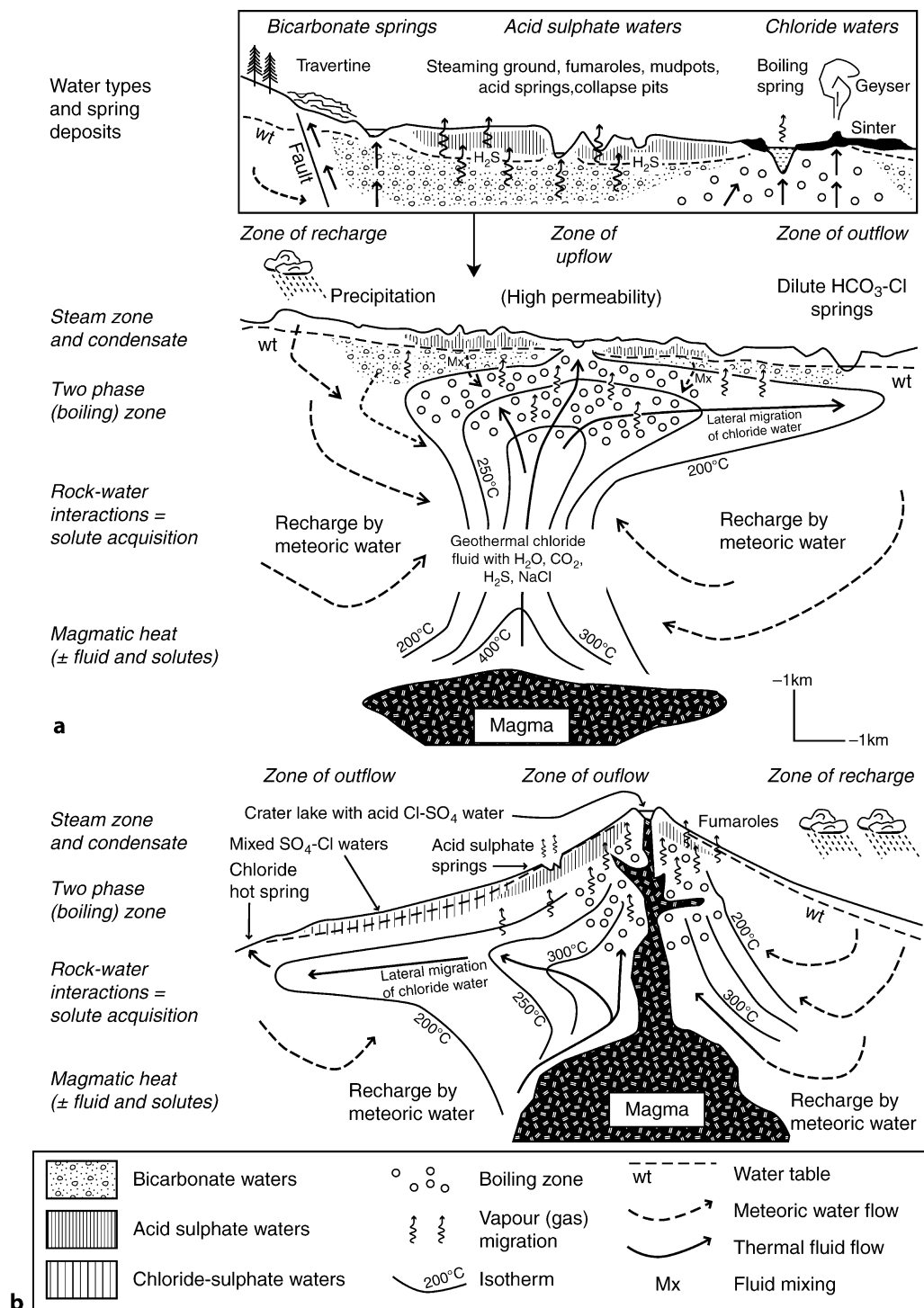
Low-temperature geothermal systems are common and of diverse origins. Most are characterized by dilute waters that are discharged as springs with maximum temperatures of about 30–60°C. They form in regions of both normal and above-average heat flow. They are common where meteoric fluids return to the surface having been heated to temperatures above ambient surface temperature. These springs commonly occur in regions of deep faulting and folding, in regions of tectonic uplift where hot rocks have been elevated to higher levels in the crust, and from the residual heat of cooling plutons (Nicholson, 1993). Meteoric fluids circulate at depth through faults, fractures, and permeable rocks. The chemical composition of the fluids reflects such factors as the bedrock mineralogical composition, depth and temperature of fluid circulation, the rates of recharge, and degree of mixing with near-surface waters before discharge. Such low-enthalpy systems are common in western Canada, Europe, Australia, and on most continents.

The surface environment in hydrothermal areas

Areas of hydrothermal discharge are distinctive features of the landscape. Where they are extensive, they can influence the local ecology at all scales, from large mammals to microbes. The impact of hydrothermal discharge at the land surface ranges from negligible, where dilute warm springs flow quietly from small vents at temperatures little above ambient, to major, where high-temperature and acidic fluids destroy vegetation, precipitate mineral deposits, or flood the landscape. In general, areas of neutral to alkaline hydrothermal discharge are mainly constructive with new rocks (sinter, travertine, and others) precipitated from hydrothermal fluids upon an existing landscape (Figure 2a, c). In contrast, where the fluids are mainly acidic, the net impact on the surface environment is often destructive and the landscape commonly becomes nearly barren and pock-marked by craters that result from collapse due to hydrothermal alteration of the host bedrock or hydrothermal explosions (Figure 2b, d). The main hydrothermal surface features are hot springs and geysers, fumaroles, steam vents, and mudpots. Some hydrothermal springs also discharge on the floors of lakes.

Hot springs

Hot springs are sites where geothermally heated groundwater discharges at the land surface (see Chapter *Hot Springs and Geysers*). There is no universally accepted definition of a hot spring (Pentecost et al., 2003). Some definitions place the lower temperature limit at an



Hydrothermal Environments, Terrestrial, Figure 3 General models of liquid-dominated magmatic geothermal systems showing relationships between subsurface fluid evolution and associated surface environments. (a) Liquid-dominated system in a low-relief setting. Inset expands the upper left half of the figure to show how surface environments and deposits vary spatially according to the type of fluid being discharged. (b) Liquid-dominated system in a high-relief (stratovolcano) setting. Adapted from figures by Henley and Ellis (1983) and Nicholson (1993).

arbitrary value (e.g., human body temperature: $\sim 37^{\circ}\text{C}$; or some fixed value: 20° , 35° , 50°C , etc.), whereas others define a hot spring by its temperature relationship to local ambient surface water temperature, mean air temperature, or a similar environmental factor. As generally used, the term hot spring is given to those springs that discharge at between $\sim 35^{\circ}\text{C}$ and the local boiling point.

Individual hot springs vary greatly in size (<10 cm to ~ 80 m in vent diameter), depth, shape, and discharge. The most common forms are circular and subcircular pools where water flows from poorly consolidated sediments or hydrothermally altered ground (Figure 4a). Very large hot springs include Grand Prismatic Spring at Yellowstone National Park and Champagne Pool at Waiotapu in New Zealand. Some large springs are composite with multiple vents discharging into a single basin (e.g., Frying Pan Lake, Waimangu, New Zealand). In contrast, shape and size can be highly irregular where flow emanates from a resistant substrate such as volcanic bedrock or from faults and fractures (Figure 4b, c). In the latter case, for example, the vents may be linear fissures without a pool. The vent morphology may change through time as pools coalesce or contract with changes, for example, in surface discharge and earthquake activity.

Geysers (Figure 4d) are hot springs characterized by intermittent turbulent discharge of water that is induced by a vapor phase of gases (mainly steam) that results from boiling. Geysers are rare (Figure 1), with more than 50% of all known geysers in Yellowstone National Park, Wyoming, USA (Bryan, 2005). Well-known geysers include Geysir in Iceland (eponym for geyser) (Torfason, 1985), Old Faithful in Yellowstone National Park (Bryan, 2008), and Pohutu at Whakarewarewa in New Zealand (Jones and Renaut, 2003b). Intermittent springs flow periodically but discharge quietly and lack the forceful ejection of a true geyser. Some springs, termed perpetual spouters (Figure 4e), erupt jets of water and steam constantly, but lack the periodicity of geysers.

Springs also change in morphology as minerals are precipitated in and around their vents (see Chapter Sinter). Mineral precipitation commonly begins around pool margins with build up of a pool-rim dam (Figure 4a), which, depending on hydrostatic pressure, may raise the water level. Water overflowing the dam may then precipitate minerals to form terraces (rimstone dams and pools), oncoids, pisoids, and a range of other spring deposits (Jones and Renaut, 2003a; Pentecost, 2005). At some springs, including many geysers, vents may grow upward forming a range of conical mound deposits (Figure 4d). Microorganisms are commonly fossilized in the spring deposits, and some have been implicated in mineral precipitation.

Fumaroles (steam vents) and mudpots

Fumaroles are vents or openings in the ground that discharge hot water vapor (steam) and (or) volcanic gases. Fumaroles are common features of active volcanoes,

where they can attain temperatures of $>900^{\circ}\text{C}$, but much cooler ($<150^{\circ}\text{C}$) vents are also present at dormant and recently extinct volcanoes and at some high-temperature spring sites, mainly in areas where the water table is relatively low. Fumaroles emit mainly steam (typically $>90\%$), carbon dioxide, and lesser amounts of sulfur (especially H_2S) and chlorine compounds. Minor carbon monoxide, ammonia, boron and fluorine compounds are present in some gases. The predominant steam is mainly superheated groundwater, whereas much of other gas is magmatically sourced. *Solfataras* are areas where fumaroles discharge large volumes of sulfur gases; *mofettes* discharge carbon dioxide, sometimes methane, from fumaroles at temperatures below boiling point of water but above those of ambient air. Mofette gases trapped in valleys and depressions can cause asphyxiation.

The impact of fumaroles on the landscape and environment reflects their abundance, gas composition, temperature, and location. Fumaroles can form on steep and low-angled slopes. On steep slopes, they typically emerge from cracks in the substrate, cm to m in scale, which may be linear, irregular, or funnel-shaped openings where gases have hydrothermally altered the bedrock. On low ground, fumaroles may pockmark the landscape with series of pits (Figure 4f), some meters in diameter. Minerals (*fumarolites*) are commonly precipitated around the vents as rock coatings, many of which are sublimates from the fumarole gases. This diverse array of minerals includes opaline silica, native sulfur, jarosite, Fe-oxyhydroxides, and many salts and rare minerals, some incorporating volatiles.

Rising steam and gases may heat the ground and condense near the land surface or may heat groundwater at shallow depth. The condensed steam and heated water may boil at the water table to produce areas of steaming ground. If H_2S is present in the vapor phase, the resulting sulfuric acid commonly attacks the bedrock and soils and may generate abundant clay minerals, especially kaolinite, producing mudpots (Figure 4g). Forceful eruption of viscous mud can produce mud volcanoes (Figure 4h). The mineralogy of mudpots has received little attention, but includes a similar suite of minerals to other acid sulfate springs.

Steam (hydrothermal) explosions

The surface environment at high-temperature hydrothermal sites occasionally undergoes major disturbances due to hydrothermal eruptions. Such eruptions have several causes but are typically related to the inability of the plumbing system to withstand high fluid pressures generated by boiling. Most eruptions result from the rapid generation of steam due to a sudden pressure reduction (Browne and Lawless, 2001). The explosive release of vapor may lead to break up of sinter or other rocks near the land surface. Blocks become strewn across the landscape, new vents may be formed, and old vents may become inactive because of the changes in the subsurface plumbing system.



Hydrothermal Environments, Terrestrial, Figure 4 Surface features in terrestrial hydrothermal environments. (a) Hot spring with sinter pool-rim dam. Oyster Pool (pH 4.5; T: 65°C), Waiotapu, New Zealand. (b) Hot spring (pH 8.8; 99°C) emerging from fractured trachyphonolite bedrock at Chemurkeu, Lake Bogoria, Kenya. (c) Low-enthalpy, dilute bicarbonate hot spring (pH: 6.6; T: 36°C) at Lobo, Lake Bogoria, Kenya. (d) Clepsydra Geyser, Yellowstone National Park. (e) Perpetually spouting hot spring (pH: 8.7; T: 98.5°C) with mound of subfossil travertine, Lake Bogoria, Kenya. (f) Large (3 m wide) fumarole vent at Sol de Mañana geothermal field, southern Bolivia. (g) Boiling mudpool at Sol de Mañana geothermal field, southern Bolivia. (h) Mud volcano, Tikitere, near Rotorua, New Zealand.

The Waimangu geothermal area on North Island, New Zealand, experienced major hydrothermal explosions on April 1, 1917 that created Frying Pan Lake, with further eruptions in 1924 and 1973 (Lloyd and Keam, 1974). The 1973 eruption, for example, distributed ejecta, which consisted of hydrothermally-altered angular blocks of country rock (up to 30 cm diameter) and fine debris, over an area of $\sim 3 \text{ km}^2$.

Sublacustrine hydrothermal discharge

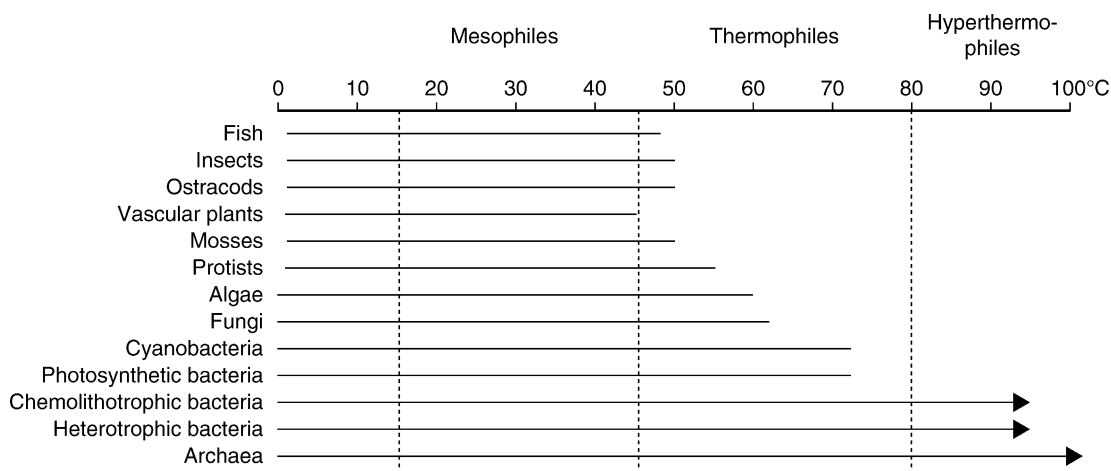
Thermal fluids can discharge directly from lake floors either as underwater springs or as steam and gas vents. Diffuse upward-moving steam and gases may also condense in lake water through permeable sediments without point-sourced vents. Sublacustrine springs are most common on the floors of caldera (crater) lakes, continental rift, and other tectonic lakes, and in lakes produced by subsidence, collapse, or hydrothermal eruptions in areas of acidic hydrothermal discharge. Their impact on life in the lake depends on the respective chemistry and temperatures of the lake water and thermal fluids, the rate and volume of thermal fluid discharge, and lake size, morphology, and climate, which can control seasonal stratification and hydrological closure. Like submarine springs, sublacustrine hot springs may host local communities of thermophilic organisms, and biofilms or microbial mats commonly cover the vent surfaces. Sublacustrine sinter, travertine (tufa), and other mineral deposits (e.g., metal sulfides) can precipitate around vents at sites where thermal fluids mix with lake water, undergo cooling, or exsolve noncondensable gases. Spring deposits include spires, chimneys, mounds, crusts, and various microbialites. Sublacustrine spring deposits commonly incorporate lacustrine organisms, especially planktonic diatoms and freshwater sponges, which can help distinguish them from morphologically similar subaerial spring deposits when examining ancient deposits.

Well-known examples of sublacustrine spring systems include those in Crater Lake (Oregon), Yellowstone Lake (Wyoming), Lakes Tanganyika, Kivu and Bogoria in the East African Rift, and Lake Taupo (North Island, New Zealand). Hot springs on the floor of Lake Taupo at depths of $\sim 140 \text{ m}$, for example, have produced small chimneys formed of opal-A and various Mn-, Fe-, and Hg-precipitates (de Ronde et al., 2002). Crater lakes in active and dormant volcanoes commonly receive discharge from acidic springs and fumaroles that produces acid lake waters.

Life in terrestrial hydrothermal environments

Terrestrial hydrothermal environments host many organisms, but some, notably thermophiles ($\sim 45^\circ\text{--}80^\circ\text{C}$) and hyperthermophiles ($>80^\circ\text{C}$), are unique to hydrothermal settings (see Chapter *Extreme Environments*). Although the ecology of hydrothermal environments has been studied extensively (e.g., Brock, 1978; Reysenbach et al., 2001; Robb et al., 2007; Wiegand, 2007), new discoveries continue to be made regularly. The distribution of microorganisms at hot springs and geysers is controlled mainly by temperature, pH, and water chemistry. Brock (1978) first established the main temperature tolerance ranges for the main groups of organisms in hot-spring systems (Figure 5).

Proximal areas under the influence of near-boiling water ejected from hot springs and geysers were once regarded as being sterile. The ornate and banded silica deposits commonly found around geyser vents were named “geyserite” because they were thought to have formed abiotically (Walter, 1976). It has since been shown that a diverse community of microorganisms inhabit these very hot waters (Blank et al., 2002) and that geyserite commonly contains abundant silicified microbes (Jones et al., 1997). Identifying these microbes is a challenge because many are difficult to culture and their



Hydrothermal Environments, Terrestrial, Figure 5 Upper temperature tolerance limits of selected organisms in hydrothermal environments (modified from Brock, 1978).

morphological diversity is low. DNA analyses, however, show that these extremophiles are biologically diverse (Barns et al., 2004). The hyperthermophiles are dominantly archaea, and some are also thermoacidophiles. In the hottest (anoxic) waters, the archaea are mainly chemolithoautotrophs and chemoorganotrophs, and include coccoid, rod-shaped, and filamentous forms (e.g., *Pyrolobus*, *Pyrococcus*, *Methanothermus*). Hyperthermophilic bacteria (e.g., *Aquifex*, *Geothermobacterium*), some of which are sulfide and hydrogen oxidizing species, also thrive in waters up to boiling point. Viruses also inhabit these high-temperature settings. Thin microbial films locally coat immersed rock and soil surfaces around the edge of the vents (Figure 6a), and filamentous stringers of hyperthermophiles are sometimes seen attached to the substrate in proximal outflow channels.

As water temperature decreases downstream from the spring and geyser vents, the species diversity usually increases. At temperatures between 75 and $\sim 40^\circ\text{C}$, microbial mats are commonly well developed in neutral and alkaline waters (Figure 6b). Varicoloured (green, orange, brown) microbial mats built by photosynthetic cyanobacteria often cover the substrate in sites that remain almost constantly moist from thermal outflow, and are commonly several cm thick (Figure 6c). Small coniform stromatolites form in some low-energy pools (Figure 6d, e) (Jones et al., 2002). Common genera include *Oscillatoria*, *Synechococcus*, *Phormidium*, *Anabaena*, and *Spirulina*. *Chloroflexus*, a green sulfur bacterium and anoxygenic photoautotroph, is commonly associated with these mats. Extensive extracellular polymeric gels are produced by some of these communities. Heterotrophic bacterial communities normally underlie the surface layers of these mats (Brock, 1978). Organisms common in waters cooler than $\sim 45^\circ\text{C}$ include the cyanobacteria *Calothrix* and *Pleurocapsa*, which often build nodular mats, eukaryotic algae, diatoms, fungi, and protists, some of which become important components of microbial mats.

Acid thermal areas, including areas around some fumaroles (and solfataras), usually have distinctive acidophilic communities. In acidic hot springs where the temperature is $<60^\circ\text{C}$, green mats built by the unicellular red alga *Cyanidium caldarium* are often present (Figure 6f) (Brock, 1978), together with a range of bacteria (e.g., *Thiobacillus*). Below pH 5, cyanobacteria are less abundant and mats composed of diatoms and fungi become common. Stromatolitic sinters built by diatoms, for example, are present at Frying Pan Flats at Waiotapu, New Zealand, where the pH is <3 (Figure 6g) (Jones et al., 2000).

Invertebrates, such as mat-grazing ostracods, are common in some hot spring waters that are $<45^\circ\text{C}$. Insects are common at some hot springs and include spiders, beetles, and flies. Adult and larval *Ephydrya* spp., for example, feed on microbial mats in hot spring effluent (Figure 6h). Few vertebrates are restricted to thermal aquatic environments, but fish (*Alcolapia grahami*) inhabit hot spring pools with temperatures up to 46°C at Lake Magadi, Kenya (Figure 6i).

Environmental impact of terrestrial hydrothermal activity

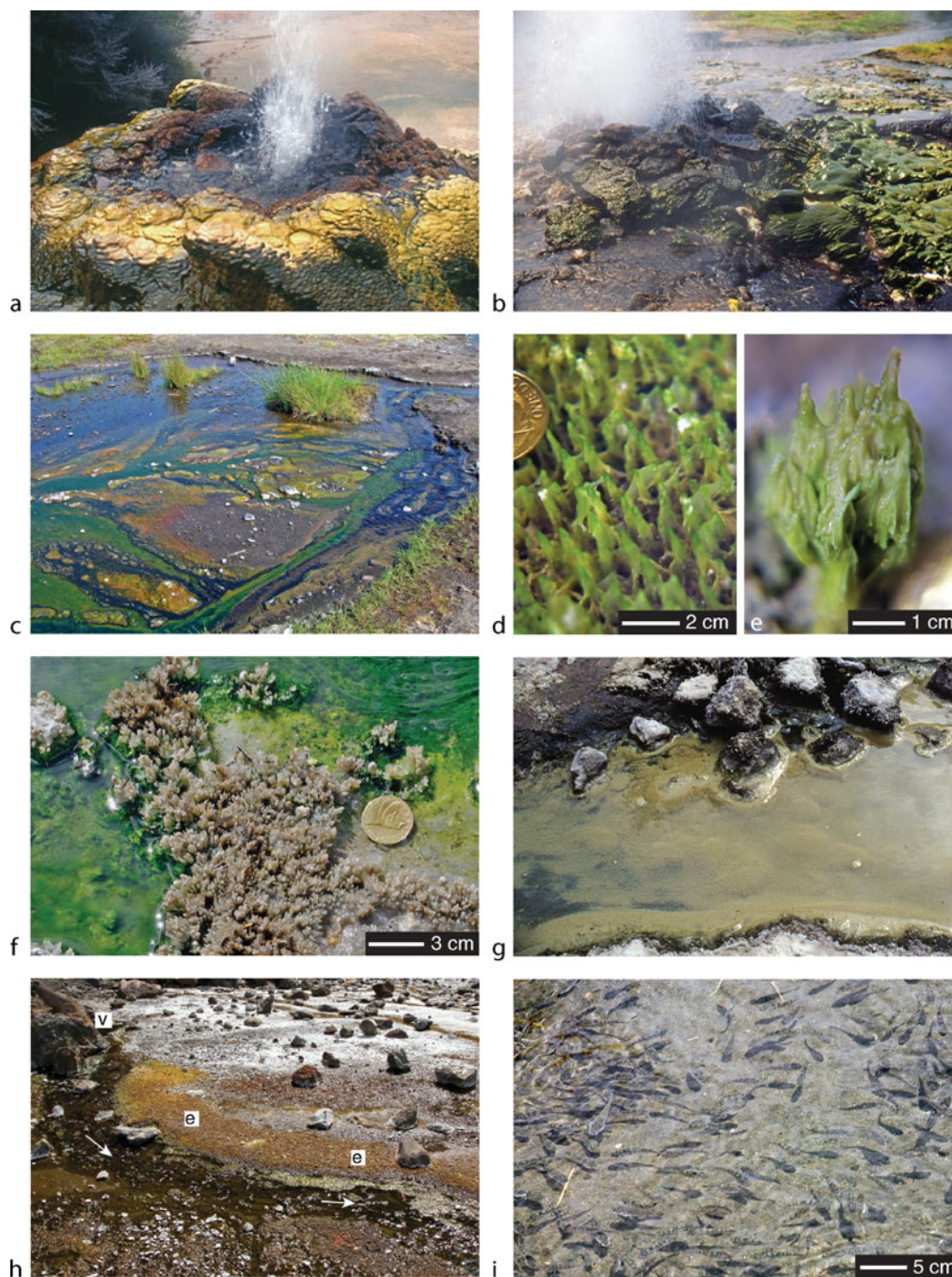
The environmental consequences of terrestrial hydrothermal activity are highly diverse, as a few examples can illustrate. High concentrations of metals such as As that are present in effluent can be absorbed by vegetation in thermal areas with variable effects (Koch et al., 1999). High concentrations of fluoride in dilute hot-spring outflow are a problem (fluorosis) for animals and humans in parts of China, Kenya, Tanzania, and elsewhere (e.g., Xu et al., 1995). Toxins in thermophilic cyanobacterial mats have been blamed for deaths (e.g., Krienitz et al., 2003) of birds. Fumarole gases are commonly toxic and have killed animals and vegetation in many locations (e.g., Baxter et al., 1999). Effluent from geothermal sites can contaminate freshwater aquifers and surface waters. In many cases, the impact of hydrothermal activity is negative from a human perspective, but this is partly balanced by the strong appeal of hot springs, geysers, and fumaroles as tourist attractions.

Temporal and anthropogenic changes in hot spring and geyser activity

Hydrothermal features at the land surface are typically ephemeral, with activity lasting from months up to several hundred thousand years (see Chapter *Hydrothermal Environments, Fossil*), but renewed activity may recur at the same location following volcanic and tectonic events (e.g., faulting) or climate change. Hot springs, geysers, and fumaroles may continue to function provided the basic requirements of a heat source, reliable fluid source, and permeable fluid pathway to the surface are met. Variations in heat source typically occur over long timescales (e.g., magmatic cooling), but water supply and fluid pathways (permeability) to the land surface can vary over much shorter timescales and induce frequent and shorter-term changes in hydrothermal activity.

The fluid recharge is especially sensitive to climate. Sturchio et al. (1993), for example, showed that periods of sinter formation in the arid northern Kenya Rift during the Pleistocene could be correlated with phases of humid climate. Springs that discharged chloride waters and precipitated sinter when water tables were regionally high during wetter periods are now acidic fumaroles or have become extinct. Even very short-term climate changes can have an impact. The activity of geysers and hot springs on the shoreline of Lake Bogoria in Kenya, for example, responds rapidly to minor changes in lake level (<30 cm) that are induced by El Niño rains (Renaut et al., 2005).

Anthropogenic activity can also cause substantial changes to the level of the water table over periods of a few years, as examples from New Zealand demonstrate. Development of the Wairakei geothermal power plant in the late 1950s led to a large fall in the water table, subsidence, and the loss of at least 100 geysers (Glover, 1965; Bryan, 2008). Decreasing geyser activity and other



Hydrothermal Environments, Terrestrial, Figure 6 Life in terrestrial hydrothermal environments. (a) Vent of small geyser (former well bore) at Tokaanu, New Zealand. Despite the boiling water spray and overflow, a diverse microbial community inhabits the area at the vent and their remains are fossilized in the sinter. (b) Thick (up to 10 cm) microbial mats near the vent of a perpetually spouting, Na-HCO₃ hot spring at Lake Bogoria, Kenya (shown in Figure 2c). The mats (*Chloroflexus* and several cyanobacterial species) receive spray from the spring but are absent where water temperature is constantly >70°C. (c) Microbial mats (cyanobacteria) in shallow outflow (pH: 8.8; T: 40–85°C) from a Na-HCO₃ hot spring at Lake Bogoria, Kenya. (d), (e) Small coniform stromatolites, built by *Phormidium* growing in Kirihero spring pool (pH: 7.5; T: 38°C), Tokaanu, New Zealand. (f) Green subaqueous *Cyanidium* mats adjacent to glassy spicular sinter in an acidic (pH 2.6; T: 48°C), shallow spring pool near Hakereteke Stream, north Waiotapu, New Zealand. (g) Soft mats built mainly by diatoms and fungi in acidic outflow channel (pH: 2.6; T: 41°C) from Frying Pan Flat, Waiotapu, New Zealand. (h) Thick accumulation of pupae of *Ephydra* sp. (e) along the edge of a small alkaline hot spring (v) (pH: 8.6; T: 47°C), Lake Magadi, Kenya. The ephydriids feed on the mats on the discharge apron. (i) *Alcolapia grahami* swimming in the vent pool (pH: 9.9; T: 46°C) of an alkaline hot spring, Lake Magadi, Kenya.

changes in the Whakarewarewa geothermal area over several decades have been attributed to natural changes in the subterranean plumbing system and to increased water extraction in nearby Rotorua.

The history of Geysir in Iceland is known from 1,294 AD because its behavior has been recorded in Icelandic literature. Historical sources show that the geyser activity has varied considerably, with periods of regular discharge alternating with periods of quiescence with eruptions. At Geysir, earthquakes rather than climate variability often seem to cause changes in its patterns of eruption (Torfason, 1985; Jones et al., 2007).

In some instances, changes in the subsurface aquifer and plumbing systems may trigger renewed activity after years of inactivity. The sudden, renewed activity of hot springs in residential areas can, for example, have a radical impact. This has happened recently (2000–2001) in Rotorua, New Zealand, where house owners awoke to find hot springs and mudpools in what used to be their back gardens, and a hydrothermal eruption (February 2001) sent mud 100 m into the air and created a pool 10 m in diameter in a local park.

Conclusions

Terrestrial hydrothermal environments occupy a small percentage of the Earth's surface but they provide a wealth of information on both geological and biological processes. These environments cover almost the entire spectrum of pH (0 to >10 in some alkaline hot springs) and water temperature range, and it is commonly possible to examine alkaline and highly acidic environments within a few kilometers of each other. For the geologists, they provide clues to mineral–water reactions occurring below the Earth's surface and the origin of some ore minerals. For the biologists, they provide opportunities to study a wide range of extremophiles in natural environments that, with care and permission, are generally accessible. With the discovery of possible siliceous hydrothermal deposits and highly acidic environments on Mars, the scientific interest in these environments is currently high and is likely to continue.

Bibliography

- Bargar, K. E., 1978. Geology and thermal history of Mammoth Hot Springs, Yellowstone National Park, Wyoming. *United States Geological Survey Bulletin*, **1444**.
- Barns, S. M., Fundyga, R. E., Jeffries, M. W., and Pace, N. R., 2004. Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 1609–1613.
- Baxter, P. J., Baubron, J. C., and Coutinho, R., 1999. Health hazards and disaster potential of ground gas emissions at Furnas volcano, São Miguel, Azores. *Journal of Volcanology and Geothermal Research*, **92**, 95–106.
- Blank, C. E., Cady, S. L., and Pace, N. R., 2002. Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park. *Applied and Environmental Microbiology*, **68**, 5123–5135.
- Bock, G. R., and Goode, J. A. (eds.), 1996. *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)* (CIBA Foundation Symposium 202). Chichester: Wiley.
- Bogie, I., Lawless, J. V., Rychagov, S., and Belousov, V., 2005. Magmatic-related hydrothermal systems: classification of the types of geothermal systems and their ore mineralization. In Rychagov, S. (ed.), *Geothermal and Mineral Resources of Modern Volcanism Areas (Proceedings of the International Kuril-Kamchatka field workshop, July 16–August 6, 2005)*. Moscow: Óotiskó, pp. 51–73.
- Brock, T. D., 1978. *Thermophilic Organisms and Life and High Temperature*. New York: Springer.
- Browne, P. R. L., and Lawless, J. V., 2001. Characteristics of hydrothermal eruptions, with examples from New Zealand and elsewhere. *Earth-Science Reviews*, **52**, 299–331.
- Bryan, T. S., 2005. *Geysers. What They Are and How They Work*, 2nd edn. Missoula, MT: Mountain Press Publishing Co.
- Bryan, T. S., 2008. *The Geysers of Yellowstone*, 4th edn. Boulder, CO: University Press of Colorado.
- Chafetz, H. S., and Folk, R. L., 1984. Travertines: depositional morphology and the bacterially constructed constituents. *Journal of Sedimentary Research*, **54**, 289–316.
- Craig, H., 1963. The isotopic geochemistry of water and carbon in geothermal areas. In Tongiorgi, E. (ed.), *Nuclear Geology in Geothermal Areas*. Pisa: Consiglio Nazionale delle Ricerche, Laboratorio di Geologia Nucleare, pp. 17–53.
- Cusicanqui, H., Mahon, W. A. J., and Ellis, A. J., 1976. The geochemistry of the El Tatio Geothermal Field, Northern Chile. In *Proceedings of the 2nd United Nations Symposium on Geothermal Fields*. Berkeley, CA, pp. 703–711.
- De Ronde, C. E. J., Stoffers, P., Garbe-Schöberg, D., Christenson, B. W., Jones, B., Manconi, R., Browne, P. R. L., Hissmann, K., Botz, R., Davy, B. W., Schmitt, M., and Battershill, C. N., 2002. Discovery of active hydrothermal venting in Lake Taupo, New Zealand. *Journal of Volcanology and Geothermal Research*, **115**, 257–275.
- Ellis, A. J., and Mahon, W. A. J., 1977. *Chemistry and Geothermal Systems*. New York: Academic Press.
- Fernandez-Turiel, J. L., Garcia-Valles, M., Gimeno-Torrente, D., Saavedra-Alonso, J., and Martinez-Anent, S., 2005. The hot-springs and geyser sinters of El Tatio, Northern Chile. *Sedimentary Geology*, **180**, 125–147.
- Giggenbach, W. F., 1994. Magma degassing and mineral deposition in hydrothermal systems along convergent plate boundaries. *Economic Geology*, **94**, 1193–1212.
- Glover, R. B., 1965. *Changes in the Wairakei Geyser Valley Springs 1962–1965*. Open File Report, Department of Scientific and Industrial Research, New Zealand.
- Heiken, G., 1982. Geology of geothermal systems. In Edwards, L. M., Chilingar, G. V., Rieke, H. H., and Fertl, W. H. (eds.), *Handbook of Geothermal Energy*. Houston, TX: Gulf Publishing, pp. 177–217.
- Henley, R. W., and Ellis, A. J., 1983. Geothermal systems, ancient and modern: a geochemical review. *Earth-Science Reviews*, **19**, 1–50.
- Jones, B., and Renaut, R. W. (eds.), 2003a. Sedimentology of hot spring systems. *Canadian Journal of Earth Sciences*, **40** (Special Issue), 1439–1738.
- Jones, B., and Renaut, R. W., 2003b. Hot spring and geyser sinters: the integrated product of precipitation, replacement, and deposition. *Canadian Journal of Earth Sciences*, **40**, 1549–1569.
- Jones, B., and Renaut, R. W., 2006. Selective mineralization of microbes in Fe-rich precipitates (jarosite, hydrous ferric oxides) from acid hot springs in the Waitotapu geothermal area, North Island, New Zealand. *Sedimentary Geology*, **194**, 77–98.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1997. Biogenicity of silica precipitation around geysers and hot-spring vents,

- North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2000. Stromatolites forming in acidic hot-spring waters, North Island, New Zealand. *Palaos*, **15**, 450–475.
- Jones, B., Renaut, R. W., Rosen, M. R., and Ansdell, K. M., 2002. Coniform stromatolites from geothermal systems, North Island, New Zealand. *Palaos*, **17**, 84–103.
- Jones, B., Renaut, R. W., Torfason, H., and Owen, R. B., 2007. The historical development of Geysir, Iceland. *Journal of the Geological Society (London)*, **164**, 1241–1252.
- Koch, I., Feldmann, J., Wang, L., Andrewes, P., Reimer, K. J., and Cullen, W. R., 1999. Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. *The Science of the Total Environment*, **236**, 101–117.
- Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J. S., Codd, G. A., and Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology*, **43**, 141–148.
- Kühn, M., 2004. *Reactive Flow Modeling of Hydrothermal Systems*. Berlin: Springer.
- Lloyd, E. F., and Keam, R. F., 1974. Trinity terrace hydrothermal eruption, Waimangu, New Zealand. *New Zealand Journal of Science*, **17**, 511–528.
- Nicholson, K., 1993. *Geothermal Fluids: Chemistry and Exploration Techniques*. Berlin: Springer.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer.
- Pentecost, A., Jones, B., and Renaut, R. W., 2003. What is a hot spring? *Canadian Journal of Earth Sciences*, **40**, 1443–1446.
- Pirajno, F., 1992. *Hydrothermal Mineral Deposits*. Berlin: Springer.
- Renaut, R. W., and Jones, B., 1997. Controls on aragonite and calcite precipitation in hot spring travertines at Chemurkeu, Lake Bogoria, Kenya. *Canadian Journal of Earth Sciences*, **34**, 801–814.
- Renaut, R. W., and Jones, B., 2000. Microbial precipitates around continental hot springs and geysers. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 187–195.
- Renaut, R. W., and Owen, R. B., 2005. The geysers of Lake Bogoria, Kenya Rift Valley, Africa. *GOSA Transactions*, **9**, 4–18.
- Reysenbach, A. L., Voytek, M., and Mancinelli, R. (eds.), 2001. *Thermophiles: Biodiversity, Ecology and Evolution*. New York: Springer.
- Robb, F., Antranikian, G., Grogan, D., and Driessen, A. (eds.), 2007. *Thermophiles: Biology and Technology at High Temperatures*. London: Taylor and Francis.
- Rodgers, K. A., Cook, K. L., Browne, P. R. L., and Campbell, K. A., 2002. The mineralogy, texture and significance of silica derived from alteration by steam condensate in three New Zealand geothermal fields. *Clay Minerals*, **37**, 299–322.
- Sturchio, N. C., Dunkley, P. N., and Smith, M., 1993. Climate-driven variations in geothermal activity in the northern Kenya Rift Valley. *Nature*, **362**, 233–234.
- Torfason, H., 1985. *The Great Geysir*. Reykjavík: Geysir Conservation Committee.
- Walter, M. R., 1976. Geysers of Yellowstone National Park: an example of abiogenic “stromatolites”. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 87–112.
- Wiegel, J., 2007. *Thermophiles: The Keys to Molecular Evolution and the Origin of Life?* London: Taylor and Francis.
- Xu, R. H., Yuan, H. H., and Fan, A., 1995. Endemic fluorosis in China from ingestion of food immersed in hot spring water. *Bulletin of Environmental Contamination and Toxicology*, **54**, 337–341.

Cross-references

[Astrobiology](#)
[Extreme Environments](#)
[Hot Springs and Geysers](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Marine](#)
[Sinter](#)

HYPERHALINE ENVIRONMENTS

Please refer to “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Salinity History of the Earth’s Ocean](#),” “[Saline Lakes](#),” “[Soda Lakes](#).”

- North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2000. Stromatolites forming in acidic hot-spring waters, North Island, New Zealand. *Palaio*, **15**, 450–475.
- Jones, B., Renaut, R. W., Rosen, M. R., and Ansdell, K. M., 2002. Coniform stromatolites from geothermal systems, North Island, New Zealand. *Palaio*, **17**, 84–103.
- Jones, B., Renaut, R. W., Torfason, H., and Owen, R. B., 2007. The historical development of Geysir, Iceland. *Journal of the Geological Society (London)*, **164**, 1241–1252.
- Koch, I., Feldmann, J., Wang, L., Andrewes, P., Reimer, K. J., and Cullen, W. R., 1999. Arsenic in the Meager Creek hot springs environment, British Columbia, Canada. *The Science of the Total Environment*, **236**, 101–117.
- Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J. S., Codd, G. A., and Pflugmacher, S., 2003. Contribution of hot spring cyanobacteria to the mysterious deaths of Lesser Flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology*, **43**, 141–148.
- Kühn, M., 2004. *Reactive Flow Modeling of Hydrothermal Systems*. Berlin: Springer.
- Lloyd, E. F., and Keam, R. F., 1974. Trinity terrace hydrothermal eruption, Waimangu, New Zealand. *New Zealand Journal of Science*, **17**, 511–528.
- Nicholson, K., 1993. *Geothermal Fluids: Chemistry and Exploration Techniques*. Berlin: Springer.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer.
- Pentecost, A., Jones, B., and Renaut, R. W., 2003. What is a hot spring? *Canadian Journal of Earth Sciences*, **40**, 1443–1446.
- Pirajno, F., 1992. *Hydrothermal Mineral Deposits*. Berlin: Springer.
- Renaut, R. W., and Jones, B., 1997. Controls on aragonite and calcite precipitation in hot spring travertines at Chemurkeu, Lake Bogoria, Kenya. *Canadian Journal of Earth Sciences*, **34**, 801–814.
- Renaut, R. W., and Jones, B., 2000. Microbial precipitates around continental hot springs and geysers. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 187–195.
- Renaut, R. W., and Owen, R. B., 2005. The geysers of Lake Bogoria, Kenya Rift Valley, Africa. *GOSA Transactions*, **9**, 4–18.
- Reysenbach, A. L., Voytek, M., and Mancinelli, R. (eds.), 2001. *Thermophiles: Biodiversity, Ecology and Evolution*. New York: Springer.
- Robb, F., Antranikian, G., Grogan, D., and Driessen, A. (eds.), 2007. *Thermophiles: Biology and Technology at High Temperatures*. London: Taylor and Francis.
- Rodgers, K. A., Cook, K. L., Browne, P. R. L., and Campbell, K. A., 2002. The mineralogy, texture and significance of silica derived from alteration by steam condensate in three New Zealand geothermal fields. *Clay Minerals*, **37**, 299–322.
- Sturchio, N. C., Dunkley, P. N., and Smith, M., 1993. Climate-driven variations in geothermal activity in the northern Kenya Rift Valley. *Nature*, **362**, 233–234.
- Torfason, H., 1985. *The Great Geysir*. Reykjavík: Geysir Conservation Committee.
- Walter, M. R., 1976. Geysers of Yellowstone National Park: an example of abiogenic “stromatolites”. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 87–112.
- Wiegel, J., 2007. *Thermophiles: The Keys to Molecular Evolution and the Origin of Life?* London: Taylor and Francis.
- Xu, R. H., Yuan, H. H., and Fan, A., 1995. Endemic fluorosis in China from ingestion of food immersed in hot spring water. *Bulletin of Environmental Contamination and Toxicology*, **54**, 337–341.

Cross-references

[Astrobiology](#)
[Extreme Environments](#)
[Hot Springs and Geysers](#)
[Hydrothermal Environments, Fossil](#)
[Hydrothermal Environments, Marine](#)
[Sinter](#)

HYPERHALINE ENVIRONMENTS

Please refer to “[Deep Biosphere of Salt Deposits](#),” “[Extreme Environments](#),” “[Halobacteria – Halophiles](#),” “[Salinity History of the Earth’s Ocean](#),” “[Saline Lakes](#),” “[Soda Lakes](#).”

the ingestion of refractory organic carbon can lead to the excretion of more labile fecal pellets (Lee, 1992).

With most intrusive bioturbation, the sediment–water interface increases in surface area as a result of infaunal animal colonization. The larger (and deepened) sediment–water interface alters chemical fluxes between the sediment and water column. Animals that pump water through their burrow (bioirrigation) increase the rate of chemical flux. Most notably, irrigation of worm burrows with oxygenated seawater results in increased downward and lateral diffusion of O₂, which then affects the oxidation of redox-sensitive elements. For the aforementioned reasons, the accepted scheme of biogeochemical zones in sediment is an oversimplification. Bioturbation influences the solid phase and pore-water properties of sediment by increasing the transport of diagenetic reactants and products across the sediment–water interface. Consequently, surficial sediments cannot be considered a homogeneous medium characterized by one-dimensional vertical diffusion. They are, instead, heterogeneous and intensively mixed with a complex spatial distribution of biogeochemical zones that are difficult to accurately resolve (Aller, 1980).

Metal ion enrichment in ichnofossils

Cation enrichment in burrow linings is the result of sorptive processes. Metals concentrate in burrow linings in three ways: (1) adsorption, as oxide or oxyhydroxide coatings on to the high-energy surfaces provided by fine-grained particles (silt and clay); (2) as sulfide or phosphate phases under reducing conditions; or (3) associated with organic material (as adsorbed coatings or forming organo-metallic complexes) (Over, 1990). The relative importance of each type of enrichment mechanism varies with the depth of burrow penetration, the grain size/composition of the sediment, and bottom-water and pore-water composition.

Ichnofossils and cementation

Trace fossils commonly influence local cementation patterns by changing the sediment texture, its redistribution, and local composition (Gingras et al., 2004). They also facilitate the accumulation of microbial communities, and through their chemoheterotrophic metabolisms, the microbes can alter pore-water chemistry and saturation states. Ichnofossils also possess higher metal concentrations which can serve as nucleation sites for authigenic mineral formation. Although ichnofossils have been strongly linked to the distribution of microorganisms, there is no existing model associating trace fossil diagenesis to microorganic process.

Summary

The presence of ichnofossils/bioturbation in a sedimentary substrate has the effect of altering sediment distribution and permeability pathways. Moreover, the redistribution and stowage of organics in and around

ichnofossils strongly influence the distribution of microorganisms associated with the sedimentary surface. This has an impact on the nature and distribution of biochemical zonations in sediment. The mechanical and (bio)chemical nature of trace fossils influences diagenetic processes.

Bibliography

- Aller, R. C., 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. *Geochimica et Cosmochimica Acta*, **44**, 1955–1965.
- Bromley, R. G., 1996. *Trace Fossils: Biology, Taphonomy and Applications*, 2nd edn. London: Chapman and Hall, p. 349.
- Chow, N., and Longstaffe, F., 1995. Dolomites of the Middle Devonian Elk Point Formation, Southern Manitoba: intrinsic controls on early dolomitization. *Bulletin of Canadian Petroleum Geology*, **43**, 214–225.
- Gingras, M. K., Pemberton, S. G., Muelenbachs, K., and Machel, H., 2004. Conceptual models for burrow-related, selective dolomitization with textural and isotopic evidence from the Tyndall Stone, Canada. *Geobiology*, **2**, 21–30.
- Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochimica et Cosmochimica Acta*, **56**, 3323–3335.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Seilacher, A., 1967. Bathymetry of trace fossils. *Marine Geology*, **5**, 413–428.

Cross-references

[Biosignatures in Rocks](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Trace Fossils: Neoproterozoic](#)

IMMUNOLocalization

Michael Hoppert, Christoph Wrede
 University of Göttingen, Göttingen, Germany

Synonyms

Immunocytochemistry; Immunodetection; Immunohistochemistry; Immunolabeling

Definition

Immunolocalization: Technique using specific antibodies to localize macromolecules (proteins, polysaccharides) within biological material (subcellular fractions, cells, tissues, biofilms).

Introduction

Immunolocalization is based on the detection of specific targets (“antigens”; mostly proteins or polysaccharides) in biological samples (cells, tissue, biofilms) by antibodies

as probes. Regularly, antibodies of IgG subtype (Figure 1a) and IgM and IgA subtypes are used. The antibodies may be monoclonal (i.e., identical molecules that recognize only one epitope, which is a short stretch of amino acids or sugar molecules) or polyclonal (a set of antibodies directed against a mostly undefined variety of epitopes on the surface of a larger molecule or a cellular component). Specific non-covalent binding is brought about by the hypervariable region of the antibody (paratope), exactly fitting to an epitope. The hypervariable regions are the tip parts of the Y-shaped arm of the antibody (Figure 1a). All other parts, including the Fc-part, are structurally identical within one antibody subclass. Localization of the antigen is only possible when the specific (or primary) antibody ("probe") binds to a marker. A variety of antibody-marker systems are available for different applications (Figure 1b; see below). The marker may be coupled by different methods to the probe (Hoppert, 2003):

- By direct coupling of the marker to the probe prior to use in a localization experiment. The detection procedure requires just one binding step. The method is also termed *direct immunolocalization*.
- By binding of a secondary probe (mostly a secondary antibody) to the Fc-part of a primary antibody after this primary antibody is already bound to the specific target (two-step procedure). The secondary probe is coupled to the marker. The method is also termed *indirect immunolocalization*.
- By binding of tertiary probes to the secondary probe (for signal amplification). Tertiary probes are responsible for binding or deposition of the respective marker.

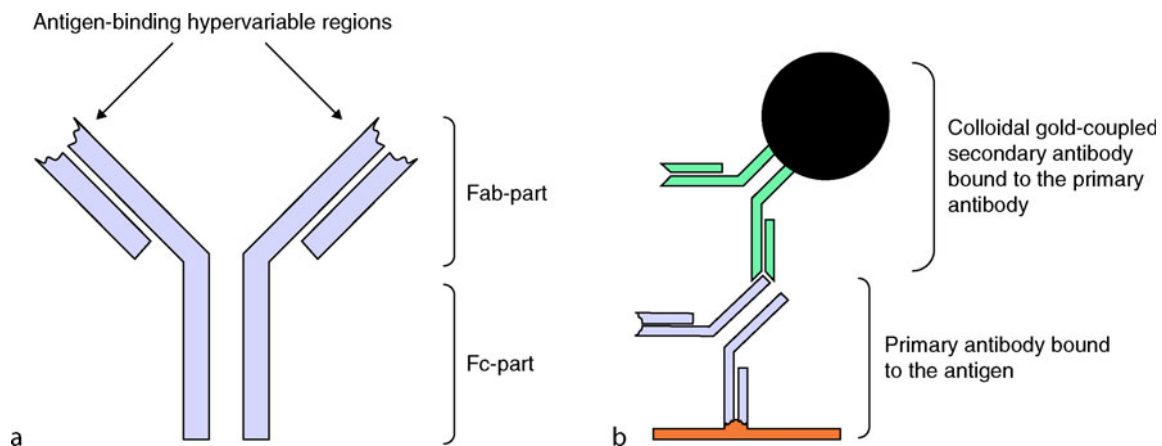
Immunolocalization in light and electron microscopy

For immunolocalization in (fluorescence) light microscopy, fluorescent dyes are used as markers

(immunofluorescence microscopy). The marker consists of the dye covalently coupled to a protein (e.g., the secondary antibody) that binds to the primary marker, the antigen-specific antibody. It is also possible to detect more than one target in a sample by the use of marker-probe systems with specificities for different targets coupled to fluorescence markers with discernable absorption–emission spectra. Access to the antigen in tissues or embedded samples can be achieved by (cryo-) sectioning or by permeabilization (Goldenthal et al., 1985). For (nonfluorescent) bright field microscopy, marker systems that catalyze enzymatic reactions are used, leading to deposition of dark-stained precipitates at the antigen binding sites (Ramos-Vara, 2005).

For electron microscopy, probes are coupled to colloidal gold as an electron dense marker. As stated for light microscopy, the marker can be directly coupled to the primary antibody, but regularly, a secondary system is combined with a primary antibody. Also, tertiary systems for signal amplifications are used. In equivalence to multiple detections of targets in light microscopy, it is possible to detect more than one target in a sample by use of marker-probe systems with discernable particle sizes of gold colloids. Particle sizes between 5 and 25 nm are used for experiments at the cellular level. Small gold particles may also be enlarged with silver or gold salt solutions for easier localization (Humbel et al., 1995). For epitope mapping in macromolecules, 1-nm markers ("nanogold") are available (Hainfeld and Powell, 2000).

Several modifications of the original immunogold marker technique can increase the resolution and sensitivity of the method. Dodecaborane clusters may be coupled to antibodies or Fab-fragments via polylysine dendrimers. The boronated antibodies have a lower detection limit in immunolocalization experiments than conventional gold conjugates. Detection of boron is performed by element-specific imaging in energy-filtering transmission electron



Immunolocalization, Figure 1 Antibody probes and colloidal gold markers: (a) Schematized structure of an IgG subtype antibody. (b) Primary antibody with bound secondary antibody and colloidal gold marker.

microscopes (Bendayan et al., 1989; Qualmann et al., 1996; Kessels et al., 1996).

Techniques like catalyzed reporter deposition ("CARD") use tertiary markers. CARD is a technique for amplification of a signal, originally designed for Western blotting, but modified for light and electron microscopy. In the first step, a specific primary antibody is coupled to the target. Then, in a second step, a secondary, biotin-tagged antibody is coupled to the primary antibody. A streptavidin–peroxidase conjugate is then bound to the biotin tag. Peroxidase catalyzes the deposition of tyramides indirectly. The tyramide itself is bound to a detectable marker (e.g., again biotin), and may be localized by the use of streptavidin-coupled colloidal gold for detection in the electron microscope (Mayer and Bendayan, 1997). For light microscopy, fluorescent dye-coupled tyramides are used (Wasielewski et al., 1997).

Related techniques

Besides the widely used antibodies, other specific probes for localization of a target may be used. Table 1 gives an overview of the relevant techniques. Lectin proteins have defined specificities to short carbohydrate stretches and are therefore an alternative to antibodies for detection of polysaccharides. They are mainly used for detection of glycosylated residues or polysaccharides on cell surfaces and/or in microbial biofilms (Kämper et al., 2004).

Especially when macromolecules are present in high concentrations, a target based on a substrate-specific enzyme may be applied. Enzyme–substrate interaction may also be detected with a substrate inducing the precipitation of an electron-dense marker to localize an enzyme activity.

Equivalent to in situ hybridization in light microscopy, the electron microscopic approach can allow high-resolution mapping of specific mRNA. The technique

Immunolocalization, Table 1 Techniques for the localization of biological macromolecules by light (LM) and electron microscopy (EM)

Problem	Specimen treatment	Marker system (technique; references)
Localization of specific proteins (antibody-gold/antibody-fluorescent probe) or carbohydrate complexes (lectin-gold/lectin-fluorescent probe) inside cells (LM/EM)	Ultrathin sections of resin-embedded samples subjected to immunolocalization	Antibody-colloidal gold, lectin-colloidal gold (Roth et al., 1980; Horisberger, 1985; Danguy et al., 1988)
Localization of specific proteins on the cell surface or on subcellular particles (EM)	Incubation of cells or subcellular fractions with the marker system prior to resin embedding or direct adsorption on specimen support films	Antibody-colloidal gold, lectin-colloidal gold (Acker, 1988; Rohde et al., 1988)
Localization of single protein epitopes and their relative position to each other on the surface of the object (EM)	Homogeneous protein preparations (especially large proteins or viruses) subjected to localization of epitopes	Antibody, Fab-fragment, (epitope-labeling; Hermann et al., 1991)
Detection of enzyme activities (EM)	Incubation of cells or subcellular fractions with the marker system	Specific enzyme substrate coupled to precipitation of electron dense marker (Hayat, 1973ff; Wohlrab and Gossrau, 1992)
Detection of macromolecular components acting as enzyme substrates (e.g., starch, lipids) (EM)	Labeling of enzyme substrates with gold particles coated with the relevant enzymes in ultrathin sections of samples	Enzyme-colloidal gold (Bendayan, 1985)
Localization of metabolites in the cell (EM)	Incubation of metabolically active cells with the precursor, embedding in conventional resin or by application of cryotechniques, treatment with a specific photoemulsion	Metabolic precursors (e.g., sugars or aminoacids) labeled with radioactive markers (autoradiography; Gregg and Reznik-Schüller, 1984)
Localization of specific DNA or RNA (preferably mRNA) sequences in cells (LM/EM)	Fluorescent in situ hybridization for light microscopy, hybridisation of DNA/RNA of fixed samples with the marker system followed by embedding and sectioning, detection of the marker system by autoradiography or with streptavidin-colloidal gold for electron microscopy	RNA or DNA oligonucleotide labeled with different markers (in situ hybridization; Amann et al., 1995; Egger et al., 1994)
Comparison of immunolocalization targets from overview (low resolution) to subcellular structures (high resolution) (EM)	Ultrathin (and semithin) sections of resin-embedded samples subjected to immunolocalization	Antibody-colloidal gold and antibody-fluorescent probe, lectin-colloidal gold and lectin-fluorescent probe (Takizawa and Robinson, 2003).

may be combined with specific signal enhancement methods (CARD, see above) and is suitable for high-resolution in situ hybridization (Cheung et al., 1999).

For microautoradiography, radiolabeled markers, incorporated into cell structures, are located by exposure to photographic emulsion. The deposition of silver bromide grains on the photoemulsion is detectable by light and electron microscopy. The technique is demanding and difficult to reproduce, though it gives information on processing and distribution of metabolites that cannot be obtained, on a structural level, by other methods (Gregg and Reznik-Schüller, 1984).

Correlative microscopy

The technique of correlative light/electron microscopy allows examination of the same sample at micrometer scale for overview images and at nanometer scale for imaging of features at high resolution. Regularly, (fluorescence) light microscopy and electron microscopy of samples is combined. The marker/probe systems are either suitable for both microscopic techniques at the same time or for combinations of different systems that are detectable either by light or by electron microscopy.

Quantum dots (qdots) are colloidal semiconductor nanocrystals and are detectable in the electron microscope, either as electron dense particles or via element specific imaging. Since qdots exhibit specific absorption/emission spectra in the light, they are also detectable as fluorescent markers. When qdots are coupled to primary antibodies, they are marker-probe systems suitable for both imaging techniques (Nisman et al., 2004).

Though it is possible to visualize ultrathin sections by (fluorescence) light microscopy, sections of several micrometer in thickness (semithin sections) are regularly more suitable. It is possible to obtain nearly identical features of a specimen when one ultrathin and one semithin is

consecutively cut from a specimen. Semithin and ultrathin sections are then processed in different ways, either with marker-probe-systems for immunofluorescence or for immunoelectron microscopy (Figure 2; Takizawa and Robinson, 2003; Wrede et al., 2008).

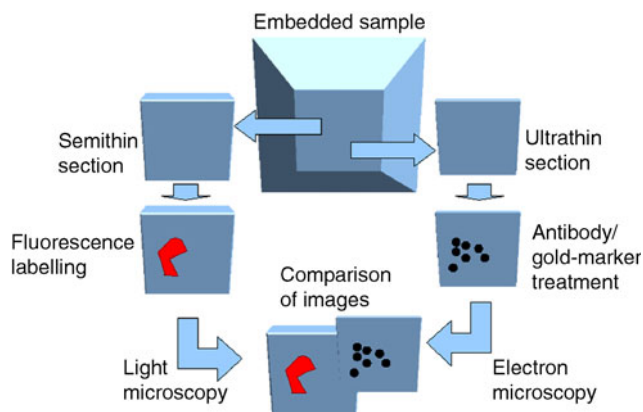
Correlation of data from immunolocalization may also be performed with other microscopic techniques, such as Raman microscopy, scanning electron microscopy, or atomic force microscopy.

Conclusions

It has already been understood in the first half of the past century that, besides comprehending biochemical or molecular reactions in organisms, the understanding of their organization is of equal importance (Hopkins, 1933). Tools for understanding organization of these processes have been developed consecutively, when methods became available to keep organisms alive or at least well-preserved down to molecular scale. The techniques revealed that the presumption of some early biochemists was right, i.e., metabolic pathways, growth and cell division, even of apparently non-compartmented bacteria, are organized in high ordered, large macromolecular complexes. Future direction of localization techniques will include the application of marker systems for high resolving microscopy systems that will allow observation of living cells down to nanometer scale (Donnert et al., 2006).

Bibliography

- Acker, G., 1988. Immunoelectron microscopy of surface antigens (polysaccharides) of gram-negative bacteria using pre- and post-embedding techniques. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 147–174.
- Amann, R. I., Ludwig, W., and Schleifer, K. H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Bendayan, M., 1985. The enzyme-gold technique: a new cytochemical approach for the ultrastructural localization of macromolecules. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol 3, pp. 179–201.
- Bendayan, M., Barth, R. F., Gingras, D., Londono, I., Robinson, P. T., Alam, F., Adams, D. M., and Mattiazzi, L., 1989. Electron spectroscopic imaging for high-resolution immunocytochemistry: use of boronated Protein A. *Journal of Histochemistry and Cytochemistry*, **37**, 573–580.
- Cheung, A. L., Graf, A. H., Hauser-Kronberger, C., Dietze, O., Tubbs, R. R., and Hacker, G. W., 1999. Detection of human papillomavirus in cervical carcinoma: comparison of peroxidase, Nanogold, and catalyzed reporter deposition. *Modern Pathology*, **12**, 689–696.
- Danguy, A., Kiss, R., and Pasteels, J. L., 1988. Lectins in histochemistry. A survey. *Biological Structures and Morphogenesis*, **1**, 93–106.
- Donnert, G., Keller, J., Medda, R., Andrei, M. A., Rizzoli, S. O., Lüthmann, R., Jahn, R., Eggeling, C., and Hell, S. W., 2006. Macromolecular-scale resolution in biological fluorescence microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 11440–11445.
- Egger, D., Troxler, M., and Bienz, K., 1994. Light and electron microscopic in situ hybridization: non-radioactive labeling and



Immunolocalization, Figure 2 Specimen Preparation for correlative Microscopy. From the embedded sample, alternating semithin and ultrathin sections are prepared and processed either by fluorescence labeling for light microscopy or treatment with antibodies and colloidal gold. In light and electron microscopy, corresponding areas are identified and compared.

- detection, double hybridization, and combined hybridization-immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **42**, 815–822.
- Goldenthal, K. L., Hedman, K., Chen, J. W., August, J. T., and Willingham, M. C., 1985. Postfixation detergent treatment for immunofluorescence suppresses localization of some integral membrane proteins. *Journal of Histochemistry and Cytochemistry*, **33**, 813–820.
- Gregg, M., and Reznik-Schüller, H. M., 1984. An improved method for rapid electron microscopic autoradiography. *Journal of Microscopy*, **135**, 115–118.
- Hainfeld, J. F., and Powell, R. D., 2000. New frontiers in gold labeling. *Journal of Histochemistry and Cytochemistry*, **48**, 471–480.
- Hayat, M. A., 1973–1977. *Electron Microscopy of Enzymes: Principles and Methods*. New York: Van Nostrand Reinhold, Vol. 1 (1973); Vols. 2 and 3 (1974); Vol. 4 (1976); Vol. 5 (1977).
- Hermann, R., Schwarz, H., and Müller, M., 1991. High precision immunoscanning electron microscopy using Fab fragments coupled to ultra-small colloidal gold. *Journal of Structural Biology*, **107**, 38–47.
- Hopkins, F. G., 1933. Some chemical aspects of life. *Nature Supplement*, **3332**, 381–394.
- Hoppert, M., 2003. *Microscopic Techniques in Biotechnology*. Weinheim: Wiley-VCH.
- Horisberger, M., 1985. The gold method as applied to lectin cytochemistry in transmission and scanning electron microscopy. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol. 3, pp. 155–178.
- Humbel, B. M., Sibon, O. C. M., Stierhof, Y.-D., and Schwarz, H., 1995. Ultra-small gold particles and silver enhancement as a detection system in immunolabeling and in situ hybridization experiments. *Journal of Histochemistry and Cytochemistry*, **43**, 735–737.
- Kämper, M., Vetterkind, S., Berker, R., and Hoppert, M., 2004. Methods for in situ detection and characterization of extracellular polymers in biofilms by electron microscopy. *Journal of Microbiological Methods*, **57**, 55–64.
- Kessels, M. M., Qualmann, B., Klobasa, F., and Sierralta, W. D., 1996. Immunocytochemistry by electron spectroscopic imaging using a homogeneously boronated peptide. *Cell and Tissue Research*, **284**, 239–245.
- Mayer, G., and Bendayan, M., 1997. Biotinyl–tyramide: a novel approach for electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1449–1454.
- Nisman, R., Dellaire, G., Ren, Y., Li, R., and Bazett, D. P., 2004. Application of quantum dots as probes for correlative fluorescence, conventional, and energy - filtered transmission electron microscopy. *Journal of Histochemistry and Cytochemistry*, **52**, 13–18.
- Qualmann, B., Kessels, M. M., Klobasa, F., Jungblut, P. W., and Sierralta, W. D., 1996. Electron spectroscopic imaging of antigens by reaction with boronated antibodies. *Journal of Microscopy*, **183**, 69–77.
- Ramos-Vara, J. A., 2005. Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**, 405–426.
- Rohde, M., Gerberding, H., Mund, T., and Kohring, G. W., 1988. Immunelectron microscopic localization of bacterial enzymes: pre- and postembedding labeling techniques on resin-embedded samples. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 175–210.
- Roth, J., Bendayan, M., and Orci, L., 1980. FITC-Protein A-Gold complex for light and electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **28**, 55–57.
- Takizawa, T., and Robinson, J. M., 2003. Ultrathin cryosections: an important tool for immunofluorescence and correlative microscopy. *Journal of Histochemistry and Cytochemistry*, **51**, 707–714.
- Wasielowski, R., Mengel, M., Gignac, S., Wilkens, L., Werner, M., and Georgii, A., 1997. Tyramine amplification technique in routine immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1455–1459.
- Wohlrab, F., and Gossrau, R., 1992. *Katalytische Enzymhistochemie*. Jena: G. Fischer Verlag.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold Seeps. *Journal of Microbiological Methods*, **73**, 85–91.

Cross-references

Fluorescence in Situ Hybridization (FISH)
Histology

IRON ISOTOPES

Please refer to “*Isotope Fractionation (Metal)*.”

IRON SULFIDE FORMATION

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

There has been a great deal of research into the formation of sedimentary iron sulfide minerals because their history is intertwined with the biogeochemical cycles of iron, sulfur, carbon, and oxygen (Berner, 2001). Iron sulfides occur in sediments from a wide range of depositional environments, from the deep sea to the nonmarine, and the principal mineral in the rock record is pyrite (FeS₂, cubic). Its dimorph marcasite (orthorhombic) is considered metastable and apt to invert to pyrite over geologic time (Murowchick, 1992), but is probably more widespread than commonly acknowledged (Schieber, 2007). Presumed precursor minerals of sedimentary pyrite are mackinawite (FeS_{1-x}) and greigite (Fe₃S₄), minerals that impart a black coloration to modern reducing sediments (Goldhaber and Kaplan, 1974). Both are metastable and transform over time into pyrite or a mixture of pyrite and pyrrhotite (Berner, 1967). Pyrrhotite, hexagonal FeS, is thermodynamically stable in sediments (Berner, 1967), but extremely rare in modern or ancient sediments (Kobayashi and Nomura, 1972) due to kinetic limitations (Canfield and Raiswell, 1991).

Mackinawite and greigite are also known as acid-volatile iron sulfides (AVS) because in contrast to pyrite and marcasite they are readily soluble in HCl. Mineralogically, greigite is the sulfur analog of magnetite and is indeed strongly ferromagnetic (Dekkers and Schoonen, 1996). It can be formed by magnetotactic bacteria and dominates the magnetic properties of some modern sediments and

[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Deep Biosphere of Sediments](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Dolomite, Microbial](#)
[Extreme Environments](#)
[Halobacteria – Halophiles](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Methane, Origin](#)
[Microbial Biomineralization](#)
[Nitrogen](#)
[Sulfate-Reducing Bacteria](#)
[Symbiosis](#)
[Terrestrial Deep Biosphere](#)

ARSENIC

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Arsenic, element 33, is a metalloid in row VB of the periodic table. The main oxidation states are arsine (−3) elemental As(0), arsenite (+3), and arsenate (+5). Arsenate, As(V), and arsenite, As(III), are most common, with HAsO_4^{2-} and $\text{H}_2\text{AsO}_4^{1-}$ predominating under oxic conditions and H_3AsO^0 and $\text{H}_2\text{AsO}_3^{2-}$ prevalent in anoxic environments. Arsenic is found in minerals such as arsenosulfides (e.g., arsenopyrite, realgar), iron hydroxides and aluminosilicates (e.g., clays), fossil fuels, geothermal sources, and weathered volcanic and evaporitic deposits. Anthropogenic inputs may also be considerable as organoarsenicals such as roxarsone (3-nitro-4-hydroxy benzene arsonic acid) and monosodium and disodium methyl arsonate continue to see wide spread use. The toxicology of arsenic is well known; As(V) is an analog of phosphate and can disrupt oxidative phosphorylation, while As(III) binds to sulfhydryl groups and can inhibit respiratory enzymes. Nevertheless, arsenic is readily metabolized by microorganisms, undergoing redox reactions involved in resistance or energy generation. Organarsenicals are used for energy generation or as a carbon source. Environments with elevated concentrations can support a diverse arsenic metabolizing microbial community and a robust biogeochemical cycle (Oremland and Stolz, 2003).

As(III)-oxidizing prokaryotes are found among the alpha-, beta-, and gamma-proteobacteria, Deinocci (i.e., *Thermus*), and Crenarchaeota. Physiologically diverse, they include heterotrophic and chemolithoautotrophic species. The oxidation of arsenite involves the arsenite oxidase (AoxAB or AroBA), a member of the molybdenum family of enzymes. The gene sequence of the catalytic subunit (*aoxB* or *aroA*) is highly conserved, and oligonucleotide primers have been used to investigate the occurrence and distribution of As(III)-oxidizing

species. The dissimilatory reduction of As(V) has been demonstrated in Crenarchaeota, thermophilic bacteria, gamma-, delta-, and epsilon-proteobacteria, and low and high mole %GC gram positive bacteria. The enzyme, ArrAB, is also a molybdoenzyme, but differs significantly from AoxBA. The *arrA* gene has also been used as an environmental probe. Arsenic resistance involves a completely different type of arsenate reductase (ArsC, ACR2). Three different but comparable resistance systems (e.g., *E. coli*, *Staphylococcus*, *Saccharomyces*) have been described. ArsC catalyzes the reduction of arsenate to arsenite, while an arsenite-specific efflux pump (ArsB, ACR3) expels As(III) from the cell. Additional components include an ATPase (ArsA) and regulatory elements (ArsR, ArsD). Far less is known about arsenic methylation and demethylation, however, a SAM-dependent methylase has recently been described (Stolz et al., 2006).

Different microbially mediated processes can result in arsenic contaminated aquifers including (1) the oxidation of arsenic-containing pyrites, (2) the release of As(V) from reduction of iron oxides by autochthonous organic matter, (3) the reduction of iron oxides by allochthonous organic matter from dissolved organics in recharging waters, (4) the exchange of adsorbed As(V) with fertilizer phosphates, and (5) recharge with water contaminated with inorganic arsenic derived from the application or production of wood preservatives, pesticides (e.g., calcium arsenate), and organoarsenicals (e.g., roxarsone). Thus organisms that metabolize arsenic can impact the speciation, mobility, and toxicity of arsenic in the environment (Oremland and Stolz, 2005).

Bibliography

- Oremland, R. S., and Stolz, J. F., 2003. The ecology of arsenic. *Science*, **300**, 939–944.
 Oremland, R. S., and Stolz, J. F., 2005. Arsenic, microbes, and contaminated aquifers. *Trends in Microbiology*, **13**, 45–49.
 Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

Cross-references

[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Geomycology](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Shewanella](#)
[Sulfide Mineral Oxidation](#)

ASTEROID AND COMET IMPACTS

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The impact of an extraterrestrial object (either an asteroid or comet) with the Earth's surface.

Introduction

There are now over 170 asteroid and comet impact craters that have been identified on the surface of the Earth (Table 1). These craters represent a small subset of the total impacts that have occurred during the history of life. Today, impact craters probably represent something on the order of 50,000 km² of Earth's surface. Although this is a small habitat area, impacts are the only extraterrestrial mechanism capable of causing localized ecological disturbance. Because impacts are a mechanism capable of delivering a pulse of energy into ecosystems, from a geobiological point of view they present an important addition to the overall picture of how ecosystems are disturbed and how they recover through time. Comparison to other processes involving ecological disturbance and recovery including volcanism, glaciation/deglaciation, storm damage, fire, landslides, and coastal erosion allows for a more complete picture of the role of catastrophic change in shaping the geobiology of the Earth.

Effects of impacts on the local environment

The kinetic energy associated with the collision of an asteroid or comet with the Earth is enormous. The collision of a rock with a 10-km diameter could release the equivalent energy of 10 million tonnes of TNT. An event on such a scale is expected to occur, on average, once in every 100 million years, and may be capable of causing global-scale disruption and extinction. Such an event is implicated in the Cretaceous–Paleogene extinctions. It is perhaps not surprising, therefore, that the biological destructiveness of impact events has overwhelmingly influenced our view of these objects and their role in ecological and evolutionary processes. However, it is important to realize that the great majority of impacts only cause effects at the local scale, although they may distribute material from the point of impact over wide areas. This contribution will focus on the local geobiological effects of impacts.

The impact of an asteroid and comet with the surface of the Earth has two profound effects that are relevant for life. First, the impact delivers energy into the target area with important geological consequences and second, it rearranges the hydrologic cycle, with implications for the availability of liquid water. Temperatures generated in

the impact cratering process can exceed several thousand degrees centigrade (Melosh, 1989). Most biologically important macronutrients volatilize at temperatures much lower than those associated with temperatures generated in the crater during impact (e.g., nitrates, 200°C; organic phosphorus, 350°C; inorganic phosphorus, 750°C; sulphur, 450°C; potassium, 570°C; and sodium, 880°C). In some parts of the crater, the substrate will not only be sterilized (made devoid of life), but will also be rendered oligotrophic (i.e., lacking in nutrients). The process and/or the extent of recolonization on melted and shocked substrates might be very different to that taking place on unprocessed substrate either inside or outside the crater and it might be very different from that associated with other agents of local ecological disturbance.

Recovery from impacts

Following the impact, there will be a well-defined sequence of biological recovery within the crater (Cockell and Lee, 2002). As there has been no large scale impact event in recorded human history, there has been no opportunity to observe these changes directly unlike post-volcanic recovery. However, observations on the present-day colonization of impact craters, which are in various states of preservation, can yield insights into the likely sequence of events (Cockell and Lee, 2002) and particularly the long-term influence of impacts on the geobiology of a region. Some general phases can be identified that are likely to occur. Following impact, the thermal pulse delivered by the impactor will heat the surrounding rock. High-temperature hydrothermal fluids generated in the modified thermal environment (Osinski et al., 2001) may, in the early stages following impact, provide a habitat for thermophilic and hyperthermophilic (heat-loving) microorganisms. For example, in the Houghton impact structure, a 24-km diameter structure on Devon Island in the Canadian High Arctic, the hydrothermal system may have lasted for at least 10,000 years. The crater cavity formed during the impact acts as a hydrologic depression and will provide a new habitat for aquatic life (either freshwater on land or marine if the impact occurred in the ocean and excavated a new salt-water filled environment).

Following impact, intracrater lacustrine ecosystems become established, depending upon the size of the structure. Even today, many ancient impact craters support lakes. The biota associated with impact crater lakes has now been studied in several cases. For example, the low biomass and poor diversity of the 3.4-km-diameter New Quebec Crater in Canada and the 18-km-diameter El'gygytyn crater in Siberia (Gronlund et al., 1990; Cremer and Wagner, 2003), contrast with the rich, abundant bacterial and algal ecosystem associated with the 1-km-diameter Tswaing impact crater in South Africa (Ashton and Schoeman, 1983; Schoeman and Ashton, 1982) (Figure 1). The Tswaing crater is situated in South African bushveld and receives organic input from the rich

Asteroid and Comet Impacts, Table 1 Information on the craters discussed in this article

Name of crater	Country	Size of crater (km)	Age of crater (Myr)
Chesapeake	Virginia, USA	90	35.5 ± 0.3
El'gygytyn	Russia	18	3.5 ± 0.5
Houghton	Canada	24	39.0
Lockne	Sweden	7.5	455
Mjølner	Norway	40	142.0 ± 2.6
New Quebec	Quebec, Canada	3.44	1.4 ± 0.1
Ries	Germany	24	15.1 ± 0.1
Tswaing	South Africa	1.13	0.22 ± 0.05



Asteroid and Comet Impacts, Figure 1 Impact crater lakes. Two examples of lakes formed in the cavity excavated by two impact events. El'gygytgyn in Siberia (left) and New Quebec in Canada (right).

vegetation that grows within the impact crater cavity. The area is characterized by erratic rainfall (between 400 and 750 mm a year) and summer temperatures that vary between a mean minimum and maximum of 14.2 and 35.2°C, respectively. The New Quebec crater is in a cold environment; temperatures are relatively constant at approximately 2.5–3°C down to the bottom (267 m) of the lake. Although the biota that takes advantage of an intra-crater lake will be influenced by the local climatic regimen, these examples show how impact excavation provides benefits for life by forming a cavity, in which water collects.

Once the crater begins to cool, exposed melt rocks can provide a substrate for primary succession (Cockell et al., 2001). The drainage of the lake by the breach of the crater wall will expose impact lithologies which then provide a substrate on which colonization can occur. Thus the sequence of geobiological events that occur after impact will be influenced by the general characteristics of impact craters (e.g., presence of a thermal pulse, an impact cavity) and the specifics of any given crater (e.g., duration of thermal pulse, local climate, size and depth of intracrater lake, substrate into which impact occurs, and the scale of the impact).

Ultimately a crater will become buried or eroded to point where the internal ecology may be indistinguishable from the exterior ecology.

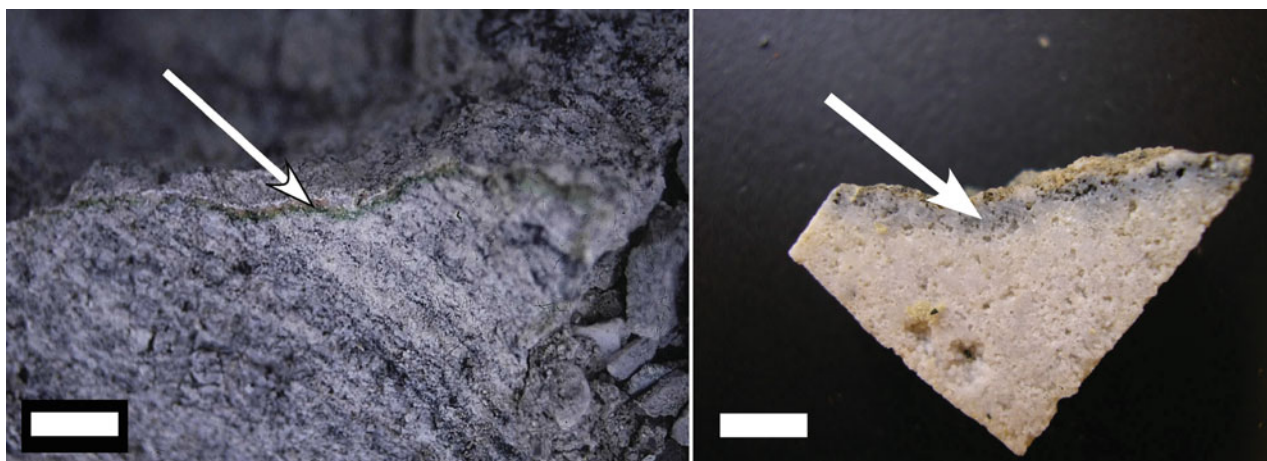
As three-quarters of the Earth is ocean, most impacts occur in water and one initial effect is the large-scale movement of water bodies. Investigations of strata in a corehole 30 km northeast of the Mjølnir crater, formed in the late Jurassic by the impact of an object into the 300–400-m deep paleo-Barents Sea, suggest that the impact event mixed water masses and caused a large introduction of nutrients into the Sea (Smelror et al., 2002). High concentrations of marine algae suggest that the increase in the availability of ammonia, nitrates, phosphates, and iron contributed to a postimpact bloom. The backwash of a tsunami, caused by the impact, is postulated

to be the reason for the high concentrations of freshwater algae in the strata. The mixing of the water, caused directly by impact, would also have been exacerbated by the collapse of the crater after formation.

Geomicrobiological influence of impacts

In the sense that impacts merely create a “hole in the ground,” their aquatic ecosystems may be indistinguishable from any topographic low in a similar location. However, it is evident that asteroid and comet impacts can have an important influence on the target lithology that is quite specific to impact and can alter the habitat for rock-dwelling microorganisms, making impact craters an important site for geomicrobiological investigations (Cockell et al., 2002). The temperatures and pressures of impact commonly increase the fracture space within rocks, which provide a greater abundance of habitats for endoliths, i.e., organisms which inhabit cracks within rocks. In some instances, the increase in permeable space within the target material in combination with an increase in the translucence of the rock in the visible part of the spectrum can yield new habitats suitable for phototrophs. Impact-shocked Precambrian gneiss in the Haughton crater has been shown to preferentially offer cryptoendolithic habitats for photosynthetic cyanobacterial colonists (Figure 2). The rock is not otherwise a suitable habitat for them (Cockell et al., 2002). This finding constituted empirical evidence for the formation of a microbial habitat specifically by an asteroid or comet impact.

The fate of highly porous substrates versus low porosity substrates during impact is different (Kieffer, 1971). In the former case more of the impact energy must be taken up in pore collapse. Thus, an examination of the effects of asteroid and comet impacts on the colonization of porous sedimentary target lithologies can provide a model with which to understand more generally the effects of impacts on the colonization potential of rocks (Cockell et al., 2005). In a study of impact-shocked sandstones from the



Asteroid and Comet Impacts, Figure 2 Cyanobacterial communities living in impact-shocked gneiss from the Houghton impact crater, Devon Island, Canadian High Arctic (left) and within class 3 shocked sandstones from the same crater (right). Scale bars 1 cm.

Houghton impact structure, Canadian High Arctic, it was shown that shock pressures from 0 to ~ 10 GPa caused the pore structure to collapse, thus rendering the material less amenable to microbial colonization. However, as shock pressures are increased to ~ 50 GPa the material becomes successively better for colonization on account of the formation of vesicular regions in the rock from rock fracturing and melting, improving conditions for colonization. At higher shock pressures colonization potential is again decreased as a solid glass is formed which is impermeable, although potentially still accessible to rock-boring microorganisms.

Thus, in summary, we can say that in general impact events may have the effect of turning lithic habitats on their head. Habitats that were previously impermeable become better sites for colonization as the rocks are fractured. Habitats that were good sites for colonization are rendered less permeable because of pore collapse, although, as the sandstones of Houghton illustrate, the effects on any given lithology tend to be complex.

Within and around impact craters new minerals can be generated in hydrothermal systems that later become habitats for life. Gypsum deposits laid down in the Houghton crater have become a habitat for cyanobacteria which inhabit the inter-sheet spaces between the sulphate crystals (Parnell et al., 2004). The extent to which these organisms are active is uncertain, but they must certainly be halotolerant.

During impact, the target lithology, especially if it is stratified and diverse, will be fragmented and mixed, leading to a heterogeneous substrate in which diverse geochemical sources of nutrients are brought into close proximity. A study of the weathering of a tsunami surge deposit in the Lockne impact crater in Sweden (formed ~ 455 million years ago) showed how lichens preferentially first colonized the soft chlorite clasts incorporated into the impactite (Cockell et al., 2007). The dissolution of the calcium carbonate clasts by fungi and other

organisms creates cavities in the rock, enhancing weathering and causing the rock to exfoliate in sheets. Previous studies on the chemical weathering of impact-shocked rocks from the Ries crater, Germany, showed that the deformation features introduced into quartz were sites of preferential weathering (Leroux, 2005). Diverse impact-generated lithologies can be used as a model to understand microbial weathering of other mixed sedimentary lithologies such as greywackes and microbe–mineral interactions in extremely disturbed geological settings in general.

Most of the geobiological studies conducted to date pertain to the surface environment. The first drill core retrieved from an impact crater with robust microbiological contamination control came from the Chesapeake Bay Impact Crater and was retrieved during the ICDP (International Continental Drilling Project)-USGS (United States Geological Survey) deep drilling project in 2005 (Gohn et al., 2008). A core of length 1.8 km was retrieved. The sequence included a 600 m sequence of postimpact sediments laid down since the impact in the late Eocene and 1.2 km of postimpact tsunami and “fall-back” deposits laid down in the crater cavity immediately after impact. This work has shown that the microbial diversity and abundance of microorganisms correlates to the lithologies in the crater cavity, showing that impacts can significantly disrupt the characteristics of the deep biosphere.

Impact events and astrobiology

The study of the geomicrobiology of impact craters provides useful clues to the opportunities for life on the Archean Earth since the emergence of life ~ 3.5 Gya ago, when the impact flux were higher than today. The formation of hydrothermal systems and the fracturing of rocks are two such opportunities that impact craters might have offered to early life. Insofar as asteroid and comet

impacts are universal (in that no solar system-forming process is known that is completely free from remnant material), habitats created in craters yield insights into the possible habitats for life on other planets, such as Mars. In particular, the likelihood that impacts melt permafrost, or generate deep fracturing into regions of a planet where liquid water is available, suggests that they offer intriguing possibilities as sites to examine the potential habitability of Mars, past and present.

Summary

Asteroid and comet impacts can cause global environmental perturbations through gross changes in atmospheric chemistry. However, much more commonly they alter geobiology at local scales. Through local disruptions of hydrology and geology, they generate new habitats and substrates, the most conspicuous being intracrater lakes. The energy delivered during impacts can also change habitats at the micron scale through changes in target lithology creating entirely novel geomicrobiological environments which, through pressure and temperature effects, are distinguishable from volcanic and other disturbed environments. The geobiological study of asteroid and comet impacts completes our picture of the role of catastrophic disturbance in influencing life on Earth alongside terrestrially mediated mechanisms of environmental change.

Bibliography

- Ashton, P. J., and Schoeman, F. R., 1983. Limnological studies on the Pretoria Salt Pan, a hypersaline maar lake. 1. Morphometric, physical and chemical features. *Hydrobiologia*, **99**, 61–73.
- Cockell, C. S., and Lee, P., 2002. The biology of impact craters – a review. *Biological Reviews*, **77**, 279–310.
- Cockell, C. S., Lee, P., Schuerger, A., Hidalgo, L., Jones, J., and Stokes, D., 2001. Microbiology and vegetation of micro-oases and polar desert, Haughton impact crater, Devon Island, Canadian High Arctic. *Arctic, Alpine and Antarctic Research*, **33**, 306–318.
- Cockell, C. S., Lee, P., Osinski, G., Horneck, G., and Broady, P., 2002. Impact-induced microbial endolithic habitats. *Meteoritics and Planetary Science*, **37**, 1287–1298.
- Cockell, C. S., Lee, P., Broady, P., Lim, D. S. S., Osinski, G. R., Parnell, J., Koeberl, C., Pesonen, L., and Salminen, J., 2005. Effects of asteroid and comet impacts on habitats for lithophytic organisms – a synthesis. *Meteoritics and Planetary Science*, **40**, 1901–1914.
- Cockell, C. S., Kennerley, N., Lindstrom, M., Watson, J., Ragnarsdottir, V., Sturkell, E., Ott, S., and Tindle, A. G., 2007. Geomicrobiology of a weathering crust from an impact crater and a hypothesis for its formation. *Geomicrobiology Journal*, **24**, 425–440.
- Cremer, H., and Wagner, B., 2003. The diatom flora in the ultra-oligotrophic Lake El'gygytgyn, Chukotka. *Polar Biology*, **26**, 105–114.
- Gohn, G., Koeberl, C., Miller, K. G., Reimold, U., Browning, J. C., Cockell, C. S., Horton, J. W., Kenkman, T., Kulpecz, A. A., Powars, D. S., Sanford, W. E., and Voytek, M. A., 2008. Deep drilling into the Chesapeake Bay impact structure. *Science*, **320**, 1740–1745.
- Gronlund, T., Lortie, G., Guilbault, J. P., Bouchard, M. A., and Saanisto, M., 1990. Diatoms and arcellaceans from lac du Cratere du Nouveau-Quebec, Ungava, Quebec, Canada. *Canadian Journal of Botany*, **68**, 1187–1200.
- Kieffer, S. W., 1971. Shock metamorphism of the Coconino sandstone at Meteor crater, Arizona. *Journal of Geophysical Research*, **76**, 5449–5473.
- Leroux, H., 2005. Weathering features in shocked quartz from the Ries impact crater: Germany. *Meteoritics and Planetary Sciences*, **40**, 1347–1352.
- Melosh, H. J., 1989. *Impact Cratering: A Geologic Process*. Oxford: Oxford University Press.
- Osinski, G. R., Spray, J. G., and Lee, P., 2001. Impact-induced hydrothermal activity within the Haughton impact structure: generation of a transient, warm, wet oasis. *Meteoritics and Planetary Science*, **36**, 731–745.
- Parnell, J., Lee, P., Cockell, C. S., and Osinski, G. R., 2004. Microbial colonization in impact-generated hydrothermal sulphate deposits, Haughton impact structure, and implications for sulphates on Mars. *International Journal of Astrobiology*, **3**, 247–256.
- Schoeman, F. R., and Ashton, P. J., 1982. The diatom flora of the Pretoria Salt Pan, Transvaal, Republic of South Africa. *Bacillaria*, **5**, 63–99.
- Smelror, M., Dypvik, H., and Mørk, A., 2002. Phytoplankton blooms in the Jurassic-cretaceous boundary beds of the Barents Sea possibly induced by the Mjølner impact. In Buffetaut, E., and Koeberl, C. (eds.), *Geological and Biological Effects of Impact Events*. New York: Springer-Verlag, pp. 69–83.

Cross-references

[Astrobiology](#)
[Biological Volcanic Rock Weathering](#)
[Cyanobacteria](#)
[Deep Biosphere of Sediments](#)
[Endoliths](#)
[Extreme Environments](#)
[Hydrothermal Environments, Terrestrial](#)
[Mass Extinctions, Phanerozoic](#)
[Meteoritics](#)

ASTROBIOLOGY

Jack D. Farmer
 Arizona State University, Tempe, AZ, USA

Synonyms

Bioastronomy; Exobiology

Definition

Astrobiology is the interdisciplinary science that studies the origin, evolution, distribution, and future of life in the Universe. It is a highly interdisciplinary field that engages many scientific disciplines, including biology, biogeochemistry, paleontology, earth and atmospheric sciences, planetology, and astrophysics among others.

Historical perspective

Astrobiology as a term was first coined in the 1940s to encompass early scientific ideas about how to explore for extraterrestrial life (Dick and Strick, 2004). During the 1960s, astrobiological research was focused more

- detection, double hybridization, and combined hybridization-immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **42**, 815–822.
- Goldenthal, K. L., Hedman, K., Chen, J. W., August, J. T., and Willingham, M. C., 1985. Postfixation detergent treatment for immunofluorescence suppresses localization of some integral membrane proteins. *Journal of Histochemistry and Cytochemistry*, **33**, 813–820.
- Gregg, M., and Reznik-Schüller, H. M., 1984. An improved method for rapid electron microscopic autoradiography. *Journal of Microscopy*, **135**, 115–118.
- Hainfeld, J. F., and Powell, R. D., 2000. New frontiers in gold labeling. *Journal of Histochemistry and Cytochemistry*, **48**, 471–480.
- Hayat, M. A., 1973–1977. *Electron Microscopy of Enzymes: Principles and Methods*. New York: Van Nostrand Reinhold, Vol. 1 (1973); Vols. 2 and 3 (1974); Vol. 4 (1976); Vol. 5 (1977).
- Hermann, R., Schwarz, H., and Müller, M., 1991. High precision immunoscanning electron microscopy using Fab fragments coupled to ultra-small colloidal gold. *Journal of Structural Biology*, **107**, 38–47.
- Hopkins, F. G., 1933. Some chemical aspects of life. *Nature Supplement*, **3332**, 381–394.
- Hoppert, M., 2003. *Microscopic Techniques in Biotechnology*. Weinheim: Wiley-VCH.
- Horisberger, M., 1985. The gold method as applied to lectin cytochemistry in transmission and scanning electron microscopy. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol. 3, pp. 155–178.
- Humbel, B. M., Sibon, O. C. M., Stierhof, Y.-D., and Schwarz, H., 1995. Ultra-small gold particles and silver enhancement as a detection system in immunolabeling and in situ hybridization experiments. *Journal of Histochemistry and Cytochemistry*, **43**, 735–737.
- Kämper, M., Vetterkind, S., Berker, R., and Hoppert, M., 2004. Methods for in situ detection and characterization of extracellular polymers in biofilms by electron microscopy. *Journal of Microbiological Methods*, **57**, 55–64.
- Kessels, M. M., Qualmann, B., Klobasa, F., and Sierralta, W. D., 1996. Immunocytochemistry by electron spectroscopic imaging using a homogeneously boronated peptide. *Cell and Tissue Research*, **284**, 239–245.
- Mayer, G., and Bendayan, M., 1997. Biotinyl–tyramide: a novel approach for electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1449–1454.
- Nisman, R., Dellaire, G., Ren, Y., Li, R., and Bazett, D. P., 2004. Application of quantum dots as probes for correlative fluorescence, conventional, and energy - filtered transmission electron microscopy. *Journal of Histochemistry and Cytochemistry*, **52**, 13–18.
- Qualmann, B., Kessels, M. M., Klobasa, F., Jungblut, P. W., and Sierralta, W. D., 1996. Electron spectroscopic imaging of antigens by reaction with boronated antibodies. *Journal of Microscopy*, **183**, 69–77.
- Ramos-Vara, J. A., 2005. Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**, 405–426.
- Rohde, M., Gerberding, H., Mund, T., and Kohring, G. W., 1988. Immunelectron microscopic localization of bacterial enzymes: pre- and postembedding labeling techniques on resin-embedded samples. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 175–210.
- Roth, J., Bendayan, M., and Orci, L., 1980. FITC-Protein A-Gold complex for light and electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **28**, 55–57.
- Takizawa, T., and Robinson, J. M., 2003. Ultrathin cryosections: an important tool for immunofluorescence and correlative microscopy. *Journal of Histochemistry and Cytochemistry*, **51**, 707–714.
- Wasielowski, R., Mengel, M., Gignac, S., Wilkens, L., Werner, M., and Georgii, A., 1997. Tyramine amplification technique in routine immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1455–1459.
- Wohlrab, F., and Gossrau, R., 1992. *Katalytische Enzymhistochemie*. Jena: G. Fischer Verlag.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold Seeps. *Journal of Microbiological Methods*, **73**, 85–91.

Cross-references

[Fluorescence in Situ Hybridization \(FISH\)](#)
[Histology](#)

IRON ISOTOPES

Please refer to “[Isotope Fractionation \(Metal\)](#).”

IRON SULFIDE FORMATION

Jürgen Schieber
 Indiana University, Bloomington, IN, USA

Definition

There has been a great deal of research into the formation of sedimentary iron sulfide minerals because their history is intertwined with the biogeochemical cycles of iron, sulfur, carbon, and oxygen (Berner, 2001). Iron sulfides occur in sediments from a wide range of depositional environments, from the deep sea to the nonmarine, and the principal mineral in the rock record is pyrite (FeS₂, cubic). Its dimorph marcasite (orthorhombic) is considered metastable and apt to invert to pyrite over geologic time (Murowchick, 1992), but is probably more widespread than commonly acknowledged (Schieber, 2007). Presumed precursor minerals of sedimentary pyrite are mackinawite (FeS_{1-x}) and greigite (Fe₃S₄), minerals that impart a black coloration to modern reducing sediments (Goldhaber and Kaplan, 1974). Both are metastable and transform over time into pyrite or a mixture of pyrite and pyrrhotite (Berner, 1967). Pyrrhotite, hexagonal FeS, is thermodynamically stable in sediments (Berner, 1967), but extremely rare in modern or ancient sediments (Kobayashi and Nomura, 1972) due to kinetic limitations (Canfield and Raiswell, 1991).

Mackinawite and greigite are also known as acid-volatile iron sulfides (AVS) because in contrast to pyrite and marcasite they are readily soluble in HCl. Mineralogically, greigite is the sulfur analog of magnetite and is indeed strongly ferromagnetic (Dekkers and Schoonen, 1996). It can be formed by magnetotactic bacteria and dominates the magnetic properties of some modern sediments and

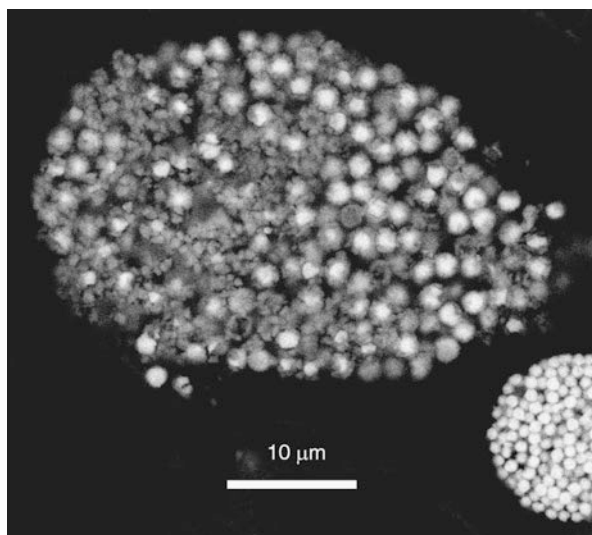
even some ancient sedimentary rocks (e.g., Krs et al., 1990).

Although they also occur in nonmarine sediments, the bulk of sedimentary iron sulfides forms in marine sediments during early diagenesis via microbial reduction of seawater sulfate. The general process of their formation has been elucidated by a number of classical studies (e.g., Kaplan et al., 1963; Berner, 1970, 1984). From a geobiological perspective, iron sulfides are of interest as their presence denotes anoxic and reducing pore waters, because they can preserve minute details of soft tissues (e.g., Briggs et al., 1991; Gabbott et al., 2004; Kammer and Ausich, 2007), because microbial life in pore spaces may be entombed in growing iron sulfide grains (Schieber, 2002a), and because the iron sulfide matrix is apparently able to preserve more complex organic molecules (such as amino acids) for long geologic time periods (Nardi et al., 1994). For an in-depth discussion of sedimentary sulfide formation and the linkage to the global sulfur cycle the reader is referred to an excellent overview by Goldhaber (2003) in the Treatise on Geochemistry.

Pathways to sedimentary iron sulfides

The understanding of iron sulfide formation under Earth surface conditions has largely been informed by extensive laboratory studies (see summary by Morse et al., 1987). Multiple potential pathways for sedimentary iron sulfide (pyrite) formation have been recognized. As summarized by Goldhaber (2003), these include (1) direct precipitation of pyrite from polysulfide bearing solutions, (2) progressive conversion of solid iron monosulfides to pyrite (Figure 1) by polysulfides, (3) reaction of H_2S with a solid iron monosulfide to form pyrite (release of H_2 gas), and (4) reactions that involve iron loss from a solid iron monosulfide precursor through oxidation.

As indicated by this summation, the body of published literature lives by the assumption that iron monosulfides (generic for mackinawite and/or greigite) are necessary precursors for pyrite formation in sediments. Thus, the normal iron sulfide paragenesis in sediments is presumed to proceed from mackinawite over greigite to pyrite (e.g., Wilkin and Barnes, 1996; Benning et al., 2000). The tacit assumption is that sedimentary pyrite cannot form in the absence of acid-volatile iron monosulfides (AVS). Yet, direct evidence for this traditional view is scant. Rickard and Morse (2005) have argued strongly that in numerous studies of modern sedimentary pyrite formation abundant pyrite is produced in the absence of detectable AVS. They argue that many of the equations that describe sedimentary pyrite formation in the literature represent net mass balances and do not describe the actual reaction processes. Rickard and Morse (2005) point out that AVS are not required for reaction pathways that involve polysulfide or H_2S (numbers (2) and (3) from above), and that aqueous FeS clusters could be involved instead of solid monosulfide phases. They propose that the products of the diagenetic sulfide machinery,

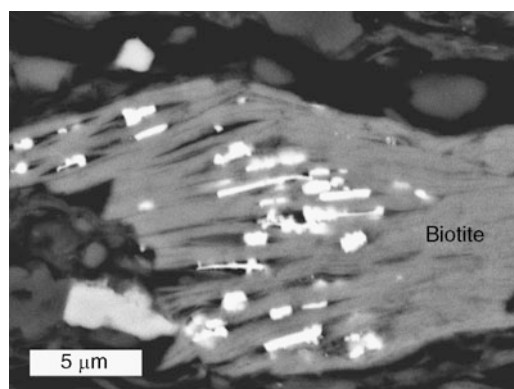


Iron Sulfide Formation, Figure 1 SEM photomicrograph of pyrite framboids from modern reducing muds of the Santa Barbara Basin, offshore southern California. The sediment was cored, stabilized in Spurr resin, and polished with diamond paste. At lower right a similar well-formed framboid might be found in ancient mudstones. In the center a less ordered framboid with bright grains that are pyrite (EDS analysis), and gray grains that have about half the sulfur peak intensity (EDS spectra) of typical pyrite grains. The interpretation is that the gray grains are the Fe-monosulfide precursor material (amorphous FeS , mackinawite, ~greigite) that has been the subject of geochemical studies. (Berner, 1970; Sweeney and Kaplan, 1973; Goldhaber and Kaplan, 1974; Murowchick and Barnes, 1987; Wilkin and Barnes, 1997.)

mackinawite, greigite, and pyrite, may form either simultaneously or in no particular order during sediment diagenesis.

The building blocks

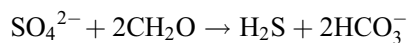
Regardless of how sedimentary iron sulfides are ultimately produced, it is instructive to examine where the requisite iron and sulfur come from. Because iron is insoluble in the presence of oxygen, the iron content of sea water and freshwater is extremely low (Stumm and Morgan, 1996). It is, therefore, practical to assume that any iron that is available for iron sulfide formation has to be part of the particulate matter that makes up the sediment. Typical deltaic and marine muds have iron contents that range from 4% to 8% (Calvert, 1976; Chester and Asten, 1976; Aller et al., 1986), but not all of that iron is equally available for diagenetic iron sulfide formation. Although there may be plenty of iron in iron silicates (e.g., biotite, chlorite), release of that iron requires alteration/destruction of the containing silicate, a process that operates more efficiently under the higher temperatures of deep burial diagenesis (Figure 2). Thus, this iron fraction is not available during early diagenesis when the bulk of sedimentary iron sulfides is formed.



Iron Sulfide Formation, Figure 2 SEM photomicrograph of late diagenetic biotite alteration associated with pyrite formation (backscatter (BS) image) in the Devonian Ohio Shale, Kentucky, USA. Pyrite (very bright) forms along cleavage planes near the end of a biotite flake because during alteration these are the places where iron is released. The surrounding pore waters must have contained abundant H_2S . Iron was precipitated as FeS_2 very close to the place where it was released from the biotite lattice, and it was not able to migrate away from its source grain.

The iron fraction that is critical in early diagenesis is in the form of coatings of iron oxyhydroxides on detrital grains (especially on clays; Carroll, 1958), as well as in the form of particulate/colloidal iron oxyhydroxides (e.g., Allard et al., 2004). Of the 4–8% of total iron that we can expect in average muddy sediments, only some 10%, and under very favorable circumstances up to about half, is made available by reducing agents (Gibbs, 1977; Trefrey and Presley, 1982; Canfield, 1989) for the formation of early diagenetic iron sulfides.

Sulfur, of course, is readily available via seawater sulfate in marine sediments, and for surficial sediments the overlying seawater represents an essentially limitless reservoir. Only upon burial, once consumed sulfate can no longer be replenished by downward diffusion, does sulfur become a limiting commodity. In all sediments, the production of H_2S for sulfide formation is accomplished by sulfate reducing prokaryotes that utilize sulfate as an electron acceptor for energy producing chemical reactions. For carbohydrate oxidation this reaction may be written as



Thus, organic matter that can be utilized by sulfate reducing microbes is essential for the operation of the sedimentary sulfide factory. Even in the presence of abundant sulfate, sulfide production will only occur if there is “food” for the microbes. Sulfate reduction equations proposed by Canfield and Raiswell (1991) suggest that it will require about 5 cm^3 of microbially “digestible” organic matter (e.g., lactate, ethanol, acetate, sugar) to precipitate 1 cm^3 of FeS_2 . Thus, low organic matter contents in sediments will tend to limit sulfide production and the amount of iron that can be converted to pyrite.

Above considerations also have implications with regard to where we most likely will find large quantities of iron sulfides in sedimentary rocks. Because pure carbonates typically accumulate far from the influence of terrigenous clastics, the average carbonate rock tends to contain only minor quantities of iron and thus, sedimentary iron sulfides are generally low in abundance. Sandstones, even though they may contain appreciable quantities of iron in the form of iron bearing detrital silicates and as iron oxyhydroxide grain coatings, are comparatively poor in organic matter and thus lack the fuel to drive extensive early diagenetic sulfate reduction. When we do find substantial accumulations of iron sulfides in carbonates or sandstones, they are in the majority of cases due to late diagenetic processes that benefited from higher temperatures and constituent bearing formation waters. Exceptions occur when carbonates or sandstones are interbedded with shales and mudstones, because the latter carry the bulk of iron in the sedimentary mass balance (Taylor and McLennan, 1985). The lion’s share of sedimentary iron sulfides is therefore contained in fine grained terrigenous rocks (shales and mudstones).

Iron sulfide textures

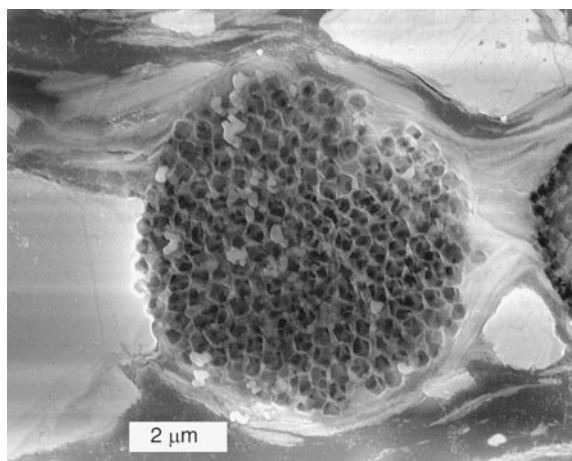
Pyrite, the most commonly reported iron sulfide in sediments, is cubic in structure, and thus cubes and octahedra are common morphologies. In addition to these fundamental forms, iron sulfides are also patterned into a wide range of textural types (e.g., single grains, framboids, pore fill cements, concretions, pyrite cemented layers, and pyritic lags) that carry useful information about conditions during deposition and early diagenesis. From a geobiological perspective, concretionary iron sulfide accumulations are of particular interest because their formation was likely driven by decomposing organic matter. Their morphology varies widely, and many types have been described, such as pyritized burrow tubes, infilling and encrustation of fossils, replacement of fossil hard parts (carbonate shells, bones), and pyritization of soft tissues.

Single grains

As single grains, pyrite may occur as a disseminated component, ranging from less than $1 \mu\text{m}$ to tens of micrometer in size. Yet, while these may indeed be grains that record a single growth episode, etching (e.g., with HNO_3) may reveal internal textures that speak to multistage growth.

Pyrite framboids

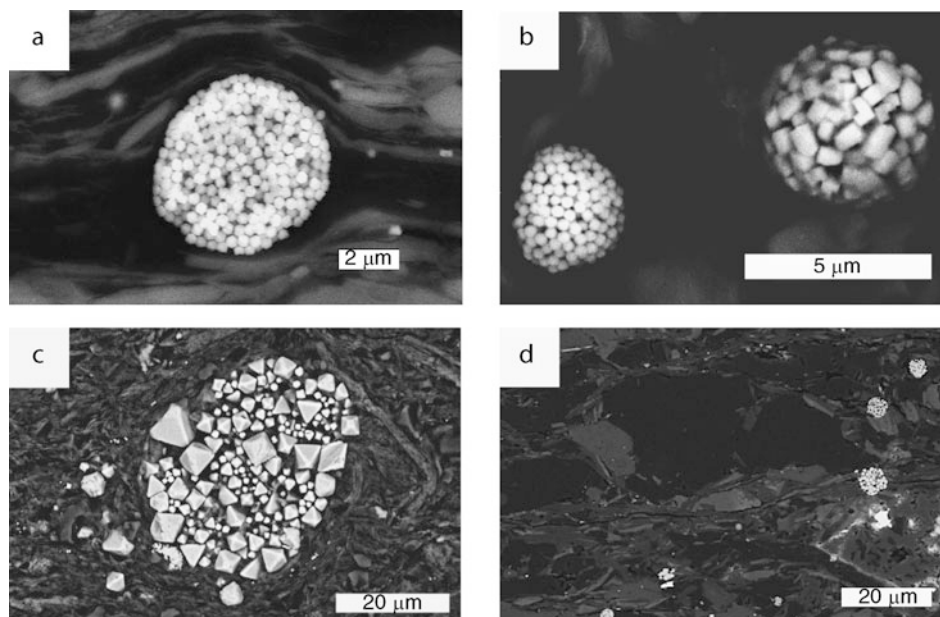
Ubiquitous in modern and ancient sediments, pyrite framboids are typically near-spherical in shape, range in diameter from about $1 \mu\text{m}$ to tens of micrometers, and are themselves composed of tiny, discrete, and equigranular pyrite crystallites. They have been the subject of numerous studies, and early on there was an assumption that organic matter (Figure 3) somehow plays a role in their formation (Schneiderhöhn, 1923; Love,



Iron Sulfide Formation, Figure 3 SEM photomicrograph of an etched pyrite framboid in the Devonian Chattanooga Shale of Tennessee, USA. The etching with HNO_3 removed the pyrite crystallites and left behind a honeycomb of organic matter that filled the space between the pyrite grains. The organic matter has the same composition as the unstructured kerogen in the surrounding sample.

1967). Yet, because they can also be produced abiotically in the laboratory (Berner, 1969; Sweeney and Kaplan, 1973), the role of organic matter remains a matter of debate (e.g., Kalliokoski, 1974). The abundantly noted close association of pyrite framboids and organic matter may well be coincidental because the reducing conditions that promote their formation are best developed in sediments with elevated contents of organic matter.

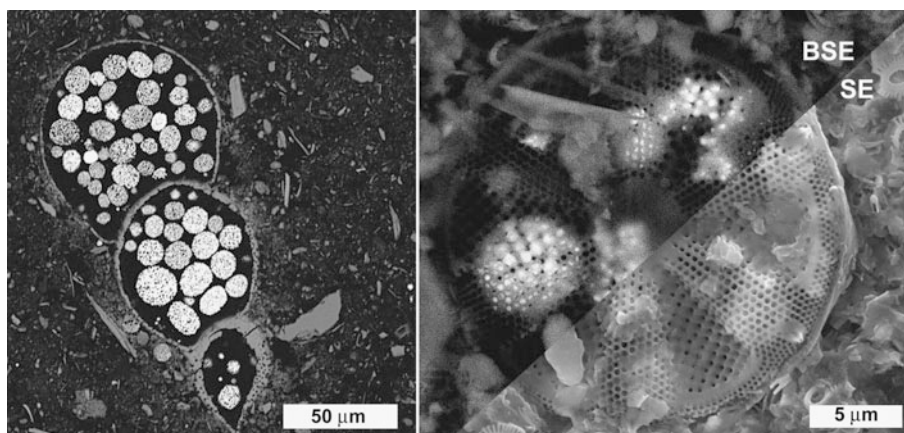
Most pyrite framboids observed in sedimentary rocks consist of microcrysts (Figure 4a; typically cubes, octahedral, or pyritohedra) that show poorly defined to highly ordered packing into spherical aggregates (Wilkin and Barnes, 1997; Ohfuji et al., 2005). The size ratio of microcrysts to framboid diameter is quite variable (Figure 4b). Directly adjacent framboids may show vast differences in diameter/microcryst size ratios (Figure 4b), and also show contrasting microcryst morphology. These textural details probably carry some information about the pore water environment during formation, but at present their significance is poorly understood. Pyrite framboids can survive intact into greenschist grade metamorphism, and can for example, identify slates that originated as marine shales (Figure 4d).



Iron Sulfide Formation, Figure 4 Pyrite framboids in the rock record. All images are SEM photomicrographs (backscatter mode). (a) Moderately well-ordered framboid from the late Devonian New Albany Shale (Indiana, USA). Note differential compaction of clays and organic matter around the framboid, indicating framboid formation prior to compaction when the sediment contained ~80% water. (b) Framboids in modern sediments from the Santa Barbara Basin (offshore southern California, USA). Note the strong differences in diameter/microcryst size ratios and the contrast in microcryst morphology (pyritohedra vs. complex cubes). (c) Framboid consisting mostly of variably sized octahedra (Ordovician Powers Steps Formation, Newfoundland). (d) Pyrite framboids that survived low-grade metamorphism and cleavage development ($\pm$ horizontal) in the Ordovician Martinsburg Slate of New Jersey, USA.

The formation processes of pyrite framboids are discussed at length by Wilkin and Barnes (1997), and further perspectives are presented by Ohfuji et al. (2003) and Butler and Rickard (2000). Fundamentally, in order to form framboids within the pore spaces of a sediment, iron should be able to migrate to the site of formation and thus, the pore waters have to be nonsulfidic (anoxic-nonsulfidic; Berner, 1981). Furthermore, in order for iron oxyhydroxides to dissolve in the pore waters, they also need to be free of oxygen. In the idealized sequence of microbial decay reactions in sediments (Brett and Allison, 1998; Curtis et al., 2000), this is known as the “suboxic” zone, and is followed by the “anoxic” zone of sulfate reduction. The boundary between the suboxic and the anoxic zone is also referred to as the redox interface, and it is here where dissolved iron meets sulfide and iron sulfides can precipitate. Pyrite framboids form in that region. Yet, whereas these zones are thought to be layered atop each other in surficial sediments, in reality it is a bit more complex. Every single framboid implies that iron migrated from a surrounding sediment volume to form this localized accumulation, and by default this means that there was a localized source of sulfide that via iron sulfide precipitation maintained the underlying concentration gradient. The sulfide source was probably a colony of sulfate reducing bacteria that prospered around a lump of easily digested organic matter. Thus, rather than simply being a narrow bedding-parallel zone in which dissolved iron interacts with dissolved sulfide, the realm of framboid formation (the redox interface) is heterogeneous. It makes more sense to think of it as “islands” of microbial sulfide production in a “sea” of iron-bearing pore waters (Figure 5), the reducing microenvironments that have been mentioned elsewhere in the literature (e.g., Hudson, 1982).

Whereas in typical sediments the redox interface is situated somewhat below the sediment water interface, there are situations where the redox interface is actually located in the overlying water column, such as in the Black Sea. In euxinic basins, with the lower portion of the water column being anoxic and sulfidic, framboids can grow in the water column near the redox interface and then settle and become part of the accumulating sediments (Wilkin et al., 1996). Wilkin et al. (1997) report that framboids of that origin are rather small (mean framboid diameters $<5\ \mu\text{m}$, narrow size distribution) when compared to those forming in sediments underlying oxic and dysoxic waters (mean framboid diameters $5\text{--}10\ \mu\text{m}$, broad size distribution). The implication of this is that one can use the framboid size distributions of sediments to determine whether they accumulated beneath an anoxic water column. Wignall and Newton (1998) applied this concept to ancient mudrocks and were able to correlate framboid size distributions with paleoecologically based reconstructions of bottom water oxygenation. However, framboid size in sediments may also be influenced by the availability of iron during early diagenesis. For example, a study of framboid size distributions in sediments from the Santa Barbara Basin (offshore southern California) indicates that framboid size distributions primarily reflect conditions within the immediate surface sediment (such as availability of readily soluble iron). Framboid size distributions in annual varves (1984–2004) from the Santa Barbara Basin suggest three euxinic interludes in the past 2 decades, even though seasonal bottom water surveys consistently recorded suboxic to dysoxic bottom water conditions (Schieber and Schimmelmann, 2006). It appears, therefore, that it may not be possible to draw conclusions about water column conditions from pyrite framboid size distributions.



Iron Sulfide Formation, Figure 5 SEM photomicrographs (backscatter mode) of localized pyrite framboid formation in reducing microenvironments of Santa Barbara Basin (offshore southern California) surface sediments. At left, a multichambered calcareous foraminifera with chambers filled by framboids. At right, a framboid filled diatom. Lower right half, imaged in secondary electron (SE) mode (framboids not visible). Upper left half, imaged in backscatter (BSE) mode, the framboids shine through the lower density opaline “lid.”

Polyframmboids

Pyrite frammboids may also occur as larger aggregates, so called polyframmboids. These have been described from sediments of various ages (Schieber and Baird, 2001) and have a tendency to form in cavities of organic remains (Figures 5 and 6). Judging from differential compaction around polyframmboids (Figure 6), they must form prior to any compaction, just like single pyrite frammboids. They also imply iron migration from a surrounding sediment volume that was anoxic but not sulfidic. The difference between polyframmboids and regular pyrite frammboids may simply be a higher iron supply from the pore waters, or alternatively that the “islands” of microbial sulfate reduction are more widely spaced and thus, focus the available iron onto fewer sites of precipitation. Because most marine sediments start out with comparable amounts of reactive iron, the latter scenario is more likely.

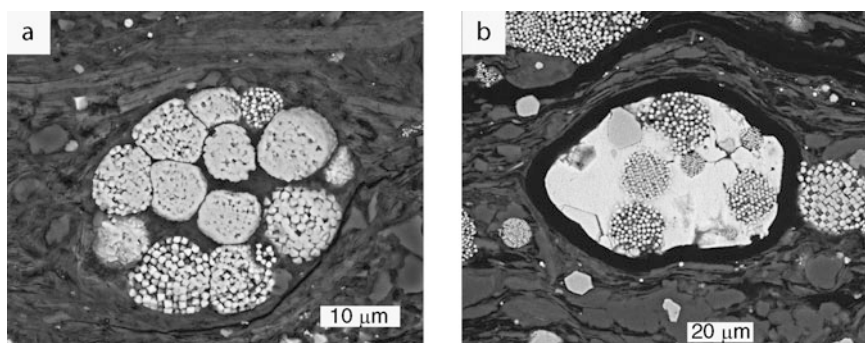
Small-scale pyrite aggregates and clusters

Pyrite bodies that consist of coalescing, irregular, rounded clots of small pyrite grains have been described as

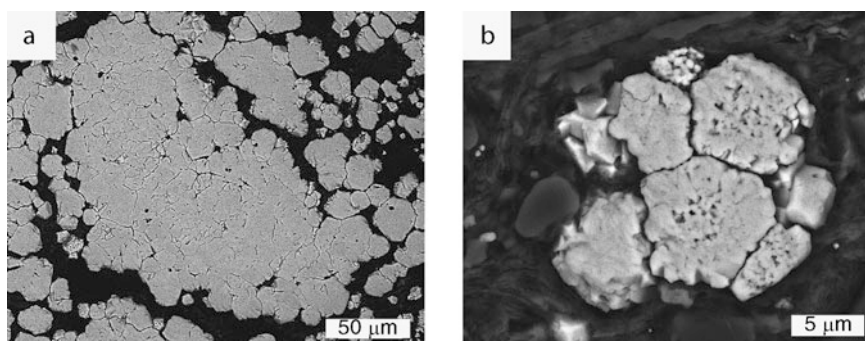
aggregated pyrite by Hudson (1982). The cores of individual clots may be small pyrite crystals or pyrite frammboids, and these cores are overgrown by coarser pyrite crystals (Figure 7). In places these overgrown grains show a radiating bladed habit. These overgrown grains tend to form somewhat later in diagenesis, at a point when downward diffusion of seawater sulfate is increasingly restricted (Strauss and Schieber, 1990). This type of pyrite aggregate ranges in size from tens of microns to several millimeters.

Concretions and pyritic layers

Iron sulfide concretions in sediments vary in size from a few millimeters to more than 10 cm. Like other occurrences of sedimentary iron sulfides they are most commonly encountered in mudstones and typically occur in distinct nodule bearing horizons (Figure 13). Although pyrite is usually reported as the sole iron sulfide mineral, careful examination reveals many examples of inverted or extant marcasite within these iron sulfide accumulations (Schieber, 2002b, 2007; Schieber and Riciputi,



Iron Sulfide Formation, Figure 6 SEM photomicrographs of pyrite polyframmboids (backscatter images). Note differential compaction around polyframmboid aggregates. (a) Ordovician Powers Steps Formation, Newfoundland, Canada. Note “squeezing” of frammboids, suggesting initial formation in a confined space (transported?). (b) Devonian Chattanooga Shale, Tennessee, USA. Here, the polyframmboid is still enclosed (black line) in an algal cyst (Tasmanites) that formed the initial reducing microenvironment. The space between frammboids is filled with secondary, coarser pyrite cement.



Iron Sulfide Formation, Figure 7 SEM photomicrographs of pyrite aggregates (backscatter images). (a) Irregularly shaped aggregate consisting of smaller pyrite clots. (b) Small cluster of pyrite clots that still have visible frammboids in their cores. The overgrown pyrite has radiating habit and may have originated as marcasite.

2005). Especially the larger concretions approximate prolate ellipsoids (Figure 8a) and show differential compaction of the surrounding mudstone (Figure 13). These features attest to growth prior to substantial compaction and growth within a stratiform chemical zone (anoxic and nonsulfidic; Berner, 1981). In order for iron to be directed to localized sites of precipitation within this zone, there need to be sites of abundant H_2S production, as, for example, provided by microbial decay of buried organisms (e.g., bivalve, fish). Thus, the interiors of iron sulfide concretions are good targets for finding well-preserved fossils. In order to allow the concretions to grow to large size, this chemical zone has to remain stationary for a long time, probably on the order of thousands of years (Canfield and Raiswell, 1991). Not uncommonly, the iron sulfide concretions in a given layer also add up to a substantial excess of iron relative to the background sediment, suggestive of prior concentration by mechanical and chemical reworking (see Section “Marcasite and its implications”). Thus, horizons with abundant iron sulfide concretions or abundant pore filling iron sulfides imply very slow sedimentation at the time of nodule formation and should also alert us to the possibility of reworking and erosion of underlying sediments (Schieber and Riciputi, 2005). Other options for the formation of iron sulfide-enriched horizons are, for example, to cover iron-oxide rich layers with an organic-rich anoxic sediment drape (Ingri and Ponter, 1986). Migration of a sulfide-rich reduction front into the iron-bearing layer would effectively convert the iron oxides to pyrite.

Hydraulic concentrations (lags)

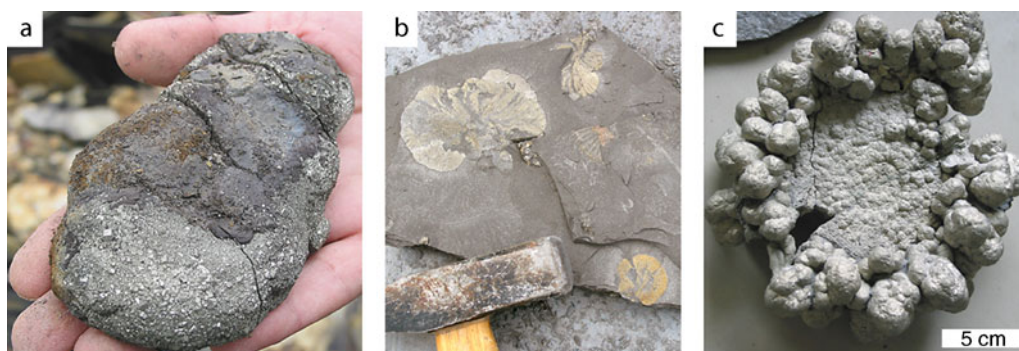
Because iron sulfide grains are of much higher density than associated silicates and organic matter, they are liable

to be concentrated into lag deposits when the easily eroded surface sediment is reworked by bottom currents and storms. Reworking can be manifest in the form of submillimeter concentrations of pyrite framboids (Figure 11c), as well as in decimeter thick concentrations of a wide variety of sedimentary pyrite grains (Figure 11). Examples are pyrite concretions (Figure 8), pyritized burrow tubes, and pyrite-filled algal cysts (Figure 6b). Lags that contain abundant iron sulfide grains have been described from the rock record in many places (e.g., Baird and Brett, 1991; Schieber, 1998a; Schieber and Baird, 2001). The mechanical enrichment of iron sulfides has two major implications and consequences. First, because of limitations on the initial soluble iron content of sediments, a substantial volume of sediment has to be sacrificed to erosion in order to concentrate all the iron into a single layer. Let us assume, for example, that typical mudstones have about 5% iron. A 5 cm thick lag with about 60% FeS_2 by volume would require that (at a minimum) a 50 cm thick (fully compacted) shale interval be winnowed to provide the necessary iron. Given that early in depositional history the uppermost meters of this shale (mud) had probably still an average porosity of at least 50%, the presence of this lag then implies that at least one meter, and more likely several meters, of sediment was removed.

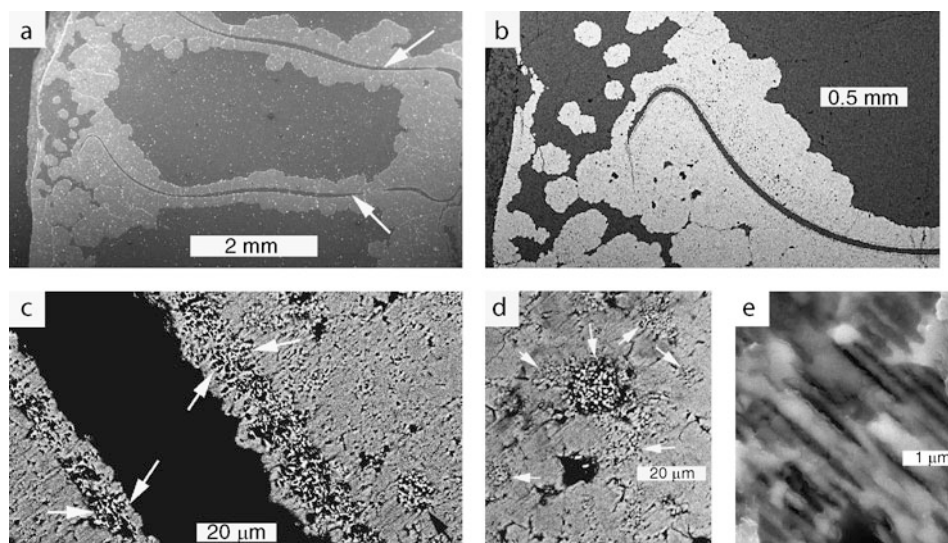
Iron sulfides and fossil preservation

Replacement and encrustation of calcareous shells

Encrustation and replacement of calcareous shells by iron sulfides are widely observed in the rock record. Typically, an assumption is made that the iron sulfide in question is pyrite, but a case can be made wherein marcasite was involved at least initially (see below). One way to think about calcite/aragonite replacement is that the saturation



Iron Sulfide Formation, Figure 8 A collection of iron sulfide concretions. (a) Oblate concretion from the Devonian Ohio Shale of eastern Kentucky, USA. Probably contains marcasite around the periphery, just like that seen in Figure 13. (b) Discoid-flattened pyrite concretions from the Jurassic Posidonia Shale near Dotternhausen, Germany. These may originally have been marcasitic. (c) Heavily pyrite (plus marcasite?) encrusted ammonite from the Lower Jurassic (Amaltheenton) near Dotternhausen, Germany. The specimen weighs at least 5 kg (courtesy of Dr. M. Jäger, Holcim GmbH). Without pre-concentration of iron in the sediment, it would require all the iron contained in a volume of 250 l of sediment (80% water content, 5% Fe in dry matter) to be concentrated into this concretion. Because iron redistribution is not that efficient, it is more likely that something on the order of 1 m³ of sediment has to be depleted.



Iron Sulfide Formation, Figure 9 Fossil preservation by iron sulfides, SEM photomicrographs. (a) Chamber lining iron sulfides in an ammonite (secondary electron image), Lias delta (Amaltheenton) near Dotternhausen, Germany. The septa of the ammonite (*white arrows*) are now calcitic, and the chamber interior is filled with calcite as well. (b) Close-up of the iron sulfide (bright, backscatter image) encrusted septum. The encrusting sulfides are a mixture of pyrite (framboids) and marcasite (overgrowth). (c) Close-up of encrustation near septum (backscatter image). The marginal areas of the septum (marked between *white arrows*) have a different texture than the areas further away. The latter areas contain pyrite framboids (*black arrow*) in a coarser matrix of blocky to radiating crystals. (d) Close-up of framboids (*white arrows*) in coarser matrix grains. The latter are anisotropic in reflected light and are either still preserved as marcasite or are marcasite relicts. (e) Details of the different textured replacement of the septum margin (between *white arrows* in (c)). The lamellar texture probably is a relict of the earlier lamellar structure of the septal aragonite.

levels of iron sulfide decline with increasing pH. When carbonates dissolve, pH increases and thus forces precipitation of iron sulfide. Chemical modeling suggests, however, that this process is unlikely during early diagenesis (Canfield and Raiswell, 1991). Nonetheless, even the shells of still living mollusks can be pyritized (Srivastava, 1975; Clark and Lutz, 1980), a process favored by pore waters with high levels of dissolved iron and excretion of microbially metabolizable organic substances by the mollusks (Reaves, 1984). In essence, the mollusks supported a sulfidic microenvironment and attracted iron from surrounding (anoxic-nonsulfidic) pore waters. This same basic scenario probably also applies to decomposing organic matter within dead mollusks, leading to shells encrusted or infilled with pyrite (Hudson, 1982). Hudson (1982) distinguished chamber linings (Figure 9), stalactitic pyrite, and over-pyrite (on outer shell surface), all of them mostly consisting of well-crystallized equant pyrite. For the potential impact of intermittent reoxidation of sedimentary iron sulfides on pH levels, carbonate precipitation, and rapid precipitation of the pyrite dimorph marcasite to promote shell encrustation, see the discussion on marcasite formation below.

Soft tissue preservation

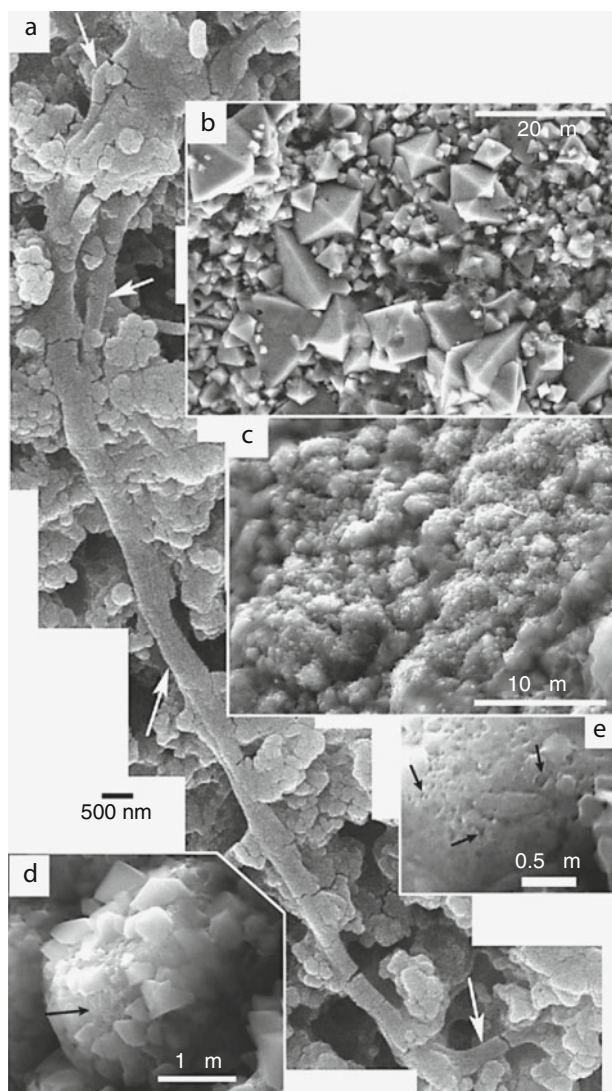
The preservation of soft tissue by pyritization is another marvel of fossilization, with well known examples from

the Hunsrück Slate (Stürmer, 1985), Beecher's trilobite bed (Cisne, 1973; Briggs et al., 1991), and the Burgess Shale (Conway Morris, 1986). Ongoing research has been producing a stream of exceptional soft bodied fossils that are preserved in pyrite, such as worms (Gabbott et al., 2004; Farrell and Briggs, 2007), crinoids (Kammer and Ausich, 2007), and plant tissues (Grimes et al., 2001). Pyritization can occur by infilling of cellular cavities, by preferential replacement of more readily decomposed components, and by pyrite coatings on easily degraded soft parts (Canfield and Raiswell, 1991). The latter process may be aided by microbial coatings (Wuttke, 1983) on the decomposing tissues that help to stabilize chemical gradients around the decomposing material. Framboidal, clustered, and aggregated pyrites are the most commonly observed type in this style of preservation. Even microbial cells and textures can be preserved under the right circumstances (Figure 10). With regard to preservation fidelity, soft tissue preservation by pyrite does not preserve as much detail as preservation in a phosphate matrix.

Degree of pyritization (DOP)

A widely used proxy for paleo-oxygenation, DOP is defined (Raiswell et al., 1988; Raiswell and Canfield, 1998) as

$$\text{DOP} = \text{pyrite Fe} / (\text{pyrite Fe} + \text{"reactive iron"})$$



Iron Sulfide Formation, Figure 10 SEM images of microbial remains preserved in early diagenetic coated iron sulfide grains (ooids and oncoids) from the Ordovician Winnipeg Formation, Saskatchewan, Canada (from Schieber and Riciputi, 2005). (a) Filamentous structure (*white arrows*) in slightly etched iron sulfide cortex. Note multiple filaments in the upper part of the picture. In the uppermost part of the picture the filaments merge into a sheet-like structure, potentially remnant of a biofilm. (b) Typical recrystallized appearance of an outermost cortex surface (unetched). Most crystals are pyrite octahedra. Between these crystal pavements one finds remnant patches of an earlier surface ((c), unetched). The latter has a lumpy appearance and consists of tightly packed clusters (a few microns in size) of iron sulfide grains. Close-ups of these clusters ((d), unetched) show that they actually consist of an ovoid smooth walled structure (*black arrow*) that is overgrown by iron sulfide grains. These are interpreted as microbes that were preserved in an iron sulfide matrix. Image (e) (unetched) shows that the smooth surfaces of these ovoids are typically perforated by irregularly shaped openings ((e), *black arrows*), comparable to wall structures seen in experimentally pyritized microbes (Bubela and Cloud, 1983).

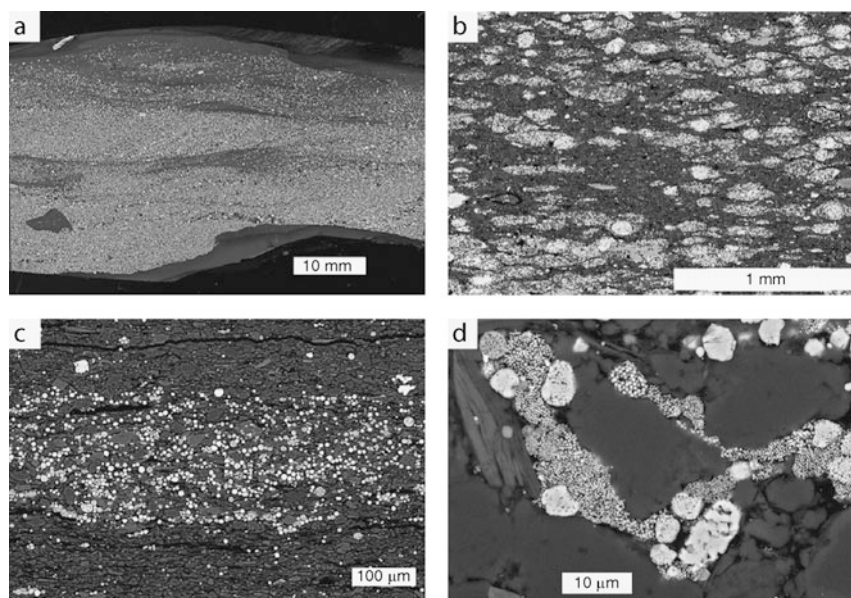
An extraction with dithionite or 1 N HCl is supposed to mimic reactive iron dissolution in reducing sediments (Canfield, 1989; Leventhal and Taylor, 1990). The highly reactive iron fraction in sediments is defined as the sum of dithionite or 1 N HCl leachable iron, and iron in acid volatile sulfides (AVS). What DOP presumably measures is the completeness of the conversion of reactive iron into pyrite via microbial sulfate reduction during early diagenesis. The underlying premise is that DOP increases as the degree of environmental oxygenation decreases. Because of the need for sufficient quantities of organic matter and reactive iron, measurements of DOP typically focus on carbonaceous mudstones. DOP values below 0.45 are considered indicative of aerobic bottom waters, and those above 0.45 are thought to mark restricted bottom water conditions. Values above 0.75 are considered to indicate anoxic or even euxinic bottom waters (Raiswell et al., 1988). However, the DOP method should not be applied uncritically, because limitations of either metabolizable organic matter or of reactive iron can lead to DOP values that are not reflective of the actual environment (as calibrated by Raiswell et al., 1988). Several other constraints on DOP application, such as outcrop weathering, maturation, and age of rock are summarized by Raiswell et al. (1988).

DOP as an environmental indicator can also be invalidated as a consequence of sedimentary processes. Any reworking and winnowing of pyrite bearing sediments will increase the pyrite content of the sediment, without simultaneously raising the reactive iron content, and as a result the DOP can rise simply as a result of wave or current reworking. In mudstones, indications of reworking at the mm to cm scale will often go undetected (Figure 11), and can lead to inflated DOP values that are incompatible with the levels of oxygen restriction suggested by observations of frequent reworking and bioturbation (Schieber, 2001, 2003).

Marcasite and its implications

Background on marcasite

Reports of marcasite in terrigenous clastics are mainly associated with coal deposits (e.g., Nayar, 1946; Read and Cook, 1969; Wiese et al., 1987). Marcasite occurs either in the coal seams themselves, or in the roof shales, prompting Krumbein and Garrels (1952) to propose an acidic bog/peat association as the characteristic environment for marcasite formation. There have, however, been a sufficient number of diagenetic marcasite occurrences reported from normal marine sediments (e.g., Maynard and Lauffenburger, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b, 2007), as well examples of marcasite that has inverted to pyrite (e.g., Bannister, 1932; Van Horn and Van Horn, 1933), to suggest that it might be more widespread in the marine realm than commonly appreciated. If this is in fact the case, and if acidic conditions are indeed a basic requirement for marcasite formation, we have an obvious problem to reconcile these



Iron Sulfide Formation, Figure 11 Reworked pyrite in shale successions. Image (a) is a photograph of a polished specimen, and all others are SEM photomicrographs (backscatter images). (a) Ripple cross-laminated lag deposit that consists mainly of spherical polyframboid fills of algal cysts (Schieber and Baird, 2001). Devonian Genesee Formation, New York, USA. (b) A horizon with abundant reworked flattened polyframboid clusters (comparable to those in Figure 6b). Devonian Chattanooga Shale, Tennessee, USA. (c) Lamina with abundant reworked pyrite framboids. Devonian Chattanooga Shale, Tennessee, USA. (d) Details of framboid-enriched lamina from (c). Shows how reworked pyrite framboids are filling spaces between larger silt grains and have been deformed due to compaction. Devonian Chattanooga Shale, Tennessee, USA.

basic realities with our current understanding of early diagenesis of marine sediments.

As pointed out above, the paragenetic relationships between sedimentary iron sulfide minerals are still poorly understood (Rickard and Morse, 2005). An essential guidepost for thinking about marcasite in sedimentary environments have been experiments conducted by Murowchick and Barnes (1986) that showed that marcasite is the dominant iron disulfide below pH 5. These experiments are consistent with results from much earlier work by Allen et al. (1914) and are also consistent with subsequent work by Schoonen and Barnes (1991a, b) and Benning et al. (2000). Thus, the relative abundance of pyrite versus marcasite in sediments should be a function of pH. Because marine waters have a slightly alkaline pH of approximately 8, it has been presumed that marcasite cannot form in marine sediments during early diagenesis (e.g., Rickard et al., 1995). Yet, regardless of this, early diagenetic marcasite unquestionably occurs in marine sedimentary rocks (Maynard and Lauffenburger, 1978; Siesser, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b; Schieber and Riciputi, 2005; Williams et al., 2003).

Detecting marcasite

Characteristic morphologies, such as “spearhead” twins and “cockscorn” crystals can be used to identify marcasite. However, morphology does not tell us whether the

marcasite has inverted to pyrite and the approach does not work for dense masses of crystals. As long as crystallites are large enough, it is comparatively easy to differentiate marcasite from pyrite with a petrographic microscope in reflected light mode (Ramdohr, 1975), and it is also possible to detect marcasite that has inverted to pyrite (Murowchick, 1992). For micron scale grains a new technique, “electron backscatter diffraction” (EBSD), can be applied for identification with an electron microscope. EBSD allows phase identification of submicron mineral grains and is widely used in material sciences (Prior et al., 1999; Schwartz et al., 2000).

Marcasite in sedimentary rocks

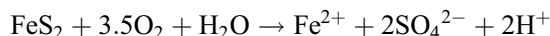
Marcasite is probably much more widespread in marine clastics than commonly assumed. It occurs as a cement mineral in marine lag deposits associated with sequence boundaries, in condensed intervals, and even in shales. Lag deposits with marcasite occur, for example, in Late Devonian shale successions of the eastern USA (Schieber, 2007). The Late Devonian Chattanooga and New Albany Shale successions contain laterally continuous erosion surfaces that compartmentalize this black shale succession into a stack of depositional sequences (Schieber, 1998a, b; Schieber and Lazar, 2004). These erosion surfaces imply substantial erosion and winnowing of previously deposited black shale during low stands of sea level (Schieber and Riciputi, 2004). The associated lag deposits contain

fish bone debris, conodonts, *Lingula* shells, quartz grains, and abundant pyrite in the form of reworked concretions and form conspicuous rust stained horizons in outcrop (Figure 12a).

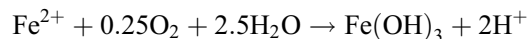
In polished thin section pore filling iron sulfides show typical marcasite morphology (Figure 12b). Overall, these lags may contain 30–40% iron sulfides by volume. Loose packing of framework grains indicates precompaction cementation with marcasite. Detrital pyrite grains in these lags are often variably rounded due to mechanical reworking and show etch and corrosion pits on the surface of detrital pyrite grains that are in turn infilled and overgrown by marcasite (Schieber, 2007).

Terrigenous clastic sediments contain at best a few percent reactive iron in the form of iron oxyhydroxide coatings on terrigenous grains (Carroll, 1958; Berner, 1969). Thus, the high iron concentrations in lag deposits with up to 40% iron sulfide require a mechanism for iron enrichment, and in the Phanerozoic at least the iron was most likely reworked from underlying strata (see above). In essence, sedimentary pyrite grains need to be eroded and winnowed in order to add more iron to the surficial layer, a process favored by stratigraphic condensation when net sedimentation rates are negative (Schieber and Riciputi, 2005; Schieber, 2007).

After mechanical enrichment, however, a portion of the sulfide iron has to be remobilized to form the observed overgrowths and pore-fill cements. Preexisting grains are destroyed, either wholly or partially, in order to supply dissolved (Fe^{2+}) or readily soluble iron (e.g., $\text{Fe}(\text{OH})_3$) for the growth of new iron sulfides. Corrosion features on reworked iron sulfide grains (Schieber and Riciputi, 2005; Schieber, 2007) are evidence for iron sulfide destruction according to the following reaction:



This reaction produces acidity and dissolved ferrous iron. Further oxidation of iron (see following equation) produces iron hydroxide and further acidity.

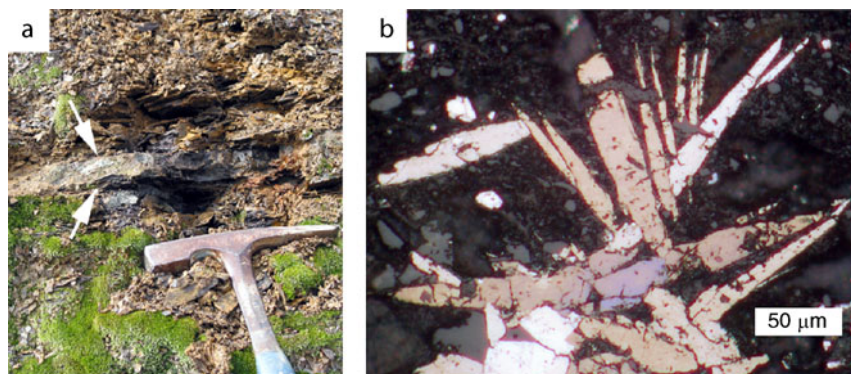


The newly formed iron hydroxides are likely to coat surface sediment grains and would remain in the surficial sediment layer. The associated lowering of pore water pH also promotes large pore water concentrations of Fe^{2+} (Maynard, 1983). Such a setting favors reprecipitation of marcasite in the presence of H_2S influx from underlying sediments (Murowchick and Barnes, 1986; Schoonen and Barnes, 1991a, b; Benning et al., 2000).

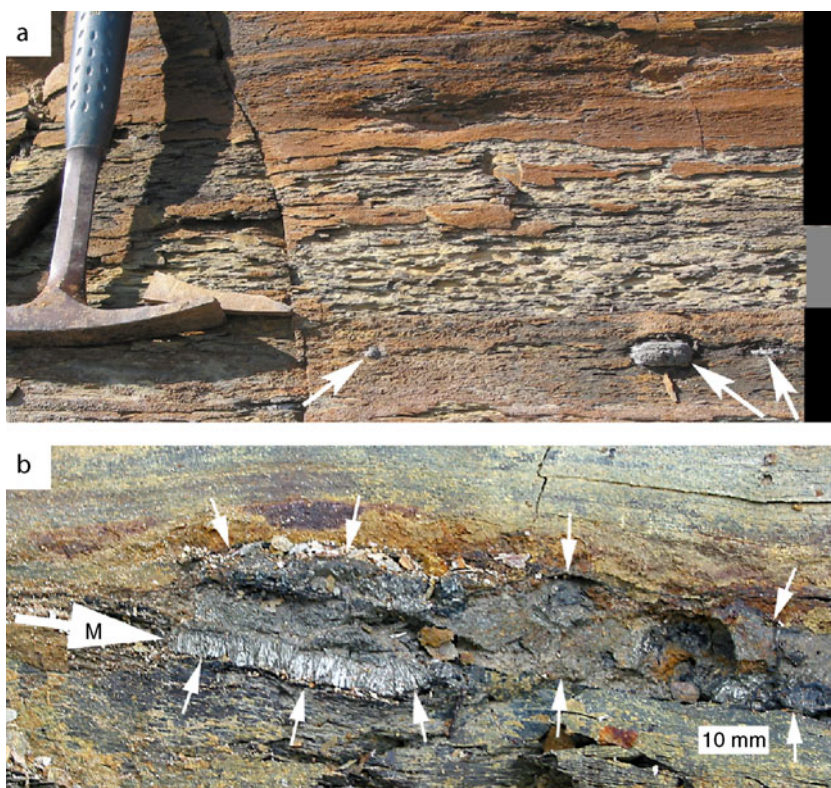
For sandy lags the presence of marcasite and partial dissolution of reworked pyrite grains are probably a good indicator that a “reoxidation” model for marcasite formation is applicable (Schieber and Riciputi, 2005; Schieber, 2007). In shales with abundant pyrite a comparable sequence of events may be caused by downward oxidation of previously deposited muds. Potential examples are stratiform horizons of iron sulfide concretions in black shale successions (Figure 13). Where these nodule horizons occur, a gray shale bed is typically found just above them (Figure 13a), and the concretions have a flattened radial fibrous habit, a morphology suggestive of marcasite (Figure 13b).

Reoxidation of reducing organic and iron sulfide-rich sediments, also termed “burndown,” has been studied extensively in the context of Quaternary Mediterranean sapropels (e.g., Jung et al., 1997), and marcasite in shales can potentially be used as a mineral-based indicator of burndown events.

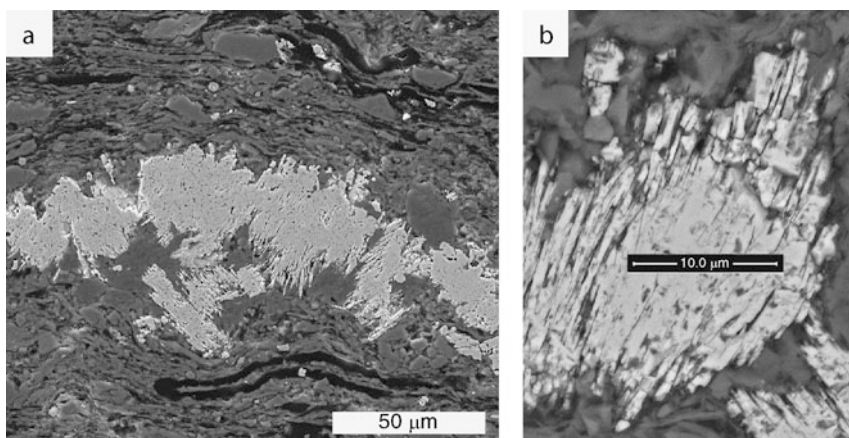
Although the generally finer grain size of diagenetic minerals in shales makes recognition of marcasite more difficult, EBSD analysis allows very tiny (micron-size)



Iron Sulfide Formation, Figure 12 Iron sulfide bearing lag deposits. (a) At left, an exposure of a sequence boundary lag (between arrows) in the late Devonian New Albany Shale. The lag is cemented by iron sulfides. Hammer for scale. (b) At right, a pore space in the lag with acicular “spearhead” twins, typical of marcasite. Electron backscatter diffraction (EBSD) analysis showed that many of the pore-fill iron sulfides still consist largely of marcasite.



Iron Sulfide Formation, Figure 13 Marcasite in late Devonian New Albany Shale, Kentucky, USA. (a) Stratiform horizon of marcasitic nodules (*white arrows*, black vs. gray shales marked by color bar at *right*). The nodules are located about 15 mm below the gray/black shale contact. The large amounts of iron sulfide accumulation at this level suggest that the redox interface stayed in that position for a long time, probably a reflection of zero to very low sedimentation. (b) Close-up of nodules that are broken through and show rims (*white arrows*) of radiating bladed marcasite (*large arrow M*). Note differential compaction.



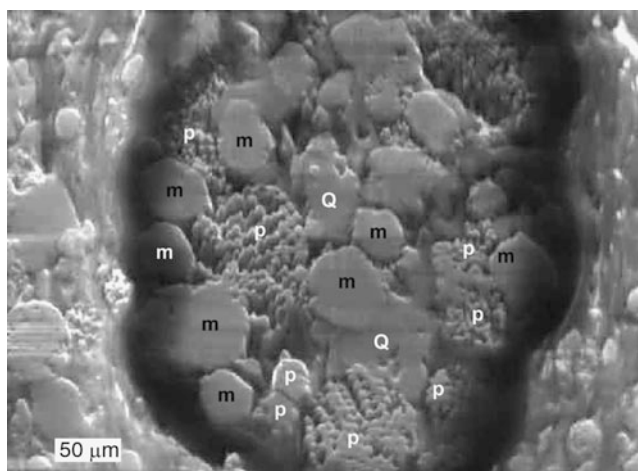
Iron Sulfide Formation, Figure 14 SEM photomicrographs (backscatter mode) of marcasite in an iron sulfide-enriched lamina in the Cleveland Shale (Upper Devonian, NE Kentucky). (a) Clusters of bladed marcasite grains that form bedding parallel lamina. Note differential compaction around marcasite clusters, suggesting formation when the sediment still had water content in excess of 80%. It also suggests that marcasite in the Cleveland Shale formed within 10–20 cm of the sediment–water interface. (b) Close-up that shows the platy habit of marcasite crystals (marcasite identification with EBSD). The reoxidation that is implicit in this observation contradicts earlier interpretations of this shale as euxinic (e.g., Jaminski et al., 1998; Rimmer, 2004).

marcasite grains to be identified in situ. A preliminary application of this technique to shales has detected marcasite in carbonaceous shales that range from Ordovician to Cretaceous in age (Figures 14 and 15).

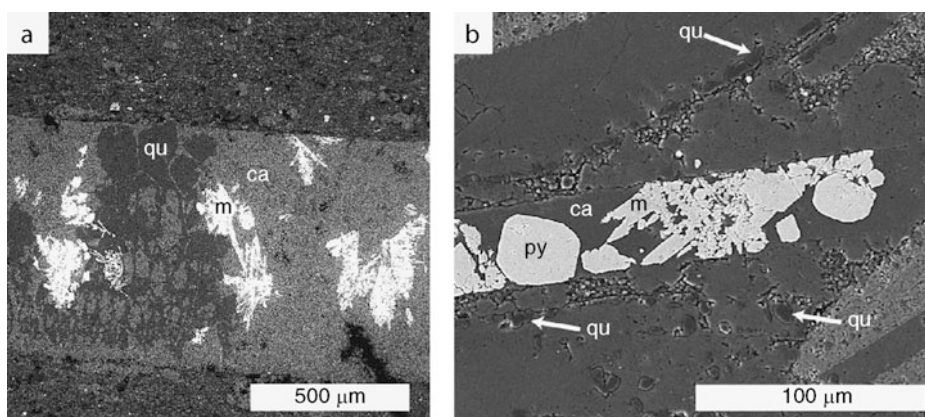
Unique fossil beds where preservation in pyrite and marcasite has been observed, such as Beecher's trilobite bed (Cisne, 1973) and pyritized ammonoids (Hudson, 1982; Seilacher et al., 1985), are another phenomenon where our "burndown" model could find application. The large stratiform iron buildup that at least some of these deposits represent requires a similar level of stratigraphic condensation as seen in some of the examples above.

Furthermore, iron needs to be mobile to form the observed mineralized and encrusted fossils. Thus, finding marcasite and dissolution features on reworked pyrite grains in pyritic Lagerstätten would support the view that such deposits may have formed during intermittent reoxygenation events.

The pH drop associated with pyrite oxidation during intermittent reoxygenation also explains textural features observed in association with iron sulfide replacement of calcareous shells. Examination of calcareous shell replacement in Carboniferous through Cretaceous mudstones showed marcasite as a common component,



Iron Sulfide Formation, Figure 15 Pyrite polyframboid cluster in Chattanooga Shale (Tennessee, USA) that formed within an algal cyst (dark rim). The infill was examined with EBSD. Marcasite (m) and quartz (Q) grew in the spaces between pyrite framboids (p). The open arrangement of framboids and the differential compaction around the cyst indicate that cementation happened prior to compaction. The marcasite observed here may well have been caused by intermittent pyrite oxidation in surface sediments. The relief in this image is an artifact, a consequence of oblique imaging (70° tilt) in EBSD mode.



Iron Sulfide Formation, Figure 16 SEM photomicrographs (backscatter) of marcasite replacing biogenic calcite. (a) Clam shell in Boquillas Formation (Cenomanian of West Texas). Skeletal marcasite crystals (marked m) grow as radiating clusters and replace calcite (ca). There is also partial replacement of the shell by quartz (marked qu). (b) Brachiopod shells in Barnett Shale (Mississippian of Texas). Shell in center is replaced by marcasite (marked m) that grows as bladed and sharply pointed crystals. Equant pyrite (py) formed toward end of iron sulfide deposition. Calcite shells marked ca, and replacing quartz marked qu.

intimately associated with calcite dissolution and precipitation of diagenetic quartz (Figure 16). This mineralogical triumvirate makes chemical sense because marcasite formation requires low pH, calcite dissolves at low pH, and dissolved silica (from opaline tests) precipitates under low pH conditions.

With regard to carbonate rocks, most published reports of marcasite are from chalks (e.g., Morgan-Jones, 1977; Kelts, 1976). This marcasite is typically in nodule form, measures up to 10 cm across, and shows crystals with marcasite morphology. Nonetheless, XRD analysis frequently shows that this marcasite has inverted to pyrite. There also seems to be an association with phosphate concretions, and thus with reworking, negative net sedimentation, and geochemical “reworking” of iron, just like that observed in marcasite occurrences in sandstones and shales.

Conclusions

The geobiological significance of sedimentary iron sulfides is far reaching. Microbial sulfate reduction as a metabolic pathway is of great antiquity and dates back to at least the early Archean (3.47-Ga; Shen and Buick, 2004). From that time onward, global cycling of sulfur and carbon has been essential for controlling the amount of oxygen in the Earth’s atmosphere (Berner, 2001), and some have even speculated that the emergence of life itself is tied to sulfur chemistry on the early Earth (e.g., Russell and Hall, 1997). It also appears that in itself oxygen buildup during the Precambrian led to the evolution of increasingly complex biochemical networks among microbes and eventually even to the evolution of complex life forms (Raymond and Segrè, 2006). The iron sulfides that we find in the rock record are a substantial part of the raw data for understanding the complex interaction of life with the atmosphere and the oceans. Every bit of that record, be it in the form of isotope shifts (e.g., Goldhaber, 2003), proportions and distribution of different iron sulfides, sulfide textures, stratal distribution, enclosed and preserved micro- and macro-fossils, as well as subsequent alterations of these minerals, is valuable for understanding chemical changes that affected the biosphere on the local, global, and temporal scales.

For the rock record, the enduring product of all these processes is the iron sulfide pyrite (FeS_2). Yet, the dimorph of pyrite, marcasite, can still be recognized in sedimentary rocks as old as Proterozoic (personal observations) and probably played a much larger role than commonly appreciated. In the minds of many geologists, the presence of iron sulfides in sedimentary rocks is associated with anoxic environmental conditions. In reality, however, once iron sulfides begin to form localized concentrations, be it in the form of micron-size framboids or fist-size concretions, one can imply that oxygen must have been close by. As elaborated repeatedly above, in order to form even microscopic features like pyrite framboids, iron should be able to migrate through the pore waters, and

those conditions only occur near the redox interface that “separates” deeper sulfidic from shallower oxygenated pore waters. Fundamentally, this holds true even in the most classical anoxic-euxinic locale of all, the Black Sea. There, pyrite framboids do not form in the sulfidic bottom waters, but form again at the redox interface where these waters come in contact with oxygenated surface waters (Wilkin et al., 1996). The bulk of sedimentary iron sulfides in the rock records marks the interface between the anoxic and oxic worlds; they should not be taken as an indicator of environmentally pervasive anoxia.

Bibliography

- Allard, T., Menguy, N., Salomon, J., Calligaro, T., Weber, T., Calas, G., and Benedetti, M. F., 2004. Revealing forms of iron in river-borne material from major tropical rivers of the Amazon Basin (Brazil). *Geochimica et Cosmochimica Acta*, **68**, 3079–3094.
- Allen, E. T., Crenshaw, J. L., and Merwin, H. E., 1914. Effect of temperature and acidity in the formation of marcasite (FeS_2) and wurzite (ZnS). *American Journal of Science*, **38**, 393–421.
- Aller, R. C., Mackin, J. E., and Cox, R. T., 1986. Diagenesis of Fe and S in Amazon inner shelf muds: apparent dominance of Fe reduction and implications for the genesis of ironstones. *Continental Shelf Research*, **6**, 263–289.
- Baird, G. C., and Brett, C. E., 1991. Submarine erosion on the anoxic sea floor: Stratigraphic, palaeoenvironmental, and temporal significance of reworked pyrite-bone deposits. In Tyson, R. V., and Pearson, T.H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. Geological Society of London, Special Publication, 58, pp. 233–257.
- Bannister, F. A., 1932. The distinction of pyrite from marcasite in nodular growths. *Mineralogical Magazine*, **23**, 179–187.
- Benning, L. G., Wilkin, R. T., and Barnes, H. L., 2000. Reaction pathways in the Fe-S system below 100°C. *Chemical Geology*, **167**, 25–51.
- Berner, R. A., 1967. Thermodynamic stabilities of sedimentary iron sulfides. *American Journal of Science*, **265**, 773–785.
- Berner, R. A., 1969. Migration of iron and sulfur within anaerobic sediments during early diagenesis. *American Journal of Science*, **267**, 19–42.
- Berner, R. A., 1970. Sedimentary pyrite formation. *American Journal of Science*, **268**, 1–23.
- Berner, R. A., 1981. A new geochemical classification of sedimentary environments. *Journal of Sedimentary Petrology*, **51**, 359–365.
- Berner, R. A., 1984. Sedimentary pyrite formation: an update. *Geochimica et Cosmochimica Acta*, **48**, 605–615.
- Berner, R. A., 2001. Modeling atmospheric O_2 over Phanerozoic time. *Geochimica et Cosmochimica Acta*, **65**, 685–694.
- Brett, C., and Allison, P. A., 1998. Paleontological approaches to the environmental interpretation of marine mudrocks. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones (Vol. 1): Basin Studies, Sedimentology and Paleontology*. Stuttgart: Schweizerbart’sche Verlagsbuchhandlung, pp. 301–349.
- Briggs, D. E. G., Bottrell, S. H., and Raiswell, R., 1991. Pyritization of soft-bodied fossils; Beecher’s Trilobite Bed, Upper Ordovician, New York State. *Geology*, **9**, 1221–1224.
- Bubela, B., and Cloud, P., 1983. Sulfide mineralization of microbial cells. *Bureau of Mineral Resources, Journal of Australian Geology and Geophysics*, **8**, 355–357.
- Butler, I. B., and Rickard, D., 2000. Framboidal pyrite formation via the oxidation of iron (II) monosulphide by hydrogen sulphide. *Geochimica et Cosmochimica Acta*, **64**, 2665–2672.

- Calvert, S. E., 1976. The mineralogy and geochemistry of near-shore sediments. In Ripley, J. P., and Chester, R. (eds.), *Chemical Oceanography*. New York: Academic, Vol. 6, pp. 187–280.
- Canfield, D. E., 1989. Reactive iron in marine sediments. *Geochimica et Cosmochimica Acta*, **53**, 619–632.
- Canfield, D. E., and Raiswell, R., 1991. Pyrite formation and fossil preservation. In Allison, P. A., and Briggs, D. E. (eds.), *Taphonomy: Releasing the Data Locked in the Fossil Record*. New York: Plenum, pp. 337–387.
- Carroll, D., 1958. Role of clay minerals in the transportation of iron. *Geochimica et Cosmochimica Acta*, **14**, 1191–1206.
- Chester, R., and Asten, S. R., 1976. The geochemistry of deep-sea sediments. In Ripley, J. P., and Chester, R. (eds.), *Chemical Oceanography*. New York: Academic, Vol. 6, pp. 281–291.
- Cisne, J. L., 1973. Anatomy of *Triarthrus* and the relationships of the Trilobita. *Fossils and Strata*, **4**, 45–64.
- Clark, G. R., and Lutz, R. A., 1980. Pyritization in the shells of living bivalves. *Geology*, **8**, 268–271.
- Conway Morris, S., 1986. The community structure of the Middle Cambrian phyllopod bed (Burgess Shale). *Paleontology*, **29**, 423–458.
- Curtis, C. D., Cope, J. C. W., Plant, D., and Macquaker, J. H. S., 2000. “Instantaneous” sedimentation, early microbial sediment strengthening and a lengthy record of chemical diagenesis preserved in Lower Jurassic ammonitiferous concretions from Dorset. *Journal of the Geological Society (London)*, **157**, 165–172.
- Dekkers, M. J., and Schoonen, M. A. A., 1996. Magnetic properties of hydrothermally synthesized greigite (Fe_3S_4): I. Rock magnetic properties at room temperature. *Geophysical Journal International*, **126**, 360–368.
- Farrell, U. C., and Briggs, D. E. G., 2007. A pyritized polychaete from the Devonian of Ontario. *Proceedings: Biological Sciences*, **274**, 499–504.
- Gabbott, S. E., Hou, X. G., Norry, J. N., and Siveter, D. J., 2004. Preservation of Early Cambrian animals of the Chengjiang biota. *Geology*, **32**, 901–904.
- Gibbs, R. J., 1977. Transport phases of transition metals in the Amazon and Yukon Rivers. *Geological Society of America Bulletin*, **88**, 829–843.
- Goldhaber, M. B., 2003. Sulfur-rich sediments. In Mackenzie, F. T. (ed.), *Treatise on Geochemistry, Vol 7, Sediments, Diagenesis, and Sedimentary Rocks*. Amsterdam: Elsevier, pp. 257–288.
- Goldhaber, M. B., and Kaplan, I. R., 1974. The sulfur cycle. In Goldberg, E. D. (ed.), *The Sea*. New York: Wiley, Vol. 5, 569–655.
- Grimes, T. G., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Hudson, J. D., 1982. Pyrite in ammonite-bearing shales from the Jurassic of England and Germany. *Sedimentology*, **29**, 639–667.
- Ingri, J., and Ponter, C., 1986. Iron and manganese layering in recent sediments of the Gulf of Bothnia. *Chemical Geology*, **56**, 105–116.
- Jaminski, J., Algeo, T. J., Maynard, J. B., and Hower, J. C., 1998. Climatic origin of dm-scale compositional cyclicity in the Cleveland Member of the Ohio Shale (Upper Devonian), central Appalachian Basin, U.S.A. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones I*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. Obermiller), pp. 217–242.
- Jowett, E. C., Roth, T., Rydzewski, A., and Oszczepalski, S., 1991. “Background” delta 34S values of Kupferschiefer sulphides in Poland: pyrite-marcasite nodules. *Mineralium Deposita*, **26**, 89–98.
- Jung, M., Ilmberger, J., Mangini, A., and Emeis, K. C., 1997. Why some Mediterranean sapropels survived burn-down (and others did not). *Marine Geology*, **141**, 51–60.
- Kalliokoski, J., 1974. Pyrite framboids: animal, vegetable, or mineral? *Geology*, **2**, 26–27.
- Kammer, T. W., and Ausich, W. I., 2007. Soft-tissue preservation of the hind gut in a new genus of cladid crinoid from the Mississippian (Visian, Asbian) at St. Andrews, Scotland. *Palaeontology*, **50**, 951–959.
- Kaplan, I. R., Emery, K. O., and Rittenberg, S. C., 1963. The distribution and isotopic abundance of sulfur in recent marine sediments off southern California. *Geochimica et Cosmochimica Acta*, **27**, 297–331.
- Kelts, K. R., 1976. *Marcasite in Miocene calcareous sediments from Hole 315A. Deep Sea Drilling Project Initial Reports*, **33**, 867–870.
- Kobayashi, K., and Nomura, M., 1972. Iron sulfides in sediment cores from the sea of Japan and their geophysical implications. *Earth and Planetary Science Letters*, **16**, 200–208.
- Krs, M., Krsova, M., Pruner, P., Zeman, A., Novak, F., and Jansa, J., 1990. A petromagnetic study of Miocene rocks bearing micro-organic material and the magnetic mineral greigite (Sokolov and Cheb basins, Czechoslovakia). *Physics of the Earth and Planetary Interiors*, **63**, 98–112.
- Krumbein, W. C., and Garrels, R. W., 1952. Origin and classification of chemical sediments in terms of pH and oxidation-reduction potentials. *The Journal of Geology*, **60**, 1–33.
- Leventhal, J. S., and Taylor, C., 1990. Comparison of methods to determine degree of pyritization. *Geochimica et Cosmochimica Acta*, **54**, 2621–2625.
- Love, L. G., 1967. Early diagenetic iron sulphide in recent sediments of the Wash (England). *Sedimentology*, **9**, 327–352.
- Maynard, J. B., 1983. *Geochemistry of Sedimentary Ore Deposits*. New York: Springer, 305 p.
- Maynard, J. B., and Lauffenburger, S. K., 1978. A marcasite layer in prodelta turbidites of the Borden Formation (Mississippian): in eastern Kentucky. *Southeastern Geology*, **20**, 47–58.
- Morgan-Jones, M., 1977. Mineralogy of the non-carbonate material from the chalk of Berkshire and Oxfordshire, England. *Clay Minerals*, **12**, 331–344.
- Morse, J. W., Millero, F. J., Cornwell, J. C., and Rickard, D., 1987. The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews*, **24**, 1–42.
- Murowchick, J. B., 1992. Marcasite inversion and the petrographic determination of pyrite ancestry. *Economic Geology*, **87**, 1141–1152.
- Murowchick, J. B., and Barnes, H., 1986. Marcasite precipitation from hydrothermal solutions. *Geochimica et Cosmochimica Acta*, **50**, 2615–2629.
- Murowchick, J. B., and Barnes, H. L., 1987. Effects of temperature and degree of supersaturation on pyrite morphology. *American Mineralogist*, **72**, 1241–1250.
- Nardi, S., Binda, P. L., Bacelle, L. S., and Concheri, G., 1994. Amino acids of Proterozoic and Ordovician sulfide-coated grains from western Canada: record of biologically mediated pyrite precipitation. *Chemical Geology*, **111**, 1–15.
- Nayar, K. V., 1946. Marcasite in Travancore lignite. *Current Science*, **5**, 229.
- Ohfuji, H., Butler, I. B., and Rickard, D., 2003. Experimental study of synthetic pyrite framboids and other morphologies. *Geochimica et Cosmochimica Acta*, **67**(Suppl. 1), A351.
- Ohfuji, H., Boyle, A. P., Prior, D. J., and Rickard, D., 2005. Structure of framboidal pyrite: An electron backscatter diffraction study. *American Mineralogist*, **90**, 1693–1704.
- Prior, D. J., Boyle, A. P., Brenker, F., Cheadle, M. C., Day, A., Lopez, G., Peruzzo, L., Potts, G. J., Reddy, S. M., Spiess, R., Timms, N. E., Trimby, P. W., Wheeler, J., and Zetterstrom, L.,

1999. The application of electron backscatter diffraction and orientation contrast imaging in the SEM to textural problems in rocks. *American Mineralogist*, **84**, 1741–1759.
- Raiswell, R., and Canfield, D. E., 1998. Sources of iron for pyrite formation. *American Journal of Science*, **298**, 219–245.
- Raiswell, R., Buckley, F., Berner, R. A., and Anderson, T. F., 1988. Degree of pyritization of iron as a paleoenvironmental indicator of bottom water oxygenation. *Journal of Sedimentary Petrology*, **58**, 812–819.
- Ramdohr, P., 1975. *Die Erzminerale und ihre Verwachsungen*. Berlin: Akademie, 1277 p.
- Raymond, J., and Segrè, D., 2006. The effect of oxygen on biochemical networks and the evolution of complex life. *Science*, **311**, 1764–1767.
- Read, H. W., and Cook, A. C., 1969. Note on coals containing marcasite plant petrifications, Yarrunga creek, Sydney Basin, New South Wales. *Journal and Proceedings of the Royal Society of New South Wales*, **102**, 197–199.
- Reaves, C. M., 1984. *The Migration of Iron and Sulfur During the Early Diagenesis of Marine Sediments*. PhD thesis, Yale University, New Haven.
- Rickard, D., and Morse, J. W., 2005. Acid volatile sulfide (AVS). *Marine Chemistry*, **97**, 141–198.
- Rickard, D., Schoonen, M. A. A., and Luther, G. W., 1995. Chemistry of iron sulfides in sedimentary environments. In Vairavamurthy, M. A., and Schoonen, M. A. A. (eds.), *Geochemical Transformations of Sedimentary Sulfur*. ACS Symposium Series 612. Washington, pp. 165–193.
- Rimmer, S. M., 2004. Geochemical paleoredox indicators in Devonian-Mississippian black shales, Central Appalachian Basin (USA). *Chemical Geology*, **206**, 373–391.
- Russell, M. J., and Hall, A. J., 1997. The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *Journal of the Geological Society (London)*, **154**, 377–402.
- Ryckart, R., 1983. “Gilded” petrifications; formation of pyrite and marcasite in bituminous shale. *Mineralien Magazin*, **7**, 280–284.
- Schieber, J., 1998a. Sedimentary features indicating erosion, condensation, and hiatuses in the Chattanooga Shale of Central Tennessee: relevance for sedimentary and stratigraphic evolution. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones I*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägelé u. Obermiller), pp. 187–215.
- Schieber, J., 1998b. Developing a sequence stratigraphic framework for the Late Devonian Chattanooga Shale of the southeastern US: relevance for the Bakken shale. In Christopher, J. E., Gilboy, C. F., Paterson, D. F., and Bend, S. L. (eds.), *Eighth International Williston Basin Symposium*. Saskatchewan Geological Society Special Publication, Vol. 13, pp. 58–68.
- Schieber, J., 2001. Ways in which organic petrology could contribute to a better understanding of black shales. *International Journal of Coal Geology*, **47**, 171–187.
- Schieber, J., 2002a. Sedimentary pyrite: a window into the microbial past. *Geology*, **30**, 531–534.
- Schieber, J., 2002b. The role of an organic slime matrix in the formation of pyritized burrow trails and pyrite concretions. *Palaios*, **17**, 104–109.
- Schieber, J., 2003. Simple gifts and hidden treasures – implications of finding bioturbation and erosion surfaces in black shales. *The Sedimentary Record*, **1**, 4–8.
- Schieber, J., 2007. Oxydation of detrital pyrite as a cause for Marcasite formation in Marine Lag Deposits from the Devonian of the Eastern US. *Deep Sea Research II*, **54**, 1312–1326.
- Schieber, J., and Baird, G., 2001. On the origin and significance of pyrite spheres in Devonian black shales of North America. *Journal of Sedimentary Research*, **71**, 155–166.
- Schieber, J., and Lazar, O. R., 2004. Devonian black shales of the eastern U.S. New insights into sedimentology and stratigraphy from the subsurface and outcrops in the Illinois and Appalachian Basins. *Indiana Geological Survey Open-File Study* 04–05, 90 p.
- Schieber, J., and Riciputi, L., 2004. Pyrite ooids in Devonian black shales record intermittent sea level drop and shallow water conditions. *Geology*, **32**, 305–308.
- Schieber, J., and Riciputi, L., 2005. Pyrite-marcasite coated grains in the Ordovician Winnipeg Formation, Canada: an intertwined record of surface conditions, stratigraphic condensation, geochemical “reworking”, and microbial activity. *Journal of Sedimentary Research*, **75**, 905–918.
- Schieber, J., and Schimmelmann, A., 2006. High resolution pyrite framboid size distribution in Santa Barbara Basin sediments: implications for the study of black shales. *Eos Transactions, AGU*, **87**(36), Ocean Science Meeting Supplement, Abstract OS46A-11.
- Schneiderhöhn, H., 1923. Chalkographische Untersuchungen des Mansfelder Kupferschiefers. *Neues Jahrbuch für Mineralogie*, **47**, 1–38.
- Schoonen, M. A. A., and Barnes, H. L., 1991a. Reactions forming pyrite and marcasite from solution. I. Nucleation of FeS₂ below 100°C. *Geochimica et Cosmochimica Acta*, **55**, 1495–1504.
- Schoonen, M. A. A., and Barnes, H. L., 1991b. Reactions forming pyrite and marcasite from solution. II. Via FeS precursor below 100°C. *Geochimica et Cosmochimica Acta*, **55**, 1505–1514.
- Schwartz, A. J., Kumar, M., and Adams, B. L., 2000. *Electron Backscatter Diffraction in Materials Science*. Berlin: Springer, 350 p.
- Seilacher, A., Reif, W. E., Westphal, F., Riding, R., Clarkson, E. N. K., and Whittington, H. B., 1985. Sedimentological, ecological and temporal patterns of fossil Lagerstätten [and Discussion]. Extraordinary fossil biotas: their ecological and evolutionary significance. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **311**(1148), 5–24.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth-Science Reviews*, **64**, 243–272.
- Siesser, W. G., 1978. Petrography and geochemistry of pyrite and marcasite in DSDP Leg 40 sediments. *Initial Reports of the Deep Sea Drilling Project*, Supplement to Vols. 38, 39, 40, and 41, 767–775.
- Srivastava, N. K., 1975. Early diagenetic changes in recent molluscan shells. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **148**, 380–403.
- Strauss, H., and Schieber, J., 1990. A sulfur isotope study of pyrite genesis: the mid-Proterozoic Newland Formation, Belt Supergroup, Montana. *Geochimica et Cosmochimica Acta*, **54**, 197–204.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry*. New York: Wiley, 1022 p.
- Stürmer, W., 1985. A small coleoid cephalopod with soft parts from the Lower Devonian discovered using X-ray radiography. *Nature*, **318**, 53–54.
- Sweeney, R. E., and Kaplan, I. R., 1973. Diagenetic sulfate reduction in marine sediments. *Economic Geology*, **68**, 618–634.
- Taylor, S. R., and McLennan, S. M., 1985. *The Continental Crust: Its Composition and Evolution*. Oxford: Blackwell, 312 p.
- Trefrey, J. H., and Presley, B. J., 1982. Manganese fluxes from Mississippi Delta sediments. *Geochimica et Cosmochimica Acta*, **46**, 1715–1726.
- Van Horn, F. R., and Van Horn, K. R., 1933. X-ray study of pyrite of marcasite concretions in the rocks of the Cleveland, Ohio, quadrangles. *American Mineralogist*, **18**, 288–294.
- Wiese, R. G., Powell, M. A., and Fyfe, W. S., 1987. Spontaneous formation of hydrated iron sulfates on laboratory samples of pyrite- and marcasite-bearing coals. *Chemical Geology*, **63**, 29–38.

- Wignall, P. B., and Newton, R., 1998. Pyrite framboid diameter as a measure of oxygen deficiency in ancient mudrocks. *American Journal of Science*, **298**, 537–552.
- Wilkin, R. T., and Barnes, H. L., 1996. Pyrite formation by reactions of iron monosulfides with dissolved inorganic and organic sulfur species. *Geochimica et Cosmochimica Acta*, **61**, 323–339.
- Wilkin, R. T., and Barnes, H. L., 1997. Formation processes of framboidal pyrite. *Geochimica et Cosmochimica Acta*, **61**, 323–339.
- Wilkin, R. T., Barnes, H. L., and Brantley, S. L., 1996. The size distribution of framboidal pyrite in modern sediments: an indicator of redox conditions. *Geochimica et Cosmochimica Acta*, **60**, 3897–3912.
- Wilkin, R. T., Arthur, M. A., and Dean, W. E., 1997. History of water-column anoxia in the Black Sea indicated by pyrite framboid size distributions. *Earth and Planetary Science Letters*, **148**, 517–525.
- Williams, M. L., Crossey, L. J., Jercinovic, M. D., Bloch, J. D., Karlstrom, K. E., Dehler, C. M., Heizler, M. T., Bowring, S. A., and Goncalves, P., 2003. Dating sedimentary sequences; in situ U/Th–Pb microprobe dating of early diagenetic monazite and Ar–Ar dating of marcasite nodules; case study from Neoproterozoic black shales in the Southwestern US. Geological Society of America, Abstracts with Programs, vol. 35/6, p. 595.
- Wuttke, M., 1983. “Weichteil-Erhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

Acid Rock Drainage
 Anaerobic Transformation Processes, Microbiology
 Biofilms and Fossilization
 Black Shales
 Isotopes and Geobiology
 Microbial Degradation
 Pore Waters
 Pyrite Oxidation
 Pyritization
 Sediment Diagenesis – Biologically Controlled Shales
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Sulfur Cycle

ISOTOPE FRACTIONATION (METAL)

Ariel D. Anbar¹, Silke Severmann²

¹Arizona State University, Tempe, AZ, USA

²Rutgers University, New Brunswick, NJ, USA

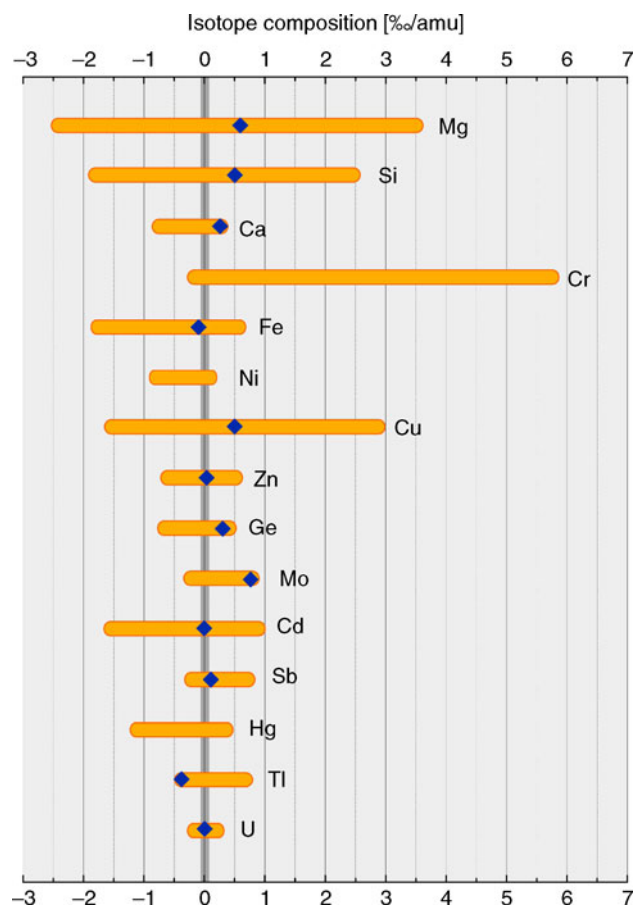
Definition

Variations in the abundances of the isotopes of metals, especially transition metals, arising from physical or chemical processes that discriminate among the isotopes of a given element.

Overview

Research into isotope fractionation of transition metals, alkaline earth metals, and metalloids is advancing rapidly

as a result of analytical developments. As of this writing, fractionations have been reported for more than a dozen such elements (Figure 1). These investigations are heavily motivated by geobiological considerations. In particular, there is wide interest in the use of these new isotope systems as biosignatures and as proxies for the



Isotope Fractionation (Metal), Figure 1 Observed range of isotopic variations in modern and ancient natural samples for a selection of metals and metalloids. All values are expressed in delta notation [parts per 1,000 (‰) per atomic mass unit], normalized to average Bulk Silicate Earth (indicated by the gray vertical line). Blue diamonds indicate the average isotope composition of modern seawater, where data are available. Data sources: Young and Galy (2004) (Mg); De La Rocha (2003); Robert and Chaussidon (2006) (Si); DePaolo (2004) (Ca); Ellis et al. (2002); Johnson and Bullen (2004); Schoenberg et al. (2008) (Cr); Dauphas and Rouxel (2006); Lacan et al. (2008); Johnson et al. (2008a) (Fe); Cameron et al. (2009) (Ni); Shields et al. (1965); Maréchal et al. (1999), Vance (2008) #1986 (Cu); Mason et al. (2005); Bermin et al. (2006); Gioia et al. (2008) (Zn); Rouxel et al. (2006a); Siebert et al. (2006a) (Ge); Anbar (2004); Poulson et al. (2006) (Mo); Wombacher et al. (2003); Ripperger and Rehkämper (2007); Schmitt et al. (2009) (Cd); Rouxel et al. (2003) (Sb); Foucher and Hintelmann (2006); Smith et al. (2008) (Hg); Rehkämper et al. (2002); Nielsen et al. (2006) (Tl); Stirling et al. (2007); Weyer et al. (2007, 2008) (U).

- detection, double hybridization, and combined hybridization-immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **42**, 815–822.
- Goldenthal, K. L., Hedman, K., Chen, J. W., August, J. T., and Willingham, M. C., 1985. Postfixation detergent treatment for immunofluorescence suppresses localization of some integral membrane proteins. *Journal of Histochemistry and Cytochemistry*, **33**, 813–820.
- Gregg, M., and Reznik-Schüller, H. M., 1984. An improved method for rapid electron microscopic autoradiography. *Journal of Microscopy*, **135**, 115–118.
- Hainfeld, J. F., and Powell, R. D., 2000. New frontiers in gold labeling. *Journal of Histochemistry and Cytochemistry*, **48**, 471–480.
- Hayat, M. A., 1973–1977. *Electron Microscopy of Enzymes: Principles and Methods*. New York: Van Nostrand Reinhold, Vol. 1 (1973); Vols. 2 and 3 (1974); Vol. 4 (1976); Vol. 5 (1977).
- Hermann, R., Schwarz, H., and Müller, M., 1991. High precision immunoscanning electron microscopy using Fab fragments coupled to ultra-small colloidal gold. *Journal of Structural Biology*, **107**, 38–47.
- Hopkins, F. G., 1933. Some chemical aspects of life. *Nature Supplement*, **3332**, 381–394.
- Hoppert, M., 2003. *Microscopic Techniques in Biotechnology*. Weinheim: Wiley-VCH.
- Horisberger, M., 1985. The gold method as applied to lectin cytochemistry in transmission and scanning electron microscopy. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol. 3, pp. 155–178.
- Humbel, B. M., Sibon, O. C. M., Stierhof, Y.-D., and Schwarz, H., 1995. Ultra-small gold particles and silver enhancement as a detection system in immunolabeling and in situ hybridization experiments. *Journal of Histochemistry and Cytochemistry*, **43**, 735–737.
- Kämper, M., Vetterkind, S., Berker, R., and Hoppert, M., 2004. Methods for in situ detection and characterization of extracellular polymers in biofilms by electron microscopy. *Journal of Microbiological Methods*, **57**, 55–64.
- Kessels, M. M., Qualmann, B., Klobasa, F., and Sierralta, W. D., 1996. Immunocytochemistry by electron spectroscopic imaging using a homogeneously boronated peptide. *Cell and Tissue Research*, **284**, 239–245.
- Mayer, G., and Bendayan, M., 1997. Biotinyl–tyramide: a novel approach for electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1449–1454.
- Nisman, R., Dellaire, G., Ren, Y., Li, R., and Bazett, D. P., 2004. Application of quantum dots as probes for correlative fluorescence, conventional, and energy - filtered transmission electron microscopy. *Journal of Histochemistry and Cytochemistry*, **52**, 13–18.
- Qualmann, B., Kessels, M. M., Klobasa, F., Jungblut, P. W., and Sierralta, W. D., 1996. Electron spectroscopic imaging of antigens by reaction with boronated antibodies. *Journal of Microscopy*, **183**, 69–77.
- Ramos-Vara, J. A., 2005. Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**, 405–426.
- Rohde, M., Gerberding, H., Mund, T., and Kohring, G. W., 1988. Immunelectron microscopic localization of bacterial enzymes: pre- and postembedding labeling techniques on resin-embedded samples. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 175–210.
- Roth, J., Bendayan, M., and Orci, L., 1980. FITC-Protein A-Gold complex for light and electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **28**, 55–57.
- Takizawa, T., and Robinson, J. M., 2003. Ultrathin cryosections: an important tool for immunofluorescence and correlative microscopy. *Journal of Histochemistry and Cytochemistry*, **51**, 707–714.
- Wasielowski, R., Mengel, M., Gignac, S., Wilkens, L., Werner, M., and Georgii, A., 1997. Tyramine amplification technique in routine immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1455–1459.
- Wohlrab, F., and Gossrau, R., 1992. *Katalytische Enzymhistochemie*. Jena: G. Fischer Verlag.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold Seeps. *Journal of Microbiological Methods*, **73**, 85–91.

Cross-references

Fluorescence in Situ Hybridization (FISH)
Histology

IRON ISOTOPES

Please refer to “*Isotope Fractionation (Metal)*.”

IRON SULFIDE FORMATION

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

There has been a great deal of research into the formation of sedimentary iron sulfide minerals because their history is intertwined with the biogeochemical cycles of iron, sulfur, carbon, and oxygen (Berner, 2001). Iron sulfides occur in sediments from a wide range of depositional environments, from the deep sea to the nonmarine, and the principal mineral in the rock record is pyrite (FeS₂, cubic). Its dimorph marcasite (orthorhombic) is considered metastable and apt to invert to pyrite over geologic time (Murowchick, 1992), but is probably more widespread than commonly acknowledged (Schieber, 2007). Presumed precursor minerals of sedimentary pyrite are mackinawite (FeS_{1-x}) and greigite (Fe₃S₄), minerals that impart a black coloration to modern reducing sediments (Goldhaber and Kaplan, 1974). Both are metastable and transform over time into pyrite or a mixture of pyrite and pyrrhotite (Berner, 1967). Pyrrhotite, hexagonal FeS, is thermodynamically stable in sediments (Berner, 1967), but extremely rare in modern or ancient sediments (Kobayashi and Nomura, 1972) due to kinetic limitations (Canfield and Raiswell, 1991).

Mackinawite and greigite are also known as acid-volatile iron sulfides (AVS) because in contrast to pyrite and marcasite they are readily soluble in HCl. Mineralogically, greigite is the sulfur analog of magnetite and is indeed strongly ferromagnetic (Dekkers and Schoonen, 1996). It can be formed by magnetotactic bacteria and dominates the magnetic properties of some modern sediments and

- Weiss, D. J., Rehkämper, M., Schoenberg, R., McLaughlin, M., Kirby, J., Campbell, P. G. C., Arnold, T., Chapman, J., Peel, K., and Gioia, A. S., 2008. Application of nontraditional stable-isotope systems to the study of sources and fate of metals in the environment. *Environmental Science and Technology*, **42**, 655–664.
- Welch, S. A., Beard, B. L., Johnson, C. M., and Bratermann, P. S., 2003. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III). *Geochimica et Cosmochimica Acta*, **67**, 4231–4250.
- Weyer, S., and Schwieters, J. B., 2003. High precision Fe isotope measurements with high mass resolution MC-ICPMS. *International Journal for Mass Spectrometry*, **226**, 355–368.
- Weyer, S., Anbar, A. D., Brey, G. P., Munker, C., Mezger, K., and Woodland, A. B., 2007. Fe-isotope fractionation during partial melting on Earth and the current view on the Fe-isotope budgets of the planets (reply to the comment of F. Poitrasson and to the comment of B. L. Beard and C. M. Johnson on “Iron isotope fractionation during planetary differentiation” by S. Weyer, A.D. Anbar, G. P. Brey, C. Munker, K. Mezger and A. B. Woodland). *Earth and Planetary Science Letters*, **256**, 638–646.
- Weyer, S., Anbar, A. D., Gerdes, A., Gordon, G. W., Algeo, T. J., and Boyle, E. A., 2008. Natural fractionation of $^{238}\text{U}/^{235}\text{U}$. *Geochimica et Cosmochimica Acta*, **72**, 345–359.
- Wiederhold, J. G., Kraemer, S. M., Teutsch, N., Borer, P. M., Halliday, A. N., and Kretzschmar, R., 2006. Iron isotope fractionation during proton-promoted, ligand-controlled, and reductive dissolution of goethite. *Environmental Science and Technology*, **40**, 3787–3793.
- Wiesli, R. A., Beard, B. L., and Johnson, C. M., 2004. Experimental determination of Fe isotope fractionation between aqueous Fe (II), Siderite, and “Green Rust” in abiotic systems. *Chemical Geology*, **211**, 343–362.
- Wille, M., Kramers, J. D., Nägler, T. F., Beukes, N. J., Schroder, S., Meisel, T., Lacassie, J. P., and Voegelin, A. R., 2007. Evidence for a gradual rise of oxygen between 2.6 and 2.5 Ga from Mo isotopes and Re-PGE signatures in shales. *Geochimica et Cosmochimica Acta*, **71**, 2417–2435.
- Wille, M., Nägler, T. F., Lehmann, B., Schroder, S., and Kramers, J. D., 2008. Hydrogen sulphide release to surface waters at the Precambrian/Cambrian boundary. *Nature*, **453**(7196), 767–769.
- Williams, H. M., McCammon, C. A., Peslier, A. H., Halliday, A. N., Teutsch, N., Levasseur, S., and Burg, J.-P., 2005. Iron isotope fractionation and the oxygen fugacity of the mantle. *Science*, **304**, 1656–1659.
- Wombacher, F., Rehkämper, M., Mezger, K., and Münker, C., 2003. Stable isotope compositions of cadmium in geological materials and meteorites determined by multiple-collector ICPMS. *Geochimica et Cosmochimica Acta*, **67**, 4639–4654.
- Yamaguchi, K. E., and Ohmoto, H., 2006. Comment on “iron isotope constraints on the Archean and paleoproterozoic ocean redox state”. *Science*, **311**, 177a.
- Yamaguchi, K. E., Johnson, C. M., Beard, B. L., and Ohmoto, H., 2005. Biogeochemical cycling of iron in the Archean-Paleoproterozoic Earth: constraints from iron isotope variations in sedimentary rocks from the Kaapvaal and Pilbara Cratons. *Chemical Geology*, **218**, 135–169.
- Young, E. D., and Galy, A., 2004. The isotope geochemistry and cosmochemistry of magnesium. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 197–230.
- Zhu, X. K., O’Nions, R. K., Guo, Y., and Reynolds, B. C., 2000. Secular variations of iron isotopes in North Atlantic Deep Water. *Science*, **287**, 2000–2002.

ISOTOPES (METHODS)

Please refer to entries “*Isotope Fractionation (Metal)*”, “*Geochronology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

ISOTOPES AND GEOBIOLOGY

Jochen Hoefs
University of Göttingen, Göttingen, Germany

Definition

It has long been recognized (e.g., Thode et al. (1949), Craig (1953) and Wellman et al. (1968)) that biological processes significantly fractionate the isotopes of C, N, and S, leading to characteristic *biosignatures* in sedimentary rocks that will be more or less preserved in the geological record. In the following, a brief overview is given of the major isotope fractionation processes in the biosphere and the geosphere. Special attention will be paid to secondary bacterial activity after deposition of the organic matter. Questions of general interest are isotopic indicators of early life on Earth and on Mars. A note of caution, however, is given whether abiogenic processes may lead to isotope compositions similar to that of biological activity.

Carbon isotope fractionation during photosynthesis

Early reviews by O’Leary (1981) and Farquhar et al. (1989) have provided the biochemical background of carbon isotope fractionations during photosynthesis, with more recent accounts by Hayes (2001) and Freeman (2001). The main isotope-discriminating steps during biological carbon fixation are (1) the uptake and intracellular diffusion of CO_2 and (2) the biosynthesis of cellular components. Such a two-step model was first proposed by Park and Epstein (1960):



From this simplified scheme, it follows that the diffusional process is reversible, whereas the enzymatic carbon fixation is irreversible. The two-step model of carbon fixation clearly suggests that isotope fractionation is dependent on the partial pressure of CO_2 , i.e., $p\text{CO}_2$ of the system. With an unlimited amount of CO_2 available to a plant, the enzymatic fractionation will determine the isotopic difference between the inorganic carbon source and the final bioproduct. Under these conditions, ^{13}C fractionations may vary from -17 to -40‰ (O’Leary, 1981). When the concentration of CO_2 is the limiting factor, the diffusion of CO_2 into the plant is the slow step in the reaction and carbon isotope fractionation of the plant decreases.

CO₂ is directly converted by the enzyme ribulose biphosphate carboxylase/oxygenase (“Rubisco”) to a 6-carbon molecule that is then cleaved into two molecules of phosphoglycerate (PGA), each with three carbon atoms (plants using this photosynthetic pathway are, therefore, called C₃ plants). Most PGA is recycled to make ribulose biphosphate, but some are used to make carbohydrates. Free exchange between external and mesophyll CO₂ makes the carbon fixation process less efficient, which causes the observed large ¹³C depletions of C₃ plants.

C₄ plants incorporate CO₂ by the carboxylation of phosphoenolpyruvate (PEP) via the enzyme PEP carboxylase to make the molecule oxaloacetate, which has four carbon atoms (hence C₄). The carboxylation product is transported from the outer layer of mesophyll cells to the inner layer of bundle sheath cells, which are able to concentrate CO₂, so that most of the CO₂ is fixed with relatively little carbon fractionation. Because mesophyll cells are permeable and bundle sheath cells are less permeable, C₃ versus C₄ plants have ¹³C depletions of −18‰ versus −4‰ relative to atmospheric CO₂. Even more complex is C-isotope fractionation in aquatic plants. Factors that control the ¹³C of phytoplankton include temperature, availability of CO₂ (aq), light intensity, nutrient availability, pH, and physiological factors such as cell size and growth rate.

Since the pioneering work of Park and Epstein (1960) and Abelson and Hoering (1961) it is well known that ¹³C is not uniformly distributed among the total organic matter of plant material, but varies between carbohydrates, proteins, and lipids. The latter class of compounds is considerably depleted in ¹³C relative to the other products of biosynthesis. Although the causes of these ¹³C differences are not entirely clear, kinetic isotope effects seem to be more plausible than thermodynamic equilibrium effects.

Organic matter in the geosphere is a complex mixture of source organisms having variable biosynthetic pathways and detrital remains. The determination of ^{δ13}C values of bulk organic matter is thus unable to distinguish among the different carbon sources. Immediately after burial of the biological organic material into sediments, complex diagenetic changes occur in the organic matter. Two processes have been proposed to explain the observed changes in carbon isotope composition: (1) preferential degradation of organic compounds which have different isotope composition compared to the preserved organic compounds. Since easily degradable organic compounds such as amino acids are enriched in ¹³C compared to the more resistant compounds such as lipids, degradation causes a shift to slightly more negative ^δ values. (2) Isotope fractionations due to metabolism of microorganisms produce new compounds having different isotopic compositions than the original source material. A classic example has been presented by Freeman et al. (1990) analyzing hydrocarbons from the Messel

Isotopes and Geobiology, Table 1 ^{δ13}C values of separated individual hydrocarbons (biomarkers) from the Messel shale (Freeman et al., 1990)

Peak	^{δ13} C (‰)	Compound
1	−22.7	Norpristane
2	−30.2	C ₁₉ acyclic isoprenoid
3	−25.4	Pristane
4	−31.8	Phytane
5	−29.1	C ₂₃ acyclic isoprenoid
8	−73.4	C ₃₂ acyclic isoprenoid
9	−24.2	Isoprenoid alkane
10	−49.9	22,29,30-Trisnorhopane
11	−60.4	Isoprenoid alkane
15	−65.3	30-Norhopane
19	−20.9	Lycopane

shale in Germany (see Table 1). While the major portion of the analyzed hydrocarbons reflects the primary biological source material, some hydrocarbons having low concentrations are extremely ¹³C depleted indicating their secondary microbial origin in a methane-rich environment. Later studies, summarized by Peckmann and Thiel (2004), have documented even larger ¹³C depletions (^{δ13}C values as low as −120‰) in various biomarkers that have been formed by diverse methanotrophic taxa.

C-isotope evidence for earliest signs of life on Earth

The idea of using C-isotope compositions of organic matter for the detection of early life dates back to the classic paper of Craig (1953). Already, at that time, it was discussed whether graphitic carbon in old rocks could be identified as being of biogenic origin (Craig, 1954; Rankama, 1954). This controversy about the meaning of graphite ^{δ13}C values from early Archean (3.5–3.8 Ga) rocks has continued till today (e.g., Bolhar et al., 2004). The validity of using carbon isotopes in the search for early life hinges on the assumption that early organic metabolism produced a C-isotope fractionation effect similar to that observed today. In addition, the thermal history of the metasediments has to be considered. In early Archean metasedimentary rocks from Greenland, ^{δ13}C values range from −22 to −50‰, which could be interpreted as biogenic (Mojzsis et al., 1996). Very negative values were measured for tiny particles of reduced carbon within apatite grains, which apparently had been shielded from metamorphic alteration. However, a case also can be made that graphite from Archean rocks may be of abiotic, hydrothermal origin. In recent years, more experimental and natural evidence has been presented that Fischer–Tropsch type reactions may produce abiogenic organic matter with ^{δ13}C values similar to biogenic organic matter (Taran et al., 2007).

Nitrogen

Microorganisms are responsible for all major conversions in the biological nitrogen cycle, which generally is divided into nitrogen fixation, nitrification, and denitrification. Other bacteria return nitrogen to the atmosphere as N_2 . *Fixation* describes the conversion of unreactive atmospheric N_2 into reactive nitrogen such as ammonium, usually involving bacteria. Fixation commonly produces organic materials with $\delta^{15}N$ values slightly less than 0‰ ranging from −3 to +1 (Fogel and Cifuentes, 1993) and is performed in the roots of plants by many bacteria. The large amount of energy needed to break the molecular nitrogen bond makes nitrogen fixation a very inefficient process with little associated N-isotope fractionation.

Nitrification is a multistep oxidation process mediated by several different autotrophic organisms. Nitrate is not the only product of nitrification, different reactions produce various nitrogen oxides as intermediate species. Nitrification can be described as two partial oxidation reactions, each of which proceeds separately. Oxidation by *Nitrosomonas* ($NH_4 \rightarrow NO_2$) followed by oxidation by *Nitrobacter* ($NO_2 \rightarrow NO_3$). Because the oxidation of nitrite to nitrate is generally rapid, most of the N-isotope fractionation is caused by the slow oxidation of ammonium by *Nitrosomonas*. In N-limited systems, fractionation is minimal.

Denitrification (reduction of more oxidized forms to more reduced forms of nitrogen) is a multistep process with various nitrogen oxides as intermediate compounds resulting from biologically mediated reduction of nitrate. Denitrification takes place in poorly aerated soil and in stratified anaerobic water bodies. Denitrification causes the $\delta^{15}N$ values of the residual nitrate to increase exponentially as nitrate concentrations decrease. Experimental investigations have demonstrated that fractionation factors may change from 10‰ to 30‰, with the largest values obtained under lowest reduction rates. Table 2, which gives a summary of observed N-isotope fractionations, clearly indicates the dependence of fractionation factors on nitrogen concentrations and demonstrates that

Isotopes and Geobiology, Table 2 Naturally observed isotope fractionation for nitrogen assimilation (after Fogel and Cifuentes, 1993)

N_2 fixation	−3 to +1‰
NH_4^+ assimilation	
Cultures	
Millimolar concentrations	0 to −15‰
Micromolar concentrations	−3 to −27‰
Field observations	
Micromolar concentrations	−10‰
NO_3^- assimilation	
Cultures	
Millimolar concentrations	0 to −24‰
Micromolar concentrations	−10‰
Field observations	
Micromolar concentrations	−4 to −5‰

at low nitrogen concentrations, fractionation is nearly zero because virtually all the nitrogen is used.

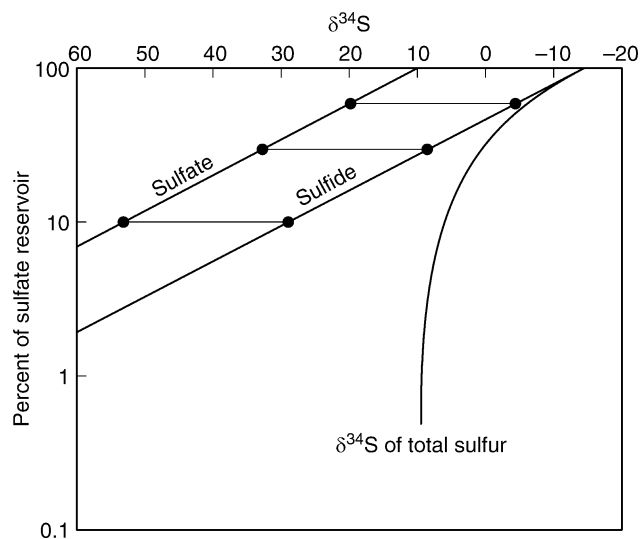
Sulfur-dissimilatory sulfate reduction

Large ^{34}S -isotope fractionations are induced during bacterial sulfate reduction. Since the early work with living cultures (Harrison and Thode, 1957a, b) it is well known that sulfate-reducing bacteria produce sulfide depleted in ^{34}S by 4–49‰. In general, the rate-limiting step is the breaking of the first S–O bond, namely the reduction of sulfate to sulfite (Goldhaber and Kaplan, 1974; Brunner et al., 2005).

Despite decades of intense research, the factors that determine the magnitude of sulfur isotope fractionation during bacterial sulfate reduction are still under debate. The magnitude of isotope fractionation depends on the rate of sulfate reduction with the highest fractionation at low rates and the lowest fractionation at high rates. One parameter which remains unclear is sulfate concentration.

Naturally occurring sulfides in sediments and euxinic waters are commonly depleted in ^{34}S by up to 70‰, far beyond the apparent capabilities of sulfate-reducing bacteria in the laboratory. Much of the sulfide produced by sulfate reduction in sediments is reoxidized, often via compounds in which sulfur has intermediate oxidation states that do not accumulate, but are readily transformed and can be further disproportionated by bacteria.

Another factor that is of great importance for the observed sulfur isotope variations of natural sulfides is whether sulfate reduction takes place in an open or a closed system. An “open” system has an infinite reservoir of sulfate in which continuous removal from the



Isotopes and Geobiology, Figure 1 Rayleigh plot for sulfur isotope fractionation during closed-system reduction of sulfate. Assumed fractionation factor 1.025; assumed initial isotope composition of sulfate +10‰.

source produces no detectable loss of material. Typical examples are the Black Sea and local oceanic deeps. In such cases, H_2S is extremely depleted in ^{34}S while consumption and change in ^{34}S remain negligible for the sulfate. In a “closed” system, the preferential loss of the lighter isotope ^{32}S from the reservoir has a feedback on the isotopic composition of the unreacted source material. The changes in the ^{34}S content of residual sulfate and of the H_2S are modeled in Figure 1, which shows that $\delta^{34}\text{S}$ values of the residual sulfate steadily increase with sulfate consumption (a linear relationship on a log-normal plot). The curve for the derivative H_2S is parallel to the sulfate curve at a distance which depends on the magnitude of the fractionation factor. As shown in Figure 1, H_2S may become isotopically heavier than the original sulfate when about two third of the reservoir has been consumed. The $\delta^{34}\text{S}$ curve for “total” sulfide asymptotically approaches the initial value of the original sulfate. It should be noted, however, that apparent “closed-system” behavior of covarying sulfate and sulfide $\delta^{34}\text{S}$ values might be also explained by “open-system” differential diffusion of the different sulfur isotope species (Jørgensen et al., 2004).

Another characteristic feature of microbial sulfate reduction is significant small-scale spatial variability in S-isotope composition of pyrite. Up to 105‰ variations in $^{34}\text{S}/^{32}\text{S}$ ratios within strongly zoned pyrite was observed by McKibben and Riciputi (1998). This zonation arises from progressive precipitation of sulfide from pore water sulfate enriched in ^{34}S providing more ^{34}S -enriched sulfide.

New insights on sulfur isotope fractionation mechanisms have been obtained from the analysis of the minor isotopes ^{33}S and ^{36}S (Farquhar et al., 2003; Johnston et al., 2005; Ono et al., 2006). By studying all sulfur isotopes with very high precision, these authors could demonstrate that bacterial sulfate reduction follows a mass-dependent relationship that is slightly different from that expected by equilibrium fractionations. As a result, samples with the same $\delta^{34}\text{S}$ value can have different $\Delta^{33}\text{S}$ and $\Delta^{36}\text{S}$ values. This opens the possibility to distinguish between different fractionation mechanisms and biosynthetic pathways (Ono et al., 2006). For instance, bacterial sulfate reduction shows slightly different fractionation relationships compared to sulfur disproportionation reactions (Johnston et al., 2005).

When did bacterial sulfate reduction start on Earth?

Considering a typical difference in $\delta^{34}\text{S}$ values of 20–60‰ between marine sulfate and bacteriogenic sulfide in present-day sedimentary environments, similar fractionations in ancient sedimentary rocks may be interpreted as an evidence for the activity of sulfate-reducing bacteria. The presence or absence of such fractionations in sedimentary rocks thus may constrain the time of emergence of sulfate-reducing bacteria. Sedimentary pyrite depleted in ^{34}S has been observed as far back in geological time as the Precambrian. There is, however,

debate about the dating of first onset of bacterial reduction in the geological record.

In early Archean sedimentary rocks, most sulfides and the rare sulfates have $\delta^{34}\text{S}$ values near 0‰ (Monster et al., 1979; Cameron, 1982), which has been interpreted as indicating an absence of bacterial reduction in the Archean. Shen et al. (2001) and Shen and Buick (2004) argued that the large spread in $\delta^{34}\text{S}$ values of microscopic pyrites aligned along growth faces of former gypsum in the 3.47 Ga North Pole barite deposit; Australia represents the oldest evidence for microbial sulfate reduction. By measuring all four stable isotopes ^{32}S , ^{33}S , ^{34}S , and ^{36}S , additional evidence can be gained to decouple hydrothermal versus biological processes (Ono et al., 2006) even when $\delta^{34}\text{S}$ values are inconclusive.

Mars

McKay et al. (1996) have claimed that Martian meteorite ALH 84001 – found in Antarctica – contains evidence of post-Martian life. Various kinds of apparent biosignatures with characteristic isotope compositions have been suggested: organic matter, carbonate minerals, magnetite grains, and sulfide minerals. After intensive investigations during the last years, none of these proposed biosignatures have survived as such.

Carbonates in Martian meteorites have been especially well studied because understanding the formation conditions of the carbonates is crucial in this connection. Despite extensive chemical and mineralogical studies, the environment of carbonate formation has remained unclear. For instance, in situ C-isotope analysis by Niles et al. (2005) gave highly zoned $\delta^{13}\text{C}$ values from +30‰ to +60‰ consistent with a derivation from the Martian atmosphere and suggesting abiotic formation.

Further evidence about a nonbiogenic origin of Martian carbonates (and even less abundant sulfates) has been presented by Farquhar et al. (1989). By measuring $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values, these authors observed an ^{17}O anomaly in the carbonates relative to the silicates which they interpreted as being produced by the photochemical decomposition of ozone just as in the Earth’s stratosphere. This finding suggests that carbonates (and sulfates) are derived from atmosphere–regolith interactions on Mars and not from biological activities.

Fe isotopes as biosignatures?

Virtually all reduction from Fe^{3+} to Fe^{2+} at the Earth’s surface is mediated by the metabolism of dissimilatory bacteria. Biological processes, therefore, may produce measurable Fe-isotopic fractionations because the metabolic processing of Fe involves a number of steps such as transport across membranes that may fractionate Fe isotopes. Experiments with dissimilatory Fe-reducing bacteria of the genus *Shewanella* indicate that iron isotope fractionations are a function of Fe(III) reduction rates: at low rates the produced Fe^{2+} is isotopically depleted by 1.3‰, whereas the depletion is up to 3‰ at high rates (Johnson et al., 2005).

Iron isotope fractionation has also been observed during bacterial Fe oxidation (Croal et al., 2004). Fe(II) oxidizing phototrophs may produce under anaerobic conditions a ferrihydrite precipitate that is 1.5‰ higher than the aqueous Fe(II) source. Controversy still exists whether the iron isotope variations observed in microbial experiments are primarily controlled by kinetic or nonbiological equilibrium factors. Since abiotic iron reduction/oxidation reactions may reveal fractionations similar in direction and magnitude to microbial reactions (Johnson et al., 2002; Skulan et al., 2002), the presence of Fe isotope variations is not in itself conclusive evidence of biotic activity. This complicates the ability to use iron isotopes to identify microbiological processing in the rock record (Balci et al., 2006).

Conclusions

“Biological processes fractionate the stable isotopes of C, N, S, and Fe in a characteristic way leading to biosignatures in the geosphere. Such isotope fingerprints have been used as indicators of early life on Earth and Mars. Since abiological processes sometimes may lead to isotope compositions comparable to biological processes, isotope biosignatures have to be applied with care.”

Bibliography

- Abelson, P. H., and Hoering, T. C., 1961. Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. *Proceedings of the National Academy of Sciences of the United States of America*, **47**, 623.
- Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W., 2006. Iron isotope fractionation during microbially substituted simulated Fe(II) oxidation and Fe(III) precipitation. *Geochimica et Cosmochimica Acta*, **70**, 622–639.
- Bolhar, R., Kamber, B. S., Moorbath, S., Fedo, C. M., and Whitehouse, M. J., 2004. Characterisation of early Archaean chemical sediments by trace element signatures. *Earth and Planetary Science Letters*, **222**, 43–60.
- Brunner, B., Bernasconi, S. M., Kleikemper, J., and Schroth, M. H., 2005. A model of oxygen and sulphur isotope fractionation in sulfate during bacterial sulfate reduction. *Geochimica et Cosmochimica Acta*, **69**, 4773–4785.
- Cameron, E. M., 1982. Sulphate and sulphate reduction in early Precambrian oceans. *Nature*, **296**, 145–149.
- Craig, H., 1953. The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 133–149.
- Craig, H., 1954. Geochemical implications of the isotopic composition of carbon in ancient rocks. *Geochimica et Cosmochimica Acta*, **6**, 186–196.
- Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K., 2004. Iron isotope fractionation by Fe(II) oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, **68**, 1227–1242.
- Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.
- Farquhar, J., Thiemens, M. H., and Jackson, T., 1998. Atmosphere-surface interactions on Mars: $\Delta^{17}\text{O}$ measurements of carbonate from ALH 84001. *Science*, **280**, 1580–1582.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulfur cycle. *Science*, **289**, 756–759.
- Farquhar, J., Johnston, D. T., Wing, B. A., Habicht, K. S., Canfield, D. E., Airieau, S., and Thiemens, M. H., 2003. Multiple sulphur isotope interpretations for biosynthetic pathways: implications for biological signatures in the sulphur isotope record. *Geobiology*, **1**, 27–36.
- Fogel, M. L., and Cifuentes, L. A., 1993. Isotope fractionation during primary production. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 73–98.
- Freeman, K. H., 2001. Isotopic biogeochemistry of marine organic carbon. *Reviews in Mineralogy and Geochemistry*, **43**, 579–605.
- Freeman, K. H., Hayes, J. M., Trendel, J. M., and Albrecht, P., 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature*, **343**, 254–256.
- Goldhaber, M. B., and Kaplan, I. R., 1974. The sedimentary sulphur cycle. In Goldberg, E. B. (ed.), *The Sea Vol IV*. New York: Wiley.
- Harrison, A. G., and Thode, H. G., 1957a. Kinetic isotope effect in chemical reduction of sulphate. *Faraday Society Transaction*, **53**, 1648–1651.
- Harrison, A. G., and Thode, H. G., 1957b. Mechanism of the bacterial reduction of sulphate from isotope fractionation studies. *Faraday Society Transaction*, **54**, 84–92.
- Hayes, J. M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: principle and evidence. *Marine Geology*, **113**, 111–125.
- Hayes, J. M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In Valley, J. W., and Cole, D. R. (eds.), *Stable Isotope Geochemistry*. Reviews in Mineralogy and Geochemistry, **43**, 225–277.
- Johnson, C. M., Skulan, J. L., Beard, B. L., Sun, H., Neelson, K. H., and Brateman, P. S., 2002. Isotopic fraction between Fe(III) and Fe(II) in aqueous solutions. *Earth and Planetary Science Letters*, **195**, 141–153.
- Johnson, C. M., Roden, E. R., Welch, S. A., and Beard, B. L., 2005. Experimental constraints on Fe isotope fractionation during magnetite and Fe carbonate formation coupled to dissimilatory hydrous ferric oxide reduction. *Geochimica et Cosmochimica Acta*, **69**, 963–993.
- Johnston, D. T., Farquhar, J., Wing, B. A., Kaufman, A. J., Canfield, D. E., and Habicht, K. S., 2005. Multiple sulphur isotope fractionations in biological systems: a case study with sulphate reducers and sulphur disproportionators. *American Journal of Science*, **305**, 645–660.
- Jørgensen, B. B., Böttcher, M. A., Lüschen, H., Neretin, L. N., and Volkov, I. I., 2004. Anaerobic methane oxidation and a deep H_2S sink generate isotopically heavy sulfides in Black Sea sediments. *Geochimica et Cosmochimica Acta*, **68**, 2095–2118.
- McKay, D. S., Gibson, E. K. Jr., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chiller, X. D. F., Maechling, C. R., and Zare, R. N., 1996. Search for past life on Mars: possible relic biogenic activity in martian meteorite ALH 84001. *Science*, **273**, 924–930.
- McKibben, M. A., and Eldridge, C. S., 1995. Microscopic sulfur isotope variations in ore minerals from the Viburnum Trend, Southeast Missouri: a shrimp study. *Economic Geology*, **90**, 228–245.
- McKibben, M. A., and Riciputi, L. R., 1998. Sulfur isotopes by ion microprobe. In: Application of microanalytical techniques to understanding mineralizing processes. *Reviews Economic Geology*, **7**, 121–140.
- Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P., and Friend, C. R. L., 1996. Evidence for life on Earth before 3,800 million years ago. *Nature*, **384**, 55–59.
- Monster, J., Appel, P. W., Thode, H. G., Schidlowski, M., Carmichael, C. W., and Bridgewater, D., 1979. Sulphur isotope studies in early Archean sediments from Isua, West Greenland: implications for the antiquity of bacterial sulfate reduction. *Geochimica et Cosmochimica Acta*, **43**, 405–413.

- Niles, P. B., Leshin, L. A., and Guan, Y., 2005. Microscale carbon isotope variability in ALH84001 carbonates and a discussion of possible formation environments. *Geochimica et Cosmochimica Acta*, **69**, 2931–2944.
- O' Leary, M. H., 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553–567.
- Ono, S., Wing, B. A., Johnston, D., Farquhar, J., and Rumble, D., 2006. Mass-dependent fractionation of quadruple sulphur isotope system as a new tracer of sulphur biogeochemical cycles. *Geochimica et Cosmochimica Acta*, **70**, 2238–2252.
- Park, R., and Epstein, S., 1960. Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta*, **21**, 110–126.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Rankama, K., 1954. Early pre-Cambrian carbon of biogenic origin from the Canadian Shield. *Science*, **119**, 506.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth Science Reviews*, **64**, 243–272.
- Shen, Y., Buick, R., and Canfield, D. E., 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature*, **410**, 77–81.
- Skulan, J. L., Beard, B. L., and Johnson, C. M., 2002. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(III) and hematite. *Geochimica et Cosmochimica Acta*, **66**, 2995–3015.
- Taran, Y. A., Kliger, G. A., and Sevastianov, V. S., 2007. Carbon isotope effect in the open system Fischer Trosch synthesis. *Geochimica et Cosmochimica Acta*, **71**, 4474–4487.
- Thode, H. G., Macnamara, J., and Collins, C. B., 1949. Natural variations in the isotopic content of sulphur and their significance. *Canadian Journal of Research*, **27B**, 361.
- Wellman, R. P., Cook, F. D., and Krouse, H. D., 1968. Nitrogen-15: microbiological alteration of abundance. *Science*, **161**, 269–270.

Cross-references

Anaerobic Oxidation of Methane with Sulfate
 Astrobiology
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Iron Sulfide Formation
 Isotope Fractionation (Metal)
 Nitrogen
 Methane, Origin
 Photosynthesis
 Sulfate-Reducing Bacteria
 Sulfur Isotopes

ISOTOPES, RADIOGENIC

Bent T. Hansen
 University of Göttingen, Göttingen, Germany

Definition

Radiogenic nuclides (more commonly referred to as “radiogenic isotopes”) are produced by a process of radioactive decay. Radioactive decay means that unstable isotopes (mother nuclides) are spontaneously transferred

into stable isotopes (daughter nuclides) by emission of particles and loss of energy (radioactive radiation).

Introduction

The radioactive decay provides an accurate method of measuring the ages of rocks and minerals. This possibility was recognized both by Rutherford (1906) and Boltwood (1907) at the beginning of the twentieth century. However, the measurement and interpretation of variations in the isotopic composition of certain elements in natural materials were not possible until modern mass spectrometers were developed based on the design by Nier (1940). After the World War II, isotope determinations became an important tool in modern geology, geochemistry, and geobiology not only for dating geological processes, but also for investigations of climate change, environmental, and biological parameters. The most frequently used systems are Rb/Sr, Sr/Sr, Sm/Nd, Nd/Nd, U/Pb, and Pb/Pb. In the following, typical examples of the most common radiogenic isotope applications are presented.

Strontium isotopes

There are four stable isotopes of Sr: ^{88}Sr , ^{87}Sr , ^{86}Sr , and ^{84}Sr . All but ^{87}Sr are nonradiogenic (that is, not the products of radioactive decay), whereas ^{87}Sr is produced by radioactive decay of ^{87}Rb (half-life 0.488×10^9 years). The $^{87}\text{Rb} \rightarrow ^{87}\text{Sr}$ radioactive decay pair has therefore produced different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios depending on the age and the amount of Rubidium in the material investigated. For more than half of the century, the Rb/Sr system is one of the most frequently used decay systems for dating geological events.

The isotopic composition of strontium is not only used for dating purposes, but also has turned out to be a universal tool for describing a variety of geological, biological, and environmental processes.

Groundwater and basement brines

Strontium is easily dissolved in water. The advantage of the strontium isotopic system is that the isotopic composition assumed till recently will not be changed by fractionation processes; it means that strontium in circulation depends on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the rocks and minerals that interact with water at or near the surface of the Earth. The Sr isotope composition of groundwater aquifers is constant over years, independent of the amount of rainfall, as demonstrated in a recent study by Klaus et al. (2007). Investigations in the Bangkok Metropolitan Area showed that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are useful tools to characterize groundwater flow and related biological and environmental processes.

Strontium isotope variations also play an important role in evaluating the suitability of crystalline rocks for geological disposal of nuclear waste. At the Hard Rock Laboratory at Äspö in southern Sweden, Sr isotope composition of groundwater has been investigated by Peterman and Walin (1999) down to a depth of 850 m from boreholes. Microbial

ICHOLOGY

Murray K. Gingras, Kurt O. Konhauser
University of Alberta, Edmonton, Alberta, Canada

Definition

Ichnology is the study of fossilized, generally macroscopic, animal–sediment interactions. This includes morphological, textural, and local compositional effects related to the animal’s activities. Similar studies in modern sediments are referred to as neoichnology.

Overview

Ichnological studies comprise a wide range of animal–sediment interactions. The scope of animal–sediment interactions was broadly established by Seilacher (1967). He showed that various animal activities, including motility, dwelling, grazing, farming, scavenging, predation, and sessile feeding, are represented by ichnofossils (also referred to as trace fossils).

Bioturbation is the displacement of sediment by animals. In marine environments, common mediators of bioturbation include worm-form animals (such as nemerteans, polychaetes, and hemichordates), marine crustaceans, bivalves, motile echinoderms, sponges, and sea anemones.

Ichnofossils can form from impressions on the sediment surface, intrusion into the sediment, or from excavation of sediment. The former can be thought of as tracks and trails, whereas intrusions and excavations are generally represented by burrows, shafts, and tunnels. Biodeformation structures may result from intrusive activities in loose or fluid-rich sediment. Intrusive, excavatory, and biodeformational structures impact the sedimentary and geochemical character of the surrounding sediment. Traces also incorporate, rework, and redistribute organics

in sediment. This influences the geochemical zonations associated with sedimentary surfaces, and in this regard, bioturbation can be viewed as a diagenetic process.

Biomechanical alteration of sediment texture

Burrowing organisms significantly alter the physical character of a substrate. The changes involved include redistribution of grain sizes (vertically and laterally), modification of grain size, sediment compaction, and sorting (summarized in Bromley, 1996). The physical manifestations of bioturbation are variable depending on the intrusive process employed by the animal (i.e., inversion, compression, excavation, or backfill).

In clastic sediments, the most important result of bioturbation is the rearrangement of coarse *versus* fine grains. This results in an alteration of permeable pathways in the sedimentary media that can influence diagenetic reactions. In carbonate sediment, grain-size reduction probably represents the most significant substrate modification (Chow and Longstaffe, 1995). Reduction of substrate grain size permits a larger surface area to interact with diagenetic fluids and provides more nucleation sites in a given volume.

Biochemical alteration of sediment texture

An important result of bioturbation is the incorporation of localized, concentrated organic material in the form of mucus or feces. Burrow-associated organics induce a geochemical microenvironment that may extend several centimeters, or even decimeters, into the substrate. In well-aerated sediments, burrow linings comprising agglutinated, fecal or primary organic material contain the most concentrated local sources of organic material. These burrows therefore provide excellent substrates for bacterial colonization. Grazing and mining of intact organic remains further lead to their disintegration and introduce larger surface areas for microbial respiration. Moreover,

the ingestion of refractory organic carbon can lead to the excretion of more labile fecal pellets (Lee, 1992).

With most intrusive bioturbation, the sediment–water interface increases in surface area as a result of infaunal animal colonization. The larger (and deepened) sediment–water interface alters chemical fluxes between the sediment and water column. Animals that pump water through their burrow (bioirrigation) increase the rate of chemical flux. Most notably, irrigation of worm burrows with oxygenated seawater results in increased downward and lateral diffusion of O₂, which then affects the oxidation of redox-sensitive elements. For the aforementioned reasons, the accepted scheme of biogeochemical zones in sediment is an oversimplification. Bioturbation influences the solid phase and pore-water properties of sediment by increasing the transport of diagenetic reactants and products across the sediment–water interface. Consequently, surficial sediments cannot be considered a homogeneous medium characterized by one-dimensional vertical diffusion. They are, instead, heterogeneous and intensively mixed with a complex spatial distribution of biogeochemical zones that are difficult to accurately resolve (Aller, 1980).

Metal ion enrichment in ichnofossils

Cation enrichment in burrow linings is the result of sorptive processes. Metals concentrate in burrow linings in three ways: (1) adsorption, as oxide or oxyhydroxide coatings on to the high-energy surfaces provided by fine-grained particles (silt and clay); (2) as sulfide or phosphate phases under reducing conditions; or (3) associated with organic material (as adsorbed coatings or forming organo-metallic complexes) (Over, 1990). The relative importance of each type of enrichment mechanism varies with the depth of burrow penetration, the grain size/composition of the sediment, and bottom-water and pore-water composition.

Ichnofossils and cementation

Trace fossils commonly influence local cementation patterns by changing the sediment texture, its redistribution, and local composition (Gingras et al., 2004). They also facilitate the accumulation of microbial communities, and through their chemoheterotrophic metabolisms, the microbes can alter pore-water chemistry and saturation states. Ichnofossils also possess higher metal concentrations which can serve as nucleation sites for authigenic mineral formation. Although ichnofossils have been strongly linked to the distribution of microorganisms, there is no existing model associating trace fossil diagenesis to microorganic process.

Summary

The presence of ichnofossils/bioturbation in a sedimentary substrate has the effect of altering sediment distribution and permeability pathways. Moreover, the redistribution and stowage of organics in and around

ichnofossils strongly influence the distribution of microorganisms associated with the sedimentary surface. This has an impact on the nature and distribution of biochemical zonations in sediment. The mechanical and (bio)chemical nature of trace fossils influences diagenetic processes.

Bibliography

- Aller, R. C., 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. *Geochimica et Cosmochimica Acta*, **44**, 1955–1965.
- Bromley, R. G., 1996. *Trace Fossils: Biology, Taphonomy and Applications*, 2nd edn. London: Chapman and Hall, p. 349.
- Chow, N., and Longstaffe, F., 1995. Dolomites of the Middle Devonian Elk Point Formation, Southern Manitoba: intrinsic controls on early dolomitization. *Bulletin of Canadian Petroleum Geology*, **43**, 214–225.
- Gingras, M. K., Pemberton, S. G., Muelenbachs, K., and Machel, H., 2004. Conceptual models for burrow-related, selective dolomitization with textural and isotopic evidence from the Tyndall Stone, Canada. *Geobiology*, **2**, 21–30.
- Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochimica et Cosmochimica Acta*, **56**, 3323–3335.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Seilacher, A., 1967. Bathymetry of trace fossils. *Marine Geology*, **5**, 413–428.

Cross-references

[Biosignatures in Rocks](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Trace Fossils: Neoproterozoic](#)

IMMUNOLocalIZATION

Michael Hoppert, Christoph Wrede
 University of Göttingen, Göttingen, Germany

Synonyms

Immunocytochemistry; Immunodetection; Immunohistochemistry; Immunolabeling

Definition

Immunolocalization: Technique using specific antibodies to localize macromolecules (proteins, polysaccharides) within biological material (subcellular fractions, cells, tissues, biofilms).

Introduction

Immunolocalization is based on the detection of specific targets (“antigens”; mostly proteins or polysaccharides) in biological samples (cells, tissue, biofilms) by antibodies

as probes. Regularly, antibodies of IgG subtype (Figure 1a) and IgM and IgA subtypes are used. The antibodies may be monoclonal (i.e., identical molecules that recognize only one epitope, which is a short stretch of amino acids or sugar molecules) or polyclonal (a set of antibodies directed against a mostly undefined variety of epitopes on the surface of a larger molecule or a cellular component). Specific non-covalent binding is brought about by the hypervariable region of the antibody (paratope), exactly fitting to an epitope. The hypervariable regions are the tip parts of the Y-shaped arm of the antibody (Figure 1a). All other parts, including the Fc-part, are structurally identical within one antibody subclass. Localization of the antigen is only possible when the specific (or primary) antibody ("probe") binds to a marker. A variety of antibody-marker systems are available for different applications (Figure 1b; see below). The marker may be coupled by different methods to the probe (Hoppert, 2003):

- By direct coupling of the marker to the probe prior to use in a localization experiment. The detection procedure requires just one binding step. The method is also termed *direct immunolocalization*.
- By binding of a secondary probe (mostly a secondary antibody) to the Fc-part of a primary antibody after this primary antibody is already bound to the specific target (two-step procedure). The secondary probe is coupled to the marker. The method is also termed *indirect immunolocalization*.
- By binding of tertiary probes to the secondary probe (for signal amplification). Tertiary probes are responsible for binding or deposition of the respective marker.

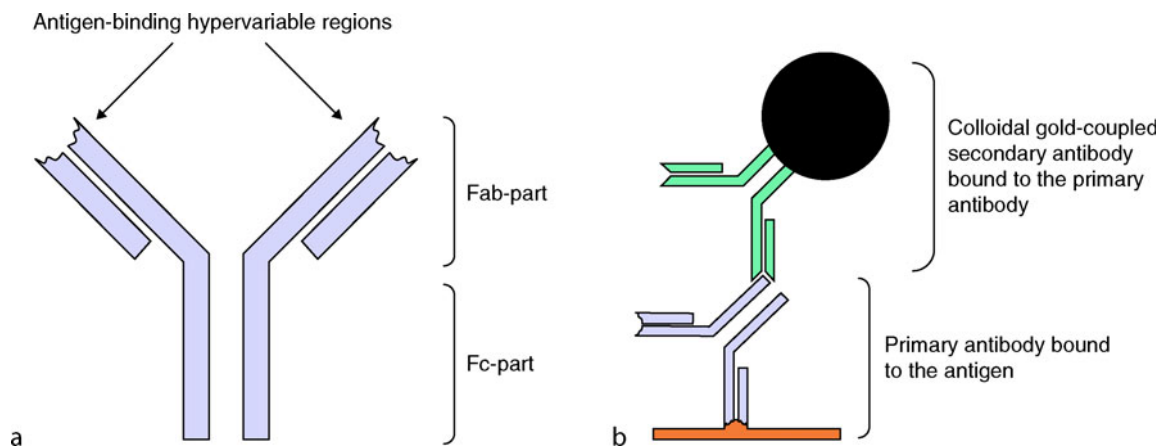
Immunolocalization in light and electron microscopy

For immunolocalization in (fluorescence) light microscopy, fluorescent dyes are used as markers

(immunofluorescence microscopy). The marker consists of the dye covalently coupled to a protein (e.g., the secondary antibody) that binds to the primary marker, the antigen-specific antibody. It is also possible to detect more than one target in a sample by the use of marker-probe systems with specificities for different targets coupled to fluorescence markers with discernable absorption–emission spectra. Access to the antigen in tissues or embedded samples can be achieved by (cryo-) sectioning or by permeabilization (Goldenthal et al., 1985). For (nonfluorescent) bright field microscopy, marker systems that catalyze enzymatic reactions are used, leading to deposition of dark-stained precipitates at the antigen binding sites (Ramos-Vara, 2005).

For electron microscopy, probes are coupled to colloidal gold as an electron dense marker. As stated for light microscopy, the marker can be directly coupled to the primary antibody, but regularly, a secondary system is combined with a primary antibody. Also, tertiary systems for signal amplifications are used. In equivalence to multiple detections of targets in light microscopy, it is possible to detect more than one target in a sample by use of marker-probe systems with discernable particle sizes of gold colloids. Particle sizes between 5 and 25 nm are used for experiments at the cellular level. Small gold particles may also be enlarged with silver or gold salt solutions for easier localization (Humbel et al., 1995). For epitope mapping in macromolecules, 1-nm markers ("nanogold") are available (Hainfeld and Powell, 2000).

Several modifications of the original immunogold marker technique can increase the resolution and sensitivity of the method. Dodecaborane clusters may be coupled to antibodies or Fab-fragments via polylysine dendrimers. The boronated antibodies have a lower detection limit in immunolocalization experiments than conventional gold conjugates. Detection of boron is performed by element-specific imaging in energy-filtering transmission electron



Immunolocalization, Figure 1 Antibody probes and colloidal gold markers: (a) Schematized structure of an IgG subtype antibody. (b) Primary antibody with bound secondary antibody and colloidal gold marker.

microscopes (Bendayan et al., 1989; Qualmann et al., 1996; Kessels et al., 1996).

Techniques like catalyzed reporter deposition ("CARD") use tertiary markers. CARD is a technique for amplification of a signal, originally designed for Western blotting, but modified for light and electron microscopy. In the first step, a specific primary antibody is coupled to the target. Then, in a second step, a secondary, biotin-tagged antibody is coupled to the primary antibody. A streptavidin–peroxidase conjugate is then bound to the biotin tag. Peroxidase catalyzes the deposition of tyramides indirectly. The tyramide itself is bound to a detectable marker (e.g., again biotin), and may be localized by the use of streptavidin-coupled colloidal gold for detection in the electron microscope (Mayer and Bendayan, 1997). For light microscopy, fluorescent dye-coupled tyramides are used (Wasielewski et al., 1997).

Related techniques

Besides the widely used antibodies, other specific probes for localization of a target may be used. Table 1 gives an overview of the relevant techniques. Lectin proteins have defined specificities to short carbohydrate stretches and are therefore an alternative to antibodies for detection of polysaccharides. They are mainly used for detection of glycosylated residues or polysaccharides on cell surfaces and/or in microbial biofilms (Kämper et al., 2004).

Especially when macromolecules are present in high concentrations, a target based on a substrate-specific enzyme may be applied. Enzyme–substrate interaction may also be detected with a substrate inducing the precipitation of an electron-dense marker to localize an enzyme activity.

Equivalent to in situ hybridization in light microscopy, the electron microscopic approach can allow high-resolution mapping of specific mRNA. The technique

Immunolocalization, Table 1 Techniques for the localization of biological macromolecules by light (LM) and electron microscopy (EM)

Problem	Specimen treatment	Marker system (technique; references)
Localization of specific proteins (antibody-gold/antibody-fluorescent probe) or carbohydrate complexes (lectin-gold/lectin-fluorescent probe) inside cells (LM/EM)	Ultrathin sections of resin-embedded samples subjected to immunolocalization	Antibody-colloidal gold, lectin-colloidal gold (Roth et al., 1980; Horisberger, 1985; Danguy et al., 1988)
Localization of specific proteins on the cell surface or on subcellular particles (EM)	Incubation of cells or subcellular fractions with the marker system prior to resin embedding or direct adsorption on specimen support films	Antibody-colloidal gold, lectin-colloidal gold (Acker, 1988; Rohde et al., 1988)
Localization of single protein epitopes and their relative position to each other on the surface of the object (EM)	Homogeneous protein preparations (especially large proteins or viruses) subjected to localization of epitopes	Antibody, Fab-fragment, (epitope-labeling; Hermann et al., 1991)
Detection of enzyme activities (EM)	Incubation of cells or subcellular fractions with the marker system	Specific enzyme substrate coupled to precipitation of electron dense marker (Hayat, 1973ff; Wohlrab and Gossrau, 1992)
Detection of macromolecular components acting as enzyme substrates (e.g., starch, lipids) (EM)	Labeling of enzyme substrates with gold particles coated with the relevant enzymes in ultrathin sections of samples	Enzyme-colloidal gold (Bendayan, 1985)
Localization of metabolites in the cell (EM)	Incubation of metabolically active cells with the precursor, embedding in conventional resin or by application of cryotechniques, treatment with a specific photoemulsion	Metabolic precursors (e.g., sugars or aminoacids) labeled with radioactive markers (autoradiography; Gregg and Reznik-Schüller, 1984)
Localization of specific DNA or RNA (preferably mRNA) sequences in cells (LM/EM)	Fluorescent in situ hybridization for light microscopy, hybridisation of DNA/RNA of fixed samples with the marker system followed by embedding and sectioning, detection of the marker system by autoradiography or with streptavidin-colloidal gold for electron microscopy	RNA or DNA oligonucleotide labeled with different markers (in situ hybridization; Amann et al., 1995; Egger et al., 1994)
Comparison of immunolocalization targets from overview (low resolution) to subcellular structures (high resolution) (EM)	Ultrathin (and semithin) sections of resin-embedded samples subjected to immunolocalization	Antibody-colloidal gold and antibody-fluorescent probe, lectin-colloidal gold and lectin-fluorescent probe (Takizawa and Robinson, 2003).

may be combined with specific signal enhancement methods (CARD, see above) and is suitable for high-resolution in situ hybridization (Cheung et al., 1999).

For microautoradiography, radiolabeled markers, incorporated into cell structures, are located by exposure to photographic emulsion. The deposition of silver bromide grains on the photoemulsion is detectable by light and electron microscopy. The technique is demanding and difficult to reproduce, though it gives information on processing and distribution of metabolites that cannot be obtained, on a structural level, by other methods (Gregg and Reznik-Schüller, 1984).

Correlative microscopy

The technique of correlative light/electron microscopy allows examination of the same sample at micrometer scale for overview images and at nanometer scale for imaging of features at high resolution. Regularly, (fluorescence) light microscopy and electron microscopy of samples is combined. The marker/probe systems are either suitable for both microscopic techniques at the same time or for combinations of different systems that are detectable either by light or by electron microscopy.

Quantum dots (qdots) are colloidal semiconductor nanocrystals and are detectable in the electron microscope, either as electron dense particles or via element specific imaging. Since qdots exhibit specific absorption/emission spectra in the light, they are also detectable as fluorescent markers. When qdots are coupled to primary antibodies, they are marker-probe systems suitable for both imaging techniques (Nisman et al., 2004).

Though it is possible to visualize ultrathin sections by (fluorescence) light microscopy, sections of several micrometer in thickness (semithin sections) are regularly more suitable. It is possible to obtain nearly identical features of a specimen when one ultrathin and one semithin is

consecutively cut from a specimen. Semithin and ultrathin sections are then processed in different ways, either with marker-probe-systems for immunofluorescence or for immunoelectron microscopy (Figure 2; Takizawa and Robinson, 2003; Wrede et al., 2008).

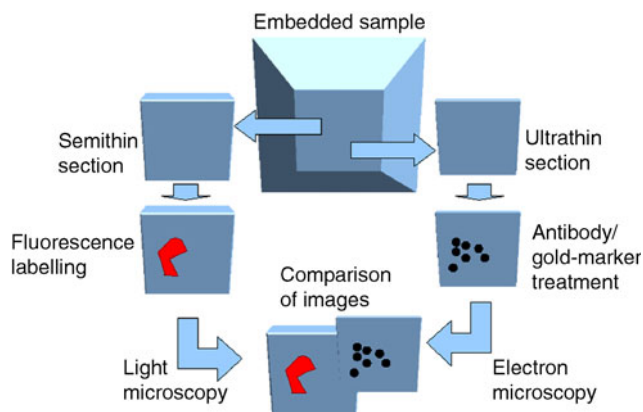
Correlation of data from immunolocalization may also be performed with other microscopic techniques, such as Raman microscopy, scanning electron microscopy, or atomic force microscopy.

Conclusions

It has already been understood in the first half of the past century that, besides comprehending biochemical or molecular reactions in organisms, the understanding of their organization is of equal importance (Hopkins, 1933). Tools for understanding organization of these processes have been developed consecutively, when methods became available to keep organisms alive or at least well-preserved down to molecular scale. The techniques revealed that the presumption of some early biochemists was right, i.e., metabolic pathways, growth and cell division, even of apparently non-compartmented bacteria, are organized in high ordered, large macromolecular complexes. Future direction of localization techniques will include the application of marker systems for high resolving microscopy systems that will allow observation of living cells down to nanometer scale (Donnert et al., 2006).

Bibliography

- Acker, G., 1988. Immunoelectron microscopy of surface antigens (polysaccharides) of gram-negative bacteria using pre- and post-embedding techniques. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 147–174.
- Amann, R. I., Ludwig, W., and Schleifer, K. H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Bendayan, M., 1985. The enzyme-gold technique: a new cytochemical approach for the ultrastructural localization of macromolecules. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol 3, pp. 179–201.
- Bendayan, M., Barth, R. F., Gingras, D., Londono, I., Robinson, P. T., Alam, F., Adams, D. M., and Mattiazzi, L., 1989. Electron spectroscopic imaging for high-resolution immunocytochemistry: use of boronated Protein A. *Journal of Histochemistry and Cytochemistry*, **37**, 573–580.
- Cheung, A. L., Graf, A. H., Hauser-Kronberger, C., Dietze, O., Tubbs, R. R., and Hacker, G. W., 1999. Detection of human papillomavirus in cervical carcinoma: comparison of peroxidase, Nanogold, and catalyzed reporter deposition. *Modern Pathology*, **12**, 689–696.
- Danguy, A., Kiss, R., and Pasteels, J. L., 1988. Lectins in histochemistry. A survey. *Biological Structures and Morphogenesis*, **1**, 93–106.
- Donnert, G., Keller, J., Medda, R., Andrei, M. A., Rizzoli, S. O., Lüthmann, R., Jahn, R., Eggeling, C., and Hell, S. W., 2006. Macromolecular-scale resolution in biological fluorescence microscopy. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 11440–11445.
- Egger, D., Troxler, M., and Bienz, K., 1994. Light and electron microscopic in situ hybridization: non-radioactive labeling and



Immunolocalization, Figure 2 Specimen Preparation for correlative Microscopy. From the embedded sample, alternating semithin and ultrathin sections are prepared and processed either by fluorescence labeling for light microscopy or treatment with antibodies and colloidal gold. In light and electron microscopy, corresponding areas are identified and compared.

- detection, double hybridization, and combined hybridization-immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **42**, 815–822.
- Goldenthal, K. L., Hedman, K., Chen, J. W., August, J. T., and Willingham, M. C., 1985. Postfixation detergent treatment for immunofluorescence suppresses localization of some integral membrane proteins. *Journal of Histochemistry and Cytochemistry*, **33**, 813–820.
- Gregg, M., and Reznik-Schüller, H. M., 1984. An improved method for rapid electron microscopic autoradiography. *Journal of Microscopy*, **135**, 115–118.
- Hainfeld, J. F., and Powell, R. D., 2000. New frontiers in gold labeling. *Journal of Histochemistry and Cytochemistry*, **48**, 471–480.
- Hayat, M. A., 1973–1977. *Electron Microscopy of Enzymes: Principles and Methods*. New York: Van Nostrand Reinhold, Vol. 1 (1973); Vols. 2 and 3 (1974); Vol. 4 (1976); Vol. 5 (1977).
- Hermann, R., Schwarz, H., and Müller, M., 1991. High precision immunoscanning electron microscopy using Fab fragments coupled to ultra-small colloidal gold. *Journal of Structural Biology*, **107**, 38–47.
- Hopkins, F. G., 1933. Some chemical aspects of life. *Nature Supplement*, **3332**, 381–394.
- Hoppert, M., 2003. *Microscopic Techniques in Biotechnology*. Weinheim: Wiley-VCH.
- Horisberger, M., 1985. The gold method as applied to lectin cytochemistry in transmission and scanning electron microscopy. In Bullock, G. R., and Petrusz, P. (eds.), *Techniques in Immunocytochemistry*. London: Academic Press, Vol. 3, pp. 155–178.
- Humbel, B. M., Sibon, O. C. M., Stierhof, Y.-D., and Schwarz, H., 1995. Ultra-small gold particles and silver enhancement as a detection system in immunolabeling and in situ hybridization experiments. *Journal of Histochemistry and Cytochemistry*, **43**, 735–737.
- Kämper, M., Vetterkind, S., Berker, R., and Hoppert, M., 2004. Methods for in situ detection and characterization of extracellular polymers in biofilms by electron microscopy. *Journal of Microbiological Methods*, **57**, 55–64.
- Kessels, M. M., Qualmann, B., Klobasa, F., and Sierralta, W. D., 1996. Immunocytochemistry by electron spectroscopic imaging using a homogeneously boronated peptide. *Cell and Tissue Research*, **284**, 239–245.
- Mayer, G., and Bendayan, M., 1997. Biotinyl–tyramide: a novel approach for electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1449–1454.
- Nisman, R., Dellaire, G., Ren, Y., Li, R., and Bazett, D. P., 2004. Application of quantum dots as probes for correlative fluorescence, conventional, and energy - filtered transmission electron microscopy. *Journal of Histochemistry and Cytochemistry*, **52**, 13–18.
- Qualmann, B., Kessels, M. M., Klobasa, F., Jungblut, P. W., and Sierralta, W. D., 1996. Electron spectroscopic imaging of antigens by reaction with boronated antibodies. *Journal of Microscopy*, **183**, 69–77.
- Ramos-Vara, J. A., 2005. Technical aspects of immunohistochemistry. *Veterinary Pathology*, **42**, 405–426.
- Rohde, M., Gerberding, H., Mund, T., and Kohring, G. W., 1988. Immunelectron microscopic localization of bacterial enzymes: pre- and postembedding labeling techniques on resin-embedded samples. In Mayer, F. (ed.), *Methods in Microbiology*, Electron microscopy in Microbiology. London: Academic Press, Vol. 20, pp. 175–210.
- Roth, J., Bendayan, M., and Orci, L., 1980. FITC-Protein A-Gold complex for light and electron microscopic immunocytochemistry. *Journal of Histochemistry and Cytochemistry*, **28**, 55–57.
- Takizawa, T., and Robinson, J. M., 2003. Ultrathin cryosections: an important tool for immunofluorescence and correlative microscopy. *Journal of Histochemistry and Cytochemistry*, **51**, 707–714.
- Wasielowski, R., Mengel, M., Gignac, S., Wilkens, L., Werner, M., and Georgii, A., 1997. Tyramine amplification technique in routine immunohistochemistry. *Journal of Histochemistry and Cytochemistry*, **45**, 1455–1459.
- Wohlrab, F., and Gossrau, R., 1992. *Katalytische Enzymhistochemie*. Jena: G. Fischer Verlag.
- Wrede, C., Heller, C., Reitner, J., and Hoppert, M., 2008. Correlative light/electron microscopy for the investigation of microbial mats from Black Sea Cold Seeps. *Journal of Microbiological Methods*, **73**, 85–91.

Cross-references

Fluorescence in Situ Hybridization (FISH)
Histology

IRON ISOTOPES

Please refer to “*Isotope Fractionation (Metal)*.”

IRON SULFIDE FORMATION

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

There has been a great deal of research into the formation of sedimentary iron sulfide minerals because their history is intertwined with the biogeochemical cycles of iron, sulfur, carbon, and oxygen (Berner, 2001). Iron sulfides occur in sediments from a wide range of depositional environments, from the deep sea to the nonmarine, and the principal mineral in the rock record is pyrite (FeS₂, cubic). Its dimorph marcasite (orthorhombic) is considered metastable and apt to invert to pyrite over geologic time (Murowchick, 1992), but is probably more widespread than commonly acknowledged (Schieber, 2007). Presumed precursor minerals of sedimentary pyrite are mackinawite (FeS_{1-x}) and greigite (Fe₃S₄), minerals that impart a black coloration to modern reducing sediments (Goldhaber and Kaplan, 1974). Both are metastable and transform over time into pyrite or a mixture of pyrite and pyrrhotite (Berner, 1967). Pyrrhotite, hexagonal FeS, is thermodynamically stable in sediments (Berner, 1967), but extremely rare in modern or ancient sediments (Kobayashi and Nomura, 1972) due to kinetic limitations (Canfield and Raiswell, 1991).

Mackinawite and greigite are also known as acid-volatile iron sulfides (AVS) because in contrast to pyrite and marcasite they are readily soluble in HCl. Mineralogically, greigite is the sulfur analog of magnetite and is indeed strongly ferromagnetic (Dekkers and Schoonen, 1996). It can be formed by magnetotactic bacteria and dominates the magnetic properties of some modern sediments and

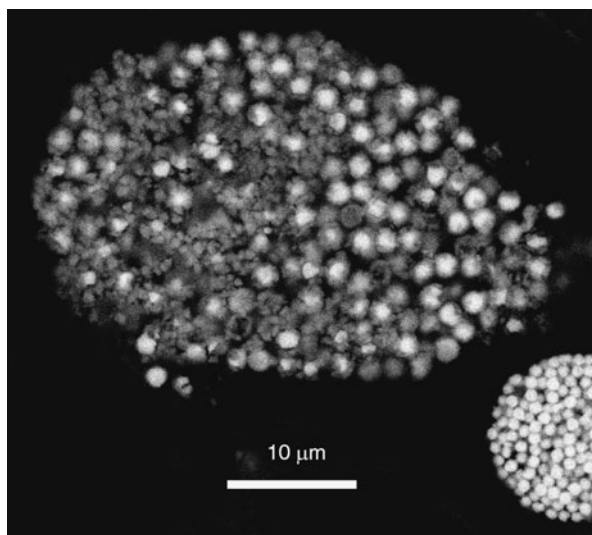
even some ancient sedimentary rocks (e.g., Krs et al., 1990).

Although they also occur in nonmarine sediments, the bulk of sedimentary iron sulfides forms in marine sediments during early diagenesis via microbial reduction of seawater sulfate. The general process of their formation has been elucidated by a number of classical studies (e.g., Kaplan et al., 1963; Berner, 1970, 1984). From a geobiological perspective, iron sulfides are of interest as their presence denotes anoxic and reducing pore waters, because they can preserve minute details of soft tissues (e.g., Briggs et al., 1991; Gabbott et al., 2004; Kammer and Ausich, 2007), because microbial life in pore spaces may be entombed in growing iron sulfide grains (Schieber, 2002a), and because the iron sulfide matrix is apparently able to preserve more complex organic molecules (such as amino acids) for long geologic time periods (Nardi et al., 1994). For an in-depth discussion of sedimentary sulfide formation and the linkage to the global sulfur cycle the reader is referred to an excellent overview by Goldhaber (2003) in the Treatise on Geochemistry.

Pathways to sedimentary iron sulfides

The understanding of iron sulfide formation under Earth surface conditions has largely been informed by extensive laboratory studies (see summary by Morse et al., 1987). Multiple potential pathways for sedimentary iron sulfide (pyrite) formation have been recognized. As summarized by Goldhaber (2003), these include (1) direct precipitation of pyrite from polysulfide bearing solutions, (2) progressive conversion of solid iron monosulfides to pyrite (Figure 1) by polysulfides, (3) reaction of H_2S with a solid iron monosulfide to form pyrite (release of H_2 gas), and (4) reactions that involve iron loss from a solid iron monosulfide precursor through oxidation.

As indicated by this summation, the body of published literature lives by the assumption that iron monosulfides (generic for mackinawite and/or greigite) are necessary precursors for pyrite formation in sediments. Thus, the normal iron sulfide paragenesis in sediments is presumed to proceed from mackinawite over greigite to pyrite (e.g., Wilkin and Barnes, 1996; Benning et al., 2000). The tacit assumption is that sedimentary pyrite cannot form in the absence of acid-volatile iron monosulfides (AVS). Yet, direct evidence for this traditional view is scant. Rickard and Morse (2005) have argued strongly that in numerous studies of modern sedimentary pyrite formation abundant pyrite is produced in the absence of detectable AVS. They argue that many of the equations that describe sedimentary pyrite formation in the literature represent net mass balances and do not describe the actual reaction processes. Rickard and Morse (2005) point out that AVS are not required for reaction pathways that involve polysulfide or H_2S (numbers (2) and (3) from above), and that aqueous FeS clusters could be involved instead of solid monosulfide phases. They propose that the products of the diagenetic sulfide machinery,

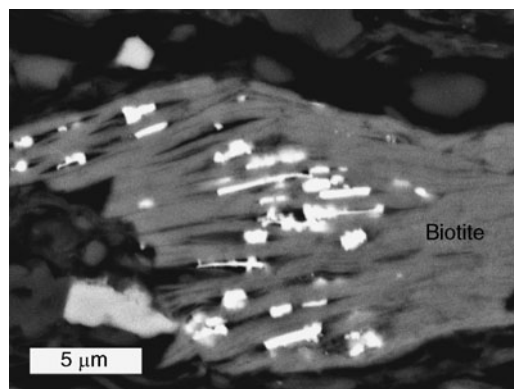


Iron Sulfide Formation, Figure 1 SEM photomicrograph of pyrite framboids from modern reducing muds of the Santa Barbara Basin, offshore southern California. The sediment was cored, stabilized in Spurr resin, and polished with diamond paste. At lower right a similar well-formed framboid might be found in ancient mudstones. In the center a less ordered framboid with bright grains that are pyrite (EDS analysis), and gray grains that have about half the sulfur peak intensity (EDS spectra) of typical pyrite grains. The interpretation is that the gray grains are the Fe-monosulfide precursor material (amorphous FeS , mackinawite, ~greigite) that has been the subject of geochemical studies. (Berner, 1970; Sweeney and Kaplan, 1973; Goldhaber and Kaplan, 1974; Murowchick and Barnes, 1987; Wilkin and Barnes, 1997.)

mackinawite, greigite, and pyrite, may form either simultaneously or in no particular order during sediment diagenesis.

The building blocks

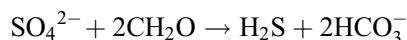
Regardless of how sedimentary iron sulfides are ultimately produced, it is instructive to examine where the requisite iron and sulfur come from. Because iron is insoluble in the presence of oxygen, the iron content of sea water and freshwater is extremely low (Stumm and Morgan, 1996). It is, therefore, practical to assume that any iron that is available for iron sulfide formation has to be part of the particulate matter that makes up the sediment. Typical deltaic and marine muds have iron contents that range from 4% to 8% (Calvert, 1976; Chester and Asten, 1976; Aller et al., 1986), but not all of that iron is equally available for diagenetic iron sulfide formation. Although there may be plenty of iron in iron silicates (e.g., biotite, chlorite), release of that iron requires alteration/destruction of the containing silicate, a process that operates more efficiently under the higher temperatures of deep burial diagenesis (Figure 2). Thus, this iron fraction is not available during early diagenesis when the bulk of sedimentary iron sulfides is formed.



Iron Sulfide Formation, Figure 2 SEM photomicrograph of late diagenetic biotite alteration associated with pyrite formation (backscatter (BS) image) in the Devonian Ohio Shale, Kentucky, USA. Pyrite (very bright) forms along cleavage planes near the end of a biotite flake because during alteration these are the places where iron is released. The surrounding pore waters must have contained abundant H_2S . Iron was precipitated as FeS_2 very close to the place where it was released from the biotite lattice, and it was not able to migrate away from its source grain.

The iron fraction that is critical in early diagenesis is in the form of coatings of iron oxyhydroxides on detrital grains (especially on clays; Carroll, 1958), as well as in the form of particulate/colloidal iron oxyhydroxides (e.g., Allard et al., 2004). Of the 4–8% of total iron that we can expect in average muddy sediments, only some 10%, and under very favorable circumstances up to about half, is made available by reducing agents (Gibbs, 1977; Trefrey and Presley, 1982; Canfield, 1989) for the formation of early diagenetic iron sulfides.

Sulfur, of course, is readily available via seawater sulfate in marine sediments, and for surficial sediments the overlying seawater represents an essentially limitless reservoir. Only upon burial, once consumed sulfate can no longer be replenished by downward diffusion, does sulfur become a limiting commodity. In all sediments, the production of H_2S for sulfide formation is accomplished by sulfate reducing prokaryotes that utilize sulfate as an electron acceptor for energy producing chemical reactions. For carbohydrate oxidation this reaction may be written as



Thus, organic matter that can be utilized by sulfate reducing microbes is essential for the operation of the sedimentary sulfide factory. Even in the presence of abundant sulfate, sulfide production will only occur if there is “food” for the microbes. Sulfate reduction equations proposed by Canfield and Raiswell (1991) suggest that it will require about 5 cm^3 of microbially “digestible” organic matter (e.g., lactate, ethanol, acetate, sugar) to precipitate 1 cm^3 of FeS_2 . Thus, low organic matter contents in sediments will tend to limit sulfide production and the amount of iron that can be converted to pyrite.

Above considerations also have implications with regard to where we most likely will find large quantities of iron sulfides in sedimentary rocks. Because pure carbonates typically accumulate far from the influence of terrigenous clastics, the average carbonate rock tends to contain only minor quantities of iron and thus, sedimentary iron sulfides are generally low in abundance. Sandstones, even though they may contain appreciable quantities of iron in the form of iron bearing detrital silicates and as iron oxyhydroxide grain coatings, are comparatively poor in organic matter and thus lack the fuel to drive extensive early diagenetic sulfate reduction. When we do find substantial accumulations of iron sulfides in carbonates or sandstones, they are in the majority of cases due to late diagenetic processes that benefited from higher temperatures and constituent bearing formation waters. Exceptions occur when carbonates or sandstones are interbedded with shales and mudstones, because the latter carry the bulk of iron in the sedimentary mass balance (Taylor and McLennan, 1985). The lion’s share of sedimentary iron sulfides is therefore contained in fine grained terrigenous rocks (shales and mudstones).

Iron sulfide textures

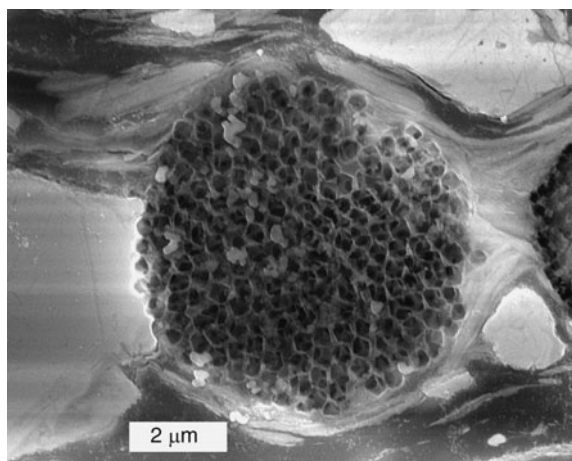
Pyrite, the most commonly reported iron sulfide in sediments, is cubic in structure, and thus cubes and octahedra are common morphologies. In addition to these fundamental forms, iron sulfides are also patterned into a wide range of textural types (e.g., single grains, framboids, pore fill cements, concretions, pyrite cemented layers, and pyritic lags) that carry useful information about conditions during deposition and early diagenesis. From a geobiological perspective, concretionary iron sulfide accumulations are of particular interest because their formation was likely driven by decomposing organic matter. Their morphology varies widely, and many types have been described, such as pyritized burrow tubes, infilling and encrustation of fossils, replacement of fossil hard parts (carbonate shells, bones), and pyritization of soft tissues.

Single grains

As single grains, pyrite may occur as a disseminated component, ranging from less than $1 \mu\text{m}$ to tens of micrometer in size. Yet, while these may indeed be grains that record a single growth episode, etching (e.g., with HNO_3) may reveal internal textures that speak to multistage growth.

Pyrite framboids

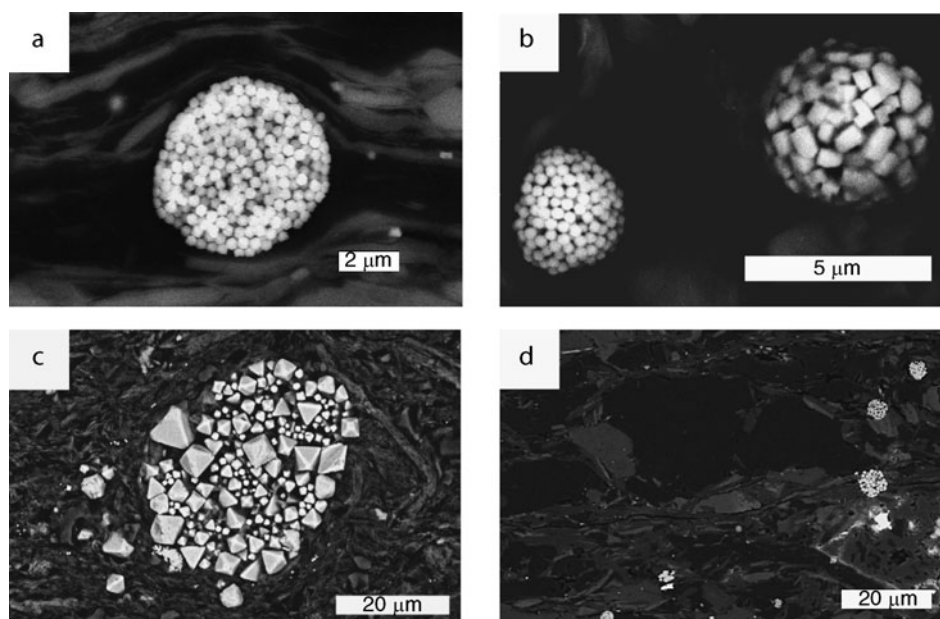
Ubiquitous in modern and ancient sediments, pyrite framboids are typically near-spherical in shape, range in diameter from about $1 \mu\text{m}$ to tens of micrometers, and are themselves composed of tiny, discrete, and equigranular pyrite crystallites. They have been the subject of numerous studies, and early on there was an assumption that organic matter (Figure 3) somehow plays a role in their formation (Schneiderhöhn, 1923; Love,



Iron Sulfide Formation, Figure 3 SEM photomicrograph of an etched pyrite framboid in the Devonian Chattanooga Shale of Tennessee, USA. The etching with HNO_3 removed the pyrite crystallites and left behind a honeycomb of organic matter that filled the space between the pyrite grains. The organic matter has the same composition as the unstructured kerogen in the surrounding sample.

1967). Yet, because they can also be produced abiotically in the laboratory (Berner, 1969; Sweeney and Kaplan, 1973), the role of organic matter remains a matter of debate (e.g., Kalliokoski, 1974). The abundantly noted close association of pyrite framboids and organic matter may well be coincidental because the reducing conditions that promote their formation are best developed in sediments with elevated contents of organic matter.

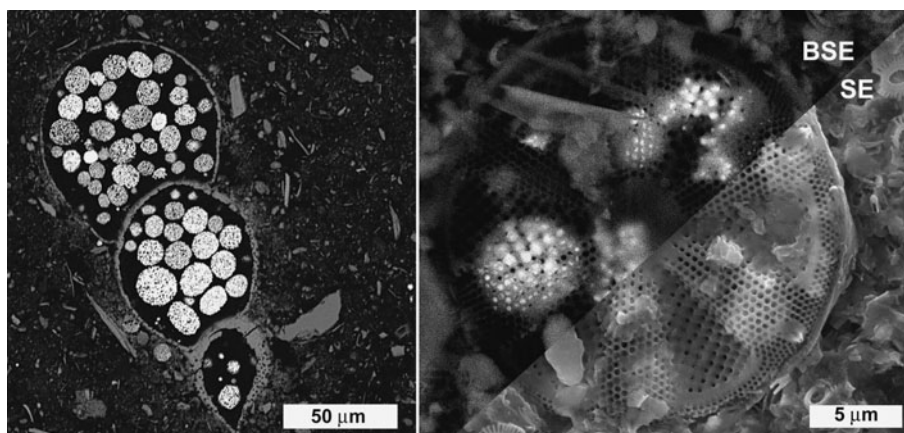
Most pyrite framboids observed in sedimentary rocks consist of microcrysts (Figure 4a; typically cubes, octahedral, or pyritohedra) that show poorly defined to highly ordered packing into spherical aggregates (Wilkin and Barnes, 1997; Ohfuji et al., 2005). The size ratio of microcrysts to framboid diameter is quite variable (Figure 4b). Directly adjacent framboids may show vast differences in diameter/microcryst size ratios (Figure 4b), and also show contrasting microcryst morphology. These textural details probably carry some information about the pore water environment during formation, but at present their significance is poorly understood. Pyrite framboids can survive intact into greenschist grade metamorphism, and can for example, identify slates that originated as marine shales (Figure 4d).



Iron Sulfide Formation, Figure 4 Pyrite framboids in the rock record. All images are SEM photomicrographs (backscatter mode). (a) Moderately well-ordered framboid from the late Devonian New Albany Shale (Indiana, USA). Note differential compaction of clays and organic matter around the framboid, indicating framboid formation prior to compaction when the sediment contained ~80% water. (b) Framboids in modern sediments from the Santa Barbara Basin (offshore southern California, USA). Note the strong differences in diameter/microcryst size ratios and the contrast in microcryst morphology (pyritohedra vs. complex cubes). (c) Framboid consisting mostly of variably size octahedra (Ordovician Powers Steps Formation, Newfoundland). (d) Pyrite framboids that survived low-grade metamorphism and cleavage development ($\pm$ horizontal) in the Ordovician Martinsburg Slate of New Jersey, USA.

The formation processes of pyrite framboids are discussed at length by Wilkin and Barnes (1997), and further perspectives are presented by Ohfuji et al. (2003) and Butler and Rickard (2000). Fundamentally, in order to form framboids within the pore spaces of a sediment, iron should be able to migrate to the site of formation and thus, the pore waters have to be nonsulfidic (anoxic-nonsulfidic; Berner, 1981). Furthermore, in order for iron oxyhydroxides to dissolve in the pore waters, they also need to be free of oxygen. In the idealized sequence of microbial decay reactions in sediments (Brett and Allison, 1998; Curtis et al., 2000), this is known as the “suboxic” zone, and is followed by the “anoxic” zone of sulfate reduction. The boundary between the suboxic and the anoxic zone is also referred to as the redox interface, and it is here where dissolved iron meets sulfide and iron sulfides can precipitate. Pyrite framboids form in that region. Yet, whereas these zones are thought to be layered atop each other in surficial sediments, in reality it is a bit more complex. Every single framboid implies that iron migrated from a surrounding sediment volume to form this localized accumulation, and by default this means that there was a localized source of sulfide that via iron sulfide precipitation maintained the underlying concentration gradient. The sulfide source was probably a colony of sulfate reducing bacteria that prospered around a lump of easily digested organic matter. Thus, rather than simply being a narrow bedding-parallel zone in which dissolved iron interacts with dissolved sulfide, the realm of framboid formation (the redox interface) is heterogeneous. It makes more sense to think of it as “islands” of microbial sulfide production in a “sea” of iron-bearing pore waters (Figure 5), the reducing microenvironments that have been mentioned elsewhere in the literature (e.g., Hudson, 1982).

Whereas in typical sediments the redox interface is situated somewhat below the sediment water interface, there are situations where the redox interface is actually located in the overlying water column, such as in the Black Sea. In euxinic basins, with the lower portion of the water column being anoxic and sulfidic, framboids can grow in the water column near the redox interface and then settle and become part of the accumulating sediments (Wilkin et al., 1996). Wilkin et al. (1997) report that framboids of that origin are rather small (mean framboid diameters $<5\ \mu\text{m}$, narrow size distribution) when compared to those forming in sediments underlying oxic and dysoxic waters (mean framboid diameters $5\text{--}10\ \mu\text{m}$, broad size distribution). The implication of this is that one can use the framboid size distributions of sediments to determine whether they accumulated beneath an anoxic water column. Wignall and Newton (1998) applied this concept to ancient mudrocks and were able to correlate framboid size distributions with paleoecologically based reconstructions of bottom water oxygenation. However, framboid size in sediments may also be influenced by the availability of iron during early diagenesis. For example, a study of framboid size distributions in sediments from the Santa Barbara Basin (offshore southern California) indicates that framboid size distributions primarily reflect conditions within the immediate surface sediment (such as availability of readily soluble iron). Framboid size distributions in annual varves (1984–2004) from the Santa Barbara Basin suggest three euxinic interludes in the past 2 decades, even though seasonal bottom water surveys consistently recorded suboxic to dysoxic bottom water conditions (Schieber and Schimmelmann, 2006). It appears, therefore, that it may not be possible to draw conclusions about water column conditions from pyrite framboid size distributions.



Iron Sulfide Formation, Figure 5 SEM photomicrographs (backscatter mode) of localized pyrite framboid formation in reducing microenvironments of Santa Barbara Basin (offshore southern California) surface sediments. At left, a multichambered calcareous foraminifera with chambers filled by framboids. At right, a framboid filled diatom. Lower right half, imaged in secondary electron (SE) mode (framboids not visible). Upper left half, imaged in backscatter (BSE) mode, the framboids shine through the lower density opaline “lid.”

Polyframmboids

Pyrite frammboids may also occur as larger aggregates, so called polyframmboids. These have been described from sediments of various ages (Schieber and Baird, 2001) and have a tendency to form in cavities of organic remains (Figures 5 and 6). Judging from differential compaction around polyframmboids (Figure 6), they must form prior to any compaction, just like single pyrite frammboids. They also imply iron migration from a surrounding sediment volume that was anoxic but not sulfidic. The difference between polyframmboids and regular pyrite frammboids may simply be a higher iron supply from the pore waters, or alternatively that the “islands” of microbial sulfate reduction are more widely spaced and thus, focus the available iron onto fewer sites of precipitation. Because most marine sediments start out with comparable amounts of reactive iron, the latter scenario is more likely.

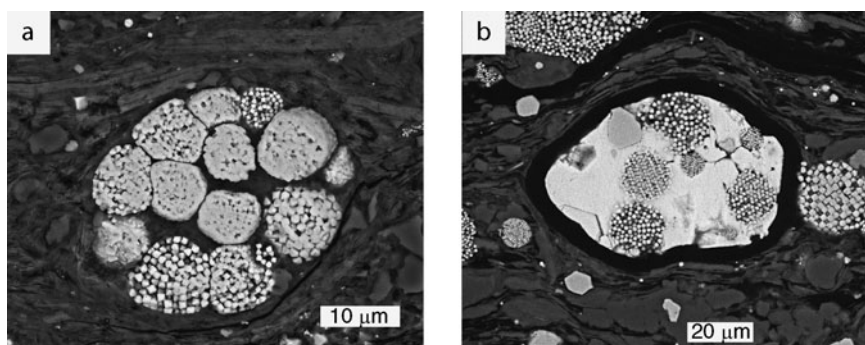
Small-scale pyrite aggregates and clusters

Pyrite bodies that consist of coalescing, irregular, rounded clots of small pyrite grains have been described as

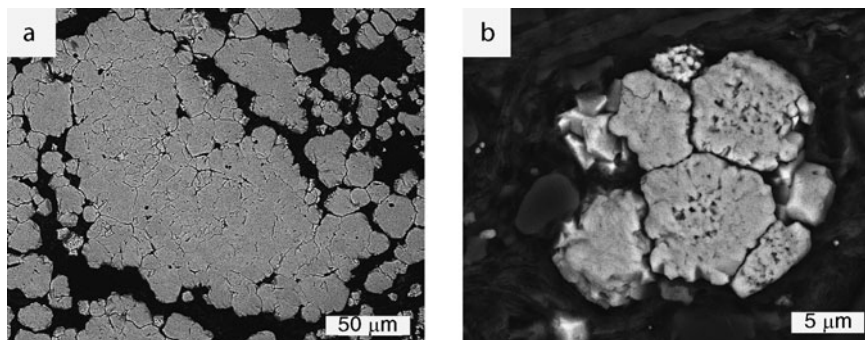
aggregated pyrite by Hudson (1982). The cores of individual clots may be small pyrite crystals or pyrite frammboids, and these cores are overgrown by coarser pyrite crystals (Figure 7). In places these overgrown grains show a radiating bladed habit. These overgrown grains tend to form somewhat later in diagenesis, at a point when downward diffusion of seawater sulfate is increasingly restricted (Strauss and Schieber, 1990). This type of pyrite aggregate ranges in size from tens of microns to several millimeters.

Concretions and pyritic layers

Iron sulfide concretions in sediments vary in size from a few millimeters to more than 10 cm. Like other occurrences of sedimentary iron sulfides they are most commonly encountered in mudstones and typically occur in distinct nodule bearing horizons (Figure 13). Although pyrite is usually reported as the sole iron sulfide mineral, careful examination reveals many examples of inverted or extant marcasite within these iron sulfide accumulations (Schieber, 2002b, 2007; Schieber and Riciputi,



Iron Sulfide Formation, Figure 6 SEM photomicrographs of pyrite polyframmboids (backscatter images). Note differential compaction around polyframmboid aggregates. (a) Ordovician Powers Steps Formation, Newfoundland, Canada. Note “squeezing” of frammboids, suggesting initial formation in a confined space (transported?). (b) Devonian Chattanooga Shale, Tennessee, USA. Here, the polyframmboid is still enclosed (black line) in an algal cyst (Tasmanites) that formed the initial reducing microenvironment. The space between frammboids is filled with secondary, coarser pyrite cement.



Iron Sulfide Formation, Figure 7 SEM photomicrographs of pyrite aggregates (backscatter images). (a) Irregularly shaped aggregate consisting of smaller pyrite clots. (b) Small cluster of pyrite clots that still have visible frammboids in their cores. The overgrown pyrite has radiating habit and may have originated as marcasite.

2005). Especially the larger concretions approximate prolate ellipsoids (Figure 8a) and show differential compaction of the surrounding mudstone (Figure 13). These features attest to growth prior to substantial compaction and growth within a stratiform chemical zone (anoxic and nonsulfidic; Berner, 1981). In order for iron to be directed to localized sites of precipitation within this zone, there need to be sites of abundant H_2S production, as, for example, provided by microbial decay of buried organisms (e.g., bivalve, fish). Thus, the interiors of iron sulfide concretions are good targets for finding well-preserved fossils. In order to allow the concretions to grow to large size, this chemical zone has to remain stationary for a long time, probably on the order of thousands of years (Canfield and Raiswell, 1991). Not uncommonly, the iron sulfide concretions in a given layer also add up to a substantial excess of iron relative to the background sediment, suggestive of prior concentration by mechanical and chemical reworking (see Section “Marcasite and its implications”). Thus, horizons with abundant iron sulfide concretions or abundant pore filling iron sulfides imply very slow sedimentation at the time of nodule formation and should also alert us to the possibility of reworking and erosion of underlying sediments (Schieber and Riciputi, 2005). Other options for the formation of iron sulfide-enriched horizons are, for example, to cover iron-oxide rich layers with an organic-rich anoxic sediment drape (Ingri and Ponter, 1986). Migration of a sulfide-rich reduction front into the iron-bearing layer would effectively convert the iron oxides to pyrite.

Hydraulic concentrations (lags)

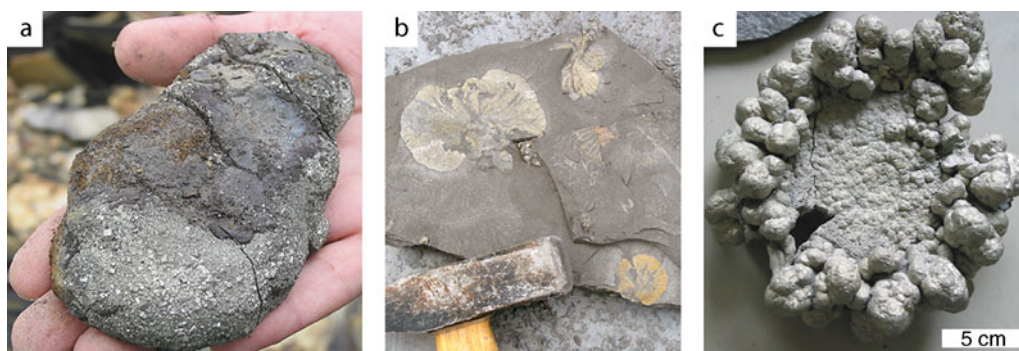
Because iron sulfide grains are of much higher density than associated silicates and organic matter, they are liable

to be concentrated into lag deposits when the easily eroded surface sediment is reworked by bottom currents and storms. Reworking can be manifest in the form of submillimeter concentrations of pyrite framboids (Figure 11c), as well as in decimeter thick concentrations of a wide variety of sedimentary pyrite grains (Figure 11). Examples are pyrite concretions (Figure 8), pyritized burrow tubes, and pyrite-filled algal cysts (Figure 6b). Lags that contain abundant iron sulfide grains have been described from the rock record in many places (e.g., Baird and Brett, 1991; Schieber, 1998a; Schieber and Baird, 2001). The mechanical enrichment of iron sulfides has two major implications and consequences. First, because of limitations on the initial soluble iron content of sediments, a substantial volume of sediment has to be sacrificed to erosion in order to concentrate all the iron into a single layer. Let us assume, for example, that typical mudstones have about 5% iron. A 5 cm thick lag with about 60% FeS_2 by volume would require that (at a minimum) a 50 cm thick (fully compacted) shale interval be winnowed to provide the necessary iron. Given that early in depositional history the uppermost meters of this shale (mud) had probably still an average porosity of at least 50%, the presence of this lag then implies that at least one meter, and more likely several meters, of sediment was removed.

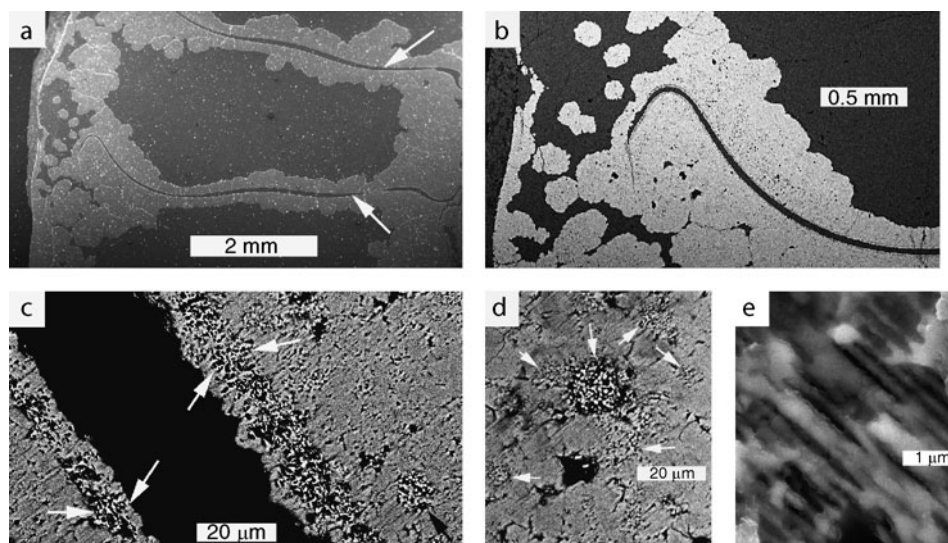
Iron sulfides and fossil preservation

Replacement and encrustation of calcareous shells

Encrustation and replacement of calcareous shells by iron sulfides are widely observed in the rock record. Typically, an assumption is made that the iron sulfide in question is pyrite, but a case can be made wherein marcasite was involved at least initially (see below). One way to think about calcite/aragonite replacement is that the saturation



Iron Sulfide Formation, Figure 8 A collection of iron sulfide concretions. (a) Oblate concretion from the Devonian Ohio Shale of eastern Kentucky, USA. Probably contains marcasite around the periphery, just like that seen in Figure 13. (b) Discoid-flattened pyrite concretions from the Jurassic Posidonia Shale near Dotternhausen, Germany. These may originally have been marcasitic. (c) Heavily pyrite (plus marcasite?) encrusted ammonite from the Lower Jurassic (Amaltheenton) near Dotternhausen, Germany. The specimen weighs at least 5 kg (courtesy of Dr. M. Jäger, Holcim GmbH). Without pre-concentration of iron in the sediment, it would require all the iron contained in a volume of 250 l of sediment (80% water content, 5% Fe in dry matter) to be concentrated into this concretion. Because iron redistribution is not that efficient, it is more likely that something on the order of 1 m³ of sediment has to be depleted.



Iron Sulfide Formation, Figure 9 Fossil preservation by iron sulfides, SEM photomicrographs. (a) Chamber lining iron sulfides in an ammonite (secondary electron image), Lias delta (Amaltheenton) near Dotternhausen, Germany. The septa of the ammonite (*white arrows*) are now calcitic, and the chamber interior is filled with calcite as well. (b) Close-up of the iron sulfide (bright, backscatter image) encrusted septum. The encrusting sulfides are a mixture of pyrite (framboids) and marcasite (overgrowth). (c) Close-up of encrustation near septum (backscatter image). The marginal areas of the septum (marked between *white arrows*) have a different texture than the areas further away. The latter areas contain pyrite framboids (*black arrow*) in a coarser matrix of blocky to radiating crystals. (d) Close-up of framboids (*white arrows*) in coarser matrix grains. The latter are anisotropic in reflected light and are either still preserved as marcasite or are marcasite relicts. (e) Details of the different textured replacement of the septum margin (between *white arrows* in (c)). The lamellar texture probably is a relict of the earlier lamellar structure of the septal aragonite.

levels of iron sulfide decline with increasing pH. When carbonates dissolve, pH increases and thus forces precipitation of iron sulfide. Chemical modeling suggests, however, that this process is unlikely during early diagenesis (Canfield and Raiswell, 1991). Nonetheless, even the shells of still living mollusks can be pyritized (Srivastava, 1975; Clark and Lutz, 1980), a process favored by pore waters with high levels of dissolved iron and excretion of microbially metabolizable organic substances by the mollusks (Reaves, 1984). In essence, the mollusks supported a sulfidic microenvironment and attracted iron from surrounding (anoxic-nonsulfidic) pore waters. This same basic scenario probably also applies to decomposing organic matter within dead mollusks, leading to shells encrusted or infilled with pyrite (Hudson, 1982). Hudson (1982) distinguished chamber linings (Figure 9), stalactitic pyrite, and over-pyrite (on outer shell surface), all of them mostly consisting of well-crystallized equant pyrite. For the potential impact of intermittent reoxidation of sedimentary iron sulfides on pH levels, carbonate precipitation, and rapid precipitation of the pyrite dimorph marcasite to promote shell encrustation, see the discussion on marcasite formation below.

Soft tissue preservation

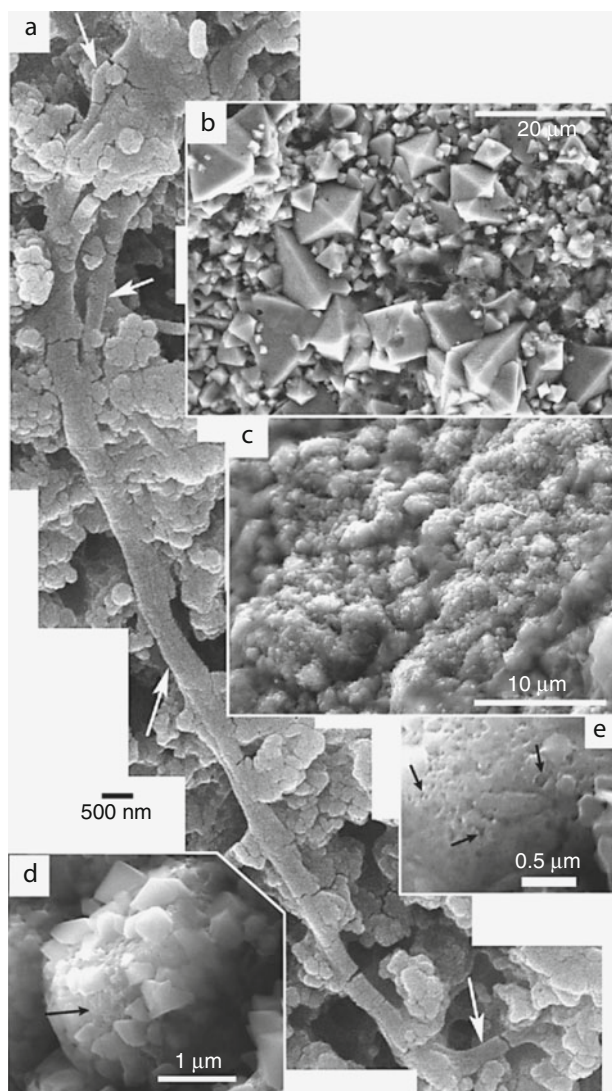
The preservation of soft tissue by pyritization is another marvel of fossilization, with well known examples from

the Hunsrück Slate (Stürmer, 1985), Beecher's trilobite bed (Cisne, 1973; Briggs et al., 1991), and the Burgess Shale (Conway Morris, 1986). Ongoing research has been producing a stream of exceptional soft bodied fossils that are preserved in pyrite, such as worms (Gabbott et al., 2004; Farrell and Briggs, 2007), crinoids (Kammer and Ausich, 2007), and plant tissues (Grimes et al., 2001). Pyritization can occur by infilling of cellular cavities, by preferential replacement of more readily decomposed components, and by pyrite coatings on easily degraded soft parts (Canfield and Raiswell, 1991). The latter process may be aided by microbial coatings (Wuttke, 1983) on the decomposing tissues that help to stabilize chemical gradients around the decomposing material. Framboidal, clustered, and aggregated pyrites are the most commonly observed type in this style of preservation. Even microbial cells and textures can be preserved under the right circumstances (Figure 10). With regard to preservation fidelity, soft tissue preservation by pyrite does not preserve as much detail as preservation in a phosphate matrix.

Degree of pyritization (DOP)

A widely used proxy for paleo-oxygenation, DOP is defined (Raiswell et al., 1988; Raiswell and Canfield, 1998) as

$$\text{DOP} = \text{pyrite Fe} / (\text{pyrite Fe} + \text{"reactive iron"})$$



Iron Sulfide Formation, Figure 10 SEM images of microbial remains preserved in early diagenetic coated iron sulfide grains (ooids and oncoids) from the Ordovician Winnipeg Formation, Saskatchewan, Canada (from Schieber and Riciputi, 2005). (a) Filamentous structure (*white arrows*) in slightly etched iron sulfide cortex. Note multiple filaments in the upper part of the picture. In the uppermost part of the picture the filaments merge into a sheet-like structure, potentially remnant of a biofilm. (b) Typical recrystallized appearance of an outermost cortex surface (unetched). Most crystals are pyrite octahedra. Between these crystal pavements one finds remnant patches of an earlier surface ((c), unetched). The latter has a lumpy appearance and consists of tightly packed clusters (a few microns in size) of iron sulfide grains. Close-ups of these clusters ((d), unetched) show that they actually consist of an ovoid smooth walled structure (*black arrow*) that is overgrown by iron sulfide grains. These are interpreted as microbes that were preserved in an iron sulfide matrix. Image (e) (unetched) shows that the smooth surfaces of these ovoids are typically perforated by irregularly shaped openings ((e), *black arrows*), comparable to wall structures seen in experimentally pyritized microbes (Bubela and Cloud, 1983).

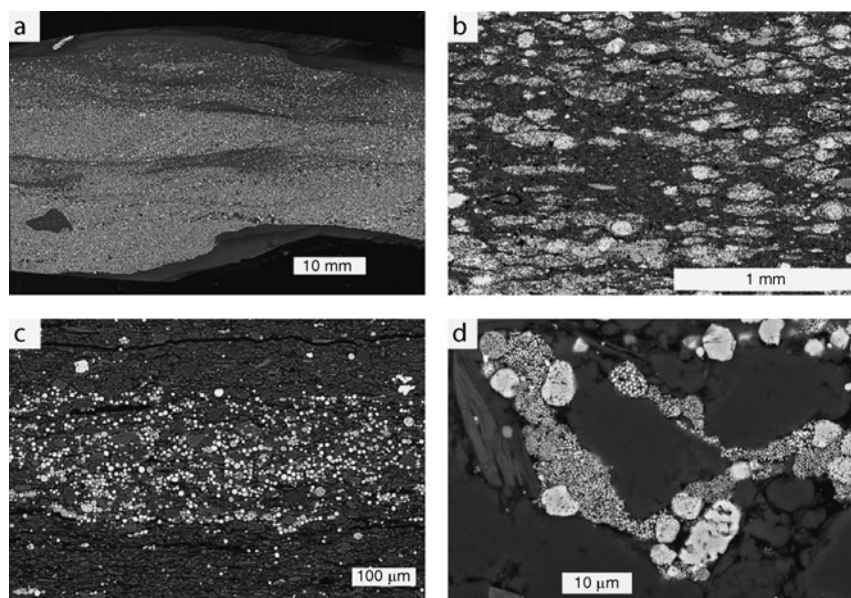
An extraction with dithionite or 1 N HCl is supposed to mimic reactive iron dissolution in reducing sediments (Canfield, 1989; Leventhal and Taylor, 1990). The highly reactive iron fraction in sediments is defined as the sum of dithionite or 1 N HCl leachable iron, and iron in acid volatile sulfides (AVS). What DOP presumably measures is the completeness of the conversion of reactive iron into pyrite via microbial sulfate reduction during early diagenesis. The underlying premise is that DOP increases as the degree of environmental oxygenation decreases. Because of the need for sufficient quantities of organic matter and reactive iron, measurements of DOP typically focus on carbonaceous mudstones. DOP values below 0.45 are considered indicative of aerobic bottom waters, and those above 0.45 are thought to mark restricted bottom water conditions. Values above 0.75 are considered to indicate anoxic or even euxinic bottom waters (Raiswell et al., 1988). However, the DOP method should not be applied uncritically, because limitations of either metabolizable organic matter or of reactive iron can lead to DOP values that are not reflective of the actual environment (as calibrated by Raiswell et al., 1988). Several other constraints on DOP application, such as outcrop weathering, maturation, and age of rock are summarized by Raiswell et al. (1988).

DOP as an environmental indicator can also be invalidated as a consequence of sedimentary processes. Any reworking and winnowing of pyrite bearing sediments will increase the pyrite content of the sediment, without simultaneously raising the reactive iron content, and as a result the DOP can rise simply as a result of wave or current reworking. In mudstones, indications of reworking at the mm to cm scale will often go undetected (Figure 11), and can lead to inflated DOP values that are incompatible with the levels of oxygen restriction suggested by observations of frequent reworking and bioturbation (Schieber, 2001, 2003).

Marcasite and its implications

Background on marcasite

Reports of marcasite in terrigenous clastics are mainly associated with coal deposits (e.g., Nayar, 1946; Read and Cook, 1969; Wiese et al., 1987). Marcasite occurs either in the coal seams themselves, or in the roof shales, prompting Krumbein and Garrels (1952) to propose an acidic bog/peat association as the characteristic environment for marcasite formation. There have, however, been a sufficient number of diagenetic marcasite occurrences reported from normal marine sediments (e.g., Maynard and Lauffenburger, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b, 2007), as well examples of marcasite that has inverted to pyrite (e.g., Bannister, 1932; Van Horn and Van Horn, 1933), to suggest that it might be more widespread in the marine realm than commonly appreciated. If this is in fact the case, and if acidic conditions are indeed a basic requirement for marcasite formation, we have an obvious problem to reconcile these



Iron Sulfide Formation, Figure 11 Reworked pyrite in shale successions. Image (a) is a photograph of a polished specimen, and all others are SEM photomicrographs (backscatter images). (a) Ripple cross-laminated lag deposit that consists mainly of spherical polyframboid fills of algal cysts (Schieber and Baird, 2001). Devonian Genesee Formation, New York, USA. (b) A horizon with abundant reworked flattened polyframboid clusters (comparable to those in Figure 6b). Devonian Chattanooga Shale, Tennessee, USA. (c) Lamina with abundant reworked pyrite framboids. Devonian Chattanooga Shale, Tennessee, USA. (d) Details of framboid-enriched lamina from (c). Shows how reworked pyrite framboids are filling spaces between larger silt grains and have been deformed due to compaction. Devonian Chattanooga Shale, Tennessee, USA.

basic realities with our current understanding of early diagenesis of marine sediments.

As pointed out above, the paragenetic relationships between sedimentary iron sulfide minerals are still poorly understood (Rickard and Morse, 2005). An essential guidepost for thinking about marcasite in sedimentary environments have been experiments conducted by Murowchick and Barnes (1986) that showed that marcasite is the dominant iron disulfide below pH 5. These experiments are consistent with results from much earlier work by Allen et al. (1914) and are also consistent with subsequent work by Schoonen and Barnes (1991a, b) and Benning et al. (2000). Thus, the relative abundance of pyrite versus marcasite in sediments should be a function of pH. Because marine waters have a slightly alkaline pH of approximately 8, it has been presumed that marcasite cannot form in marine sediments during early diagenesis (e.g., Rickard et al., 1995). Yet, regardless of this, early diagenetic marcasite unquestionably occurs in marine sedimentary rocks (Maynard and Lauffenburger, 1978; Siesser, 1978; Rykart, 1983; Jowett et al., 1991; Schieber, 2002b; Schieber and Riciputi, 2005; Williams et al., 2003).

Detecting marcasite

Characteristic morphologies, such as “spearhead” twins and “cockscorn” crystals can be used to identify marcasite. However, morphology does not tell us whether the

marcasite has inverted to pyrite and the approach does not work for dense masses of crystals. As long as crystallites are large enough, it is comparatively easy to differentiate marcasite from pyrite with a petrographic microscope in reflected light mode (Ramdohr, 1975), and it is also possible to detect marcasite that has inverted to pyrite (Murowchick, 1992). For micron scale grains a new technique, “electron backscatter diffraction” (EBSD), can be applied for identification with an electron microscope. EBSD allows phase identification of submicron mineral grains and is widely used in material sciences (Prior et al., 1999; Schwartz et al., 2000).

Marcasite in sedimentary rocks

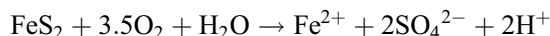
Marcasite is probably much more widespread in marine clastics than commonly assumed. It occurs as a cement mineral in marine lag deposits associated with sequence boundaries, in condensed intervals, and even in shales. Lag deposits with marcasite occur, for example, in Late Devonian shale successions of the eastern USA (Schieber, 2007). The Late Devonian Chattanooga and New Albany Shale successions contain laterally continuous erosion surfaces that compartmentalize this black shale succession into a stack of depositional sequences (Schieber, 1998a, b; Schieber and Lazar, 2004). These erosion surfaces imply substantial erosion and winnowing of previously deposited black shale during low stands of sea level (Schieber and Riciputi, 2004). The associated lag deposits contain

fish bone debris, conodonts, *Lingula* shells, quartz grains, and abundant pyrite in the form of reworked concretions and form conspicuous rust stained horizons in outcrop (Figure 12a).

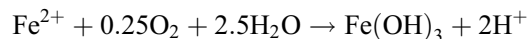
In polished thin section pore filling iron sulfides show typical marcasite morphology (Figure 12b). Overall, these lags may contain 30–40% iron sulfides by volume. Loose packing of framework grains indicates precompaction cementation with marcasite. Detrital pyrite grains in these lags are often variably rounded due to mechanical reworking and show etch and corrosion pits on the surface of detrital pyrite grains that are in turn infilled and overgrown by marcasite (Schieber, 2007).

Terrigenous clastic sediments contain at best a few percent reactive iron in the form of iron oxyhydroxide coatings on terrigenous grains (Carroll, 1958; Berner, 1969). Thus, the high iron concentrations in lag deposits with up to 40% iron sulfide require a mechanism for iron enrichment, and in the Phanerozoic at least the iron was most likely reworked from underlying strata (see above). In essence, sedimentary pyrite grains need to be eroded and winnowed in order to add more iron to the surficial layer, a process favored by stratigraphic condensation when net sedimentation rates are negative (Schieber and Riciputi, 2005; Schieber, 2007).

After mechanical enrichment, however, a portion of the sulfide iron has to be remobilized to form the observed overgrowths and pore-fill cements. Preexisting grains are destroyed, either wholly or partially, in order to supply dissolved (Fe^{2+}) or readily soluble iron (e.g., $\text{Fe}(\text{OH})_3$) for the growth of new iron sulfides. Corrosion features on reworked iron sulfide grains (Schieber and Riciputi, 2005; Schieber, 2007) are evidence for iron sulfide destruction according to the following reaction:



This reaction produces acidity and dissolved ferrous iron. Further oxidation of iron (see following equation) produces iron hydroxide and further acidity.

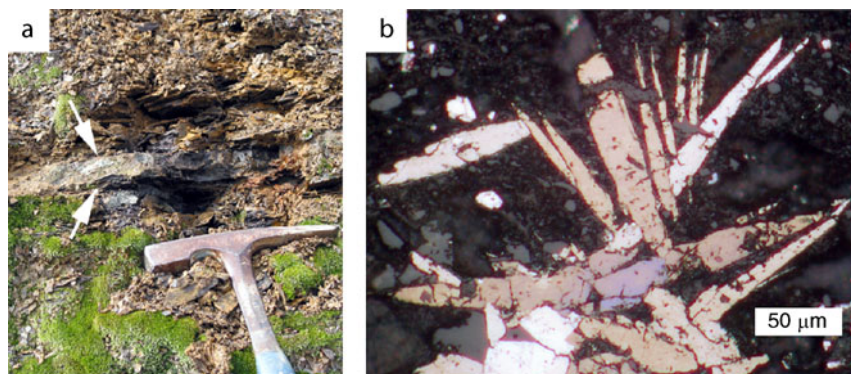


The newly formed iron hydroxides are likely to coat surface sediment grains and would remain in the surficial sediment layer. The associated lowering of pore water pH also promotes large pore water concentrations of Fe^{2+} (Maynard, 1983). Such a setting favors reprecipitation of marcasite in the presence of H_2S influx from underlying sediments (Murowchick and Barnes, 1986; Schoonen and Barnes, 1991a, b; Benning et al., 2000).

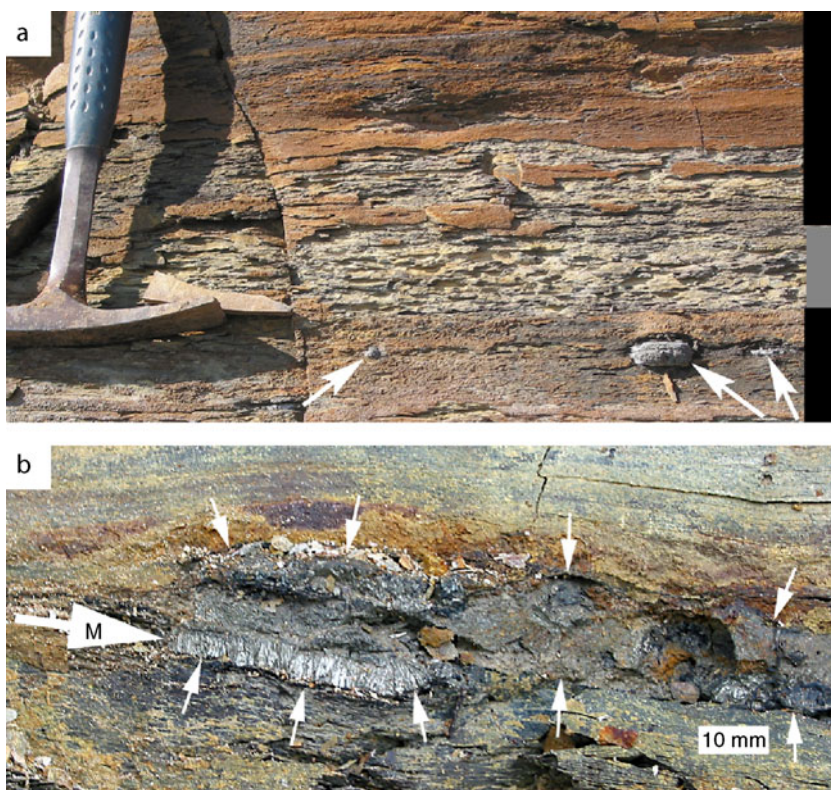
For sandy lags the presence of marcasite and partial dissolution of reworked pyrite grains are probably a good indicator that a “reoxidation” model for marcasite formation is applicable (Schieber and Riciputi, 2005; Schieber, 2007). In shales with abundant pyrite a comparable sequence of events may be caused by downward oxidation of previously deposited muds. Potential examples are stratiform horizons of iron sulfide concretions in black shale successions (Figure 13). Where these nodule horizons occur, a gray shale bed is typically found just above them (Figure 13a), and the concretions have a flattened radial fibrous habit, a morphology suggestive of marcasite (Figure 13b).

Reoxidation of reducing organic and iron sulfide-rich sediments, also termed “burndown,” has been studied extensively in the context of Quaternary Mediterranean sapropels (e.g., Jung et al., 1997), and marcasite in shales can potentially be used as a mineral-based indicator of burndown events.

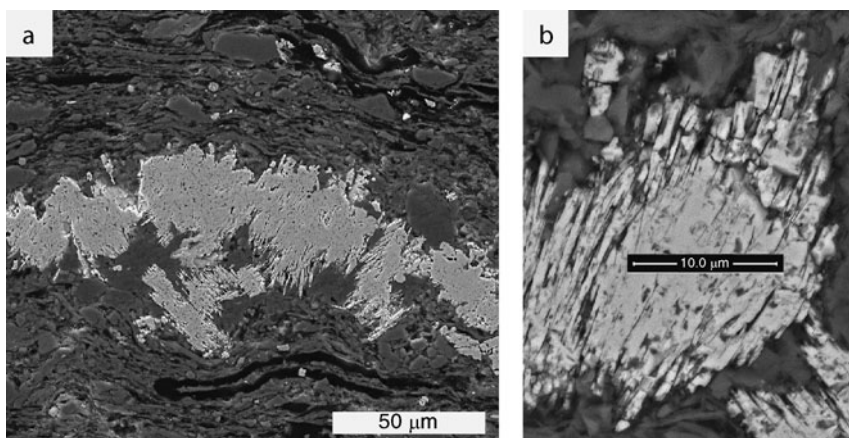
Although the generally finer grain size of diagenetic minerals in shales makes recognition of marcasite more difficult, EBSD analysis allows very tiny (micron-size)



Iron Sulfide Formation, Figure 12 Iron sulfide bearing lag deposits. (a) At left, an exposure of a sequence boundary lag (between arrows) in the late Devonian New Albany Shale. The lag is cemented by iron sulfides. Hammer for scale. (b) At right, a pore space in the lag with acicular “spearhead” twins, typical of marcasite. Electron backscatter diffraction (EBSD) analysis showed that many of the pore-fill iron sulfides still consist largely of marcasite.



Iron Sulfide Formation, Figure 13 Marcasite in late Devonian New Albany Shale, Kentucky, USA. (a) Stratiform horizon of marcasitic nodules (*white arrows*, black vs. gray shales marked by color bar at *right*). The nodules are located about 15 mm below the gray/black shale contact. The large amounts of iron sulfide accumulation at this level suggest that the redox interface stayed in that position for a long time, probably a reflection of zero to very low sedimentation. (b) Close-up of nodules that are broken through and show rims (*white arrows*) of radiating bladed marcasite (*large arrow M*). Note differential compaction.



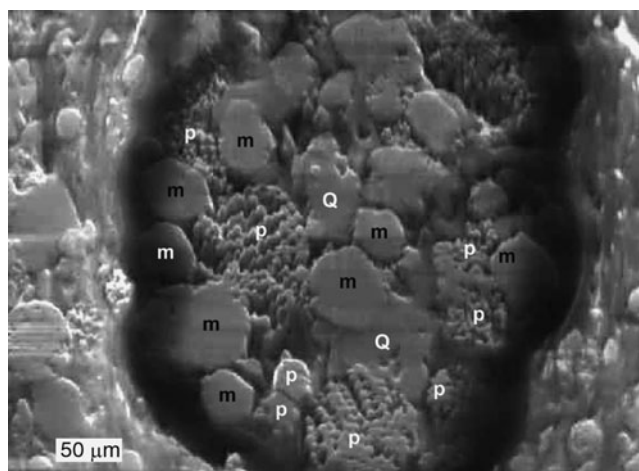
Iron Sulfide Formation, Figure 14 SEM photomicrographs (backscatter mode) of marcasite in an iron sulfide-enriched lamina in the Cleveland Shale (Upper Devonian, NE Kentucky). (a) Clusters of bladed marcasite grains that form bedding parallel lamina. Note differential compaction around marcasite clusters, suggesting formation when the sediment still had water content in excess of 80%. It also suggests that marcasite in the Cleveland Shale formed within 10–20 cm of the sediment–water interface. (b) Close-up that shows the platy habit of marcasite crystals (marcasite identification with EBSD). The reoxidation that is implicit in this observation contradicts earlier interpretations of this shale as euxinic (e.g., Jaminski et al., 1998; Rimmer, 2004).

marcasite grains to be identified *in situ*. A preliminary application of this technique to shales has detected marcasite in carbonaceous shales that range from Ordovician to Cretaceous in age (Figures 14 and 15).

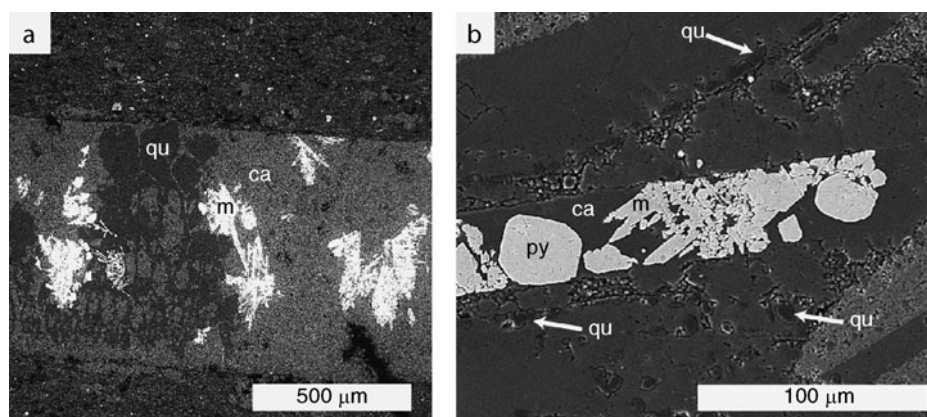
Unique fossil beds where preservation in pyrite and marcasite has been observed, such as Beecher's trilobite bed (Cisne, 1973) and pyritized ammonoids (Hudson, 1982; Seilacher et al., 1985), are another phenomenon where our "burndown" model could find application. The large stratiform iron buildup that at least some of these deposits represent requires a similar level of stratigraphic condensation as seen in some of the examples above.

Furthermore, iron needs to be mobile to form the observed mineralized and encrusted fossils. Thus, finding marcasite and dissolution features on reworked pyrite grains in pyritic Lagerstätten would support the view that such deposits may have formed during intermittent reoxygenation events.

The pH drop associated with pyrite oxidation during intermittent reoxygenation also explains textural features observed in association with iron sulfide replacement of calcareous shells. Examination of calcareous shell replacement in Carboniferous through Cretaceous mudstones showed marcasite as a common component,



Iron Sulfide Formation, Figure 15 Pyrite polyframboid cluster in Chattanooga Shale (Tennessee, USA) that formed within an algal cyst (*dark rim*). The infill was examined with EBSD. Marcasite (m) and quartz (Q) grew in the spaces between pyrite framboids (p). The open arrangement of framboids and the differential compaction around the cyst indicate that cementation happened prior to compaction. The marcasite observed here may well have been caused by intermittent pyrite oxidation in surface sediments. The relief in this image is an artifact, a consequence of oblique imaging (70° tilt) in EBSD mode.



Iron Sulfide Formation, Figure 16 SEM photomicrographs (backscatter) of marcasite replacing biogenic calcite. (a) Clam shell in Boquillas Formation (Cenomanian of West Texas). Skeletal marcasite crystals (marked m) grow as radiating clusters and replace calcite (ca). There is also partial replacement of the shell by quartz (marked qu). (b) Brachiopod shells in Barnett Shale (Mississippian of Texas). Shell in center is replaced by marcasite (marked m) that grows as bladed and sharply pointed crystals. Equant pyrite (py) formed toward end of iron sulfide deposition. Calcite shells marked ca, and replacing quartz marked qu.

intimately associated with calcite dissolution and precipitation of diagenetic quartz (Figure 16). This mineralogical triumvirate makes chemical sense because marcasite formation requires low pH, calcite dissolves at low pH, and dissolved silica (from opaline tests) precipitates under low pH conditions.

With regard to carbonate rocks, most published reports of marcasite are from chalks (e.g., Morgan-Jones, 1977; Kelts, 1976). This marcasite is typically in nodule form, measures up to 10 cm across, and shows crystals with marcasite morphology. Nonetheless, XRD analysis frequently shows that this marcasite has inverted to pyrite. There also seems to be an association with phosphate concretions, and thus with reworking, negative net sedimentation, and geochemical “reworking” of iron, just like that observed in marcasite occurrences in sandstones and shales.

Conclusions

The geobiological significance of sedimentary iron sulfides is far reaching. Microbial sulfate reduction as a metabolic pathway is of great antiquity and dates back to at least the early Archean (3.47-Ga; Shen and Buick, 2004). From that time onward, global cycling of sulfur and carbon has been essential for controlling the amount of oxygen in the Earth’s atmosphere (Berner, 2001), and some have even speculated that the emergence of life itself is tied to sulfur chemistry on the early Earth (e.g., Russell and Hall, 1997). It also appears that in itself oxygen buildup during the Precambrian led to the evolution of increasingly complex biochemical networks among microbes and eventually even to the evolution of complex life forms (Raymond and Segrè, 2006). The iron sulfides that we find in the rock record are a substantial part of the raw data for understanding the complex interaction of life with the atmosphere and the oceans. Every bit of that record, be it in the form of isotope shifts (e.g., Goldhaber, 2003), proportions and distribution of different iron sulfides, sulfide textures, stratal distribution, enclosed and preserved micro- and macro-fossils, as well as subsequent alterations of these minerals, is valuable for understanding chemical changes that affected the biosphere on the local, global, and temporal scales.

For the rock record, the enduring product of all these processes is the iron sulfide pyrite (FeS_2). Yet, the dimorph of pyrite, marcasite, can still be recognized in sedimentary rocks as old as Proterozoic (personal observations) and probably played a much larger role than commonly appreciated. In the minds of many geologists, the presence of iron sulfides in sedimentary rocks is associated with anoxic environmental conditions. In reality, however, once iron sulfides begin to form localized concentrations, be it in the form of micron-size framboids or fist-size concretions, one can imply that oxygen must have been close by. As elaborated repeatedly above, in order to form even microscopic features like pyrite framboids, iron should be able to migrate through the pore waters, and

those conditions only occur near the redox interface that “separates” deeper sulfidic from shallower oxygenated pore waters. Fundamentally, this holds true even in the most classical anoxic-euxinic locale of all, the Black Sea. There, pyrite framboids do not form in the sulfidic bottom waters, but form again at the redox interface where these waters come in contact with oxygenated surface waters (Wilkin et al., 1996). The bulk of sedimentary iron sulfides in the rock records marks the interface between the anoxic and oxic worlds; they should not be taken as an indicator of environmentally pervasive anoxia.

Bibliography

- Allard, T., Menguy, N., Salomon, J., Calligaro, T., Weber, T., Calas, G., and Benedetti, M. F., 2004. Revealing forms of iron in river-borne material from major tropical rivers of the Amazon Basin (Brazil). *Geochimica et Cosmochimica Acta*, **68**, 3079–3094.
- Allen, E. T., Crenshaw, J. L., and Merwin, H. E., 1914. Effect of temperature and acidity in the formation of marcasite (FeS_2) and wurzite (ZnS). *American Journal of Science*, **38**, 393–421.
- Aller, R. C., Mackin, J. E., and Cox, R. T., 1986. Diagenesis of Fe and S in Amazon inner shelf muds: apparent dominance of Fe reduction and implications for the genesis of ironstones. *Continental Shelf Research*, **6**, 263–289.
- Baird, G. C., and Brett, C. E., 1991. Submarine erosion on the anoxic sea floor: Stratigraphic, palaeoenvironmental, and temporal significance of reworked pyrite-bone deposits. In Tyson, R. V., and Pearson, T.H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. Geological Society of London, Special Publication, 58, pp. 233–257.
- Bannister, F. A., 1932. The distinction of pyrite from marcasite in nodular growths. *Mineralogical Magazine*, **23**, 179–187.
- Benning, L. G., Wilkin, R. T., and Barnes, H. L., 2000. Reaction pathways in the Fe-S system below 100°C. *Chemical Geology*, **167**, 25–51.
- Berner, R. A., 1967. Thermodynamic stabilities of sedimentary iron sulfides. *American Journal of Science*, **265**, 773–785.
- Berner, R. A., 1969. Migration of iron and sulfur within anaerobic sediments during early diagenesis. *American Journal of Science*, **267**, 19–42.
- Berner, R. A., 1970. Sedimentary pyrite formation. *American Journal of Science*, **268**, 1–23.
- Berner, R. A., 1981. A new geochemical classification of sedimentary environments. *Journal of Sedimentary Petrology*, **51**, 359–365.
- Berner, R. A., 1984. Sedimentary pyrite formation: an update. *Geochimica et Cosmochimica Acta*, **48**, 605–615.
- Berner, R. A., 2001. Modeling atmospheric O_2 over Phanerozoic time. *Geochimica et Cosmochimica Acta*, **65**, 685–694.
- Brett, C., and Allison, P. A., 1998. Paleontological approaches to the environmental interpretation of marine mudrocks. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones (Vol. 1): Basin Studies, Sedimentology and Paleontology*. Stuttgart: Schweizerbart’sche Verlagsbuchhandlung, pp. 301–349.
- Briggs, D. E. G., Bottrell, S. H., and Raiswell, R., 1991. Pyritization of soft-bodied fossils; Beecher’s Trilobite Bed, Upper Ordovician, New York State. *Geology*, **9**, 1221–1224.
- Bubela, B., and Cloud, P., 1983. Sulfide mineralization of microbial cells. *Bureau of Mineral Resources, Journal of Australian Geology and Geophysics*, **8**, 355–357.
- Butler, I. B., and Rickard, D., 2000. Framboidal pyrite formation via the oxidation of iron (II) monosulphide by hydrogen sulphide. *Geochimica et Cosmochimica Acta*, **64**, 2665–2672.

- Calvert, S. E., 1976. The mineralogy and geochemistry of near-shore sediments. In Ripley, J. P., and Chester, R. (eds.), *Chemical Oceanography*. New York: Academic, Vol. 6, pp. 187–280.
- Canfield, D. E., 1989. Reactive iron in marine sediments. *Geochimica et Cosmochimica Acta*, **53**, 619–632.
- Canfield, D. E., and Raiswell, R., 1991. Pyrite formation and fossil preservation. In Allison, P. A., and Briggs, D. E. (eds.), *Taphonomy: Releasing the Data Locked in the Fossil Record*. New York: Plenum, pp. 337–387.
- Carroll, D., 1958. Role of clay minerals in the transportation of iron. *Geochimica et Cosmochimica Acta*, **14**, 1191–1206.
- Chester, R., and Asten, S. R., 1976. The geochemistry of deep-sea sediments. In Ripley, J. P., and Chester, R. (eds.), *Chemical Oceanography*. New York: Academic, Vol. 6, pp. 281–291.
- Cisne, J. L., 1973. Anatomy of *Triarthrus* and the relationships of the Trilobita. *Fossils and Strata*, **4**, 45–64.
- Clark, G. R., and Lutz, R. A., 1980. Pyritization in the shells of living bivalves. *Geology*, **8**, 268–271.
- Conway Morris, S., 1986. The community structure of the Middle Cambrian phyllopod bed (Burgess Shale). *Paleontology*, **29**, 423–458.
- Curtis, C. D., Cope, J. C. W., Plant, D., and Macquaker, J. H. S., 2000. “Instantaneous” sedimentation, early microbial sediment strengthening and a lengthy record of chemical diagenesis preserved in Lower Jurassic ammonitiferous concretions from Dorset. *Journal of the Geological Society (London)*, **157**, 165–172.
- Dekkers, M. J., and Schoonen, M. A. A., 1996. Magnetic properties of hydrothermally synthesized greigite (Fe_3S_4): I. Rock magnetic properties at room temperature. *Geophysical Journal International*, **126**, 360–368.
- Farrell, U. C., and Briggs, D. E. G., 2007. A pyritized polychaete from the Devonian of Ontario. *Proceedings: Biological Sciences*, **274**, 499–504.
- Gabbott, S. E., Hou, X. G., Norry, J. N., and Siveter, D. J., 2004. Preservation of Early Cambrian animals of the Chengjiang biota. *Geology*, **32**, 901–904.
- Gibbs, R. J., 1977. Transport phases of transition metals in the Amazon and Yukon Rivers. *Geological Society of America Bulletin*, **88**, 829–843.
- Goldhaber, M. B., 2003. Sulfur-rich sediments. In Mackenzie, F. T. (ed.), *Treatise on Geochemistry, Vol 7, Sediments, Diagenesis, and Sedimentary Rocks*. Amsterdam: Elsevier, pp. 257–288.
- Goldhaber, M. B., and Kaplan, I. R., 1974. The sulfur cycle. In Goldberg, E. D. (ed.), *The Sea*. New York: Wiley, Vol. 5, 569–655.
- Grimes, T. G., Brock, F., Rickard, D., Davies, K. L., Edwards, D., Briggs, D. E. G., and Parkes, R. J., 2001. Understanding fossilization: experimental pyritization of plants. *Geology*, **29**, 123–126.
- Hudson, J. D., 1982. Pyrite in ammonite-bearing shales from the Jurassic of England and Germany. *Sedimentology*, **29**, 639–667.
- Ingri, J., and Ponter, C., 1986. Iron and manganese layering in recent sediments of the Gulf of Bothnia. *Chemical Geology*, **56**, 105–116.
- Jaminski, J., Algeo, T. J., Maynard, J. B., and Hower, J. C., 1998. Climatic origin of dm-scale compositional cyclicity in the Cleveland Member of the Ohio Shale (Upper Devonian), central Appalachian Basin, U.S.A. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones I*. Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Näglele u. Obermiller), pp. 217–242.
- Jowett, E. C., Roth, T., Rydzewski, A., and Oszczepalski, S., 1991. “Background” delta 34S values of Kupferschiefer sulphides in Poland: pyrite-marcasite nodules. *Mineralium Deposita*, **26**, 89–98.
- Jung, M., Ilmberger, J., Mangini, A., and Emeis, K. C., 1997. Why some Mediterranean sapropels survived burn-down (and others did not). *Marine Geology*, **141**, 51–60.
- Kalliokoski, J., 1974. Pyrite framboids: animal, vegetable, or mineral? *Geology*, **2**, 26–27.
- Kammer, T. W., and Ausich, W. I., 2007. Soft-tissue preservation of the hind gut in a new genus of cladid crinoid from the Mississippian (Visian, Asbian) at St. Andrews, Scotland. *Palaeontology*, **50**, 951–959.
- Kaplan, I. R., Emery, K. O., and Rittenberg, S. C., 1963. The distribution and isotopic abundance of sulfur in recent marine sediments off southern California. *Geochimica et Cosmochimica Acta*, **27**, 297–331.
- Kelts, K. R., 1976. *Marcasite in Miocene calcareous sediments from Hole 315A. Deep Sea Drilling Project Initial Reports*, **33**, 867–870.
- Kobayashi, K., and Nomura, M., 1972. Iron sulfides in sediment cores from the sea of Japan and their geophysical implications. *Earth and Planetary Science Letters*, **16**, 200–208.
- Krs, M., Krsova, M., Pruner, P., Zeman, A., Novak, F., and Jansa, J., 1990. A petromagnetic study of Miocene rocks bearing micro-organic material and the magnetic mineral greigite (Sokolov and Cheb basins, Czechoslovakia). *Physics of the Earth and Planetary Interiors*, **63**, 98–112.
- Krumbein, W. C., and Garrels, R. W., 1952. Origin and classification of chemical sediments in terms of pH and oxidation-reduction potentials. *The Journal of Geology*, **60**, 1–33.
- Leventhal, J. S., and Taylor, C., 1990. Comparison of methods to determine degree of pyritization. *Geochimica et Cosmochimica Acta*, **54**, 2621–2625.
- Love, L. G., 1967. Early diagenetic iron sulphide in recent sediments of the Wash (England). *Sedimentology*, **9**, 327–352.
- Maynard, J. B., 1983. *Geochemistry of Sedimentary Ore Deposits*. New York: Springer, 305 p.
- Maynard, J. B., and Lauffenburger, S. K., 1978. A marcasite layer in prodelta turbidites of the Borden Formation (Mississippian): in eastern Kentucky. *Southeastern Geology*, **20**, 47–58.
- Morgan-Jones, M., 1977. Mineralogy of the non-carbonate material from the chalk of Berkshire and Oxfordshire, England. *Clay Minerals*, **12**, 331–344.
- Morse, J. W., Millero, F. J., Cornwell, J. C., and Rickard, D., 1987. The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews*, **24**, 1–42.
- Murowchick, J. B., 1992. Marcasite inversion and the petrographic determination of pyrite ancestry. *Economic Geology*, **87**, 1141–1152.
- Murowchick, J. B., and Barnes, H., 1986. Marcasite precipitation from hydrothermal solutions. *Geochimica et Cosmochimica Acta*, **50**, 2615–2629.
- Murowchick, J. B., and Barnes, H. L., 1987. Effects of temperature and degree of supersaturation on pyrite morphology. *American Mineralogist*, **72**, 1241–1250.
- Nardi, S., Binda, P. L., Bacelle, L. S., and Concheri, G., 1994. Amino acids of Proterozoic and Ordovician sulfide-coated grains from western Canada: record of biologically mediated pyrite precipitation. *Chemical Geology*, **111**, 1–15.
- Nayar, K. V., 1946. Marcasite in Travancore lignite. *Current Science*, **5**, 229.
- Ohfuji, H., Butler, I. B., and Rickard, D., 2003. Experimental study of synthetic pyrite framboids and other morphologies. *Geochimica et Cosmochimica Acta*, **67**(Suppl. 1), A351.
- Ohfuji, H., Boyle, A. P., Prior, D. J., and Rickard, D., 2005. Structure of framboidal pyrite: An electron backscatter diffraction study. *American Mineralogist*, **90**, 1693–1704.
- Prior, D. J., Boyle, A. P., Brenker, F., Cheadle, M. C., Day, A., Lopez, G., Peruzzo, L., Potts, G. J., Reddy, S. M., Spiess, R., Timms, N. E., Trimby, P. W., Wheeler, J., and Zetterstrom, L.,

1999. The application of electron backscatter diffraction and orientation contrast imaging in the SEM to textural problems in rocks. *American Mineralogist*, **84**, 1741–1759.
- Raiswell, R., and Canfield, D. E., 1998. Sources of iron for pyrite formation. *American Journal of Science*, **298**, 219–245.
- Raiswell, R., Buckley, F., Berner, R. A., and Anderson, T. F., 1988. Degree of pyritization of iron as a paleoenvironmental indicator of bottom water oxygenation. *Journal of Sedimentary Petrology*, **58**, 812–819.
- Ramdohr, P., 1975. *Die Erzminerale und ihre Verwachsungen*. Berlin: Akademie, 1277 p.
- Raymond, J., and Segrè, D., 2006. The effect of oxygen on biochemical networks and the evolution of complex life. *Science*, **311**, 1764–1767.
- Read, H. W., and Cook, A. C., 1969. Note on coals containing marcasite plant petrifications, Yarrunga creek, Sydney Basin, New South Wales. *Journal and Proceedings of the Royal Society of New South Wales*, **102**, 197–199.
- Reaves, C. M., 1984. *The Migration of Iron and Sulfur During the Early Diagenesis of Marine Sediments*. PhD thesis, Yale University, New Haven.
- Rickard, D., and Morse, J. W., 2005. Acid volatile sulfide (AVS). *Marine Chemistry*, **97**, 141–198.
- Rickard, D., Schoonen, M. A. A., and Luther, G. W., 1995. Chemistry of iron sulfides in sedimentary environments. In Vairavamurthy, M. A., and Schoonen, M. A. A. (eds.), *Geochemical Transformations of Sedimentary Sulfur*. ACS Symposium Series 612. Washington, pp. 165–193.
- Rimmer, S. M., 2004. Geochemical paleoredox indicators in Devonian-Mississippian black shales, Central Appalachian Basin (USA). *Chemical Geology*, **206**, 373–391.
- Russell, M. J., and Hall, A. J., 1997. The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *Journal of the Geological Society (London)*, **154**, 377–402.
- Rykart, R., 1983. “Gilded” petrifications; formation of pyrite and marcasite in bituminous shale. *Mineralien Magazin*, **7**, 280–284.
- Schieber, J., 1998a. Sedimentary features indicating erosion, condensation, and hiatuses in the Chattanooga Shale of Central Tennessee: relevance for sedimentary and stratigraphic evolution. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones I*. Stuttgart: E. Schweizerbart’sche Verlagsbuchhandlung (Nägelé u. Obermiller), pp. 187–215.
- Schieber, J., 1998b. Developing a sequence stratigraphic framework for the Late Devonian Chattanooga Shale of the southeastern US: relevance for the Bakken shale. In Christopher, J. E., Gilboy, C. F., Paterson, D. F., and Bend, S. L. (eds.), *Eighth International Williston Basin Symposium*. Saskatchewan Geological Society Special Publication, Vol.13, pp. 58–68.
- Schieber, J., 2001. Ways in which organic petrology could contribute to a better understanding of black shales. *International Journal of Coal Geology*, **47**, 171–187.
- Schieber, J., 2002a. Sedimentary pyrite: a window into the microbial past. *Geology*, **30**, 531–534.
- Schieber, J., 2002b. The role of an organic slime matrix in the formation of pyritized burrow trails and pyrite concretions. *Palaios*, **17**, 104–109.
- Schieber, J., 2003. Simple gifts and hidden treasures – implications of finding bioturbation and erosion surfaces in black shales. *The Sedimentary Record*, **1**, 4–8.
- Schieber, J., 2007. Oxydation of detrital pyrite as a cause for Marcasite formation in Marine Lag Deposits from the Devonian of the Eastern US. *Deep Sea Research II*, **54**, 1312–1326.
- Schieber, J., and Baird, G., 2001. On the origin and significance of pyrite spheres in Devonian black shales of North America. *Journal of Sedimentary Research*, **71**, 155–166.
- Schieber, J., and Lazar, O. R., 2004. Devonian black shales of the eastern U.S. New insights into sedimentology and stratigraphy from the subsurface and outcrops in the Illinois and Appalachian Basins. *Indiana Geological Survey Open-File Study* 04–05, 90 p.
- Schieber, J., and Riciputi, L., 2004. Pyrite ooids in Devonian black shales record intermittent sea level drop and shallow water conditions. *Geology*, **32**, 305–308.
- Schieber, J., and Riciputi, L., 2005. Pyrite-marcasite coated grains in the Ordovician Winnipeg Formation, Canada: an intertwined record of surface conditions, stratigraphic condensation, geochemical “reworking”, and microbial activity. *Journal of Sedimentary Research*, **75**, 905–918.
- Schieber, J., and Schimmelmann, A., 2006. High resolution pyrite framboid size distribution in Santa Barbara Basin sediments: implications for the study of black shales. *Eos Transactions, AGU*, **87**(36), Ocean Science Meeting Supplement, Abstract OS46A-11.
- Schneiderhöhn, H., 1923. Chalkographische Untersuchungen des Mansfelder Kupferschiefers. *Neues Jahrbuch für Mineralogie*, **47**, 1–38.
- Schoonen, M. A. A., and Barnes, H. L., 1991a. Reactions forming pyrite and marcasite from solution. I. Nucleation of FeS₂ below 100°C. *Geochimica et Cosmochimica Acta*, **55**, 1495–1504.
- Schoonen, M. A. A., and Barnes, H. L., 1991b. Reactions forming pyrite and marcasite from solution. II. Via FeS precursor below 100°C. *Geochimica et Cosmochimica Acta*, **55**, 1505–1514.
- Schwartz, A. J., Kumar, M., and Adams, B. L., 2000. *Electron Backscatter Diffraction in Materials Science*. Berlin: Springer, 350 p.
- Seilacher, A., Reif, W. E., Westphal, F., Riding, R., Clarkson, E. N. K., and Whittington, H. B., 1985. Sedimentological, ecological and temporal patterns of fossil Lagerstätten [and Discussion]. Extraordinary fossil biotas: their ecological and evolutionary significance. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **311**(1148), 5–24.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth-Science Reviews*, **64**, 243–272.
- Siesser, W. G., 1978. Petrography and geochemistry of pyrite and marcasite in DSDP Leg 40 sediments. *Initial Reports of the Deep Sea Drilling Project*, Supplement to Vols. 38, 39, 40, and 41, 767–775.
- Srivastava, N. K., 1975. Early diagenetic changes in recent molluscan shells. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **148**, 380–403.
- Strauss, H., and Schieber, J., 1990. A sulfur isotope study of pyrite genesis: the mid-Proterozoic Newland Formation, Belt Supergroup, Montana. *Geochimica et Cosmochimica Acta*, **54**, 197–204.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry*. New York: Wiley, 1022 p.
- Stürmer, W., 1985. A small coleoid cephalopod with soft parts from the Lower Devonian discovered using X-ray radiography. *Nature*, **318**, 53–54.
- Sweeney, R. E., and Kaplan, I. R., 1973. Diagenetic sulfate reduction in marine sediments. *Economic Geology*, **68**, 618–634.
- Taylor, S. R., and McLennan, S. M., 1985. *The Continental Crust: Its Composition and Evolution*. Oxford: Blackwell, 312 p.
- Trefrey, J. H., and Presley, B. J., 1982. Manganese fluxes from Mississippi Delta sediments. *Geochimica et Cosmochimica Acta*, **46**, 1715–1726.
- Van Horn, F. R., and Van Horn, K. R., 1933. X-ray study of pyrite of marcasite concretions in the rocks of the Cleveland, Ohio, quadrangles. *American Mineralogist*, **18**, 288–294.
- Wiese, R. G., Powell, M. A., and Fyfe, W. S., 1987. Spontaneous formation of hydrated iron sulfates on laboratory samples of pyrite- and marcasite-bearing coals. *Chemical Geology*, **63**, 29–38.

- Wignall, P. B., and Newton, R., 1998. Pyrite framboid diameter as a measure of oxygen deficiency in ancient mudrocks. *American Journal of Science*, **298**, 537–552.
- Wilkin, R. T., and Barnes, H. L., 1996. Pyrite formation by reactions of iron monosulfides with dissolved inorganic and organic sulfur species. *Geochimica et Cosmochimica Acta*, **61**, 323–339.
- Wilkin, R. T., and Barnes, H. L., 1997. Formation processes of framboidal pyrite. *Geochimica et Cosmochimica Acta*, **61**, 323–339.
- Wilkin, R. T., Barnes, H. L., and Brantley, S. L., 1996. The size distribution of framboidal pyrite in modern sediments: an indicator of redox conditions. *Geochimica et Cosmochimica Acta*, **60**, 3897–3912.
- Wilkin, R. T., Arthur, M. A., and Dean, W. E., 1997. History of water-column anoxia in the Black Sea indicated by pyrite framboid size distributions. *Earth and Planetary Science Letters*, **148**, 517–525.
- Williams, M. L., Crossey, L. J., Jercinovic, M. D., Bloch, J. D., Karlstrom, K. E., Dehler, C. M., Heizler, M. T., Bowring, S. A., and Goncalves, P., 2003. Dating sedimentary sequences; in situ U/Th–Pb microprobe dating of early diagenetic monazite and Ar–Ar dating of marcasite nodules; case study from Neoproterozoic black shales in the Southwestern US. Geological Society of America, Abstracts with Programs, vol. 35/6, p. 595.
- Wuttke, M., 1983. “Weichteil-Erhaltung” durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus den Ölschiefern der “Grube Messel” bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

Acid Rock Drainage
 Anaerobic Transformation Processes, Microbiology
 Biofilms and Fossilization
 Black Shales
 Isotopes and Geobiology
 Microbial Degradation
 Pore Waters
 Pyrite Oxidation
 Pyritization
 Sediment Diagenesis – Biologically Controlled Shales
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Sulfur Cycle

ISOTOPE FRACTIONATION (METAL)

Ariel D. Anbar¹, Silke Severmann²

¹Arizona State University, Tempe, AZ, USA

²Rutgers University, New Brunswick, NJ, USA

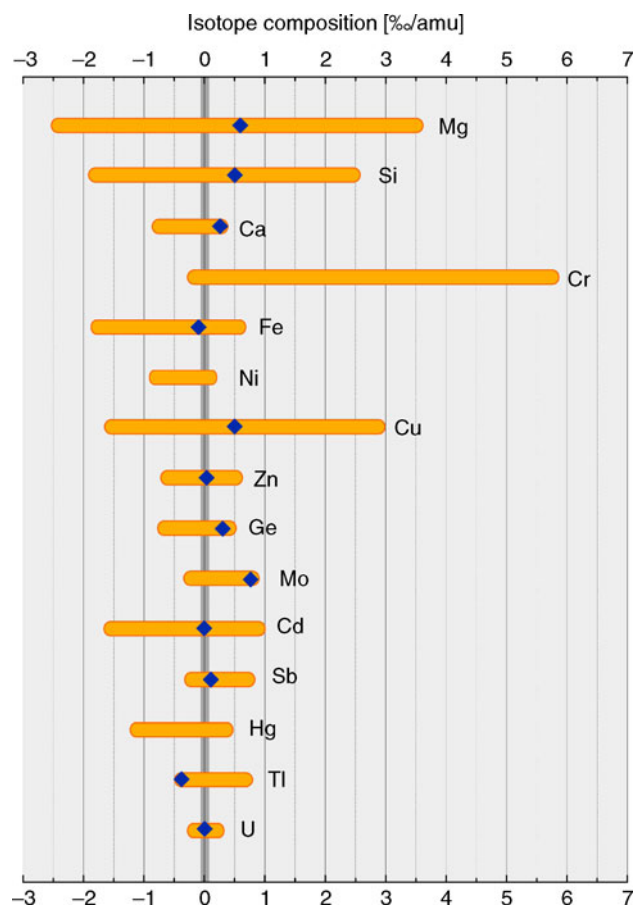
Definition

Variations in the abundances of the isotopes of metals, especially transition metals, arising from physical or chemical processes that discriminate among the isotopes of a given element.

Overview

Research into isotope fractionation of transition metals, alkaline earth metals, and metalloids is advancing rapidly

as a result of analytical developments. As of this writing, fractionations have been reported for more than a dozen such elements (Figure 1). These investigations are heavily motivated by geobiological considerations. In particular, there is wide interest in the use of these new isotope systems as biosignatures and as proxies for the



Isotope Fractionation (Metal), Figure 1 Observed range of isotopic variations in modern and ancient natural samples for a selection of metals and metalloids. All values are expressed in delta notation [parts per 1,000 (‰) per atomic mass unit], normalized to average Bulk Silicate Earth (indicated by the gray vertical line). Blue diamonds indicate the average isotope composition of modern seawater, where data are available. Data sources: Young and Galy (2004) (Mg); De La Rocha (2003); Robert and Chaussidon (2006) (Si); DePaolo (2004) (Ca); Ellis et al. (2002); Johnson and Bullen (2004); Schoenberg et al. (2008) (Cr); Dauphas and Rouxel (2006); Lacan et al. (2008); Johnson et al. (2008a) (Fe); Cameron et al. (2009) (Ni); Shields et al. (1965); Maréchal et al. (1999), Vance (2008) #1986 (Cu); Mason et al. (2005); Bermin et al. (2006); Gioia et al. (2008) (Zn); Rouxel et al. (2006a); Siebert et al. (2006a) (Ge); Anbar (2004); Poulson et al. (2006) (Mo); Wombacher et al. (2003); Ripperger and Rehkämper (2007); Schmitt et al. (2009) (Cd); Rouxel et al. (2003) (Sb); Foucher and Hintelmann (2006); Smith et al. (2008) (Hg); Rehkämper et al. (2002); Nielsen et al. (2006) (Tl); Stirling et al. (2007); Weyer et al. (2007, 2008) (U).

biogeochemical cycling of metals in modern and ancient settings. This article briefly reviews the analytical issues in historical context and the state of geobiological applications.

Historical and analytical context

Investigations into natural variations in the isotope compositions of transition metals and other metallic elements began in the middle of the twentieth century (Valley and Anderson, 1947; Shields et al., 1965), in parallel with research into isotope variations of non-metals (Urey, 1947; Bigeleisen, 1965). Most of this early metal isotope research focused on variations arising from radioactive decay for geochronology, i.e., “radiogenic isotope geochemistry,” whereas non-metal isotope research was dominated by investigations of the mechanisms and applications of mass-dependent fractionation (esp. of C, O, N, and S), which came to be called “stable isotope” geochemistry.

This bifurcation was largely a consequence of the different types of mass spectrometry (MS) most readily used for these two classes of elements. Because most metals do not readily form volatile compounds, analyses of their isotope compositions were conducted primarily using thermal ionization MS (TIMS), whereby purified samples are placed on metal filaments which are heated under vacuum to form thermal ions, which are then introduced into a mass analyzer (typically, a magnetic sector). This ionization method itself induces mass-dependent isotope fractionation of a magnitude comparable to natural variations. The effects of such fractionations can be precisely corrected by external or internal normalization schemes (Wasserburg et al., 1981), but these schemes also “correct” for any natural mass-dependent fractionation. As a result, TIMS is best suited for the analysis of isotope variations arising from non-mass-dependent processes, notably radioactive decay or nucleosynthetic anomalies. Precise measurements of mass dependent isotope fractionation using TIMS are possible, but require use of double spiking procedures (Russell et al., 1978; Albarède and Beard, 2004).

In contrast, isotope variations of non-metals were (and still are) typically measured using gas source electron impact MS, taking advantage of the ease with which many of these elements are converted to gas phase compounds such as H₂O or CO₂. An electron beam can readily ionize these gases. This MS method lends itself to an analytical scheme in which gases derived from samples and standards are introduced in rapid alternation to the same instrument. Such “sample-standard bracketing,” not possible with TIMS, corrects for mass fractionation induced by the mass spectrometer without compromising the ability to detect preexisting natural isotope variations. Hence, stable isotope studies of mass-dependent effects flourished for the most abundant non-metal elements.

In the final years of the twentieth century and the first decade of the twenty-first, the development of inductively

coupled plasma source MS coupled to multiple ion “collectors” (MC-ICP-MS) made it possible to easily ionize transition metals and other elements using procedures that allowed for precise determination of mass-dependent isotope variations (Walder and Freedman, 1992; Halliday et al., 1995; Maréchal et al., 1999). Specifically, in ICP-MS samples are introduced to the mass spectrometer by nebulization of aqueous solutions into a high-energy plasma (Houk and Thompson, 1988). This technique readily ionizes most elements, and is amenable to sample-standard bracketing analogous to that employed for traditional stable isotope analyses (as well as double spiking). The combination of ICP ionization, magnetic sector mass analyzers, and multiple ion collectors, greatly increased the precision of such analyses. High mass resolution (Weyer and Schwieters, 2003), collision cells (Turner et al., 1998), and specialized nebulizers (Belshaw et al., 2000) solved analytical problems specific to particular elements such as Fe. These developments made it practical to examine physical and chemical fractionations of the isotopes of many metals (reviewed in Johnson et al., 2004a; Anbar and Rouxel, 2007; Weiss et al., 2008). This research led to the discovery that such variations are ubiquitous in nature, easily produced in the lab, and relevant to geobiological applications.

Variations in isotope ratios of these elements are typically of order 0.1–1‰, and so isotope compositions are usually reported as ‰ deviations from a reference standard, using the classic “ δ notation” (some studies report compositions as parts-per-ten-thousand deviations, using the analogous “ ϵ notation”; e.g., Zhu et al., 2000; Rouxel et al., 2003; Rehkämper et al., 2004; Lacan et al., 2006; Stirling et al., 2007). Analytical precision is now routinely $\pm 0.05\text{‰}$ or better for most elements, although reproducibility of natural samples is often not as good as that of pure standards due to complications arising from impurities even in chemically processed samples.

Biosignatures

Much of the research into metal isotope fractionation is motivated by the possibility that Fe isotopes, in particular, could prove useful as biosignatures in ancient materials. This idea builds on an analogy with carbon, nitrogen, and sulfur isotopes. These elements are fractionated as a result of biologically mediated redox chemistry, which dominates the biogeochemical cycling of these elements (e.g., Rankama, 1948; Craig, 1953b; Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Wada and Hattori, 1976a, b; Altabet and Francois, 1994; Canfield, 2001; Horita, 2005). Fe oxidation and reduction at the Earth’s surface is likewise biologically catalyzed, by the actions of dissimilatory bacteria that derive metabolic energy from redox reactions involving Fe.

Fe isotope fractionation has been documented to occur during dissimilatory reduction of Fe(III) phases such as hydrous ferric oxide and hematite by bacteria including strains of the genera *Shewanella* and *Geobacter* (Beard

et al., 1999; Crosby et al., 2007). Fe isotopes are also fractionated during oxidation of dissolved Fe(II) and precipitation of Fe(III)-oxides mediated by *Acidithiobacillus ferrooxidans* (Balci et al., 2006) and an anoxygenic photoautotroph of the genus *Thiodictyon* (Croal et al., 2004). In all cases, Fe(II) is isotopically lighter by 1.3–3‰ relative to Fe(III)-oxide (here, Fe isotope variations are reported in terms of $^{56}\text{Fe}/^{54}\text{Fe}$). A dissolved Fe isotope profile from the Southern Ocean – the first data of its kind – shows small variations between –0.13 and 0.21‰ that appear to be consistent with biological utilization and remineralization (Lacan et al., 2008).

The specific mechanisms governing these isotope effects are complex because a multitude of processes can cause Fe isotopes to fractionate, including adsorption of dissolved Fe(II) to Fe(III)-oxide surfaces (Icopini et al., 2004; Crosby et al., 2007; Jang et al., 2008), precipitation of Fe-bearing minerals (Bullen et al., 2001; Skulan et al., 2002; Wiesli et al., 2004; Butler et al., 2005), and isotope-exchange between different Fe-ligand species or ligand-promoted dissolution (Brantley et al., 2001; Schauble et al., 2001; Wiederhold et al., 2006; Dideriksen et al., 2008; Hill and Schauble, 2008; Domagal-Goldman et al., 2009), and even diffusion (Rodushkin et al., 2004). Many of these processes occur in even the simplest experiments involving dissimilatory Fe metabolizing bacteria. However, the largest effect is the equilibrium isotope fractionation between Fe(II) and Fe(III) species. Experiments (Johnson et al., 2002; Welch et al., 2003) and theory (Schauble et al., 2001; Anbar et al., 2005; Domagal-Goldman and Kubicki, 2008) indicate fractionation of 2.5–3‰ favoring light Fe in the Fe(II) species when Fe(II) and Fe(III) aquo complexes equilibrate at $\sim 25^\circ\text{C}$. These issues and their consequences are reviewed in detail elsewhere (Johnson et al., 2004b; Dauphas and Rouxel, 2006; Johnson et al., 2008a).

A key consideration, however, is that, in contrast to microbial conversion of S or C, dissimilatory Fe reduction and oxidation are extracellular processes that take place at mineral surfaces, facilitated by electron shuttles. Therefore, isotope fractionation may result from isotope exchange between surface adsorbed Fe(II) and Fe(III) at the oxide surface (Crosby et al., 2005). This hypothesis may explain why the fractionation factors during dissimilatory Fe(III) reduction and Fe(II) oxidation are so similar, independent of the species of microbe or the substrate, and why they are also within error of the equivalent fractionation factor between dissolved Fe(II) and Fe(III)-oxide in abiotic systems (Crosby et al., 2007).

Other transition metals for which isotope fractionation during biological transformation has been demonstrated experimentally include Mo (Wasylenki et al., 2007), Zn (John, 2007), Cr (Sikora et al., 2008), Ni (Cameron et al., 2009), and Hg (Bergquist and Blum, 2007; Kritee et al., 2007). Some organisms convert metal redox states to gain metabolic energy or for detoxification, or both. In such cases, isotope fractionation occurs during dissimilatory reduction or oxidation (e.g., Fe, Cr, and Hg). In other

cases, metals are fractionated during assimilation (e.g., Zn, Mo, Fe, and Ni).

Redox changes, adsorption to inorganic and organic surfaces, precipitation, ligand exchange and diffusion can occur independent of biology in aqueous systems. Therefore, the same considerations lead to widespread nonbiological Fe isotope effects in experiments and nature (e.g., Anbar et al., 2000; Bullen et al., 2001; Johnson et al., 2002; Brantley et al., 2004; Icopini et al., 2004; Butler et al., 2005; Crosby et al., 2007; Dideriksen et al., 2008), making it difficult to differentiate biogenic and abiogenic effects in natural systems. The same is true for other metals (e.g., Ellis et al., 2002; Rehkämper et al., 2002; Larson et al., 2003; Rouxel et al., 2003; Barling and Anbar, 2004; Mathur et al., 2005; Pokrovsky et al., 2005; Siebert et al., 2006a; Malinovsky et al., 2007; Stirling et al., 2007; Balistrieri et al., 2008; John et al., 2008; Vance et al., 2008). As more is learned, multi-element isotope fractionation patterns might be developed that constitute distinctively biological fingerprints. However, as of now no definitive metal isotope “magic bullet” biosignature has been identified.

In this regard the isotopes of Fe and other metals are no different than the C and S isotope systems. For these elements, too, abiotic effects are potentially significant (Ohmoto and Rye, 1979; Chang et al., 1983; Machel et al., 1995; Horita and Berndt, 1999; Pavlov et al., 2001; McCollom and Seewald, 2006). As a result, controversy erupts whenever isotope values alone, absent other context, are used as evidence of life (Rankama, 1950; Craig, 1953a; Schidlowski, 1988; Mojzsis et al., 1996; Schidlowski, 2001; van Zuilen et al., 2002). Nevertheless, these non-metal isotope systems are widely used to build logical, if not definitive, arguments about biological influence over C and S cycling in modern and ancient environments (Goldhaber and Kaplan, 1980; Canfield and Teske, 1996; Rosing, 1999; Shanks, 2001; Shen and Buick, 2004; Hinrichs et al., 2006). Similarly, for Fe and other metals. For example, it has been proposed that isotopically light Fe isotope values observed in anoxic sediment pore fluids are indicative of the importance of bacterial dissimilatory Fe reduction in such settings (Bergquist and Boyle, 2006; Severmann et al., 2006). Hence, $\delta^{56}\text{Fe}$ measurements in rocks derived from ancient anoxic sediments may provide information about the evolution of this metabolism (Johnson et al., 2008a). Such applications are discussed below.

Biogeochemistry and paleoenvironmental proxies

In addition to their growing use in biosignature research, metal stable isotopes are evolving into powerful proxies of biogeochemical cycling and paleoenvironmental conditions (Anbar and Rouxel, 2007; Johnson et al., 2008a). Most of this research centers on paleoredox applications, particularly on understanding changes in ocean oxygenation in response to rising atmospheric O_2 before the Cenozoic era – a topic of intense interest to geobiologists.

Initial efforts have emphasized isotopes of Fe and Mo. These elements are both found at the Earth's surface in two or more redox states, and the geochemical behaviors of both elements are strongly affected by the prevailing redox conditions encountered in the environment. For example, a recent study of the Black Sea indicates that bulk sedimentary Fe isotope compositions are related to the availability of dissolved O_2 and the presence of H_2S in the water column (Severmann et al., 2008). Apparently, an isotopically distinct pool of Fe(II) is generated during early diagenetic processes, including dissimilatory Fe reduction of Fe(III) oxides, in sediment pore waters on basin shelves. If the water column is anoxic, this isotopically distinct Fe can migrate to the deeper basin, where it is immobilized in H_2S -rich waters as pyrite. Isotopically light pyrite therefore can provide a paleorecord of basin anoxia and dissimilatory Fe metabolism. Similar systematic Fe isotope variations have been observed in the sediments of other modern (Fehr et al., 2008) and ancient (Czaja et al., 2010; Duan et al., 2010) anoxic depositional settings. Hence, under certain conditions the Fe isotope compositions in ancient sediments record evidence of ancient redox conditions.

This concept has been applied to the interpretation of systematic changes in sedimentary $\delta^{56}Fe$ through time (Matthews et al., 2004; Rouxel et al., 2005; Yamaguchi et al., 2005; Archer and Vance, 2006; Jenkyns et al., 2007). In Archean black shales dating to at least 3 Ga, Fe isotope values of -1 to -3‰ relative to average igneous rocks are common (e.g., Rouxel et al., 2005; Yamaguchi et al., 2005; Archer and Vance, 2006); variations among igneous rocks and minerals are much smaller; e.g., Beard and Johnson, 2004; Williams et al., 2005; Poitrasson, 2006; Weyer et al., 2007; Teng et al., 2008). After ~ 1.8 Ga, fractionation in such sediments is negligible (Rouxel et al., 2005). These values may reflect the importance of dissimilatory Fe reduction in marine sediments as a source of dissolved Fe(II) to Archean ocean basins (Yamaguchi et al., 2005; Johnson et al., 2008b; Severmann et al., 2008; Czaja et al., 2010 but see also Rouxel et al., 2005). If so, they provide strong evidence that this metabolism was important in Archean oceans, consistent with the notion that the deep oceans at that time were anoxic and that Fe(III) was being produced in surface oceans and delivered to sediments (e.g., Walker, 1987; Kappler et al., 2005; Konhauser et al., 2005).

The production of Fe(III) may itself be biologically catalyzed (Walker, 1987; Kappler et al., 2005; Konhauser et al., 2005), and so it has been hypothesized that Precambrian Banded Iron Formations (BIFs) are manifestation of biogenic Fe(II) oxidation. So far it has not been possible to identify a unique organic biomarker for microbial Fe(II) oxidation in the rock record, which is why hopes are high for Fe isotopes to confirm a biogenic origin of BIFs (Yamaguchi et al., 2005; Johnson et al., 2008b). However, given the complexity of the often overlapping biotic and abiotic fractionations, it remains doubtful that Fe isotope evidence on its own will be able to deliver such

a “smoking gun” (see discussion in Rouxel et al., 2006b; Yamaguchi and Ohmoto, 2006). Instead, Dauphas et al. (2004) have used Fe isotopes to make the case that the controversial early Archean Akilia Island rocks are indeed metamorphosed chemical precipitates (possibly biogenic) rather than a heavily metamorphosed igneous protolith (Fedo and Whitehouse, 2002).

Although, Mo isotopes, like Fe, presumably fractionate during Mo reduction or oxidation, this is paradoxically not the basis of the use of Mo isotopes as a redox proxy. Instead, Mo isotope paleoredox applications follow from the large isotope fractionation that occurs when Mo adsorbs to some oxide mineral surfaces. The best-studied effect, seen in natural samples and laboratory experiments, occurs on the surfaces of birnessite and other Mn oxyhydroxides. A fractionation of $\sim 2.7\text{‰}$ is observed in $^{98}Mo/^{95}Mo$ ratios, favoring adsorption of light Mo isotopes (Barling and Anbar, 2004) that seems largely independent of temperature or solution composition (Wasylenski et al., 2008c). The origin of this surprisingly large effect remains under investigation (Tossell, 2005; Weeks et al., 2007; Wasylenski et al., 2008b). A fractionation approximately half as large is seen during adsorption to ferric oxyhydroxides (Canfield et al., 2008; Wasylenski et al., 2008a).

In contrast, Mo isotope fractionation is muted during removal into sediments accumulating under euxinic (H_2S -rich) bottom waters. This finding probably reflects near-quantitative scavenging of Mo when the concentration of H_2S exceeds $\sim 10 \mu M$ (Helz et al., 1996); sediments accumulating under conditions that are anoxic but not sulfidic exhibit modest isotope fractionation (Poulson et al., 2006; Neubert et al., 2008), as do suboxic sediments (McManus et al., 2002; Siebert et al., 2006b). In both cases, light isotopes are preferentially removed.

The net result of these processes is that $\delta^{98/95}Mo$ of seawater – in which the residence time of Mo today exceeds 500,000 year – reflects the balance between oxic and reducing sedimentary sinks. Today, most Mo is removed to oxic and suboxic sedimentary sinks, shifting the dissolved Mo pool in the oceans to heavy values as compared to riverine and other inputs (Barling et al., 2001; Siebert et al., 2003); $\delta^{98/95}Mo$ of the modern oceans is $\sim 2.4\text{‰}$ vs. an assumed continental crust value of $\sim 0\text{‰}$ and rivers carrying Mo with an average isotopic composition of $\sim 0.7\text{‰}$ (Archer and Vance, 2008). The balance of fluxes in ancient, less oxygenated oceans would have been shifted toward euxinic and suboxic sinks that cause less fractionation than Mn oxides. Hence, Mo in such oceans would be lighter than today. Ancient ocean $\delta^{98/95}Mo$ can be reconstructed from measurements in black shales (Barling et al., 2001; Siebert et al., 2003), so long as care is taken to ensure that conditions were locally euxinic during deposition (Gordon et al., 2008; Neubert et al., 2008). This concept has been used to examine the extent of ocean anoxia in the Archean and Paleoproterozoic (Siebert et al., 2005; Wille et al., 2007),

mid-Proterozoic (Arnold et al., 2004), early Cambrian (Wille et al., 2008), and Jurassic (Pearce et al., 2008).

Building on these successful examples, other transition metal isotope systems are being explored for paleoenvironmental possibilities. These include U and Th isotopes (Rehkämper et al., 2004; Weyer et al., 2008), which are like Mo in that they fractionate during adsorption to metal oxides but are driven by an unusual fractionation mechanism based on differences in nuclear volume rather than mass (Schauble, 2007). Other novel isotope systems that are currently being explored include (Miller et al., 2008), Cr (Amor et al., 2008; Frei and Gaucher, 2008), and Ce (Weber et al., 2008).

In addition to transition metals, there has been a surge of activity examining the mass-dependent isotope variations of alkaline earth metals, including Mg (Young and Galy, 2004; Tipper et al., 2006) and Ca (Skulan et al., 1997; De La Rocha and DePaolo, 2000; Nägler et al., 2000) and a number of metalloids such as B (Pearson and Palmer, 1999; Pearson and Palmer, 2000; Hönisch and Hemming, 2005; Pagani et al., 2005), Si (De La Rocha et al., 1998; Varela et al., 2004; Reynolds et al., 2006), Ge (Rouxel et al., 2006a; Siebert et al., 2006a), and Sb (Rouxel et al., 2003). Most of this research is focused on paleoenvironmental questions of direct interest to geobiologists. In several cases, the isotope compositions are measured in materials, such as carbonates, phosphates, or silica, of biogenic origin.

Finally, many of these isotope systems are also being used to unravel issues in modern biogeochemical cycling. In some cases such research is an essential foundation for paleoenvironmental applications, since isotope variations in ancient sediments cannot be interpreted without an understanding of processes that are best studied and calibrated in modern settings. However, in at least one case modern applications are the primary motivation: Hg. This new isotope system is characterized by surprisingly large fractionations up to 2.5‰ (in terms of $^{199}\text{Hg}/^{198}\text{Hg}$) that are strikingly non-mass-dependent (Bergquist and Blum, 2007). Hg isotopes are finding their initial applications as a tool to shed light on Hg sources (Foucher and Hintelmann, 2006), on microbially mediated transformations of Hg in the environment (Kritee et al., 2007), and on the mechanisms of Hg bioaccumulation in food chains (Bergquist and Blum, 2007). It is likely that more isotope systems will find similar uses in modern settings, such as to trace sources of bioessential elements to the oceans (Severmann et al., 2006), or terrestrial environments (Borrok et al., 2008; Chen et al., 2008; Gioia et al., 2008), or to understand the pathways of uptake of these elements by microbiota in nature (Cameron et al., 2009; John, 2007; Wasylenki et al., 2007).

Summary

Technological advances in MS have led to the emergence of a new subdiscipline in the field of stable isotope geochemistry over the past decade: natural variations in

isotope compositions have now been shown to occur for many metals that were previously very difficult or impossible to measure with sufficient precision and accuracy. Isotope fractionations have been linked to a wide range of processes, including biological utilization (dissimilatory and assimilatory), surface adsorption, redox-exchange, mineral precipitation, ligand exchange, or diffusion. A clear distinction between biological and abiotic processes based on metal isotope variations alone is often not possible, because both processes may result in isotope fractionations of similar magnitude, and may occur simultaneously. This apparent complication can be resolved by integrating these novel proxies with more traditional proxies that can provide context for their interpretation. The two isotope systems that have so far received the most attention are Fe and Mo, and this contribution briefly reviews examples of their application in biogeochemical or paleoenvironmental research.

Bibliography

- Albarède, F., and Beard, B. L., 2004. Analytical methods for non-traditional isotopes. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 113–152.
- Altabet, M. A., and Francois, R., 1994. Sedimentary nitrogen isotopic ratio as a recorder for surface ocean nitrate utilization. *Global Biogeochemical Cycles*, **8**, 103–116.
- Amor, K., Al-Suwaidi, A., Hesselbo, S., Jenkyns, H., and Porcelli, D., 2008. Chromium isotope stratigraphy of the Toarcian OAE from Yorkshire, NE England. *Eos Trans AGU*, **89**, PP31C–1495.
- Anbar, A. D., 2004. Molybdenum stable isotopes: observations, interpretations and directions. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 429–454.
- Anbar, A. D., and Rouxel, O., 2007. Metal stable isotopes in paleoceanography. *Annual Review of Earth and Planetary Sciences*, **35**, 717–746.
- Anbar, A. D., Roe, J. E., Barling, J., and Nealson, K. H., 2000. Nonbiological fractionation of iron isotopes. *Science*, **288**, 126–128.
- Anbar, A. D., Jazecki, A. A., and Spiro, T. G., 2005. Theoretical investigation of iron isotope fractionation between $\text{Fe}(\text{H}_2\text{O})_6^{3+}$ and $\text{Fe}(\text{H}_2\text{O})_6^{2+}$: implications for stable isotope geochemistry. *Geochimica et Cosmochimica Acta*, **69**, 825–837.
- Archer, C., and Vance, D., 2006. Coupled Fe and S isotope evidence for Archean microbial Fe(III) and sulphate reduction. *Geology*, **34**, 153–156.
- Archer, C., and Vance, D., 2008. The isotopic signature of the global riverine molybdenum flux and anoxia in the ancient oceans. *Nature Geoscience*, **1**, 597–600.
- Arnold, G. L., Anbar, A. D., Barber, T., and Lyons, T. W., 2004. Molybdenum isotope evidence for widespread anoxia in mid-Proterozoic oceans. *Science*, **304**, 87–90.
- Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W., 2006. Iron isotope fractionation during microbially stimulated Fe(II) oxidation and Fe(III) precipitation. *Geochimica et Cosmochimica Acta*, **70**, 622–639.
- Balistreri, L. S., Borrok, D. M., Wanty, R. B., and Ridley, W. I., 2008. Fractionation of Cu and Zn isotopes during adsorption onto amorphous Fe(III) oxyhydroxide: experimental mixing of

- acid rock drainage and ambient river water. *Geochimica et Cosmochimica Acta*, **72**, 311–328.
- Barling, J., and Anbar, A. D., 2004. Molybdenum isotope fractionation during adsorption by manganese oxides. *Earth and Planetary Science Letters*, **217**, 315–329.
- Barling, J., Arnold, G. L., and Anbar, A. D., 2001. Natural mass-dependent variations in the isotopic composition of molybdenum. *Earth and Planetary Science Letters*, **193**, 447–457.
- Beard, B. L., and Johnson, C. M., 2004. Inter-mineral Fe isotope variations in mantle-derived rocks and implications for the Fe geochemical cycle. *Geochimica et Cosmochimica Acta*, **68**, 4727–4743.
- Beard, B., Johnson, C. M., Cox, L., Sun, H., Neilson, K. H., and Aguilar, C., 1999. Iron isotope biosignatures. *Science*, **285**, 1889–1896.
- Belshaw, N. S., Zhu, X. K., Guo, Y., and O’Nions, R. K., 2000. High precision measurement of iron isotopes by plasma source mass spectrometry. *International Journal for Mass Spectrometry*, **197**, 191–195.
- Bergquist, B. A., and Blum, J. D., 2007. Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems. *Science*, **318**, 417–420.
- Bergquist, B. A., and Boyle, E. A., 2006. Iron isotopes in the Amazon River system: weathering and transport signatures. *Earth and Planetary Science Letters*, **248**, 39.
- Bermin, J., Vance, D., Archer, C., and Statham, P. J., 2006. The determination of the isotopic composition of Cu and Zn in seawater. *Chemical Geology Special Issue in Honour of R. K. O’Nions*, **226**, 280–297.
- Bigeleisen, J., 1965. Chemistry of isotopes: isotope chemistry has opened new areas of chemical physics, geochemistry, and molecular biology. *Science*, **147**, 463–471.
- Borrok, D. M., Nimick, D. A., Wanty, R. B., and Ridley, W. I., 2008. Isotopic variations of dissolved copper and zinc in stream waters affected by historical mining. *Geochimica et Cosmochimica Acta*, **72**, 329–344.
- Brantley, S. L., Liermann, L., and Bullen, T. D., 2001. Fractionation of Fe isotopes by soil microbes and organic acids. *Geology*, **29**, 535–538.
- Brantley, S. L., Liermann, L., Guynn, R. L., Anbar, A. D., Icopini, G. A., and Barling, J., 2004. Fe isotopic fractionation during mineral dissolution with and without bacteria. *Geochimica et Cosmochimica Acta*, **68**, 3189–3204.
- Bullen, T. D., White, A. F., Childs, C. W., Vivit, D. V., and Schulz, M. S., 2001. Demonstration of significant abiotic iron isotope fractionation in nature. *Geology*, **29**, 699–702.
- Butler, I. B., Archer, C., Vance, D., Olroyd, A., and Rickard, D. T., 2005. Fe isotope fractionation on FeS formation in ambient aqueous solution. *Earth and Planetary Science Letters*, **236**, 430–442.
- Cameron, V., Vance, D., Archer, C., and House, C. H., 2009. A biomarker based on the stable isotopes of nickel. *Proceedings of the National Academy of Sciences*, **106**, 10944–10948.
- Canfield, D. E., 2001. Isotope fractionation by natural populations of sulfate-reducing bacteria. *Geochimica et Cosmochimica Acta*, **65**, 1117–1124.
- Canfield, D. E., and Teske, A., 1996. Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulphur-isotope studies. *Nature*, **382**, 127–132.
- Canfield, D. E., Poulton, S. W., Knoll, A. H., Narbonne, G. M., Ross, G., Goldberg, T., and Strauss, H., 2008. Ferruginous conditions dominated later Neoproterozoic Deep-water chemistry. *Science*, **321**, 949–952.
- Chang, S., Des Marais, D. J., Mack, R., Miller, S. L., and Strathearn, G. E., 1983. Prebiotic organic synthesis and the origin of life. In Schopf, J. W. (ed.), *Earth’s Earliest Biosphere: its Origin and Evolution*. Princeton, NJ: Princeton University Press, pp. 53–92.
- Chen, J., Gaillardet, J. M., and Louvat, P., 2008. Zinc isotopes in the Seine River waters, France: a probe of anthropogenic contamination. *Environmental Science and Technology*, **42**, 6494–6501.
- Craig, H., 1953a. Corycium defunctum: the non-indicative properties of isotopes and review articles. *Economic Geology*, **48**, 600–603.
- Craig, H., 1953b. The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 53–92.
- Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K., 2004. Iron isotope fractionation by Fe(II)-oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, **68**, 1227–1242.
- Crosby, H. A., Johnson, C. M., Roden, E. E., and Beard, B. L., 2005. Fe(II)-Fe(III) electron/atom exchange as a mechanism for Fe isotope fractionation during dissimilatory iron oxide reduction. *Environmental Science and Technology*, **39**, 6698–6704.
- Crosby, H. A., Roden, E. E., Johnson, C. M., and Beard, B. L., 2007. The mechanisms of iron isotope fractionation produced during dissimilatory Fe(III) reduction by *Shewanella putrefaciens* and *Geobacter sulfurreducens*. *Geobiology*, **5**, 169–189.
- Czaja, A. D., Johnson, C. M., Beard, B. L., Eigenbrode, J. L., Freeman, K. H., and Yamaguchi, K. E., 2010. Iron and carbon isotope evidence for ecosystem and environmental diversity in the ~2.7 to 2.5 Ga Hamersley Province, Western Australia. *Earth and Planetary Science Letters*, doi:10.1016/j.epsl.2010.01.032, in press.
- Dauphas, N., and Rouxel, O. J., 2006. Mass spectrometry and natural variations of iron isotopes. *Mass Spectrometry Reviews*, **25**, 515–550.
- Dauphas, N., Van Zuilen, M., Wadhwa, M., Davis, A. M., Marty, B., and Janney, P. E., 2004. Clues from Fe isotope variations on the origin of early Archean BIFs from Greenland. *Science*, **306**, 2077–2080.
- De La Rocha, C. L., 2003. Silicon isotope fractionation by marine sponges and the reconstruction of the silicon isotope composition of ancient deep water. *Geology*, **31**, 423–426.
- De La Rocha, C. L., and DePaolo, D. J., 2000. Isotopic evidence for variations in the marine calcium cycle over the Cenozoic. *Science*, **289**, 1176–1178.
- De La Rocha, C. L., Brzezinski, M. A., DeNiro, M. J., and Shemesh, A., 1998. Silicon-isotope composition of diatoms as an indicator of past oceanic change. *Nature*, **395**, 680–683.
- DePaolo, D. J., 2004. Calcium isotopic variations produced by biological, kinetic, radiogenic and nucleosynthetic processes. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 255–288.
- Diderksen, K., Baker, J. A., and Stipp, S. L. S., 2008. Equilibrium Fe isotope fractionation between inorganic aqueous Fe(III) and the siderophore complex, Fe(III)-desferrioxamine B. *Earth and Planetary Science Letters*, **269**, 280–290.
- Domagal-Goldman, S. D., and Kubicki, J. D., 2008. Density functional theory predictions of equilibrium isotope fractionation of iron due to redox changes and organic complexation. *Geochimica et Cosmochimica Acta*, **72**, 5201–5216.
- Domagal-Goldman, S. D., Paul, K. W., Sparks, D. L., and Kubicki, J. D., 2009. Quantum chemical study of the Fe(III)-desferrioxamine B siderophore complex – electronic structure, vibrational frequencies, and equilibrium Fe-isotope fractionation. *Geochimica et Cosmochimica Acta*, **73**, 1–12.
- Duan, Y., Severmann, S., Anbar, A. D., Lyons, T. W., Gordon, G. W., and Sageman, B. B., 2010. Isotopic evidence for Fe cycling and repartitioning in ancient oxygen-deficient settings: Examples from black shales of the mid-to-late Devonian Appalachian basin. *Earth and Planetary Science Letters*, **290**, 244–253.

- Ellis, A. S., Johnson, T. M., and Bullen, T. D., 2002. Chromium isotopes and the fate of hexavalent chromium in the environment. *Science*, **295**, 2060–2062.
- Fedo, C. M., and Whitehouse, M. J., 2002. Metasomatic origin of quartz-pyroxene rock, Akilia, Greenland, and implications for earth's earliest life. *Science*, **296**, 1448–1452.
- Fehr, M. A., Andersson, P. S., Halenius, U., and Morth, C.-M., 2008. Iron isotope variations in Holocene sediments of the Gotland Deep, Baltic Sea. *Geochimica et Cosmochimica Acta*, **72**, 807–826.
- Foucher, D., and Hintelmann, H., 2006. High-precision measurement of mercury isotope ratios in sediments using cold-vapor generation multi-collector inductively coupled plasma mass spectrometry. *Analytical and Bioanalytical Chemistry*, **384**, 1470–1478.
- Frei, R., Gaucher, C., Poulton, S. W., and Canfield, D. E., 2009. Fluctuations in Precambrian atmospheric oxygenation recorded by chromium isotopes. *Nature*, **461**, 250–253.
- Gioia, S., Weiss, D., Coles, B., Arnold, T., and Babinski, M., 2008. Accurate and precise zinc isotope ratio measurements in urban aerosols. *Analytical Chemistry*, **80**, 9776–9780.
- Goldhaber, M. B., and Kaplan, I. R., 1980. Mechanism of sulfur incorporation and isotopic fractionation during early diagenesis in sediments of the Gulf of California. *Marine Chemistry*, **9**, 95–143.
- Gordon, G. W., Lyons, T. W., Arnold, G. L., Roe, J., Sageman, B. B., and Anbar, A. D., 2009. When do black shales tell molybdenum isotope tales? *Geology*, **37**, 535–538.
- Halliday, A. N., Lee, D.-C., Christensen, J. N., Walder, A. J., Freedman, P. A., Jones, C. E., Hall, C. M., Yi, W., and Teagle, D., 1995. Recent developments in inductively coupled plasma magnetic sector multiple collector mass spectrometry. *International Journal of Mass Spectrometry and Ion Processes*, **146–147**, 21–33.
- Harrison, A. G., and Thode, H. G., 1958. Mechanism of bacterial reduction of sulfate from isotope fractionation studies. *Transactions of the Faraday Society*, **53**, 84–92.
- Helz, G. R., Miller, C. V., Charnock, J. M., Mosselmans, J. F. W., Patrick, R. A. D., Garner, C. D., and Vaughan, D. J., 1996. Mechanism of molybdenum removal from the sea and its concentration in black shales: EXAFS evidence. *Geochimica et Cosmochimica Acta*, **60**, 3631–3642.
- Hill, P. S., and Schauble, E. A., 2008. Modeling the effects of bond environment on equilibrium iron isotope fractionation in ferric aquo-chloro complexes. *Geochimica et Cosmochimica Acta*, **72**, 1939–1958.
- Hinrichs, K.-U., Hayes, J. M., Bach, W., Spivack, A. J., Hmelo, L. R., Holm, N. G., Johnson, C. G., and Sylva, S. P., 2006. Biological formation of ethane and propane in the deep marine subsurface. *Proceedings of the National Academy of Science*, **103**, 14684–14689.
- Hönisch, B., and Hemming, N. G., 2005. Surface ocean pH response to variations in pCO₂ through two full glacial cycles. *Earth and Planetary Science Letters*, **236**, 305–314.
- Horita, J., 2005. Some perspectives on isotope biosignatures for early life. *Chemical Geology*, **218**, 171–186.
- Horita, J., and Berndt, M. E., 1999. Abiogenic methane formation and isotopic fractionation under hydrothermal conditions. *Science*, **285**, 1055–1057.
- Houk, R. S., and Thompson, J. J., 1988. Inductively coupled plasma mass spectrometry. *Mass Spectrometry Reviews*, **7**, 425–461.
- Icopini, G. A., Anbar, A. D., Ruebush, S. S., Tien, M., and Brantley, S. L., 2004. Iron isotope fractionation during microbial reduction of iron: the importance of adsorption. *Geology*, **32**, 205–208.
- Jang, J.-H., Mathur, R., Liermann, L. J., Ruebush, S., and Brantley, S. L., 2008. An iron isotope signature related to electron transfer between aqueous ferrous iron and goethite. *Chemical Geology*, **250**, 40–48.
- Jenkyns, H. C., Matthews, A., Tsikos, H., and Erel, Y., 2007. Nitrate reduction, sulfate reduction, and sedimentary iron isotope evolution during the Cenomanian-Turonian oceanic anoxic event. *Paleoceanography*, **22**, PA3208, doi:10.1029/2006PA001355.
- John, S. G., 2007. *The Marine Biogeochemistry of Zinc Isotopes*. PhD, MA, Institute of Technology/Woods Hole Oceanographic Institution.
- John, S. G., Rouxel, O. J., Craddock, P. R., Engwall, A. M., and Boyle, E. A., 2008. Zinc stable isotopes in seafloor hydrothermal vent fluids and chimneys. *Earth and Planetary Science Letters*, **269**, 17–28.
- Johnson, T. M., and Bullen, T. D., 2004. Mass-dependent fractionation of selenium and chromium isotopes in low-temperature environments. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 289–317.
- Johnson, C. M., Skulan, J. L., Beard, B. L., Sun, H., Nealson, K. H., and Bratermann, P. S., 2002. Isotopic fractionation between Fe (III) and Fe(II) in aqueous solutions. *Earth and Planetary Science Letters*, **195**, 141–153.
- Johnson, C. M., Beard, B. L., and Albarède, F., 2004a. *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, 454 p.
- Johnson, C. M., Beard, B. L., Roden, E. E., Newman, D. K., and Nealson, K. H., 2004b. Isotopic constraints on biogeochemical cycling of Fe. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 359–408.
- Johnson, C. M., Beard, B. L., and Roden, E. E., 2008a. The iron isotope fingerprints of redox and biogeochemical cycling in modern and ancient earth. *Annual Review of Earth and Planetary Sciences*, **36**, 457–493.
- Johnson, C. M., Beard, B. L., Klein, C., Beukes, N. J., and Roden, E. E., 2008b. Iron isotopes constrain biologic and abiologic processes in banded iron formation genesis. *Geochimica et Cosmochimica Acta*, **72**, 151–169.
- Kaplan, I. R., and Rittenberg, S. C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology*, **34**, 195–212.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, **33**, 865–868.
- Konhauser, K. O., Newman, D. K., and Kappler, A., 2005. The potential significance of microbial Fe(III) reduction during deposition of Precambrian banded iron formations. *Geobiology*, **3**, 167–177.
- Kritee, K., Blum, J. D., Johnson, M. W., Bergquist, B. A., and Barkay, T., 2007. Mercury stable isotope fractionation during reduction of Hg(II) to Hg(0) by mercury resistant microorganisms. *Environmental Science and Technology*, **41**, 1889–1895.
- Lacan, F., Francois, R., Ji, Y., and Sherrell, R. M., 2006. Cadmium isotopic composition in the ocean. *Geochimica et Cosmochimica Acta*, **70**, 5104.
- Lacan, F., Radic, A., Jeandel, C., Poitras, F., Sarthou, G., Pradoux, C., and Freydis, R., 2008. Measurement of the isotopic composition of dissolved iron in the open ocean. *Geophysical Research Letters*, **35**, L24610-1–L24610-5.
- Larson, P. B., Maher, K., Ramos, F. C., Chang, Z., Gaspar, M., and Meinert, L. D., 2003. Copper isotope ratios in magmatic and hydrothermal ore-forming environments. *Chemical Geology*, **201**, 337–350.
- Machel, H. G., Krouse, H. R., and Sassen, R., 1995. Products and distinguishing criteria of bacterial and thermochemical sulfate reduction. *Applied Geochemistry*, **10**, 373–389.

- Malinovsky, D., Baxter, D. C., and Rodushkin, I., 2007. Ion-specific isotopic fractionation of molybdenum during diffusion in aqueous solutions. *Environmental Science and Technology*, **41**, 1596–1600.
- Maréchal, C. N., Télouk, P., and Albarède, F., 1999. Precise analysis of copper and zinc isotopic compositions by plasma-source mass spectrometry. *Chemical Geology*, **156**, 251–273.
- Mason, T. F. D., Weiss, D. J., Chapman, J. B., Wilkinson, J. J., Tessalina, S. G., Spiro, B., Horstwood, M. S. A., Spratt, J., and Coles, B. J., 2005. Zn and Cu isotopic variability in the Alexandrinka volcanic-hosted massive sulphide (VHMS) ore deposit, Urals, Russia. *Chemical Geology*, **221**, 170–187.
- Mathur, R., Ruiz, J., Titley, S., Liermann, L., Buss, H., and Brantley, S., 2005. Cu isotopic fractionation in the supergene environment with and without bacteria. *Geochimica et Cosmochimica Acta*, **69**, 5233–5246.
- Matthews, A., Morhans-Bell, H. S., Emmanuel, S., Jenkyns, H., Erel, Y., and Halciz, L., 2004. Controls of iron-isotope fractionation in organic-rich sediments (Kimmeridge Clay, Upper Jurassic, southern England). *Geochimica et Cosmochimica Acta*, **68**, 3107–3123.
- McCollom, T. M., and Seewald, J. S., 2006. Carbon isotope composition of organic compounds produced by abiotic synthesis under hydrothermal conditions. *Earth and Planetary Science Letters*, **243**, 74–84.
- McManus, J., Nägler, T. F., Siebert, C., Wheat, C. G., and Hammond, D. E., 2002. Oceanic molybdenum isotope fractionation: diagenesis and hydrothermal ridge-flank alteration. *Geochemistry Geophysics Geosystems*, **3**, 1078, doi:10.1029/2002GC000356.
- Miller, C. A., Peuker-Ehrenbrink, B., and Ball, L., 2008. Rhenium isotopes in natural systems. *Geochimica et Cosmochimica Acta*, **72**, A628.
- Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P., and Friend, C. R. L., 1996. Evidence for life on Earth before 3,800 million years ago. *Nature*, **384**, 55–59.
- Nägler, T. F., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -temperature calibration on fossil and cultured *Globigerinoides sacculifer*: new tool for reconstruction of past sea surface temperatures. *Geochemistry Geophysics Geosystems*, **1**, 1052, doi:10.1029/2000GC000091.
- Neubert, N., Nägler, T. F., and Böttcher, M. E., 2008. Solidity controls molybdenum isotope fractionation into exotic sediments: evidence from the modern Black Sea. *Geology*, **36**, 775–778.
- Nielsen, S. G., Rehkämper, M., Teagle, D. A. H., Butterfield, D. A., Alt, J. C., and Halliday, A. N., 2006. Hydrothermal fluid fluxes calculated from the isotopic mass balance of thallium in the ocean crust. *Earth and Planetary Science Letters*, **251**, 120–133.
- Ohmoto, H., and Rye, R. O., 1979. Isotopes of sulfur and carbon. In Barnes, H. L. (ed.), *Geochemistry of Hydrothermal Ore Deposits*. New York: Wiley, pp. 509–567.
- Pagani, M., Lemarchand, D., Spivack, A., and Gaillardet, J., 2005. A critical evaluation of the boron isotope-pH proxy: the accuracy of ancient ocean pH estimates. *Geochimica et Cosmochimica Acta*, **69**, 953–961.
- Pavlov, A. A., Kasting, J. F., Eigenbrode, J. L., and Freeman, K. H., 2001. Organic haze in Earth's early atmosphere: source of low- ^{13}C late Archean Kerogens? *Geology*, **29**, 1003–1006.
- Pearce, C. R., Cohen, A. S., Coe, A. L., and Burton, K. W., 2008. Molybdenum isotope evidence for global ocean anoxia coupled with perturbations to the carbon cycle during the Early Jurassic. *Geology*, **36**, 231–234.
- Pearson, P. N., and Palmer, M. R., 1999. Middle Eocene seawater pH and atmospheric carbon dioxide concentrations. *Science*, **284**, 1824–1826.
- Pearson, P. N., and Palmer, M. R., 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature*, **406**, 695–699.
- Poitrasson, F., 2006. On the iron isotope homogeneity level of the continental crust. *Chemical Geology*, **235**, 195–200.
- Pokrovsky, O. S., Viers, J., and Freydis, R., 2005. Zinc stable isotope fractionation during its adsorption on oxides and hydroxides. *Journal of Colloid and Interface Science*, **291**, 192–200.
- Poulson, R. L., McManus, J., Siebert, C., and Berelson, W. M., 2006. Authigenic molybdenum isotope signatures in marine sediments. *Geology*, **34**, 617–620.
- Rankama, K., 1948. New evidence of the origin of Pre-cambrian carbon. *Bulletin of the Geological Society of America*, **59**, 389–416.
- Rankama, K., 1950. *Corycium resuscitatum*: a discussion. *Journal of Geology*, **58**, 75–79.
- Rehkämper, M., Frank, M., Hein, J. R., Porcelli, D., Halliday, A., Ingri, J., and Liebetrau, V., 2002. Thallium isotope variations in seawater and hydrogenetic, diagenetic, and hydrothermal ferromanganese deposits. *Earth and Planetary Science Letters*, **197**, 65–81.
- Rehkämper, M., Frank, M., Hein, J. R., and Halliday, A., 2004. Cenozoic marine geochemistry of thallium deduced from isotopic studies of ferromanganese crusts and pelagic sediments. *Earth and Planetary Science Letters*, **219**, 77–91.
- Reynolds, B. C., Frank, M., and Halliday, A. N., 2006. Silicon isotope fractionation during nutrient utilization in the North Pacific. *Earth and Planetary Science Letters*, **244**, 431–443.
- Ripperger, S., and Rehkämper, M., 2007. Precise determination of cadmium isotope fractionation in seawater by double spike MC-ICPMS. *Geochimica et Cosmochimica Acta*, **71**, 631–642.
- Robert, F., and Chaussidon, M., 2006. A palaeotemperature curve for the Precambrian oceans based on silicon isotopes in cherts. *Nature*, **443**, 969–972.
- Rodushkin, I., Stenberg, A., Andren, H., Malinovsky, D., and Baxter, D. C., 2004. Isotopic fractionation during diffusion of transition metal ions in solution. *Analytical Chemistry*, **76**, 2148–2151.
- Rosing, M. T., 1999. ^{13}C -Depleted carbon microparticles in $\geq 3700\text{-Ma}$ sea-floor sedimentary rocks from West Greenland. *Science*, **283**, 674–676.
- Rouxel, O., Ludden, J., and Fouquet, Y., 2003. Antimony isotope variations in natural systems and implications for their use as geochemical tracers. *Chemical Geology*, **200**, 25–40.
- Rouxel, O., Bekker, A., and Edwards, K. J., 2005. Iron isotope constraints on the Archean and Paleoproterozoic ocean redox state. *Science*, **307**, 1088–1091.
- Rouxel, O., Galy, A., and Elderfield, H., 2006a. Germanium isotopic variations in igneous rocks and marine sediments. *Geochimica et Cosmochimica Acta*, **70**, 3387.
- Rouxel, O. J., Bekker, A., and Edwards, K. J., 2006b. Response to comment on “iron isotope constraints on the Archean and Paleoproterozoic ocean redox state”. *Science*, **311**, 177.
- Russell, W. A., Papanastassiou, D. A., and Tombrello, T. A., 1978. Ca isotope fractionation on the earth and other solar system materials. *Geochimica et Cosmochimica Acta*, **42**, 1075–1090.
- Schauble, E. A., 2007. Role of nuclear volume in driving equilibrium stable isotope fractionation of mercury, thallium, and other very heavy elements. *Geochimica et Cosmochimica Acta*, **71**, 2170–2189.
- Schauble, E. A., Rossman, G. R., and Taylor, H. P., 2001. Theoretical estimates of equilibrium Fe-isotope fractionations from vibrational spectroscopy. *Geochimica et Cosmochimica Acta*, **65**, 2487–2497.

- Schidlowski, M., 1988. A 3,800-million-year isotopic record of life from carbon in sedimentary rocks. *Nature*, **333**, 313–318.
- Schidlowski, M., 2001. Carbon isotopes as biogeochemical recorders of life over 3.8 Ga of Earth history: evolution of a concept. *Precambrian Research*, **106**, 117–134.
- Schmitt, A.-D., Galer, S. J. G., and Abouchami, W., 2009. Mass-dependent cadmium isotopic variations in nature with emphasis on the marine environment. *Earth and Planetary Science Letters*, **277**, 262–272.
- Schoenberg, R., Zink, S., Staubwasser, M., and von Blanckenburg, F., 2008. The stable Cr isotope inventory of solid earth reservoirs determined by double spike MC-ICP-MS. *Chemical Geology*, **249**, 294–306.
- Severmann, S., Johnson, C. M., Beard, B. L., and McManus, J., 2006. The effect of early diagenesis on the Fe isotope compositions of porewaters and authigenic minerals in continental margin sediments. *Geochimica et Cosmochimica Acta*, **70**, 2006–2022.
- Severmann, S., Lyons, T. W., Anbar, A., McManus, J., and Gordon, G., 2008. Modern iron isotope perspective on Fe shuttling in the Archean and the redox evolution of ancient oceans. *Geology*, **36**, 487–490.
- Shanks, W. C. III., 2001. Stable isotopes in Seafloor Hydrothermal Systems: vent fluids, hydrothermal deposits, hydrothermal alteration, and microbial processes. *Reviews in Mineralogy and Geochemistry*, **43**, 469–525.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth Science Reviews*, **64**, 243–272.
- Shields, W. R., Goldich, S. S., Garner, E. L., and Murphy, T. J., 1965. Natural variations in the abundance ratio and the atomic weight of copper. *Journal of Geophysical Research*, **70**, 479–491.
- Siebert, C., Nögler, T. F., von Blanckenburg, F., and Kramers, J. D., 2003. Molybdenum isotope records as a potential new proxy for paleoceanography. *Earth and Planetary Science Letters*, **211**, 159–171.
- Siebert, C., Kramers, J. D., Meisel, T., Morel, P., and Nögler, T. F., 2005. PGE, Re-Os, and Mo isotope systematics in Archean and early Proterozoic sedimentary systems as proxies for redox conditions of the early Earth. *Geochimica et Cosmochimica Acta*, **69**, 1787–1801.
- Siebert, C., Ross, A., and McManus, J., 2006a. Germanium isotope measurements of high-temperature geothermal fluids using double-spike hydride generation MC-ICP-MS. *Geochimica et Cosmochimica Acta*, **70**, 3986–3995.
- Siebert, C., McManus, J., Bice, A., Poulson, R., and Berelson, W. M., 2006b. Molybdenum isotope signatures in continental margin marine sediments. *Earth and Planetary Science Letters*, **241**, 723–733.
- Sikora, E. R., Johnson, T. M., and Bullen, T. D., 2008. Microbial mass-dependent fractionation of chromium isotopes. *Geochimica et Cosmochimica Acta*, **72**, 3631–3641.
- Skulan, J., DePaolo, D. J., and Owens, T. L., 1997. Biological control of calcium isotopic abundances in the global calcium cycle. *Geochimica et Cosmochimica Acta*, **61**, 2505–2510.
- Skulan, J. L., Beard, B. L., and Johnson, C. M., 2002. Kinetic and equilibrium isotope fractionation between aqueous Fe(III) and hematite. *Geochimica et Cosmochimica Acta*, **66**, 2995–3015.
- Smith, C. N., Kesler, S. E., Blum, J. D., and Rytuba, J. J., 2008. Isotope geochemistry of mercury in source rocks, mineral deposits and spring deposits of the California Coast Ranges, USA. *Earth and Planetary Science Letters*, **269**, 399–407.
- Stirling, C. H., Andersen, M. B., Potter, E.-K., and Halliday, A. N., 2007. Low-temperature isotopic fractionation of uranium. *Earth and Planetary Science Letters*, **264**, 208–225.
- Teng, F.-Z., Dauphas, N., and Helz, R. T., 2008. Iron isotope fractionation during magmatic differentiation in Kilauea Iki lava lake. *Science*, **320**, 1620–1622.
- Tipper, E. T., Galy, A., Gaillardet, J., Bickle, M. J., Elderfield, H., and Carder, E. A., 2006. The magnesium isotope budget of the modern ocean: constraints from riverine magnesium isotope ratios. *Earth and Planetary Science Letters*, **250**, 241.
- Tossell, J. A., 2005. Calculating the partitioning of the isotopes of Mo between oxidic and sulfidic species in aqueous solution. *Geochimica et Cosmochimica Acta*, **69**, 2981–2993.
- Turner, P. J., Mills, D. J., Schroder, E., Lapitajs, G., Jung, G., Lacone, L. A., Haydar, D. A., and Montaser, A., 1998. Instrumentation for low- and high-resolution ICPMS. In Montaser, A. (ed.), *Inductively Coupled Plasma Mass Spectrometry*. New York, NY: Wiley-VCH Verlag, pp. 421–501.
- Urey, H. C., 1947. The thermodynamic properties of isotopic substances. *Journal of the Chemical Society*, **1947**, 562–581.
- Valley, G. E., and Anderson, H. H., 1947. A comparison of the abundance ratios of the isotopes of terrestrial and of meteoritic iron. *Journal of the American Chemical Society*, **69**, 1871–1873.
- Vance, D., Archer, C., Bermin, J., Perkins, J., Statham, P. J., Lohan, M. C., Ellwood, M. J., and Mills, R. A., 2008. The copper isotope geochemistry of rivers and the oceans. *Earth and Planetary Science Letters*, **274**, 204–213.
- van Zuilen, M. A., Lepland, A., and Arrhenius, G., 2002. Reassessing the evidence for the earliest traces of life. *Nature*, **418**, 627–630.
- Varela, D. E., Pride, C. J., and Brzezinski, M. A., 2004. Biological fractionation of silicon isotopes in Southern Ocean surface waters. *Global Biogeochemical Cycles*, **18**, GB1047, doi:10.1029/2003GB002140.
- Wada, E., and Hattori, A., 1976a. Natural abundance of ^{15}N in particulate organic matter in the North Pacific Ocean. *Geochimica et Cosmochimica Acta*, **40**, 249–251.
- Wada, E., and Hattori, A., 1976b. Hattori, natural abundance of $\delta^{15}\text{N}$ in particulate organic matter in the North Pacific Ocean. *Geomicrobiology Journal*, **1**, 85–101.
- Walder, A. J., and Freedman, P. A., 1992. Isotopic ratio measurement using a double focusing magnetic sector mass analyser with an inductively coupled plasma as an ion source. *Journal of Analytical Atomic Spectrometry*, **7**, 571–575.
- Walker, J. C. G., 1987. Was the Archean biosphere upside down? *Nature*, **329**, 710–712.
- Wasserburg, G. J., Jacobsen, S. B., DePaolo, D. J., McCulloch, M. T., and Wen, T., 1981. Precise determination of ratios, Sm and Nd isotopic abundances in standard solutions. *Geochimica et Cosmochimica Acta*, **45**, 2311–2323.
- Wasylenki, L. E., Lund, T. J., and Anbar, A. D., 2008a. Mo isotopes fractionate during adsorption to hydrous ferric oxide: implications for Mo isotopes in Archean and Proterozoic oceans. *Astrobiology*, **6**(325), 8–26-O.
- Wasylenki, L. E., Weeks, C. L., Spiro, T. G., Barga, J. R., and Anbar, A. D., 2008b. How does metal adsorption cause isotopes to apportion? *Geochimica et Cosmochimica Acta*, **72**, A1007.
- Wasylenki, L. E., Rolfe, B. A., Weeks, C. L., Spiro, T. G., and Anbar, A. D., 2008c. Experimental investigation of the effects of temperature and ionic strength on Mo isotope fractionation during adsorption to manganese oxides. *Geochimica et Cosmochimica Acta*, **72**, 5997–6005.
- Wasylenki, L. E., Anbar, A. D., Liermann, L. J., Mathur, R., Gordon, G. W., and Brantley, S. L., 2007. Isotope fractionation during microbial metal uptake measured by MC-ICP-MS. *Journal of Analytical Atomic Spectrometry*, **22**, 905–910.
- Weber, T., Frei, R., and Rosing, M., 2008. Assessing mass-dependent isotope fractionation of cerium. *Eos Trans AGU*, **89**, PP31C–1514.
- Weeks, C. L., Anbar, A. D., Wasylenki, L. E., and Spiro, T. G., 2007. Density functional theory analysis of molybdenum isotope fractionation†. *The Journal of Physical Chemistry A*, **111**, 12434–12438.

- Weiss, D. J., Rehkämper, M., Schoenberg, R., McLaughlin, M., Kirby, J., Campbell, P. G. C., Arnold, T., Chapman, J., Peel, K., and Gioia, A. S., 2008. Application of nontraditional stable-isotope systems to the study of sources and fate of metals in the environment. *Environmental Science and Technology*, **42**, 655–664.
- Welch, S. A., Beard, B. L., Johnson, C. M., and Bratermann, P. S., 2003. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III). *Geochimica et Cosmochimica Acta*, **67**, 4231–4250.
- Weyer, S., and Schwieters, J. B., 2003. High precision Fe isotope measurements with high mass resolution MC-ICPMS. *International Journal for Mass Spectrometry*, **226**, 355–368.
- Weyer, S., Anbar, A. D., Brey, G. P., Munker, C., Mezger, K., and Woodland, A. B., 2007. Fe-isotope fractionation during partial melting on Earth and the current view on the Fe-isotope budgets of the planets (reply to the comment of F. Poitrasson and to the comment of B. L. Beard and C. M. Johnson on “Iron isotope fractionation during planetary differentiation” by S. Weyer, A.D. Anbar, G. P. Brey, C. Munker, K. Mezger and A. B. Woodland). *Earth and Planetary Science Letters*, **256**, 638–646.
- Weyer, S., Anbar, A. D., Gerdes, A., Gordon, G. W., Algeo, T. J., and Boyle, E. A., 2008. Natural fractionation of $^{238}\text{U}/^{235}\text{U}$. *Geochimica et Cosmochimica Acta*, **72**, 345–359.
- Wiederhold, J. G., Kraemer, S. M., Teutsch, N., Borer, P. M., Halliday, A. N., and Kretzschmar, R., 2006. Iron isotope fractionation during proton-promoted, ligand-controlled, and reductive dissolution of goethite. *Environmental Science and Technology*, **40**, 3787–3793.
- Wiesli, R. A., Beard, B. L., and Johnson, C. M., 2004. Experimental determination of Fe isotope fractionation between aqueous Fe (II), Siderite, and “Green Rust” in abiotic systems. *Chemical Geology*, **211**, 343–362.
- Wille, M., Kramers, J. D., Nägler, T. F., Beukes, N. J., Schroder, S., Meisel, T., Lacassie, J. P., and Voegelin, A. R., 2007. Evidence for a gradual rise of oxygen between 2.6 and 2.5 Ga from Mo isotopes and Re-PGE signatures in shales. *Geochimica et Cosmochimica Acta*, **71**, 2417–2435.
- Wille, M., Nägler, T. F., Lehmann, B., Schroder, S., and Kramers, J. D., 2008. Hydrogen sulphide release to surface waters at the Precambrian/Cambrian boundary. *Nature*, **453**(7196), 767–769.
- Williams, H. M., McCammon, C. A., Peslier, A. H., Halliday, A. N., Teutsch, N., Levasseur, S., and Burg, J.-P., 2005. Iron isotope fractionation and the oxygen fugacity of the mantle. *Science*, **304**, 1656–1659.
- Wombacher, F., Rehkämper, M., Mezger, K., and Münker, C., 2003. Stable isotope compositions of cadmium in geological materials and meteorites determined by multiple-collector ICPMS. *Geochimica et Cosmochimica Acta*, **67**, 4639–4654.
- Yamaguchi, K. E., and Ohmoto, H., 2006. Comment on “iron isotope constraints on the Archean and paleoproterozoic ocean redox state”. *Science*, **311**, 177a.
- Yamaguchi, K. E., Johnson, C. M., Beard, B. L., and Ohmoto, H., 2005. Biogeochemical cycling of iron in the Archean-Paleoproterozoic Earth: constraints from iron isotope variations in sedimentary rocks from the Kaapvaal and Pilbara Cratons. *Chemical Geology*, **218**, 135–169.
- Young, E. D., and Galy, A., 2004. The isotope geochemistry and cosmochemistry of magnesium. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 197–230.
- Zhu, X. K., O’Nions, R. K., Guo, Y., and Reynolds, B. C., 2000. Secular variations of iron isotopes in North Atlantic Deep Water. *Science*, **287**, 2000–2002.

ISOTOPES (METHODS)

Please refer to entries “*Isotope Fractionation (Metal)*”, “*Geochronology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

ISOTOPES AND GEOBIOLOGY

Jochen Hoefs
University of Göttingen, Göttingen, Germany

Definition

It has long been recognized (e.g., Thode et al. (1949), Craig (1953) and Wellman et al. (1968)) that biological processes significantly fractionate the isotopes of C, N, and S, leading to characteristic *biosignatures* in sedimentary rocks that will be more or less preserved in the geological record. In the following, a brief overview is given of the major isotope fractionation processes in the biosphere and the geosphere. Special attention will be paid to secondary bacterial activity after deposition of the organic matter. Questions of general interest are isotopic indicators of early life on Earth and on Mars. A note of caution, however, is given whether abiogenic processes may lead to isotope compositions similar to that of biological activity.

Carbon isotope fractionation during photosynthesis

Early reviews by O’Leary (1981) and Farquhar et al. (1989) have provided the biochemical background of carbon isotope fractionations during photosynthesis, with more recent accounts by Hayes (2001) and Freeman (2001). The main isotope-discriminating steps during biological carbon fixation are (1) the uptake and intracellular diffusion of CO_2 and (2) the biosynthesis of cellular components. Such a two-step model was first proposed by Park and Epstein (1960):



From this simplified scheme, it follows that the diffusional process is reversible, whereas the enzymatic carbon fixation is irreversible. The two-step model of carbon fixation clearly suggests that isotope fractionation is dependent on the partial pressure of CO_2 , i.e., $p\text{CO}_2$ of the system. With an unlimited amount of CO_2 available to a plant, the enzymatic fractionation will determine the isotopic difference between the inorganic carbon source and the final bioproduct. Under these conditions, ^{13}C fractionations may vary from -17 to -40‰ (O’Leary, 1981). When the concentration of CO_2 is the limiting factor, the diffusion of CO_2 into the plant is the slow step in the reaction and carbon isotope fractionation of the plant decreases.

CO₂ is directly converted by the enzyme ribulose biphosphate carboxylase/oxygenase (“Rubisco”) to a 6-carbon molecule that is then cleaved into two molecules of phosphoglycerate (PGA), each with three carbon atoms (plants using this photosynthetic pathway are, therefore, called C₃ plants). Most PGA is recycled to make ribulose biphosphate, but some are used to make carbohydrates. Free exchange between external and mesophyll CO₂ makes the carbon fixation process less efficient, which causes the observed large ¹³C depletions of C₃ plants.

C₄ plants incorporate CO₂ by the carboxylation of phosphoenolpyruvate (PEP) via the enzyme PEP carboxylase to make the molecule oxaloacetate, which has four carbon atoms (hence C₄). The carboxylation product is transported from the outer layer of mesophyll cells to the inner layer of bundle sheath cells, which are able to concentrate CO₂, so that most of the CO₂ is fixed with relatively little carbon fractionation. Because mesophyll cells are permeable and bundle sheath cells are less permeable, C₃ versus C₄ plants have ¹³C depletions of −18‰ versus −4‰ relative to atmospheric CO₂. Even more complex is C-isotope fractionation in aquatic plants. Factors that control the ¹³C of phytoplankton include temperature, availability of CO₂ (aq), light intensity, nutrient availability, pH, and physiological factors such as cell size and growth rate.

Since the pioneering work of Park and Epstein (1960) and Abelson and Hoering (1961) it is well known that ¹³C is not uniformly distributed among the total organic matter of plant material, but varies between carbohydrates, proteins, and lipids. The latter class of compounds is considerably depleted in ¹³C relative to the other products of biosynthesis. Although the causes of these ¹³C differences are not entirely clear, kinetic isotope effects seem to be more plausible than thermodynamic equilibrium effects.

Organic matter in the geosphere is a complex mixture of source organisms having variable biosynthetic pathways and detrital remains. The determination of ^{δ¹³C} values of bulk organic matter is thus unable to distinguish among the different carbon sources. Immediately after burial of the biological organic material into sediments, complex diagenetic changes occur in the organic matter. Two processes have been proposed to explain the observed changes in carbon isotope composition: (1) preferential degradation of organic compounds which have different isotope composition compared to the preserved organic compounds. Since easily degradable organic compounds such as amino acids are enriched in ¹³C compared to the more resistant compounds such as lipids, degradation causes a shift to slightly more negative ^δ values. (2) Isotope fractionations due to metabolism of microorganisms produce new compounds having different isotopic compositions than the original source material. A classic example has been presented by Freeman et al. (1990) analyzing hydrocarbons from the Messel

Isotopes and Geobiology, Table 1 ^{δ¹³C} values of separated individual hydrocarbons (biomarkers) from the Messel shale (Freeman et al., 1990)

Peak	^{δ¹³C} (‰)	Compound
1	−22.7	Norpristane
2	−30.2	C ₁₉ acyclic isoprenoid
3	−25.4	Pristane
4	−31.8	Phytane
5	−29.1	C ₂₃ acyclic isoprenoid
8	−73.4	C ₃₂ acyclic isoprenoid
9	−24.2	Isoprenoid alkane
10	−49.9	22,29,30-Trisnorhopane
11	−60.4	Isoprenoid alkane
15	−65.3	30-Norhopane
19	−20.9	Lycopane

shale in Germany (see Table 1). While the major portion of the analyzed hydrocarbons reflects the primary biological source material, some hydrocarbons having low concentrations are extremely ¹³C depleted indicating their secondary microbial origin in a methane-rich environment. Later studies, summarized by Peckmann and Thiel (2004), have documented even larger ¹³C depletions (^{δ¹³C} values as low as −120‰) in various biomarkers that have been formed by diverse methanotrophic taxa.

C-isotope evidence for earliest signs of life on Earth

The idea of using C-isotope compositions of organic matter for the detection of early life dates back to the classic paper of Craig (1953). Already, at that time, it was discussed whether graphitic carbon in old rocks could be identified as being of biogenic origin (Craig, 1954; Rankama, 1954). This controversy about the meaning of graphite ^{δ¹³C} values from early Archean (3.5–3.8 Ga) rocks has continued till today (e.g., Bolhar et al., 2004). The validity of using carbon isotopes in the search for early life hinges on the assumption that early organic metabolism produced a C-isotope fractionation effect similar to that observed today. In addition, the thermal history of the metasediments has to be considered. In early Archean metasedimentary rocks from Greenland, ^{δ¹³C} values range from −22 to −50‰, which could be interpreted as biogenic (Mojzsis et al., 1996). Very negative values were measured for tiny particles of reduced carbon within apatite grains, which apparently had been shielded from metamorphic alteration. However, a case also can be made that graphite from Archean rocks may be of abiotic, hydrothermal origin. In recent years, more experimental and natural evidence has been presented that Fischer–Tropsch type reactions may produce abiogenic organic matter with ^{δ¹³C} values similar to biogenic organic matter (Taran et al., 2007).

Nitrogen

Microorganisms are responsible for all major conversions in the biological nitrogen cycle, which generally is divided into nitrogen fixation, nitrification, and denitrification. Other bacteria return nitrogen to the atmosphere as N_2 . *Fixation* describes the conversion of unreactive atmospheric N_2 into reactive nitrogen such as ammonium, usually involving bacteria. Fixation commonly produces organic materials with $\delta^{15}N$ values slightly less than 0‰ ranging from −3 to +1 (Fogel and Cifuentes, 1993) and is performed in the roots of plants by many bacteria. The large amount of energy needed to break the molecular nitrogen bond makes nitrogen fixation a very inefficient process with little associated N-isotope fractionation.

Nitrification is a multistep oxidation process mediated by several different autotrophic organisms. Nitrate is not the only product of nitrification, different reactions produce various nitrogen oxides as intermediate species. Nitrification can be described as two partial oxidation reactions, each of which proceeds separately. Oxidation by *Nitrosomonas* ($NH_4 \rightarrow NO_2$) followed by oxidation by *Nitrobacter* ($NO_2 \rightarrow NO_3$). Because the oxidation of nitrite to nitrate is generally rapid, most of the N-isotope fractionation is caused by the slow oxidation of ammonium by *Nitrosomonas*. In N-limited systems, fractionation is minimal.

Denitrification (reduction of more oxidized forms to more reduced forms of nitrogen) is a multistep process with various nitrogen oxides as intermediate compounds resulting from biologically mediated reduction of nitrate. Denitrification takes place in poorly aerated soil and in stratified anaerobic water bodies. Denitrification causes the $\delta^{15}N$ values of the residual nitrate to increase exponentially as nitrate concentrations decrease. Experimental investigations have demonstrated that fractionation factors may change from 10‰ to 30‰, with the largest values obtained under lowest reduction rates. Table 2, which gives a summary of observed N-isotope fractionations, clearly indicates the dependence of fractionation factors on nitrogen concentrations and demonstrates that

Isotopes and Geobiology, Table 2 Naturally observed isotope fractionation for nitrogen assimilation (after Fogel and Cifuentes, 1993)

N_2 fixation	−3 to +1‰
NH_4^+ assimilation	
Cultures	
Millimolar concentrations	0 to −15‰
Micromolar concentrations	−3 to −27‰
Field observations	
Micromolar concentrations	−10‰
NO_3^- assimilation	
Cultures	
Millimolar concentrations	0 to −24‰
Micromolar concentrations	−10‰
Field observations	
Micromolar concentrations	−4 to −5‰

at low nitrogen concentrations, fractionation is nearly zero because virtually all the nitrogen is used.

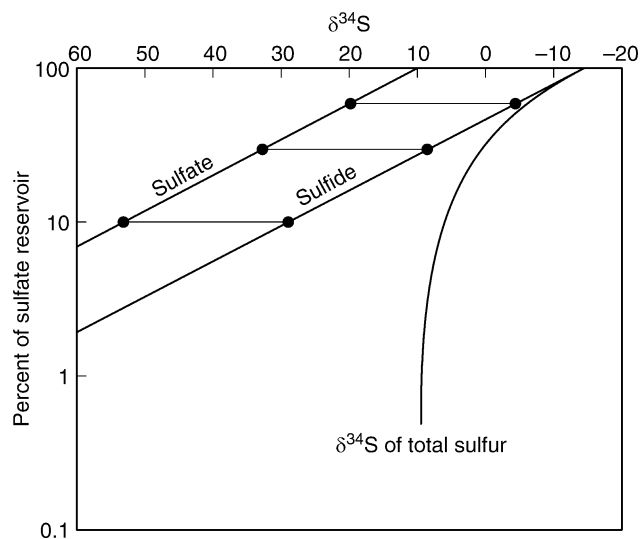
Sulfur-dissimilatory sulfate reduction

Large ^{34}S -isotope fractionations are induced during bacterial sulfate reduction. Since the early work with living cultures (Harrison and Thode, 1957a, b) it is well known that sulfate-reducing bacteria produce sulfide depleted in ^{34}S by 4–49‰. In general, the rate-limiting step is the breaking of the first S–O bond, namely the reduction of sulfate to sulfite (Goldhaber and Kaplan, 1974; Brunner et al., 2005).

Despite decades of intense research, the factors that determine the magnitude of sulfur isotope fractionation during bacterial sulfate reduction are still under debate. The magnitude of isotope fractionation depends on the rate of sulfate reduction with the highest fractionation at low rates and the lowest fractionation at high rates. One parameter which remains unclear is sulfate concentration.

Naturally occurring sulfides in sediments and euxinic waters are commonly depleted in ^{34}S by up to 70‰, far beyond the apparent capabilities of sulfate-reducing bacteria in the laboratory. Much of the sulfide produced by sulfate reduction in sediments is reoxidized, often via compounds in which sulfur has intermediate oxidation states that do not accumulate, but are readily transformed and can be further disproportionated by bacteria.

Another factor that is of great importance for the observed sulfur isotope variations of natural sulfides is whether sulfate reduction takes place in an open or a closed system. An “open” system has an infinite reservoir of sulfate in which continuous removal from the



Isotopes and Geobiology, Figure 1 Rayleigh plot for sulfur isotope fractionation during closed-system reduction of sulfate. Assumed fractionation factor 1.025; assumed initial isotope composition of sulfate +10‰.

source produces no detectable loss of material. Typical examples are the Black Sea and local oceanic deeps. In such cases, H_2S is extremely depleted in ^{34}S while consumption and change in ^{34}S remain negligible for the sulfate. In a “closed” system, the preferential loss of the lighter isotope ^{32}S from the reservoir has a feedback on the isotopic composition of the unreacted source material. The changes in the ^{34}S content of residual sulfate and of the H_2S are modeled in Figure 1, which shows that $\delta^{34}\text{S}$ values of the residual sulfate steadily increase with sulfate consumption (a linear relationship on a log-normal plot). The curve for the derivative H_2S is parallel to the sulfate curve at a distance which depends on the magnitude of the fractionation factor. As shown in Figure 1, H_2S may become isotopically heavier than the original sulfate when about two third of the reservoir has been consumed. The $\delta^{34}\text{S}$ curve for “total” sulfide asymptotically approaches the initial value of the original sulfate. It should be noted, however, that apparent “closed-system” behavior of covarying sulfate and sulfide $\delta^{34}\text{S}$ values might be also explained by “open-system” differential diffusion of the different sulfur isotope species (Jørgensen et al., 2004).

Another characteristic feature of microbial sulfate reduction is significant small-scale spatial variability in S-isotope composition of pyrite. Up to 105‰ variations in $^{34}\text{S}/^{32}\text{S}$ ratios within strongly zoned pyrite was observed by McKibben and Riciputi (1998). This zonation arises from progressive precipitation of sulfide from pore water sulfate enriched in ^{34}S providing more ^{34}S -enriched sulfide.

New insights on sulfur isotope fractionation mechanisms have been obtained from the analysis of the minor isotopes ^{33}S and ^{36}S (Farquhar et al., 2003; Johnston et al., 2005; Ono et al., 2006). By studying all sulfur isotopes with very high precision, these authors could demonstrate that bacterial sulfate reduction follows a mass-dependent relationship that is slightly different from that expected by equilibrium fractionations. As a result, samples with the same $\delta^{34}\text{S}$ value can have different $\Delta^{33}\text{S}$ and $\Delta^{36}\text{S}$ values. This opens the possibility to distinguish between different fractionation mechanisms and biosynthetic pathways (Ono et al., 2006). For instance, bacterial sulfate reduction shows slightly different fractionation relationships compared to sulfur disproportionation reactions (Johnston et al., 2005).

When did bacterial sulfate reduction start on Earth?

Considering a typical difference in $\delta^{34}\text{S}$ values of 20–60‰ between marine sulfate and bacteriogenic sulfide in present-day sedimentary environments, similar fractionations in ancient sedimentary rocks may be interpreted as an evidence for the activity of sulfate-reducing bacteria. The presence or absence of such fractionations in sedimentary rocks thus may constrain the time of emergence of sulfate-reducing bacteria. Sedimentary pyrite depleted in ^{34}S has been observed as far back in geological time as the Precambrian. There is, however,

debate about the dating of first onset of bacterial reduction in the geological record.

In early Archean sedimentary rocks, most sulfides and the rare sulfates have $\delta^{34}\text{S}$ values near 0‰ (Monster et al., 1979; Cameron, 1982), which has been interpreted as indicating an absence of bacterial reduction in the Archean. Shen et al. (2001) and Shen and Buick (2004) argued that the large spread in $\delta^{34}\text{S}$ values of microscopic pyrites aligned along growth faces of former gypsum in the 3.47 Ga North Pole barite deposit; Australia represents the oldest evidence for microbial sulfate reduction. By measuring all four stable isotopes ^{32}S , ^{33}S , ^{34}S , and ^{36}S , additional evidence can be gained to decouple hydrothermal versus biological processes (Ono et al., 2006) even when $\delta^{34}\text{S}$ values are inconclusive.

Mars

McKay et al. (1996) have claimed that Martian meteorite ALH 84001 – found in Antarctica – contains evidence of post-Martian life. Various kinds of apparent biosignatures with characteristic isotope compositions have been suggested: organic matter, carbonate minerals, magnetite grains, and sulfide minerals. After intensive investigations during the last years, none of these proposed biosignatures have survived as such.

Carbonates in Martian meteorites have been especially well studied because understanding the formation conditions of the carbonates is crucial in this connection. Despite extensive chemical and mineralogical studies, the environment of carbonate formation has remained unclear. For instance, in situ C-isotope analysis by Niles et al. (2005) gave highly zoned $\delta^{13}\text{C}$ values from +30‰ to +60‰ consistent with a derivation from the Martian atmosphere and suggesting abiotic formation.

Further evidence about a nonbiogenic origin of Martian carbonates (and even less abundant sulfates) has been presented by Farquhar et al. (1989). By measuring $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values, these authors observed an ^{17}O anomaly in the carbonates relative to the silicates which they interpreted as being produced by the photochemical decomposition of ozone just as in the Earth’s stratosphere. This finding suggests that carbonates (and sulfates) are derived from atmosphere–regolith interactions on Mars and not from biological activities.

Fe isotopes as biosignatures?

Virtually all reduction from Fe^{3+} to Fe^{2+} at the Earth’s surface is mediated by the metabolism of dissimilatory bacteria. Biological processes, therefore, may produce measurable Fe-isotopic fractionations because the metabolic processing of Fe involves a number of steps such as transport across membranes that may fractionate Fe isotopes. Experiments with dissimilatory Fe-reducing bacteria of the genus *Shewanella* indicate that iron isotope fractionations are a function of Fe(III) reduction rates: at low rates the produced Fe^{2+} is isotopically depleted by 1.3‰, whereas the depletion is up to 3‰ at high rates (Johnson et al., 2005).

Iron isotope fractionation has also been observed during bacterial Fe oxidation (Croal et al., 2004). Fe(II) oxidizing phototrophs may produce under anaerobic conditions a ferrihydrite precipitate that is 1.5‰ higher than the aqueous Fe(II) source. Controversy still exists whether the iron isotope variations observed in microbial experiments are primarily controlled by kinetic or nonbiological equilibrium factors. Since abiotic iron reduction/oxidation reactions may reveal fractionations similar in direction and magnitude to microbial reactions (Johnson et al., 2002; Skulan et al., 2002), the presence of Fe isotope variations is not in itself conclusive evidence of biotic activity. This complicates the ability to use iron isotopes to identify microbiological processing in the rock record (Balci et al., 2006).

Conclusions

“Biological processes fractionate the stable isotopes of C, N, S, and Fe in a characteristic way leading to biosignatures in the geosphere. Such isotope fingerprints have been used as indicators of early life on Earth and Mars. Since abiological processes sometimes may lead to isotope compositions comparable to biological processes, isotope biosignatures have to be applied with care.”

Bibliography

- Abelson, P. H., and Hoering, T. C., 1961. Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. *Proceedings of the National Academy of Sciences of the United States of America*, **47**, 623.
- Balci, N., Bullen, T. D., Witte-Lien, K., Shanks, W. C., Motelica, M., and Mandernack, K. W., 2006. Iron isotope fractionation during microbially substituted simulated Fe(II) oxidation and Fe(III) precipitation. *Geochimica et Cosmochimica Acta*, **70**, 622–639.
- Bolhar, R., Kamber, B. S., Moorbath, S., Fedo, C. M., and Whitehouse, M. J., 2004. Characterisation of early Archaean chemical sediments by trace element signatures. *Earth and Planetary Science Letters*, **222**, 43–60.
- Brunner, B., Bernasconi, S. M., Kleikemper, J., and Schroth, M. H., 2005. A model of oxygen and sulphur isotope fractionation in sulfate during bacterial sulfate reduction. *Geochimica et Cosmochimica Acta*, **69**, 4773–4785.
- Cameron, E. M., 1982. Sulphate and sulphate reduction in early Precambrian oceans. *Nature*, **296**, 145–149.
- Craig, H., 1953. The geochemistry of the stable carbon isotopes. *Geochimica et Cosmochimica Acta*, **3**, 133–149.
- Craig, H., 1954. Geochemical implications of the isotopic composition of carbon in ancient rocks. *Geochimica et Cosmochimica Acta*, **6**, 186–196.
- Croal, L. R., Johnson, C. M., Beard, B. L., and Newman, D. K., 2004. Iron isotope fractionation by Fe(II) oxidizing photoautotrophic bacteria. *Geochimica et Cosmochimica Acta*, **68**, 1227–1242.
- Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T., 1989. Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503–537.
- Farquhar, J., Thiemens, M. H., and Jackson, T., 1998. Atmosphere-surface interactions on Mars: $\Delta^{17}\text{O}$ measurements of carbonate from ALH 84001. *Science*, **280**, 1580–1582.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulfur cycle. *Science*, **289**, 756–759.
- Farquhar, J., Johnston, D. T., Wing, B. A., Habicht, K. S., Canfield, D. E., Airieau, S., and Thiemens, M. H., 2003. Multiple sulphur isotope interpretations for biosynthetic pathways: implications for biological signatures in the sulphur isotope record. *Geobiology*, **1**, 27–36.
- Fogel, M. L., and Cifuentes, L. A., 1993. Isotope fractionation during primary production. In Engel, M. H., and Macko, S. A. (eds.), *Organic Geochemistry*. New York: Plenum, pp. 73–98.
- Freeman, K. H., 2001. Isotopic biogeochemistry of marine organic carbon. *Reviews in Mineralogy and Geochemistry*, **43**, 579–605.
- Freeman, K. H., Hayes, J. M., Trendel, J. M., and Albrecht, P., 1990. Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons. *Nature*, **343**, 254–256.
- Goldhaber, M. B., and Kaplan, I. R., 1974. The sedimentary sulphur cycle. In Goldberg, E. B. (ed.), *The Sea Vol IV*. New York: Wiley.
- Harrison, A. G., and Thode, H. G., 1957a. Kinetic isotope effect in chemical reduction of sulphate. *Faraday Society Transaction*, **53**, 1648–1651.
- Harrison, A. G., and Thode, H. G., 1957b. Mechanism of the bacterial reduction of sulphate from isotope fractionation studies. *Faraday Society Transaction*, **54**, 84–92.
- Hayes, J. M., 1993. Factors controlling ^{13}C contents of sedimentary organic compounds: principle and evidence. *Marine Geology*, **113**, 111–125.
- Hayes, J. M., 2001. Fractionation of carbon and hydrogen isotopes in biosynthetic processes. In Valley, J. W., and Cole, D. R. (eds.), *Stable Isotope Geochemistry*. Reviews in Mineralogy and Geochemistry, **43**, 225–277.
- Johnson, C. M., Skulan, J. L., Beard, B. L., Sun, H., Neelson, K. H., and Brateman, P. S., 2002. Isotopic fraction between Fe(III) and Fe(II) in aqueous solutions. *Earth and Planetary Science Letters*, **195**, 141–153.
- Johnson, C. M., Roden, E. R., Welch, S. A., and Beard, B. L., 2005. Experimental constraints on Fe isotope fractionation during magnetite and Fe carbonate formation coupled to dissimilatory hydrous ferric oxide reduction. *Geochimica et Cosmochimica Acta*, **69**, 963–993.
- Johnston, D. T., Farquhar, J., Wing, B. A., Kaufman, A. J., Canfield, D. E., and Habicht, K. S., 2005. Multiple sulphur isotope fractionations in biological systems: a case study with sulphate reducers and sulphur disproportionators. *American Journal of Science*, **305**, 645–660.
- Jørgensen, B. B., Böttcher, M. A., Lüschen, H., Neretin, L. N., and Volkov, I. I., 2004. Anaerobic methane oxidation and a deep H_2S sink generate isotopically heavy sulfides in Black Sea sediments. *Geochimica et Cosmochimica Acta*, **68**, 2095–2118.
- McKay, D. S., Gibson, E. K. Jr., Thomas-Keprta, K. L., Vali, H., Romanek, C. S., Clemett, S. J., Chiller, X. D. F., Maechling, C. R., and Zare, R. N., 1996. Search for past life on Mars: possible relic biogenic activity in martian meteorite ALH 84001. *Science*, **273**, 924–930.
- McKibben, M. A., and Eldridge, C. S., 1995. Microscopic sulfur isotope variations in ore minerals from the Viburnum Trend, Southeast Missouri: a shrimp study. *Economic Geology*, **90**, 228–245.
- McKibben, M. A., and Riciputi, L. R., 1998. Sulfur isotopes by ion microprobe. In: Application of microanalytical techniques to understanding mineralizing processes. *Reviews Economic Geology*, **7**, 121–140.
- Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P., and Friend, C. R. L., 1996. Evidence for life on Earth before 3,800 million years ago. *Nature*, **384**, 55–59.
- Monster, J., Appel, P. W., Thode, H. G., Schidlowski, M., Carmichael, C. W., and Bridgewater, D., 1979. Sulphur isotope studies in early Archean sediments from Isua, West Greenland: implications for the antiquity of bacterial sulfate reduction. *Geochimica et Cosmochimica Acta*, **43**, 405–413.

- Niles, P. B., Leshin, L. A., and Guan, Y., 2005. Microscale carbon isotope variability in ALH84001 carbonates and a discussion of possible formation environments. *Geochimica et Cosmochimica Acta*, **69**, 2931–2944.
- O' Leary, M. H., 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553–567.
- Ono, S., Wing, B. A., Johnston, D., Farquhar, J., and Rumble, D., 2006. Mass-dependent fractionation of quadruple sulphur isotope system as a new tracer of sulphur biogeochemical cycles. *Geochimica et Cosmochimica Acta*, **70**, 2238–2252.
- Park, R., and Epstein, S., 1960. Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta*, **21**, 110–126.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Rankama, K., 1954. Early pre-Cambrian carbon of biogenic origin from the Canadian Shield. *Science*, **119**, 506.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth Science Reviews*, **64**, 243–272.
- Shen, Y., Buick, R., and Canfield, D. E., 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature*, **410**, 77–81.
- Skulan, J. L., Beard, B. L., and Johnson, C. M., 2002. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(III) and hematite. *Geochimica et Cosmochimica Acta*, **66**, 2995–3015.
- Taran, Y. A., Kliger, G. A., and Sevastianov, V. S., 2007. Carbon isotope effect in the open system Fischer Trosch synthesis. *Geochimica et Cosmochimica Acta*, **71**, 4474–4487.
- Thode, H. G., Macnamara, J., and Collins, C. B., 1949. Natural variations in the isotopic content of sulphur and their significance. *Canadian Journal of Research*, **27B**, 361.
- Wellman, R. P., Cook, F. D., and Krouse, H. D., 1968. Nitrogen-15: microbiological alteration of abundance. *Science*, **161**, 269–270.

Cross-references

Anaerobic Oxidation of Methane with Sulfate
 Astrobiology
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Iron Sulfide Formation
 Isotope Fractionation (Metal)
 Nitrogen
 Methane, Origin
 Photosynthesis
 Sulfate-Reducing Bacteria
 Sulfur Isotopes

ISOTOPES, RADIOGENIC

Bent T. Hansen
 University of Göttingen, Göttingen, Germany

Definition

Radiogenic nuclides (more commonly referred to as “radiogenic isotopes”) are produced by a process of radioactive decay. Radioactive decay means that unstable isotopes (mother nuclides) are spontaneously transferred

into stable isotopes (daughter nuclides) by emission of particles and loss of energy (radioactive radiation).

Introduction

The radioactive decay provides an accurate method of measuring the ages of rocks and minerals. This possibility was recognized both by Rutherford (1906) and Boltwood (1907) at the beginning of the twentieth century. However, the measurement and interpretation of variations in the isotopic composition of certain elements in natural materials were not possible until modern mass spectrometers were developed based on the design by Nier (1940). After the World War II, isotope determinations became an important tool in modern geology, geochemistry, and geobiology not only for dating geological processes, but also for investigations of climate change, environmental, and biological parameters. The most frequently used systems are Rb/Sr, Sr/Sr, Sm/Nd, Nd/Nd, U/Pb, and Pb/Pb. In the following, typical examples of the most common radiogenic isotope applications are presented.

Strontium isotopes

There are four stable isotopes of Sr: ^{88}Sr , ^{87}Sr , ^{86}Sr , and ^{84}Sr . All but ^{87}Sr are nonradiogenic (that is, not the products of radioactive decay), whereas ^{87}Sr is produced by radioactive decay of ^{87}Rb (half-life 0.488×10^9 years). The $^{87}\text{Rb} \rightarrow ^{87}\text{Sr}$ radioactive decay pair has therefore produced different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios depending on the age and the amount of Rubidium in the material investigated. For more than half of the century, the Rb/Sr system is one of the most frequently used decay systems for dating geological events.

The isotopic composition of strontium is not only used for dating purposes, but also has turned out to be a universal tool for describing a variety of geological, biological, and environmental processes.

Groundwater and basement brines

Strontium is easily dissolved in water. The advantage of the strontium isotopic system is that the isotopic composition assumed till recently will not be changed by fractionation processes; it means that strontium in circulation depends on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the rocks and minerals that interact with water at or near the surface of the Earth. The Sr isotope composition of groundwater aquifers is constant over years, independent of the amount of rainfall, as demonstrated in a recent study by Klaus et al. (2007). Investigations in the Bangkok Metropolitan Area showed that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are useful tools to characterize groundwater flow and related biological and environmental processes.

Strontium isotope variations also play an important role in evaluating the suitability of crystalline rocks for geological disposal of nuclear waste. At the Hard Rock Laboratory at Äspö in southern Sweden, Sr isotope composition of groundwater has been investigated by Peterman and Walin (1999) down to a depth of 850 m from boreholes. Microbial

biofilms found in the deeper part of the tunnel are controlled in their Sr composition by highly saline brines (58 mg/l and $\delta^{87}\text{Sr}$ values as large as +13.9‰). Mixing with Baltic seawater from the surface (1.5 mg/l and $\delta^{87}\text{Sr}$ values of +0.3‰) is negligible; nevertheless, the method is sensitive enough to show the very small input of Baltic seawater even in the deepest parts of the Äspö tunnel.

Sr isotope investigations on high saline brines within the German Continental Deep Drilling Program (KTB) showed that the Sr isotopic compositions of paleofluids and recent fluids in the upper continental crust can be distinguished by means of $^{87}\text{Sr}/^{86}\text{Sr}$ versus $1/\text{Sr}$ mixture curves (Möller et al., 1997). Similar to saline brines, mesophilic and (hyper) thermophilic microorganisms living in shallow or deeper crust showed different Sr isotope compositions. Hence, Sr isotopic compositions may comprise a promising tool for the correlation of deep biosphere systems with depth and temperature regimes.

Strontium in animal and human skeletons

As the ionic radius of Sr (1.13 Å) is similar to that of Ca (0.99 Å), strontium replaces calcium in organic matters and can therefore also be used as a tracer for animal and human mobility. Different geographic (geological) regions yield different Sr isotopic compositions and as natural variations are not changed through the food chain, the strontium composition measured in skeletal elements can be used to identify the region where an animal or a human being lived (Brian and Johnson, 2000). The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios reflect the Sr that was ingested while a specific skeletal element grew. Because different skeletal elements grow and exchange Sr at different stages during the lifetime, Sr isotopic analyses of different skeletal parts can be used to infer changes in geographic location at different stages in the life of an organism. Regarding a human being, this means that the Sr isotopic composition of the enamel in the teeth will reflect the composition as a child, due to the immobile nature of Sr and Ca in teeth, whereas the Sr composition in a bone (e.g., femur) will reflect the average composition over the last 10 years of life, due to continuous processing of Sr and Ca in bone. These conclusions, however, are only valid for skeletons which are of "High Middle Age" or older. In the time of globalization, no relationship between living place and geogenic Sr input in the food chain can be reconstructed anymore.

Strontium in seawater

Biogenic carbonates are fairly resistant to diagenetic alteration. As they are secreted directly from seawater by the organisms, they contain no detrital material, and therefore reflect the isotopic composition of the seawater at the time of deposition. Biogenic carbonates are nearly free of Rb and can therefore not be dated directly. However, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of these carbonates can be used to define the evolution of the seawater isotopic composition by known stratigraphic age deduced from fossils.

Fundamental studies on the Sr seawater evolution curve were published between 1970 (Peterman et al., 1970) and 1982 (Burke et al., 1982). The obtained curves were all based on Sr isotope data from Phanerozoic carbonates. The seawater Sr data set was later extended back to the Late Proterozoic (Derry et al., 1989; Asmerom et al., 1991; Kaufman et al., 1993). In the absence of fossils, these studies were carried out on leached whole-rock carbonate samples to avoid contamination by detrital material (Figure 1).

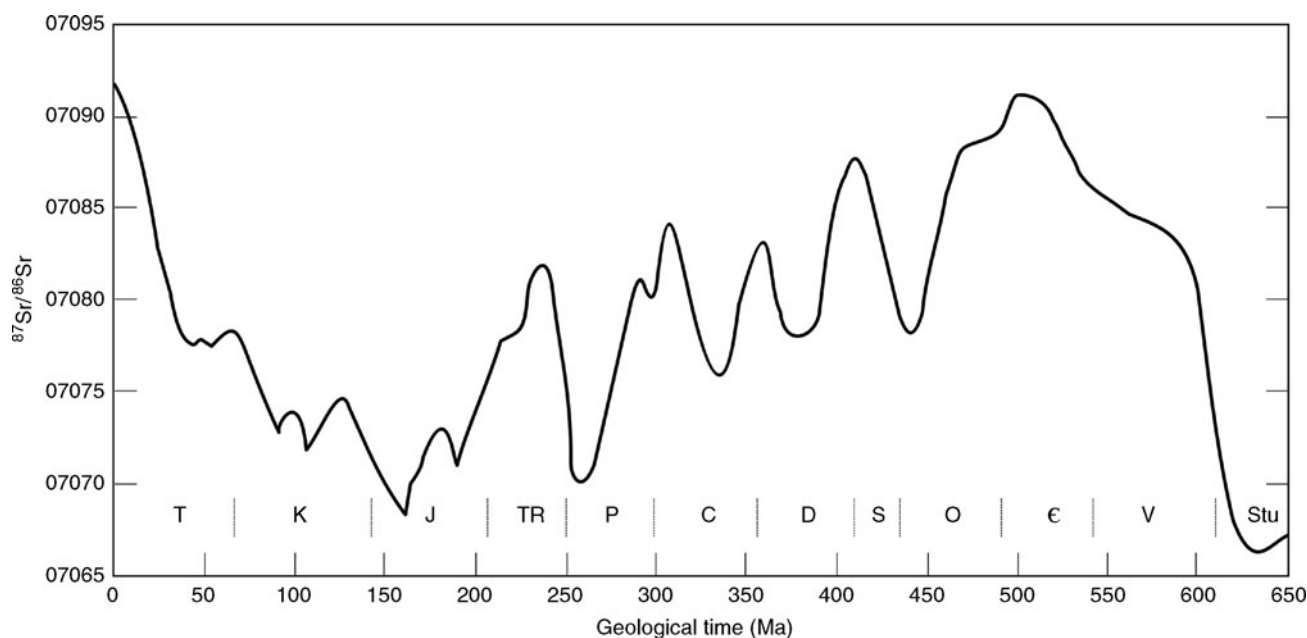
The seawater evolution curve can not only be used as an indirect dating tool for biogenic precipitations but may also be used as a geobiological tool, e.g., for indicating the setting of carbonate precipitations accompanying methane seeps. Investigations on methane-derived carbonates from the northwestern Black Sea showed $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of microcrystalline carbonates of 0.70927 and aragonitic cement of 0.70918. Both ratios are indistinguishable from ambient seawater (0.70917), and therefore indicate a shallow Sr source (Peckmann et al., 2001). The higher ratio of 0.71005 in the background sediments is related to detrital mica.

Paleotemperatures

Corals are primarily known as archives not only for changes in the sea surface temperature, but also for the variability in salinity and productivity. The latter are reflected in the common proxies such as $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. However, the temperature equations are only valid for shallow-water corals. Further, proxies such as Sr/Ca, Mg/Ca, and U/Ca are dependent on growth parameters which are linked to specific coral species. As mentioned above, up to now it is assumed that geological processes do not cause any fractionation of the Sr isotopes. Recently, new techniques (Fietzke and Eisenhauer, 2006) have shown that this may not always hold true for calcium carbonate precipitation, including inorganic aragonite precipitation. Temperature-controlled precipitation of aragonite shows only minor variations in the Sr isotope fractionation (0.0054‰/°C) in the $\delta^{88}\text{Sr}/^{86}\text{Sr}$ ratios, whereas a natural coral sample (*Pavona clavus*) shows a fractionation nearly ten times higher (0.033‰/°C) in the $\delta^{88}\text{Sr}/^{86}\text{Sr}$ ratios. This means that the strontium isotope system can also be used as a paleothermometer for the identification of different fluid sources involved in calcium carbonate precipitation at low temperatures and to characterize processes of biomineralization. The consequence of these findings is that the seawater evolution curve discussed above also has to be corrected. The high potential of this method for studying climate-dependent variables especially for cold-water corals is demonstrated in a recent paper by Rüggeberg et al. (2008).

Neodymium isotopes

There are seven stable isotopes of Nd: ^{150}Nd , ^{148}Nd , ^{146}Nd , ^{145}Nd , ^{144}Nd , ^{143}Nd , and ^{142}Nd . All but ^{142}Nd and ^{143}Nd are nonradiogenic (that is, not the products of



Isotopes, Radiogenic, Figure 1 Time-dependent variation of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of seawater. T = Tertiary, K = Cretaceous, J = Jurassic, TR = Triassic, P = Permian, C = Carboniferous, D = Devonian, S = Silurian, O = Ordovician, ε = Cambrian, V = Vendian, Stu = Sturtian. (Data compiled after McArthur et al., 2001 and Kaufmann et al., 1993.)

radioactive decay). ^{143}Nd is produced by radioactive decay of ^{147}Sm (half-life 10.6×10^{10} years). The $^{147}\text{Sm} \rightarrow ^{143}\text{Nd}$ radioactive decay pair has therefore produced different $^{143}\text{Nd}/^{144}\text{Nd}$ ratios in the investigated materials, depending on the age and the amount of Sm. Due to the short half-life (1.0×10^8 years) of $^{146}\text{Sm} \rightarrow ^{142}\text{Nd}$, this system so far has played no significant role in interpreting geological processes.

Neodymium is, like strontium, released by selective chemical weathering of polycrystalline rocks, and therefore dissolved Nd does not have the same isotopic composition as in the weathered rock. However, particulate matter transported into the sea is a characteristic product of eroded rocks in a given source area on the continent. Bock et al. (2005) showed that young sediments (4300 and 1000 year BP) in the Baltic Sea reflect a substantial change of Nd isotopic composition. This can be related either to changing inputs from the source areas or as different inflow of North Sea water. Variations of both, erosive continental input and North Sea inflow indicate a direct response of the Nd isotope signal in the Baltic Sea to climate change. The authors show that the Nd signal can be related to the cyclic shifts in the atmospheric circulation as well as demographic changes affecting agricultural activities. The reduction of farmland would also cause a reduction of soil erosion that changes the amount and composition of river-transported sediments into the Baltic Sea. This example shows that neodymium isotopic composition of sediment cores is a valuable tool for tracing climate changes.

Glacial-interglacial cycles are reflected in both Sr and Nd isotopic compositions (Tütken et al., 2002). The investigation of Arctic marine sediment cores revealed that it is possible to correlate ice rafted sediments with their source rocks, and therefore the glacial distribution as a function of time. The fact that Sr isotope composition is anticorrelated to the Nd isotopic composition makes such combined isotopic studies very sensitive to climate changes, and therefore also contributes to elucidate biological processes.

Lead isotopes

There are four stable isotopes of lead: ^{208}Pb , ^{207}Pb , ^{206}Pb , and ^{204}Pb . Only ^{204}Pb is not a product of radioactive decay, whereas ^{208}Pb , ^{207}Pb , and ^{206}Pb are produced by radioactive decay of ^{232}Th (half-life 14.010×10^9 years), ^{235}U (half-life 0.7038×10^9 years), and ^{238}U (half-life 4.468×10^9 years), respectively.

The so-called “common lead method” is used for dating minerals whose U/Pb and Th/Pb ratios are so low that their isotopic compositions do not change with time. The obtained ages, however, may vary with the evolution models used for the common lead method. One of the models, “the single-stage model” assumes that the primordial lead isotope ratios evolved in different reservoirs with different U/Pb ratios. This model is named the “Holmes–Hautermans Model” (Holmes, 1946; Hautermans, 1946). The measured isotopic compositions of the lead often resulted in ages which were to be obtained in the future

if the single-stage model was applied. Therefore, the “two-stage” Pb evolution model was proposed by Stacey and Kramers (1975). In this model, Pb evolves from primordial isotope ratios between 4.57 Ga and 3.7 Ga in a reservoir with $^{238}\text{U}/^{204}\text{Pb}$ of 7.192. At 3.7 Ga, the $^{238}\text{U}/^{204}\text{Pb}$ ratio of the reservoir changed by chemical differentiation to 9.735. Thereafter, lead evolution continued without change until present. A line connecting measured isotope ratios ($^{207}\text{Pb}/^{204}\text{Pb}$ vs. $^{206}\text{Pb}/^{204}\text{Pb}$) and the corresponding ratios at 3.7 Ga will intersect with the evolution line; these lines are called isochrones. The slope of these isochrones are related to the time elapsed since a lead sample was extracted from the reservoir, and therefore the age since separation can be calculated. Even if this model gives more geological relevant ages, it is still insufficient for geological dating. Nonetheless, lead isotopes may reveal excellent tracers for environmental investigations and, as lead is a toxic element, for characterizing biological processes. Lead isotope compositions do not change during industrial and environmental processes, and therefore reflect the lead source, thus allowing to distinguish between natural and anthropogenic origins (e.g., Cicchella et al., 2008). The anthropogenic lead has until recently mainly been contributed by leaded gasoline, with additional inputs caused by the combustion of coal. The lead isotopic signature of coal is more radiogenic, as coal is found in sedimentary rocks. Lead from basement rocks, which are the sources of ore deposits, show a different (lower) isotopic signature. Isotope analyses of, e.g., snow and surface waters can therefore contribute to unravel the sources and dynamics of lead in the oceans and the atmosphere and contribute to the database concerning climate change (Hamelin et al., 1997).

Exposure to lead as well as other heavy metals in the environment is a matter of public health concern. The lead isotopes play an important role in the development of biomarkers. Lead transfer across the placental barrier and injections of marked lead (^{206}Pb and ^{204}Pb , respectively) result in deposition in the enamel. Analyses of the enamels can be used to observe biological removal and assimilation of prenatal and postnatal tracers, respectively. This technique can be used to demonstrate the toxicokinetics of incorporating lead into fetal and neonatal steady-state system processes (Rinderknecht et al., 2005). The accumulation of heavy metals in aquatic organisms through the food chain may lead to serious health problems. Investigations of lead isotopes showed that the bioaccumulation of Pb in fish is due to a wide variety of food sources and/or exposure pathways, particularly anthropogenic inputs (Ip et al., 2005). These are only a few examples of the usefulness of lead isotopes in modern environmental research which becomes more important with increasing pollution.

Conclusions

Summarizing the applicability of selected radiogenic isotope systems, it can be concluded that a very wide field of applications can be covered, not only traditional dating

of geological events but also numerous applications in geological, (geo)biological, environmental, archeological, and forensic processes can be answered by means of radiogenic isotope investigations (further application references can be found in Hansen, 2005).

Bibliography

- Asmerom, Y., Jacobseb, S. B., Knoll, A. H., Butterfield, N. J., and Swett, K., 1991. Strontium variations of Neoproterozoic seawater: implications for crustal evolution. *Geochimica Cosmochimica Acta*, **55**, 2883–2894.
- Bock, B., Liebetrau, V., Eisenhauer, A., Frei, R., and Leipe, T., 2005. Nd isotope signature of Holocene Baltic Mn/Fe precipitates as a monitor of climate change during the Little Ice Age. *Geochimica et Cosmochimica Acta*, **68**, 2253–2263.
- Boltwood, B. B., 1907. On the ultimate disintegration products of the radioactive elements. *American Journal of Science*, **23**(4), 77–88.
- Brian, L., and Johnson, C., 2000. Strontium isotope Composition of skeletal material can determine the birth place and geographic mobility of humans and animals. *Journal of Forensic Science*, **45**, 1049–1061.
- Burke, W. H., Denison, R. E., Hetherington, E. A., Koepnick, R. B., Nelson, H. F., and Otto, J. B., 1982. Variations of seawater $^{87}\text{Sr}/^{86}\text{Sr}$ throughout Phanerozoic time. *Geology*, **10**, 516–519.
- Cicchella, D., De Vivo, B., Lima, A., Albanese, S., McGill, R. A. R., and Parrish, R. R., 2008. Heavy metal pollution and Pb isotopes in urban soils of Napoli, Italy. *Geochemistry: Exploration, Environment, Analyses*, **8**, 103–112.
- Derry, L. A., Keto, L. S., Jacobsen, S. B., Knoll, A. H., and Swett, K., 1989. Sr Isotope variations in Upper Proterozoic carbonates from Svalbard and East Greenland. *Geochimica et Cosmochimica Acta*, **53**, 2331–2339.
- Fietzke, J., and Eisenhauer, A., 2006. Determination of temperature-dependent stable strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS. *Geochemistry Geophysics Geosystems*, **7**, Q08009, doi 10.1029/2006GC001243.
- Hamelin, B., Ferrand, J. L., Alleman, L., Nicolas, E., and Veron, A., 1997. Isotopic evidence of pollutant lead transport from North America to the subtropical North Atlantic gyre. *Geochimica Cosmochimica Acta*, **61**, 4423–4428.
- Hansen, B. T., 2005. Isotope, die Gene der Gesteine. In Reitner, J., Weber, K., and Karg, U. (eds.), *In Das System Erde – Was bewegt die Welt? Lebensraum und Zukunftsperspektiven*. Göttingen: Universitätsverlag Göttingen, pp. 73–87.
- Hautermans, F. G., 1946. Die Isotopenhäufigkeiten im natürlichen Blei und das Alter des Urans. *Naturwissenschaften*, **33**, 185–186.
- Holmes, A., 1946. An estimate of the age of the earth. *Nature*, **157**, 680–684.
- Ip, C. C. M., Li, X. D., Zhang, G., Wong, C. S. C., and Zhang, W. L., 2005. Heavy metal and Pb isotopic composition of aquatic organisms in the Pearl River Estuary, South China. *Environmental Pollution*, **138**, 494–504.
- Kaufman, A. J., Jacobseb, S. B., and Knoll, A. H., 1993. The Vendian record of Sr and C isotopic variations in seawater: implications for tectonics and paleoclimate. *Earth and Planetary Science Letters*, **120**, 409–430.
- Klaus, J. S., Hansen, B. T., and Buapeng, S., 2007. $^{87}\text{Sr}/^{86}\text{Sr}$ ratio: a natural tracer to monitor groundwater flow paths during artificial recharge in the Bangkok area, Thailand, with respect to Public Water Supply and Freshwater Reinjection. *Hydrology Journal*, **15**, 745–758.
- McArthur, J. M., Howarth, R. J., and Bailey, T. R., 2001. Strontium isotope stratigraphy: LOWESS version 3: best fit to the marine

- Sr-isotope curve for 0–509 Ma and accompanying look-up table for deriving numerical age. *The Journal of Geology*, **109**, 155–170.
- Möller, P., Weise, S. M., Althaus, E., Bach, W., Behr, H. J., Rochardt, R., Bräuer, K., Drescher, J., Erzinger, J., Faber, E., Hansen, B. T., Horn, E. E., Huenges, E., Kämpf, H., Kessels, W., Kirsten, T., Landwehr, D., Lodemann, M., Machon, L., Peckdecker, A., Pielow, H. U., Reutel, C., Simon, K., Walter, J., Weinlich, F. H., and Zimmer, M., 1997. Paleofluids and resent fluids in the upper continental crust: results from the German Continental Deep Drilling Program (KTB). *Journal of Geophysical Research*, **102B8**, 18233–18254.
- Möller, P., Woith, H., Dulski, P., Lüders, V., Erzinger, J., Kämpf, H., Pekdeger, A., Hansen, B., Lodemann, M., and Banks, D., 2005. Main and trace elements in KTB-VB fluid: composition and hints to its origin. *Geofluids*, **5**, 28–41.
- Nier, A. O., 1940. A mass spectrometer for routine isotope abundance measurements. *Review of Scientific Instruments*, **11**, 212–216.
- Peckmann, P., Reimer, A., Luth, C., Hansen, B. T., Heinicke, C., Hoefs, J., and Reitner, J., 2001. Methane-derived carbonates and authigenic pyrite from the northwestern Black Sea. *Marine Geology*, **117**, 129–150.
- Peterman, Z. E., and Walin, B., 1999. Synopsis of strontium isotope variations in groundwater at Äspö, southern Sweden. *Applied Geochemistry*, **14**, 939–951.
- Peterman, Z. E., Hedge, C. E., and Tourtelot, H. A., 1970. Isotopic composition of strontium in seawater throughout Phanerozoic time. *Geochimica et Cosmochimica Acta*, **34**, 105–120.
- Rinderknecht, A. L., Kleinman, M. T., and Ericson, J. E., 2005. Pb enamel biomarker: deposition of pre- and postnatal Pb isotope injection in reconstructed time points along rat enamel transect. *Environmental Research*, **99**, 169–176.
- Rüggeberg, A., Fietzke, J., Liebetrau, V., Eisenhauer, A., Dullo, W.-C., and Freiwald, A., 2008. Stable strontium isotopes $\delta^{88/86}\text{Sr}$ in cold-water corals – a new proxy for reconstruction of intermediate ocean water temperatures. *Earth and Planetary Science Letters*, **269**, 569–574.
- Rutherford, E., 1906. *Radioaktive Transformations*. New York: Charles Scribner, p. 287.
- Stacey, J. S., and Kramers, J. D., 1975. Approximation of terrestrial lead isotope evolution by a two-stage model. *Earth and Planetary Science Letters*, **26**, 207–221.
- Tütken, T., Eisenhauer, A., Wiegand, B., and Hansen, B. T., 2002. Glacial-interglacial cycles in Sr and Nd isotopic composition of Arctic marine sediments triggered by the Svalbard/Barent Sea ice sheet. *Marine Geology*, **182**, 351–372.

Cross-references

[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Geochronology](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

- Weiss, D. J., Rehkämper, M., Schoenberg, R., McLaughlin, M., Kirby, J., Campbell, P. G. C., Arnold, T., Chapman, J., Peel, K., and Gioia, A. S., 2008. Application of nontraditional stable-isotope systems to the study of sources and fate of metals in the environment. *Environmental Science and Technology*, **42**, 655–664.
- Welch, S. A., Beard, B. L., Johnson, C. M., and Bratermann, P. S., 2003. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(II) and Fe(III). *Geochimica et Cosmochimica Acta*, **67**, 4231–4250.
- Weyer, S., and Schwieters, J. B., 2003. High precision Fe isotope measurements with high mass resolution MC-ICPMS. *International Journal for Mass Spectrometry*, **226**, 355–368.
- Weyer, S., Anbar, A. D., Brey, G. P., Munker, C., Mezger, K., and Woodland, A. B., 2007. Fe-isotope fractionation during partial melting on Earth and the current view on the Fe-isotope budgets of the planets (reply to the comment of F. Poitrasson and to the comment of B. L. Beard and C. M. Johnson on “Iron isotope fractionation during planetary differentiation” by S. Weyer, A.D. Anbar, G. P. Brey, C. Munker, K. Mezger and A. B. Woodland). *Earth and Planetary Science Letters*, **256**, 638–646.
- Weyer, S., Anbar, A. D., Gerdes, A., Gordon, G. W., Algeo, T. J., and Boyle, E. A., 2008. Natural fractionation of $^{238}\text{U}/^{235}\text{U}$. *Geochimica et Cosmochimica Acta*, **72**, 345–359.
- Wiederhold, J. G., Kraemer, S. M., Teutsch, N., Borer, P. M., Halliday, A. N., and Kretzschmar, R., 2006. Iron isotope fractionation during proton-promoted, ligand-controlled, and reductive dissolution of goethite. *Environmental Science and Technology*, **40**, 3787–3793.
- Wiesli, R. A., Beard, B. L., and Johnson, C. M., 2004. Experimental determination of Fe isotope fractionation between aqueous Fe (II), Siderite, and “Green Rust” in abiotic systems. *Chemical Geology*, **211**, 343–362.
- Wille, M., Kramers, J. D., Nägler, T. F., Beukes, N. J., Schroder, S., Meisel, T., Lacassie, J. P., and Voegelin, A. R., 2007. Evidence for a gradual rise of oxygen between 2.6 and 2.5 Ga from Mo isotopes and Re-PGE signatures in shales. *Geochimica et Cosmochimica Acta*, **71**, 2417–2435.
- Wille, M., Nägler, T. F., Lehmann, B., Schroder, S., and Kramers, J. D., 2008. Hydrogen sulphide release to surface waters at the Precambrian/Cambrian boundary. *Nature*, **453**(7196), 767–769.
- Williams, H. M., McCammon, C. A., Peslier, A. H., Halliday, A. N., Teutsch, N., Levasseur, S., and Burg, J.-P., 2005. Iron isotope fractionation and the oxygen fugacity of the mantle. *Science*, **304**, 1656–1659.
- Wombacher, F., Rehkämper, M., Mezger, K., and Münker, C., 2003. Stable isotope compositions of cadmium in geological materials and meteorites determined by multiple-collector ICPMS. *Geochimica et Cosmochimica Acta*, **67**, 4639–4654.
- Yamaguchi, K. E., and Ohmoto, H., 2006. Comment on “iron isotope constraints on the Archean and paleoproterozoic ocean redox state”. *Science*, **311**, 177a.
- Yamaguchi, K. E., Johnson, C. M., Beard, B. L., and Ohmoto, H., 2005. Biogeochemical cycling of iron in the Archean-Paleoproterozoic Earth: constraints from iron isotope variations in sedimentary rocks from the Kaapvaal and Pilbara Cratons. *Chemical Geology*, **218**, 135–169.
- Young, E. D., and Galy, A., 2004. The isotope geochemistry and cosmochemistry of magnesium. In Johnson, C. M., Beard, B. L., and Albarède, F. (eds.), *Geochemistry of Non-traditional Stable Isotopes*. Washington: Mineralogical Society of America and Geochemical Society, pp. 197–230.
- Zhu, X. K., O’Nions, R. K., Guo, Y., and Reynolds, B. C., 2000. Secular variations of iron isotopes in North Atlantic Deep Water. *Science*, **287**, 2000–2002.

ISOTOPES (METHODS)

Please refer to entries “*Isotope Fractionation (Metal)*”, “*Geochronology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

ISOTOPES AND GEOBIOLOGY

Jochen Hoefs
University of Göttingen, Göttingen, Germany

Definition

It has long been recognized (e.g., Thode et al. (1949), Craig (1953) and Wellman et al. (1968)) that biological processes significantly fractionate the isotopes of C, N, and S, leading to characteristic *biosignatures* in sedimentary rocks that will be more or less preserved in the geological record. In the following, a brief overview is given of the major isotope fractionation processes in the biosphere and the geosphere. Special attention will be paid to secondary bacterial activity after deposition of the organic matter. Questions of general interest are isotopic indicators of early life on Earth and on Mars. A note of caution, however, is given whether abiogenic processes may lead to isotope compositions similar to that of biological activity.

Carbon isotope fractionation during photosynthesis

Early reviews by O’Leary (1981) and Farquhar et al. (1989) have provided the biochemical background of carbon isotope fractionations during photosynthesis, with more recent accounts by Hayes (2001) and Freeman (2001). The main isotope-discriminating steps during biological carbon fixation are (1) the uptake and intracellular diffusion of CO_2 and (2) the biosynthesis of cellular components. Such a two-step model was first proposed by Park and Epstein (1960):



From this simplified scheme, it follows that the diffusional process is reversible, whereas the enzymatic carbon fixation is irreversible. The two-step model of carbon fixation clearly suggests that isotope fractionation is dependent on the partial pressure of CO_2 , i.e., $p\text{CO}_2$ of the system. With an unlimited amount of CO_2 available to a plant, the enzymatic fractionation will determine the isotopic difference between the inorganic carbon source and the final bioproduct. Under these conditions, ^{13}C fractionations may vary from -17 to -40‰ (O’Leary, 1981). When the concentration of CO_2 is the limiting factor, the diffusion of CO_2 into the plant is the slow step in the reaction and carbon isotope fractionation of the plant decreases.

- carbon from small mountainous and upland rivers. *Global Biogeochemical Cycles*, **20**, GB3022, doi:10.1029/2005GB002677.
- Longworth, B. E., Petsch, S. T., Raymond, P. A., and Bauer, J. E., 2007. Linking lithology and land use to sources of dissolved and particulate organic matter in headwaters of a temperate, passive-margin river system. *Geochimica et Cosmochimica Acta*, **71**, 4233–4250.
- Martens, C. S., Haddad, R. I., and Chanton, J. P., 1992. Organic matter accumulation, remineralization, and burial in an anoxic coastal sediment. In Whelan, J. K., and Farrington, J. W. (eds.), *Productivity, Accumulation and Preservation of Organic Matter in Recent and Ancient Sediments*. New York: Columbia University Press, p. 82.
- Petsch, S. T., Berner, R. A., and Eglinton, T. I., 2000. A field study of the chemical weathering of ancient sedimentary organic matter. *Organic Geochemistry*, **31**, 475–487.
- Petsch, S. T., Smernik, R. J., Eglinton, T. I., and Oades, J. M., 2001. A solid state ^{13}C -NMR study of kerogen degradation during black shale weathering. *Geochimica et Cosmochimica Acta*, **65**, 1867–1882.
- Sabine, C. L., Heiman, M., Artaxo, P., Bakker, D. C. E., Chen, C. T. A., Field, C. B., Gruber, N., LeQuéré, C., Prinn, R. G., Richey, J. E., Romero-Lankao, P., Sathaye, J. A., and Valentini, R., 2004. Current status and past trends of the carbon cycle. In Field, C. B., and Raupach, M. R. (eds.), *The Global Carbon Cycle: Integrating Humans, Climate, and the Natural World*. Washington, DC: Island Press, pp. 17–44.
- Schmidt, M. W. I., and Noack, A. G., 2000. Black carbon in soils and sediments: analysis, distribution, implications and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.
- Sheridan, C. C., Lee, C., Wakeham, S. G., and Bishop, J. K. B., 2002. Suspended particle organic composition and cycling in surface and midwaters of the equatorial Pacific ocean. *Deep-Sea Research I*, **49**, 1983–2008.
- Suess, E., 1980. Particulate organic carbon fluxes in the oceans – surface productivity and oxygen utilization. *Nature*, **288**, 260–263.
- Van Mooy, B. A. S., Keil, R. G., and Devol, A. H., 2002. Impact of suboxia on sinking particulate organic carbon: enhanced carbon flux and preferential degradation of amino acids via denitrification. *Geochimica et Cosmochimica Acta*, **66**, 457–465.
- Wakeham, S. G., Lee, C., Hedges, J. I., Hernes, P. J., and Peterson, M. L., 1997. Molecular indicators of diagenetic status in marine organic matter. *Geochimica et Cosmochimica Acta*, **61**, 5363–5369.
- Wakeham, S. G., Lee, C., and Hedges, J. I., 2002. Composition and flux of particulate amino acids and chloropigments in equatorial Pacific seawater and sediments. *Deep-Sea Research I*, **47**, 1535–1568.
- Waldron, P., Petsch, S. T., Martini, A. M., and Nüsslein, K., 2007. Archaeal community structure and metabolic activity across a salinity gradient within a methane-producing sedimentary basin. *Applied and Environmental Microbiology*, **73**, 4171–4179.
- Wellsbury, P., Mather, I., and Parkes, R. J., 2002. Geomicrobiology of deep, low organic carbon sediments in the Woodlark Basin, Pacific Ocean. *FEMS Microbiology Ecology*, **42**, 59–70.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Black Shales](#)
[Carbon \(Organic, Cycling\)](#)

[Deep Biosphere of Sediments](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Methane, Origin](#)
[Microbial Degradation](#)
[Nitrogen](#)
[Shales](#)
[Soils](#)
[Sulfate-Reducing Bacteria](#)

CARBON CYCLE

The carbon cycle is a biogeochemical cycle that describes the movement of carbon between its different compartments (reservoirs) on the Earth, namely biosphere, atmosphere, hydrosphere, pedosphere, and geosphere. Please refer to entries “*Carbon (Organic, Cycling)*” and “*Carbon (Organic, Degradation)*.”

CARBON ISOTOPES

Please refer to “*Isotopes and Geobiology*” and “*Biomarkers (Organic, Compound-Specific Isotopes)*.”

CARBONATE ENVIRONMENTS

Eberhard Gischler

J.W. Goethe-Universität, Frankfurt am Main, Germany

Definition

Carbonate environments occur both in the terrestrial and marine realms as well as in transitional zones between the land and the sea. The variety of environments spans from high-elevation continental lakes to the deep sea, from the equator to latitudes of about 60°, and they include dry and wet climate realms. The majority of carbonates are produced in the sea, currently in more or less similar proportions in neritic and pelagic settings. Carbonate production is to a large part a consequence of biological activity, either directly as in shell and skeleton formation or indirectly as a result of metabolic reactions, which trigger precipitation. The highest rates of carbonate production per unit time and space are probably reached in tropical coral reefs where several kilograms of calcium carbonate form on 1 m² during 1 year's time.

Introduction

Modern carbonate sediments are largely a product of biologic activity, either enzymatically controlled like in calcium carbonate (CaCO₃) shell and skeleton formation of aquatic organisms or indirectly by the metabolic activity

- Niles, P. B., Leshin, L. A., and Guan, Y., 2005. Microscale carbon isotope variability in ALH84001 carbonates and a discussion of possible formation environments. *Geochimica et Cosmochimica Acta*, **69**, 2931–2944.
- O' Leary, M. H., 1981. Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553–567.
- Ono, S., Wing, B. A., Johnston, D., Farquhar, J., and Rumble, D., 2006. Mass-dependent fractionation of quadruple sulphur isotope system as a new tracer of sulphur biogeochemical cycles. *Geochimica et Cosmochimica Acta*, **70**, 2238–2252.
- Park, R., and Epstein, S., 1960. Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta*, **21**, 110–126.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**, 443–467.
- Rankama, K., 1954. Early pre-Cambrian carbon of biogenic origin from the Canadian Shield. *Science*, **119**, 506.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth Science Reviews*, **64**, 243–272.
- Shen, Y., Buick, R., and Canfield, D. E., 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. *Nature*, **410**, 77–81.
- Skulan, J. L., Beard, B. L., and Johnson, C. M., 2002. Kinetic and equilibrium Fe isotope fractionation between aqueous Fe(III) and hematite. *Geochimica et Cosmochimica Acta*, **66**, 2995–3015.
- Taran, Y. A., Kliger, G. A., and Sevastianov, V. S., 2007. Carbon isotope effect in the open system Fischer Trosch synthesis. *Geochimica et Cosmochimica Acta*, **71**, 4474–4487.
- Thode, H. G., Macnamara, J., and Collins, C. B., 1949. Natural variations in the isotopic content of sulphur and their significance. *Canadian Journal of Research*, **27B**, 361.
- Wellman, R. P., Cook, F. D., and Krouse, H. D., 1968. Nitrogen-15: microbiological alteration of abundance. *Science*, **161**, 269–270.

Cross-references

Anaerobic Oxidation of Methane with Sulfate
 Astrobiology
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Iron Sulfide Formation
 Isotope Fractionation (Metal)
 Nitrogen
 Methane, Origin
 Photosynthesis
 Sulfate-Reducing Bacteria
 Sulfur Isotopes

ISOTOPES, RADIOGENIC

Bent T. Hansen
 University of Göttingen, Göttingen, Germany

Definition

Radiogenic nuclides (more commonly referred to as “radiogenic isotopes”) are produced by a process of radioactive decay. Radioactive decay means that unstable isotopes (mother nuclides) are spontaneously transferred

into stable isotopes (daughter nuclides) by emission of particles and loss of energy (radioactive radiation).

Introduction

The radioactive decay provides an accurate method of measuring the ages of rocks and minerals. This possibility was recognized both by Rutherford (1906) and Boltwood (1907) at the beginning of the twentieth century. However, the measurement and interpretation of variations in the isotopic composition of certain elements in natural materials were not possible until modern mass spectrometers were developed based on the design by Nier (1940). After the World War II, isotope determinations became an important tool in modern geology, geochemistry, and geobiology not only for dating geological processes, but also for investigations of climate change, environmental, and biological parameters. The most frequently used systems are Rb/Sr, Sr/Sr, Sm/Nd, Nd/Nd, U/Pb, and Pb/Pb. In the following, typical examples of the most common radiogenic isotope applications are presented.

Strontium isotopes

There are four stable isotopes of Sr: ^{88}Sr , ^{87}Sr , ^{86}Sr , and ^{84}Sr . All but ^{87}Sr are nonradiogenic (that is, not the products of radioactive decay), whereas ^{87}Sr is produced by radioactive decay of ^{87}Rb (half-life 0.488×10^9 years). The $^{87}\text{Rb} \rightarrow ^{87}\text{Sr}$ radioactive decay pair has therefore produced different $^{87}\text{Sr}/^{86}\text{Sr}$ ratios depending on the age and the amount of Rubidium in the material investigated. For more than half of the century, the Rb/Sr system is one of the most frequently used decay systems for dating geological events.

The isotopic composition of strontium is not only used for dating purposes, but also has turned out to be a universal tool for describing a variety of geological, biological, and environmental processes.

Groundwater and basement brines

Strontium is easily dissolved in water. The advantage of the strontium isotopic system is that the isotopic composition assumed till recently will not be changed by fractionation processes; it means that strontium in circulation depends on the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the rocks and minerals that interact with water at or near the surface of the Earth. The Sr isotope composition of groundwater aquifers is constant over years, independent of the amount of rainfall, as demonstrated in a recent study by Klaus et al. (2007). Investigations in the Bangkok Metropolitan Area showed that the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are useful tools to characterize groundwater flow and related biological and environmental processes.

Strontium isotope variations also play an important role in evaluating the suitability of crystalline rocks for geological disposal of nuclear waste. At the Hard Rock Laboratory at Äspö in southern Sweden, Sr isotope composition of groundwater has been investigated by Peterman and Walin (1999) down to a depth of 850 m from boreholes. Microbial

biofilms found in the deeper part of the tunnel are controlled in their Sr composition by highly saline brines (58 mg/l and $\delta^{87}\text{Sr}$ values as large as +13.9‰). Mixing with Baltic seawater from the surface (1.5 mg/l and $\delta^{87}\text{Sr}$ values of +0.3‰) is negligible; nevertheless, the method is sensitive enough to show the very small input of Baltic seawater even in the deepest parts of the Äspö tunnel.

Sr isotope investigations on high saline brines within the German Continental Deep Drilling Program (KTB) showed that the Sr isotopic compositions of paleofluids and recent fluids in the upper continental crust can be distinguished by means of $^{87}\text{Sr}/^{86}\text{Sr}$ versus $1/\text{Sr}$ mixture curves (Möller et al., 1997). Similar to saline brines, mesophilic and (hyper) thermophilic microorganisms living in shallow or deeper crust showed different Sr isotope compositions. Hence, Sr isotopic compositions may comprise a promising tool for the correlation of deep biosphere systems with depth and temperature regimes.

Strontium in animal and human skeletons

As the ionic radius of Sr (1.13 Å) is similar to that of Ca (0.99 Å), strontium replaces calcium in organic matters and can therefore also be used as a tracer for animal and human mobility. Different geographic (geological) regions yield different Sr isotopic compositions and as natural variations are not changed through the food chain, the strontium composition measured in skeletal elements can be used to identify the region where an animal or a human being lived (Brian and Johnson, 2000). The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios reflect the Sr that was ingested while a specific skeletal element grew. Because different skeletal elements grow and exchange Sr at different stages during the lifetime, Sr isotopic analyses of different skeletal parts can be used to infer changes in geographic location at different stages in the life of an organism. Regarding a human being, this means that the Sr isotopic composition of the enamel in the teeth will reflect the composition as a child, due to the immobile nature of Sr and Ca in teeth, whereas the Sr composition in a bone (e.g., femur) will reflect the average composition over the last 10 years of life, due to continuous processing of Sr and Ca in bone. These conclusions, however, are only valid for skeletons which are of "High Middle Age" or older. In the time of globalization, no relationship between living place and geogenic Sr input in the food chain can be reconstructed anymore.

Strontium in seawater

Biogenic carbonates are fairly resistant to diagenetic alteration. As they are secreted directly from seawater by the organisms, they contain no detrital material, and therefore reflect the isotopic composition of the seawater at the time of deposition. Biogenic carbonates are nearly free of Rb and can therefore not be dated directly. However, the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of these carbonates can be used to define the evolution of the seawater isotopic composition by known stratigraphic age deduced from fossils.

Fundamental studies on the Sr seawater evolution curve were published between 1970 (Peterman et al., 1970) and 1982 (Burke et al., 1982). The obtained curves were all based on Sr isotope data from Phanerozoic carbonates. The seawater Sr data set was later extended back to the Late Proterozoic (Derry et al., 1989; Asmerom et al., 1991; Kaufman et al., 1993). In the absence of fossils, these studies were carried out on leached whole-rock carbonate samples to avoid contamination by detrital material (Figure 1).

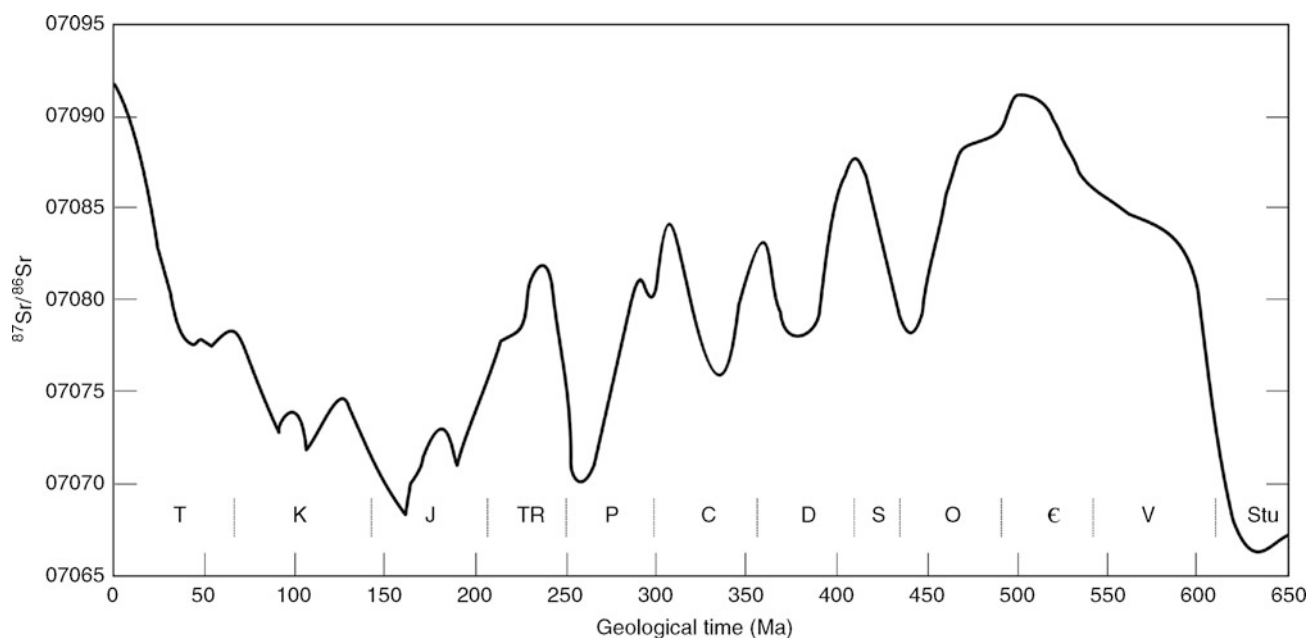
The seawater evolution curve can not only be used as an indirect dating tool for biogenic precipitations but may also be used as a geobiological tool, e.g., for indicating the setting of carbonate precipitations accompanying methane seeps. Investigations on methane-derived carbonates from the northwestern Black Sea showed $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of microcrystalline carbonates of 0.70927 and aragonitic cement of 0.70918. Both ratios are indistinguishable from ambient seawater (0.70917), and therefore indicate a shallow Sr source (Peckmann et al., 2001). The higher ratio of 0.71005 in the background sediments is related to detrital mica.

Paleotemperatures

Corals are primarily known as archives not only for changes in the sea surface temperature, but also for the variability in salinity and productivity. The latter are reflected in the common proxies such as $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$. However, the temperature equations are only valid for shallow-water corals. Further, proxies such as Sr/Ca, Mg/Ca, and U/Ca are dependent on growth parameters which are linked to specific coral species. As mentioned above, up to now it is assumed that geological processes do not cause any fractionation of the Sr isotopes. Recently, new techniques (Fietzke and Eisenhauer, 2006) have shown that this may not always hold true for calcium carbonate precipitation, including inorganic aragonite precipitation. Temperature-controlled precipitation of aragonite shows only minor variations in the Sr isotope fractionation (0.0054‰/°C) in the $\delta^{88}\text{Sr}/^{86}\text{Sr}$ ratios, whereas a natural coral sample (*Pavona clavus*) shows a fractionation nearly ten times higher (0.033‰/°C) in the $\delta^{88}\text{Sr}/^{86}\text{Sr}$ ratios. This means that the strontium isotope system can also be used as a paleothermometer for the identification of different fluid sources involved in calcium carbonate precipitation at low temperatures and to characterize processes of biomineralization. The consequence of these findings is that the seawater evolution curve discussed above also has to be corrected. The high potential of this method for studying climate-dependent variables especially for cold-water corals is demonstrated in a recent paper by Rüggeberg et al. (2008).

Neodymium isotopes

There are seven stable isotopes of Nd: ^{150}Nd , ^{148}Nd , ^{146}Nd , ^{145}Nd , ^{144}Nd , ^{143}Nd , and ^{142}Nd . All but ^{142}Nd and ^{143}Nd are nonradiogenic (that is, not the products of



Isotopes, Radiogenic, Figure 1 Time-dependent variation of the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of seawater. T = Tertiary, K = Cretaceous, J = Jurassic, TR = Triassic, P = Permian, C = Carboniferous, D = Devonian, S = Silurian, O = Ordovician, ε = Cambrian, V = Vendian, Stu = Sturtian. (Data compiled after McArthur et al., 2001 and Kaufmann et al., 1993.)

radioactive decay). ^{143}Nd is produced by radioactive decay of ^{147}Sm (half-life 10.6×10^{10} years). The $^{147}\text{Sm} \rightarrow ^{143}\text{Nd}$ radioactive decay pair has therefore produced different $^{143}\text{Nd}/^{144}\text{Nd}$ ratios in the investigated materials, depending on the age and the amount of Sm. Due to the short half-life (1.0×10^8 years) of $^{146}\text{Sm} \rightarrow ^{142}\text{Nd}$, this system so far has played no significant role in interpreting geological processes.

Neodymium is, like strontium, released by selective chemical weathering of polycrystalline rocks, and therefore dissolved Nd does not have the same isotopic composition as in the weathered rock. However, particulate matter transported into the sea is a characteristic product of eroded rocks in a given source area on the continent. Bock et al. (2005) showed that young sediments (4300 and 1000 year BP) in the Baltic Sea reflect a substantial change of Nd isotopic composition. This can be related either to changing inputs from the source areas or as different inflow of North Sea water. Variations of both, erosive continental input and North Sea inflow indicate a direct response of the Nd isotope signal in the Baltic Sea to climate change. The authors show that the Nd signal can be related to the cyclic shifts in the atmospheric circulation as well as demographic changes affecting agricultural activities. The reduction of farmland would also cause a reduction of soil erosion that changes the amount and composition of river-transported sediments into the Baltic Sea. This example shows that neodymium isotopic composition of sediment cores is a valuable tool for tracing climate changes.

Glacial-interglacial cycles are reflected in both Sr and Nd isotopic compositions (Tütken et al., 2002). The investigation of Arctic marine sediment cores revealed that it is possible to correlate ice rafted sediments with their source rocks, and therefore the glacial distribution as a function of time. The fact that Sr isotope composition is anticorrelated to the Nd isotopic composition makes such combined isotopic studies very sensitive to climate changes, and therefore also contributes to elucidate biological processes.

Lead isotopes

There are four stable isotopes of lead: ^{208}Pb , ^{207}Pb , ^{206}Pb , and ^{204}Pb . Only ^{204}Pb is not a product of radioactive decay, whereas ^{208}Pb , ^{207}Pb , and ^{206}Pb are produced by radioactive decay of ^{232}Th (half-life 14.010×10^9 years), ^{235}U (half-life 0.7038×10^9 years), and ^{238}U (half-life 4.468×10^9 years), respectively.

The so-called “common lead method” is used for dating minerals whose U/Pb and Th/Pb ratios are so low that their isotopic compositions do not change with time. The obtained ages, however, may vary with the evolution models used for the common lead method. One of the models, “the single-stage model” assumes that the primordial lead isotope ratios evolved in different reservoirs with different U/Pb ratios. This model is named the “Holmes–Hautermans Model” (Holmes, 1946; Hautermans, 1946). The measured isotopic compositions of the lead often resulted in ages which were to be obtained in the future

if the single-stage model was applied. Therefore, the “two-stage” Pb evolution model was proposed by Stacey and Kramers (1975). In this model, Pb evolves from primordial isotope ratios between 4.57 Ga and 3.7 Ga in a reservoir with $^{238}\text{U}/^{204}\text{Pb}$ of 7.192. At 3.7 Ga, the $^{238}\text{U}/^{204}\text{Pb}$ ratio of the reservoir changed by chemical differentiation to 9.735. Thereafter, lead evolution continued without change until present. A line connecting measured isotope ratios ($^{207}\text{Pb}/^{204}\text{Pb}$ vs. $^{206}\text{Pb}/^{204}\text{Pb}$) and the corresponding ratios at 3.7 Ga will intersect with the evolution line; these lines are called isochrones. The slope of these isochrones are related to the time elapsed since a lead sample was extracted from the reservoir, and therefore the age since separation can be calculated. Even if this model gives more geological relevant ages, it is still insufficient for geological dating. Nonetheless, lead isotopes may reveal excellent tracers for environmental investigations and, as lead is a toxic element, for characterizing biological processes. Lead isotope compositions do not change during industrial and environmental processes, and therefore reflect the lead source, thus allowing to distinguish between natural and anthropogenic origins (e.g., Cicchella et al., 2008). The anthropogenic lead has until recently mainly been contributed by leaded gasoline, with additional inputs caused by the combustion of coal. The lead isotopic signature of coal is more radiogenic, as coal is found in sedimentary rocks. Lead from basement rocks, which are the sources of ore deposits, show a different (lower) isotopic signature. Isotope analyses of, e.g., snow and surface waters can therefore contribute to unravel the sources and dynamics of lead in the oceans and the atmosphere and contribute to the database concerning climate change (Hamelin et al., 1997).

Exposure to lead as well as other heavy metals in the environment is a matter of public health concern. The lead isotopes play an important role in the development of biomarkers. Lead transfer across the placental barrier and injections of marked lead (^{206}Pb and ^{204}Pb , respectively) result in deposition in the enamel. Analyses of the enamels can be used to observe biological removal and assimilation of prenatal and postnatal tracers, respectively. This technique can be used to demonstrate the toxicokinetics of incorporating lead into fetal and neonatal steady-state system processes (Rinderknecht et al., 2005). The accumulation of heavy metals in aquatic organisms through the food chain may lead to serious health problems. Investigations of lead isotopes showed that the bioaccumulation of Pb in fish is due to a wide variety of food sources and/or exposure pathways, particularly anthropogenic inputs (Ip et al., 2005). These are only a few examples of the usefulness of lead isotopes in modern environmental research which becomes more important with increasing pollution.

Conclusions

Summarizing the applicability of selected radiogenic isotope systems, it can be concluded that a very wide field of applications can be covered, not only traditional dating

of geological events but also numerous applications in geological, (geo)biological, environmental, archeological, and forensic processes can be answered by means of radiogenic isotope investigations (further application references can be found in Hansen, 2005).

Bibliography

- Asmerom, Y., Jacobseb, S. B., Knoll, A. H., Butterfield, N. J., and Swett, K., 1991. Strontium variations of Neoproterozoic seawater: implications for crustal evolution. *Geochimica Cosmochimica Acta*, **55**, 2883–2894.
- Bock, B., Liebetrau, V., Eisenhauer, A., Frei, R., and Leipe, T., 2005. Nd isotope signature of Holocene Baltic Mn/Fe precipitates as a monitor of climate change during the Little Ice Age. *Geochimica et Cosmochimica Acta*, **68**, 2253–2263.
- Boltwood, B. B., 1907. On the ultimate disintegration products of the radioactive elements. *American Journal of Science*, **23**(4), 77–88.
- Brian, L., and Johnson, C., 2000. Strontium isotope Composition of skeletal material can determine the birth place and geographic mobility of humans and animals. *Journal of Forensic Science*, **45**, 1049–1061.
- Burke, W. H., Denison, R. E., Hetherington, E. A., Koepnick, R. B., Nelson, H. F., and Otto, J. B., 1982. Variations of seawater $^{87}\text{Sr}/^{86}\text{Sr}$ throughout Phanerozoic time. *Geology*, **10**, 516–519.
- Cicchella, D., De Vivo, B., Lima, A., Albanese, S., McGill, R. A. R., and Parrish, R. R., 2008. Heavy metal pollution and Pb isotopes in urban soils of Napoli, Italy. *Geochemistry: Exploration, Environment, Analyses*, **8**, 103–112.
- Derry, L. A., Keto, L. S., Jacobsen, S. B., Knoll, A. H., and Swett, K., 1989. Sr Isotope variations in Upper Proterozoic carbonates from Svalbard and East Greenland. *Geochimica et Cosmochimica Acta*, **53**, 2331–2339.
- Fietzke, J., and Eisenhauer, A., 2006. Determination of temperature-dependent stable strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS. *Geochemistry Geophysics Geosystems*, **7**, Q08009, doi 10.1029/2006GC001243.
- Hamelin, B., Ferrand, J. L., Alleman, L., Nicolas, E., and Veron, A., 1997. Isotopic evidence of pollutant lead transport from North America to the subtropical North Atlantic gyre. *Geochimica Cosmochimica Acta*, **61**, 4423–4428.
- Hansen, B. T., 2005. Isotope, die Gene der Gesteine. In Reitner, J., Weber, K., and Karg, U. (eds.), *In Das System Erde – Was bewegt die Welt? Lebensraum und Zukunftsperspektiven*. Göttingen: Universitätsverlag Göttingen, pp. 73–87.
- Hautermans, F. G., 1946. Die Isotopenhäufigkeiten im natürlichen Blei und das Alter des Urans. *Naturwissenschaften*, **33**, 185–186.
- Holmes, A., 1946. An estimate of the age of the earth. *Nature*, **157**, 680–684.
- Ip, C. C. M., Li, X. D., Zhang, G., Wong, C. S. C., and Zhang, W. L., 2005. Heavy metal and Pb isotopic composition of aquatic organisms in the Pearl River Estuary, South China. *Environmental Pollution*, **138**, 494–504.
- Kaufman, A. J., Jacobseb, S. B., and Knoll, A. H., 1993. The Vendian record of Sr and C isotopic variations in seawater: implications for tectonics and paleoclimate. *Earth and Planetary Science Letters*, **120**, 409–430.
- Klaus, J. S., Hansen, B. T., and Buapeng, S., 2007. $^{87}\text{Sr}/^{86}\text{Sr}$ ratio: a natural tracer to monitor groundwater flow paths during artificial recharge in the Bangkok area, Thailand, with respect to Public Water Supply and Freshwater Reinjection. *Hydrology Journal*, **15**, 745–758.
- McArthur, J. M., Howarth, R. J., and Bailey, T. R., 2001. Strontium isotope stratigraphy: LOWESS version 3: best fit to the marine

- Sr-isotope curve for 0–509 Ma and accompanying look-up table for deriving numerical age. *The Journal of Geology*, **109**, 155–170.
- Möller, P., Weise, S. M., Althaus, E., Bach, W., Behr, H. J., Rochardt, R., Bräuer, K., Drescher, J., Erzinger, J., Faber, E., Hansen, B. T., Horn, E. E., Huenges, E., Kämpf, H., Kessels, W., Kirsten, T., Landwehr, D., Lodemann, M., Machon, L., Peckdecker, A., Pielow, H. U., Reutel, C., Simon, K., Walter, J., Weinlich, F. H., and Zimmer, M., 1997. Paleofluids and resent fluids in the upper continental crust: results from the German Continental Deep Drilling Program (KTB). *Journal of Geophysical Research*, **102B8**, 18233–18254.
- Möller, P., Woith, H., Dulski, P., Lüders, V., Erzinger, J., Kämpf, H., Pekdeger, A., Hansen, B., Lodemann, M., and Banks, D., 2005. Main and trace elements in KTB-VB fluid: composition and hints to its origin. *Geofluids*, **5**, 28–41.
- Nier, A. O., 1940. A mass spectrometer for routine isotope abundance measurements. *Review of Scientific Instruments*, **11**, 212–216.
- Peckmann, P., Reimer, A., Luth, C., Hansen, B. T., Heinicke, C., Hoefs, J., and Reitner, J., 2001. Methane-derived carbonates and authigenic pyrite from the northwestern Black Sea. *Marine Geology*, **117**, 129–150.
- Peterman, Z. E., and Walin, B., 1999. Synopsis of strontium isotope variations in groundwater at Äspö, southern Sweden. *Applied Geochemistry*, **14**, 939–951.
- Peterman, Z. E., Hedge, C. E., and Tourtelot, H. A., 1970. Isotopic composition of strontium in seawater throughout Phanerozoic time. *Geochimica et Cosmochimica Acta*, **34**, 105–120.
- Rinderknecht, A. L., Kleinman, M. T., and Ericson, J. E., 2005. Pb enamel biomarker: deposition of pre- and postnatal Pb isotope injection in reconstructed time points along rat enamel transect. *Environmental Research*, **99**, 169–176.
- Rüggeberg, A., Fietzke, J., Liebetrau, V., Eisenhauer, A., Dullo, W.-C., and Freiwald, A., 2008. Stable strontium isotopes $\delta^{88/86}\text{Sr}$ in cold-water corals – a new proxy for reconstruction of intermediate ocean water temperatures. *Earth and Planetary Science Letters*, **269**, 569–574.
- Rutherford, E., 1906. *Radioaktive Transformations*. New York: Charles Scribner, p. 287.
- Stacey, J. S., and Kramers, J. D., 1975. Approximation of terrestrial lead isotope evolution by a two-stage model. *Earth and Planetary Science Letters*, **26**, 207–221.
- Tütken, T., Eisenhauer, A., Wiegand, B., and Hansen, B. T., 2002. Glacial-interglacial cycles in Sr and Nd isotopic composition of Arctic marine sediments triggered by the Svalbard/Barent Sea ice sheet. *Marine Geology*, **182**, 351–372.

Cross-references

[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Geochronology](#)
[Isotope Fractionation \(Metal\)](#)
[Isotopes and Geobiology](#)

K

KARST ECOSYSTEMS

Annette S. Engel
Louisiana State University, Baton Rouge, LA, USA

Definition

Karst. A landscape in and on the Earth's surface produced by the natural processes of solution and leaching of soluble rocks, generally carbonate rocks (e.g., limestone), in which the ensuing topography is mainly characterized by sinkholes, sinking streams, underground drainage networks, and caves.

Introduction

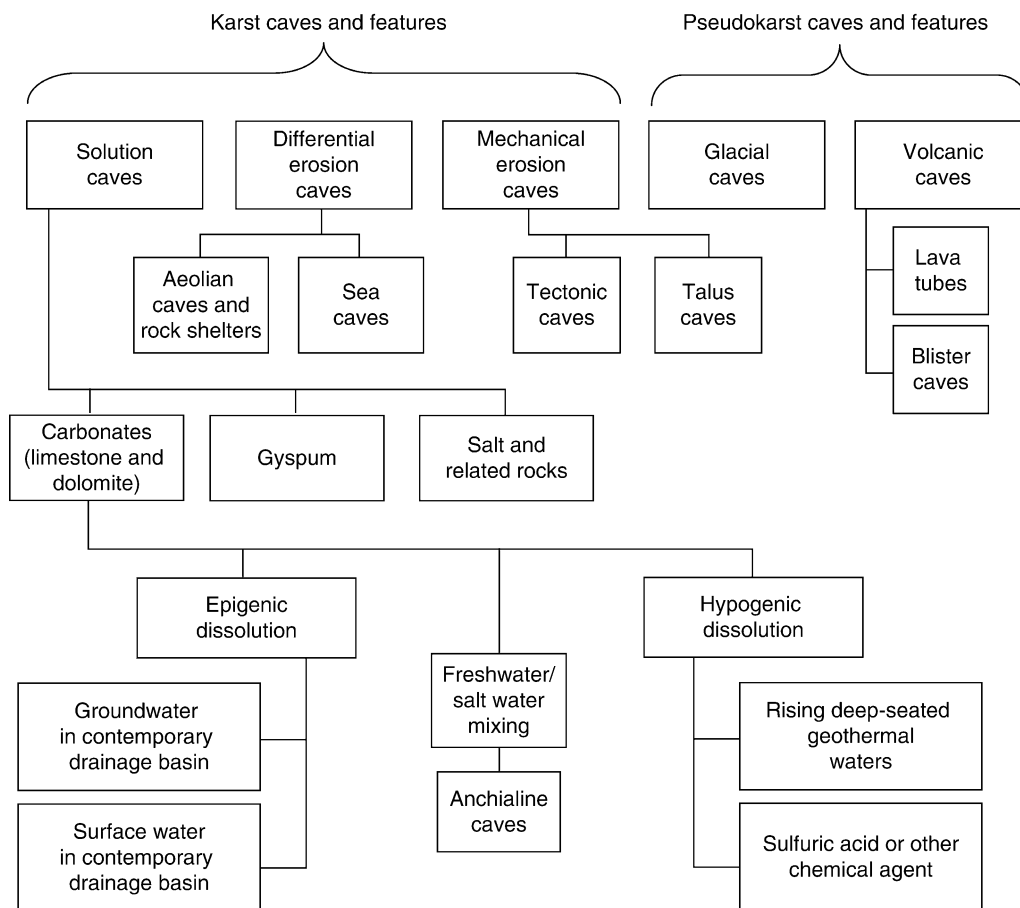
Karst landscapes comprise ~15–20% of the Earth's ice-free land surface. Because karst forms in soluble rocks, the global occurrence coincides roughly with the distribution of carbonate sedimentary rocks (e.g., Ford and Williams, 2007) (see Chapters *Carbonates* and *Carbonate Environments*). Karst landscapes link the Earth's surface to the subsurface, being characterized by features such as sinking streams, sinkholes, caves, and extensive underground water flow systems. Caves, which can extend up to 100 m into the subsurface, are solutionally- or collapse-enlarged discontinuous openings in rock. Surface karst features neither have to be extensively developed, nor present, for subsurface karst development and aquifer processes to be operative. Approximately, 25% of the global population depends upon the health of karst terrains and karst aquifers for its water supply (Ford and Williams, 2007).

Air, water, and rock within karst landscapes offer reactive interfaces for microbial activity. Considering the extent to which carbonate rocks comprise the rock record, and the depths to which subsurface carbonates can be karstified, the microbial biomass within karst settings

and at karst interfaces is potentially tremendous. Hence, microbial diversity and microbial processes are central to all of the karst-related sciences. Moreover, as some karst landscapes have remained relatively unchanged for thousands, if not millions, of years (e.g., Gale, 1992), the longevity of the karst habitat makes karst a potentially long-term reservoir for microbial communities and subsurface ecosystems (see Chapter *Deep Biosphere of Continental Rocks*). The goals of this review are two-fold: (1) to summarize the geobiology of karst ecosystems by describing the range of karst habitats that are important to maintain biological (and microbiological) diversity, and (2) to examine the range of geochemical, mineralogic, and ecological processes that are influenced by microbes in karst.

Types of caves and karst

Karst development, and subsequently cave formation (speleogenesis), is primarily due to rock dissolution, mechanical weathering, volcanic activity, or the melting of glacial ice (Figure 1). Karst can be classified in a number of ways, including from determining the solid that a feature has developed within (e.g., limestone, dolomite, gypsum, basalt, granite), the proximity to the groundwater table (e.g., above, at, or below it), the origin of the feature, and the morphology of the feature (e.g., cave passage shape, cave length, passage arrangement, passage levels). As Figure 1 demonstrates, there are a number of karst, pseudokarst, and cave types. One of the dominant karst-forming processes is the dissolution of soluble rock by the action of water, forming solution, or dissolution caves (White and Culver, 2000; Palmer, 2007); consequently, most caves are dissolutional in origin. Erosion caves also develop by moving water, but from mechanical weathering (e.g., scouring or wave active) rather than from dissolution. Dissolution caves can transit into erosion caves through time. Sea caves form



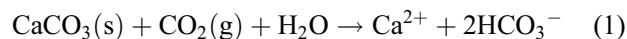
Karst Ecosystems, Figure 1 Common types of karst and processes responsible for forming a variety of caves. The processes of dissolution (epigenic or hypogenic) and weathering can also modify rejuvenated paleokarst. (Modified from White and Culver, 2000.)

as erosional caves along sea coasts in carbonate or basaltic rock, but anchialine caves typically form from dissolution along the sea coasts (see Chapter *Caves, Submarine*). Volcanic caves, or lava tubes, are starkly different than dissolution caves, resulting from a crust that formed at the surface of flowing lava while the remaining fluid (e.g., lava) continued to flow beneath the crust. The reader should review Palmer (2007) and Ford and Williams (2007) for more information about cave and karst types and speleogenetic processes. Some karst features are rare (e.g., White and Culver, 2000; Palmer, 2007), and few geobiological studies have been conducted in those settings. Therefore, this chapter focuses on dissolution caves, but briefly describes some research from lava tubes.

For dissolution caves, the proximity to the groundwater table (i.e., whether within the phreatic or vadose zone) and hydrologic conditions (i.e., confined or unconfined aquifer) are critical to the development of the karst system. When formed from dissolution at or above the water table, perhaps due to underground stream flow and/or the gravity-driven movement of meteoric waters, these

features are termed epigenic. Mammoth Cave in Kentucky (USA), at over 580 km of mapped cave passages, is the longest cave currently known and epitomizes epigenic systems worldwide (Palmer, 2007).

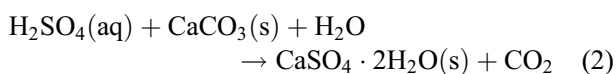
Epigenic development proceeds due to carbonic acid (H_2CO_3^0) promoted dissolution of carbonate rocks, such as those consisting of calcite (CaCO_3), whereby $\text{H}_2\text{O} + \text{CO}_2 \rightarrow \text{H}_2\text{CO}_3^0$, and so



These reactions are generally considered fast, and are usually modeled as being at equilibrium (e.g., White, 1997). Through time, epigenic karst development is controlled by the hydrological connectivity to the surface, as well as the complexity of flow path and storativity in the subsurface, rate of water movement, antecedent geochemical conditions, as well as the composition, function, and activities of microbes within the groundwater system. The location and arrangement of epigenic cave passages relative to overall landscape and surface drainage basin evolution

reflect local to regional base level, hydrological characteristics, and climate. Climate change can directly affect karst development because carbonate dissolution is directly related to CO_2 solubility, which is proportional to partial pressure (P_{CO_2}) and inversely proportional to temperature.

In contrast, hypogenic caves and karst form at or below the water table due to the action of rising fluids (water or gases), with little or no relationship to surface karst features or processes (Klimchouk, 2007). Various types of hypogenic caves can develop (Figure 1), with one of the most dramatic being due to the activity of sulfuric acid. At least 10% of carbonate caves worldwide have been attributed to this process (Palmer, 1991; Engel, 2007), including some ancient caves such as Carlsbad Cavern, New Mexico (USA) (Hill, 1996; Polyak et al., 1998). Egemeier (1981) was one of the first to examine in detail the sulfuric acid process. By observing hydrogen sulfide (H_2S)-bearing thermal springs in Lower Kane Cave in Wyoming (USA), extensive gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) deposits, and gypsum-replaced carbonate rock walls, he hypothesized that volatilization of H_2S from the sulfidic groundwater to the cave atmosphere, and H_2S autooxidation to sulfuric acid on the moist cave walls, caused the carbonate rock to be replaced with gypsum through the dissolution-replacement mechanism,



Because most natural low-temperature waters are undersaturated with respect to gypsum, the net result is mass removal and an increase in void volume. Nearly all of the subsequent studies regarding sulfuric acid speleogenesis assumed that H_2S was oxidized and consequently the sulfuric acid was produced by strictly abiotic processes. However, sulfur-oxidizing bacteria (see Chapter *Thiotrophic Bacteria*), which can generate sulfuric acid as a by-product of their metabolism, play an important role in carbonate dissolution (Engel et al., 2004b). Based on work from several sulfidic caves and the Edwards Aquifer in Central Texas (USA), it has been demonstrated that microbes focus acidity at the site of colonization and locally dissolve carbonates to a greater extent than the bulk karst fluids are capable of, because they are usually supersaturated with respect to carbonate phases (Engel, 2007).

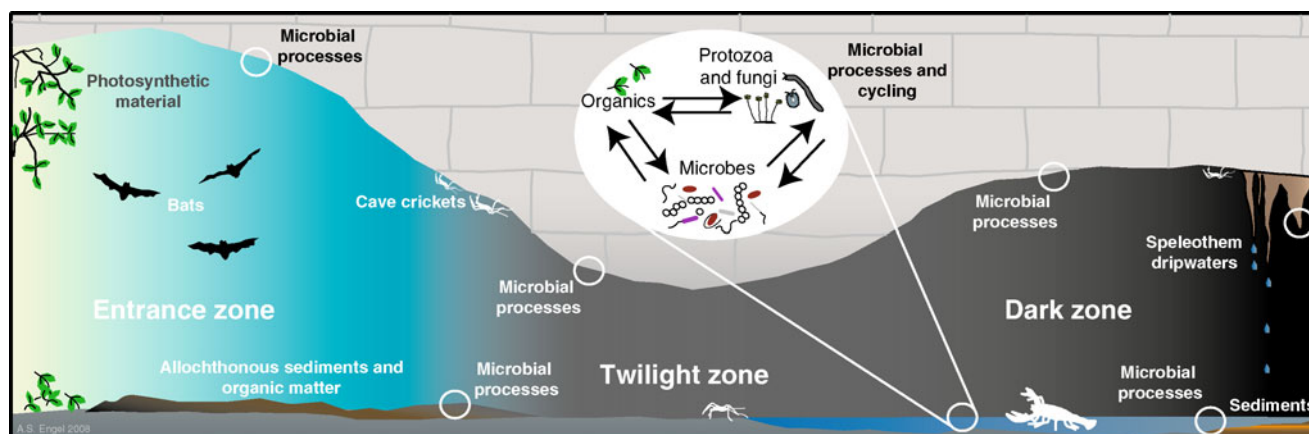
For the most part, however, recognizing whether a cave has formed from hypogenic processes or not is still a matter of debate (Klimchouk, 2007). Hypogenic caves are considered rare, even if hypogenic processes are perhaps important to nascent karst development (e.g., Palmer, 1991; Palmer, 1995; Klimchouk, 2007). The debate is perpetuated because most caves and karst terrains have polyphasic speleogenetic histories; this means that inception may have been due to hypogenic processes, but that tectonic activities or climate change may force a shift to epigenic development due to evolving base-level conditions or fluid geochemistry (Figure 1). Hypogenic development is usually not influenced by

surface hydrologic processes, as passages do not reflect contemporaneous surface drainage basin patterns. But, in some instances, polyphasic development, and epigenic processes specifically (e.g., sediment deposition due to surface-subsurface drainage, speleothem formation, etc.), may completely overprint evidence pointing to past hypogenic development. From a geobiological perspective, the role of microbes in both epigenic and hypogenic processes also confounds interpretation. Moreover, changes from one development type to another may impact how microbes come to colonize and become distributed within the subsurface. Microbial populations could potentially become isolated from each other during habitat modification. Similarly, changes in geochemical regime will impact the types of organisms that can grow, or tolerate, the new conditions, such as being thrust from oxygenated conditions to sulfidic and reducing conditions, or from neutral pH to acidic conditions (see Chapter *Extreme Environments*).

The cave habitat

The cave habitat is divided into three major zones based on light intensity: entrance, twilight, and dark zone (Figure 2). Each habitat zone has a certain set of physicochemical and nutrient conditions that control the types of organisms present in that zone. Cave and karst habitats have nearly constant temperatures and stable geochemical conditions, although some sections of a cave may change rapidly, such as in a stream due to seasonal or periodic flooding, or even within the entrance zone from daily changes in light level. But, it is the subtle physicochemical variability, availability, and speciation of redox-sensitive elements from one site to another within a cave, and the types and loading of carbon and other nutrients that can influence the diversity and metabolism of (micro) organisms that will colonize karst. Within the different zones, aquatic and terrestrial (or subaerial) habitat conditions are distinctive, and the stresses exerted on organisms will be specific. There is potentially less noticeable stress for aquatic organisms compared to the desiccation or nutritional stresses for organism living on dry cave-wall surfaces (e.g., Barton et al., 2007), although it was the juxtaposition of perceived stable physicochemical conditions to possibly extreme environmental circumstances that originally attracted biologists to study cave and karst organisms.

Consequently, speleologists who first began investigating the life of caves were predominately interested in the strange and exotic animals that they found. Troglobites are cave-adapted terrestrial animals, and stygobites are groundwater-adapted animals. Cave-adapted animals have evolved unique characteristics suitable for the subsurface habitat. Some of the organisms that live in caves are able to move freely in and out of a cave (e.g., troglonenes, such as bats and cave crickets), whereas other organisms (e.g., microbes) are more likely to be dependent on transport or translocation to move them into or



Karst Ecosystems, Figure 2 Cave habitat zones, using an epigenic, dissolution cave as the example. Open circles represent sites where microbial processes can occur, such as on rock surfaces and at interfaces, including within sediment or water. A schematic of microbial carbon cycling is shown from the dark zone, within a cave stream, where the by-products of microbial carbon degradation and processing would be consumed by various higher trophic level organisms. Specific chemolithoautotrophic processes are not shown.

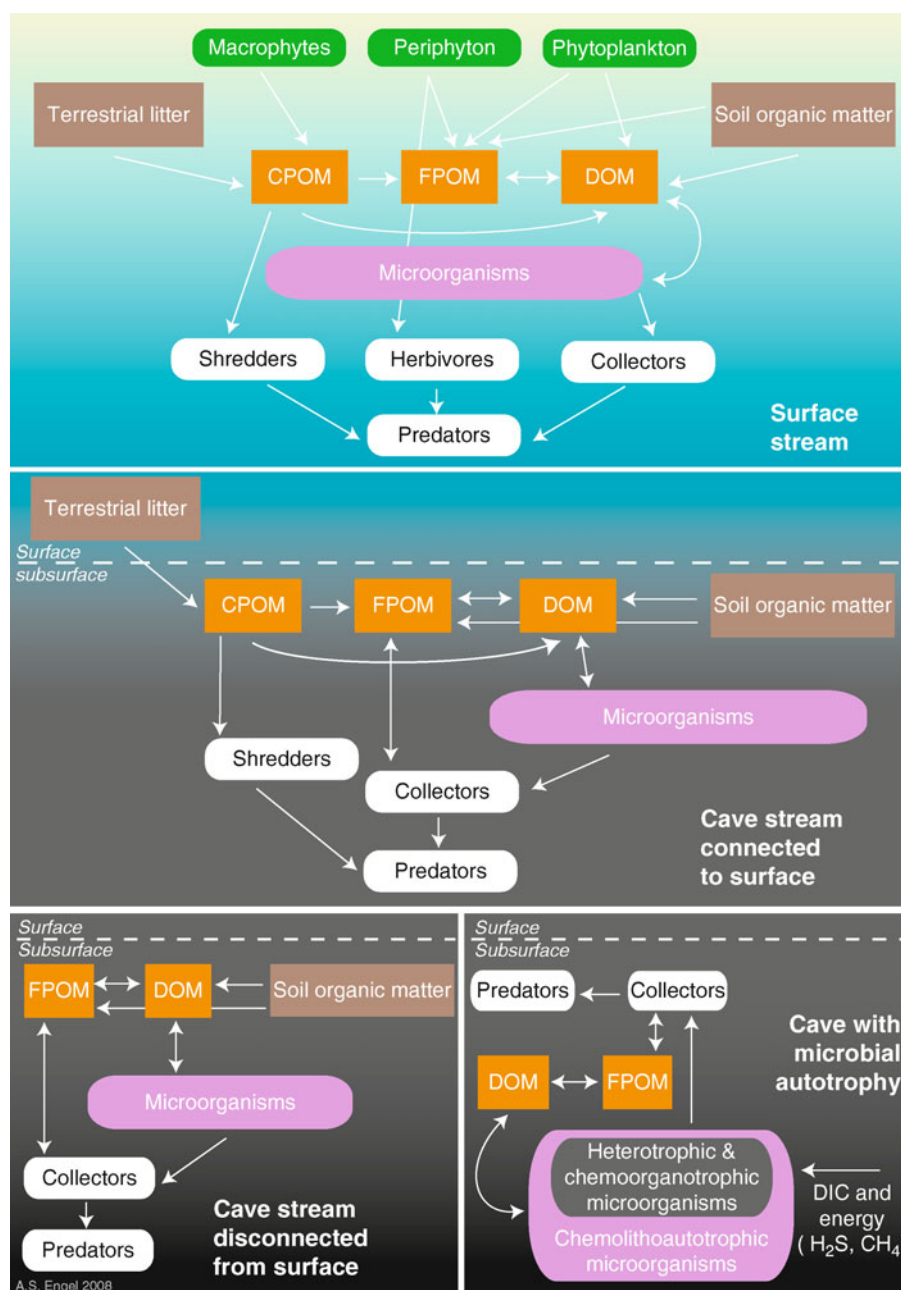
throughout a cave. Unlike the obvious transport mechanism from surface-derived drip waters, however, the extent to which air currents and groundwaters bring microbes into the cave environment is unknown. But, because microbes can also colonize virtually every surface and interface in all zones from a cave system (Figure 2), it is clear that understanding their diversity and their activities are central issues to all of the karst-related sciences (e.g., Rusterholtz and Mallory, 1994; Mahler et al., 2000; Jones, 2001; Northup and Lavoie, 2001; Simon et al., 2003; Cacchio et al., 2004; Farnleitner et al., 2005; Barton, 2006; Goldscheider et al., 2006; Ikner et al., 2007).

As photosynthesis is not possible in the dark zone, most cave and karst ecosystems are assumed to be dependent on allochthonous material brought into the system by wind, speleothem drip waters, stream drainage, as guano, and by the processing of the organic matter by microorganisms. Karst ecosystem energy flow has been poorly investigated (e.g., Simon, 2000; Simon et al., 2003), summarized in Figure 3, where cave ecosystem energy flow is compared to open surface stream energy flow. The most common type of allochthonous material is as dissolved and particulate (coarse and fine) organic matter (DOM, CPOM, and FPOM) (Figure 3), although it is possible that a wide size range can be found in caves, depending on the complexity of passages (e.g., when cave entrances and passages are sufficiently large, catastrophic flooding can bring tree trunks and automobiles deep inside caves). In some shallow settings, including in lava tubes, plant roots (rootsicles) may penetrate cave passages or aquifers and rich biota have been described from these settings (e.g., Howarth, 1973; Jasinska et al., 1996).

Some researchers have suggested that depauperate allochthonous food sources have influenced the evolution

of organisms within the subsurface and could be one reason for the diversity of organisms in epigenic caves. In general, troglobitic and stygobitic diversity is generally low compared to the diversity of surface organisms; in fairness, however, other reasons could include reduced habitat diversity (e.g., Sket, 1999), as well as fragmentation of the habitat and restricted opportunities for dispersal (Culver, 1970; Gibert, 1986; Culver and Sket, 2000). Similarly, trophic structure diversity may also be impacted by the reduced energy within the ecosystem, demonstrated by the elimination of shredders from food webs with increasing isolation from the surface and allochthonous energy input (Figure 3). Unfortunately, very little research has been conducted that addresses the mechanisms that modulate trophic structure in karst ecosystems, or that characterizes organism behavior to understand trophic functional roles.

Based on the research that has been done from caves and karst, however, the “world is green” view is beginning to change. Microbes are not just translocated soil heterotrophic, chemoorganotrophic, or fecal coliform communities that are food sources for higher trophic level organisms (Figure 3). In the absence of sunlight, groundwater rich in redox-sensitive compounds and reactive mineral surfaces (e.g., methane, hydrogen sulfide, or other reduced substances such as iron or manganese) can be used as energy sources by chemolithoautotrophs (Figure 3) (see Chapter *Chemolithotrophy*). In 1986, it was the discovery of the chemolithoautotrophically based cave ecosystem from Movile Cave in southeastern Romania that started to change our perceptions of what types of microbial activities could occur in the subsurface, as well as what types of ecosystem could be expected from such activities (Sarbu et al., 1996). Movile Cave is an access point to a large sulfidic aquifer and currently,



Karst Ecosystems, Figure 3 Energy and carbon flow in aquatic habitats, including surface streams and caves streams that may or may not be connected to the surface, modified from Simon (2000). CPOM represents coarse particulate organic matter, FPOM is fine particulate organic matter, and DOM represents dissolved organic matter. The role of microbes in these ecosystems is predominately as degraders or allochthonous organic material. However, there is the possibility that microbial primary productivity may augment the cave ecosystems carbon and energy budget. There are some cave and karst cave systems where energy and organic matter are sourced from chemolithoautotrophic primary productivity, whereby the ecosystem is entirely dependent on microbial activities. DIC represents dissolved inorganic carbon.

there have been 33 new cave-adapted taxa identified from 30 terrestrial species (24 endemic troglobites) and 18 aquatic species (nine endemic stygobites). Chemolithoautotrophic primary productivity estimated for Movile Cave was as high as $281 \text{ g C m}^{-2} \text{ year}^{-1}$, being comparable to

rates estimated from other aquatic surface systems (Porter, 1999). Based on the carbon to nitrogen ratios, the microbial mats in these sulfidic caves are also a high-quality food source (Porter, 1999; Engel et al., 2004a), although there have been relatively few studies that explore the

assimilation efficiency of higher trophic level organisms who feed on the mats in the sulfidic caves. The diversity and endemism of stygobites and troglobites from sulfidic ecosystems, compared to epigenic cave ecosystems dependent on allochthonous energy, may be related to the rates of autotrophic primary productivity and the quality and quantity of the food source (Engel, 2007).

Most of the research describing chemolithoautotrophy, in caves and karst has been from hypogenic systems and anchialine caves (e.g., Sarbu et al., 1996; Humphreys, 1999a; Opsahl and Chanton, 2006; Engel, 2007). For example, the sulfur-based ecosystems from the Frasassi Caves (Italy) (e.g., Vlasceanu et al., 2000; Macalady et al., 2006, p 59), the Cueva de Villa Luz (Mexico) (Hose et al., 2000), and some stratified sinkholes around the world (e.g., Stoessell et al., 1993; Pohlman et al., 1997), are also based in chemolithoautotrophy. Similarly, microbes have been implicated in sustaining the ecosystems within the Edwards Aquifer in Central Texas (USA) (e.g., Longley, 1981) and the Cape Range or Pilbara aquifers in Australia (e.g., Humphreys, 1999b; Eberhard et al., 2005), although the role of autotrophy is currently not known. The recent discovery of the Ayalon Cave ecosystem in Israel, with possibly a new chemosynthetically based ecosystem and eight invertebrates new to science, is testimony that substantial and unknown subterranean diversity still exists (Por, 2007). Evidence that microbial autotrophy from methane cycling serves as a base for the Upper Floridan Aquifer (USA) ecosystem trophic structure has been suggested from isotope systematics (Opsahl and Chanton, 2006). Recognition of methane-based cave and karst ecosystems can transform what we thought we knew of ecosystem function and the geobiology of karst. Lastly, some work on microbial autotrophy has also been done from epigenic systems, finding that sediments and biofilms on cave walls and in streams also contain chemolithoautotrophic microbial communities (e.g., Simon et al., 2003; Farnleitner et al., 2005) and that karst waters can have high microbial cell abundances and DOM is associated with autochthonous microbial activities rather than plant- or soil-derived humic substance (e.g., Goldscheider et al., 2006). This research direction will impact our understanding of contaminant transport in karst (e.g., Mahler et al., 2000; van Beynen and Townsend, 2005).

Geobiology and microbial activities

Despite knowing little about microeukaryotes and viruses from karst, we do know that all three domains of life and viruses occur in caves and karst. Figure 4 summarizes the variety of cave habitats with microbial communities, and associated microbial processes that have been studied to date. Details regarding the geomicrobiology of caves and karst have been reviewed in a variety of venues (e.g., Jones, 2001; Northup and Lavoie, 2001; Barton, 2006; Barton and Northup, 2007), so the reader should refer to these papers for more information. Here,

discussion begins with lava tubes because comparatively little is known about the microbial diversity, activities, and ecosystem parameters. Some of the most recent research using molecular techniques that have elucidated microbial activities and diversity of hypogenic and epigenic caves and karst, in the context of key processes, is also described (see Chapters *Geomicrobiology*, *Molecular Aspects*, *Molecular Geomicrobiology*, and *Phylogenetics*). The chapter is concluded with future perspectives on cave and karst geobiological studies.

Lava tubes and basaltic caves

There are few studies of microbes from basaltic lava tube or sea caves. Several investigations focus on humid lava tubes, rather than dry basaltic systems (e.g., Northup and Welbourn, 1997, in press), and on rootsicles from lava tubes that support complex fungi and arthropod diversity (e.g., Howarth, 1973, 1981) (Figure 4). From rRNA gene surveys and culture-based growth studies, humid lava tubes contain a variety of slime deposits, consisting of actinomycetes and members within the *Chloroflexi*, *Verrucomicrobia*, and *Proteobacteria* (Ashmole et al., 1992; Northup and Welbourn, 1997). Somewhat related has been the geomicrobiological and mineralogical work on microbial biofilms in the photic zone of basaltic sea caves in Hawaii (USA) (Leveille et al., 2000).

Hypogenic caves

Early research was based on microscopy and culturing (e.g., Hubbard et al., 1986; Olson and Thompson, 1988; Stoessell et al., 1993; Brigmon et al., 1994), but more recently the known microbial diversity from these systems has been expanded by molecular genetics, and specifically from 16S rRNA gene sequence surveys. The biodiversity and geomicrobiology of active sulfidic caves have been more extensively studied than from other types of hypogenic karst, and the reader should refer to these publications for specifics (e.g., Brigmon et al., 1994; Angert et al., 1998; Hose et al., 2000; Barton, 2006; Macalady et al., 2006; Barton and Northup, 2007; Engel, 2007). Microbes in hypogenic systems occur in microbial mats in streams and pools, sediments, elemental sulfur and other mineral deposits, and as snottites or microbial draperies on cave-wall surfaces (e.g., Hose et al., 2000; Macalady et al., 2007), as well as in corrosion residues (e.g., Northup et al., 2000, 2003) (Figure 4). The role of microbes in sulfuric acid speleogenesis was described from Lower Kane Cave (Engel et al., 2004b), and microbial communities have also been shown to be important to sulfidic karst aquifer development (Engel, 2007). From 16S rRNA gene surveys, *Proteobacteria* are one of the most prevalent groups studied to date from hypogenic caves and karst. Most of the focus has also been on sulfur-oxidizing bacteria belonging to the classes *Epsilonproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* (see Chapters *Thiotrophic Bacteria*

Karst Ecosystems, Figure 4 Schematic representation of the three common cave types discussed in this article, including lava tubes, hypogenic caves, and epigenic caves. For each cave type, specific habitats are associated with microbial activities and deposits are produced because of these activities (e.g., corrosion residues in hypogenic systems).

and *Sulfur Cycle*). Stable isotope ratio systematics and radiolabeled isotope experiments have unveiled possible ecosystem function for some of the microbes in these systems (e.g., Sarbu et al., 1996; Pohlman et al., 1997; Vlasceanu et al., 1997; Humphreys, 1999a; Porter, 1999; Engel et al., 2001; Hutchens et al., 2004; Herbert et al., 2005), but more research is needed.

One of the most interesting habitats for microbes in active sulfidic caves is on cave-wall surfaces. These habitats are acidic, due in part to gypsum mineralization and microbial activity, with pH of condensation droplets on biofilms and snottites being as low as 0 (range pH 0–4) (Hose et al., 2000; Vlasceanu et al., 2000; Macalady et al., 2007) (see Chapter *Acidophiles*). Interestingly, the phylogenetic diversity of these biofilms is similar, consisting of extremophilic groups belonging to the *Proteobacteria*, actinomycetes, the bacterial candidate lineage TM6, as well filamentous fungi and protists. The biofilms from the Frasassi and Lower Kane caves have $\delta^{13}\text{C}$ values that range between -36‰ and -39‰ , suggesting that carbon within the biofilms has been produced from chemolithoautotrophy (Vlasceanu et al., 2000; Engel, 2007).

The role of microbes in iron and manganese cycling has been studied from both hypogenic and epigenic systems (e.g., Peck, 1986), although most of the current research has been done from caves that had formed from hypogenic processes but are currently under epigenic, if any, modification processes. Specifically, a diverse, metabolically active microbial communities, some of which were related to known manganese- and iron-oxidizing bacteria, are present in corrosion residues on cave-wall surfaces (Figure 4) underlying ferromanganese deposits from Lechuguilla Cave and other caves in the Guadalupe Mountains of West Texas and New Mexico (Northup et al., 2003). Among the diverse bacterial groups identified, clones belonging to both the Crenarchaeota and Euryarchaeota have been retrieved from corrosion residues (Northup et al., 2003). Similar archaeal groups have been retrieved from paleofill sediment deposits in Wind Cave in South Dakota (USA) (Chelius and Moore, 2004). Several research groups have interpreted a variety of poorly crystalline manganese oxide and hydroxide minerals in caves to be microbially induced (e.g., Northup et al., 2000).

Methanotrophic and methanogenic microbial groups, while expected to occur in some groundwater systems with high concentrations of dissolved methane (e.g., Opsahl and Chanton, 2006), have almost exclusively been studied in detail from Mobile Cave using enrichment and isolation techniques, $^{13}\text{CH}_4$ -labeling incorporation studies, and ^{13}C -DNA analysis (Vlasceanu, 1999; Hutchens et al., 2004). The contribution, if any, of methanogenic or methanotrophic activity to speleogenesis is unknown.

Epigenic caves

Because epigenic caves are geologically more common, the numbers and ranges of geobiological studies are vast.

Most studies from epigenic systems can be generally categorized into three groups: (1) those that attempt to understand the abundance, diversity, and activities of microbial communities; (2) those that attempt to relate microbial diversity to ecosystem function; and (3) those that attempt to use microbes to explain mineralogical phenomena such as precipitation and dissolution of minerals or deposits found in caves. The majority of cave and karst geomicrobiological studies can be summarized as fitting into the first category.

The health of cave and karst ecosystems can be better understood by recognizing that there is an intimate hydrologic and ecologic connection of dissolution caves between the surface and subsurface. In particular, because the geochemistry and hydrology of most karst systems respond dramatically over short periods of time following disturbances, changes in the major taxonomic and metabolic potential of a system can alert to possible water quality or sustainability issues (van Beynen and Townsend, 2005). As such, considerable effort has been made to characterize microbial communities, including heterotrophic groups, within epigenic caves and from karst aquifers (e.g., Rusterholtz and Mallory, 1994; Mahler et al., 2000; Bates et al., 2006; Goldscheider et al., 2006; Pronk et al., 2006). Results have shown that pulses of different microbial groups over time, or even from episodic inoculation events, may disturb overall ecosystem balance (e.g., Mahler et al., 2000; van Beynen and Townsend, 2005; Goldscheider et al., 2006).

Similarly, identifying the microbes in vadose zone cave habitats, such as in river sediments and from exposed cave-wall surfaces, has been done to compare pristine communities to those that may be influenced by human impact, such as from tourism (Chelius and Moore, 2004; Ikner et al., 2007). Some of the microbes found from high impact zones in tour caves are comparable to communities found in association with Paleolithic cave paintings (e.g., Schabereiter-Gurtner et al., 2002a, b, 2004; Zimmermann et al., 2005). The microbial diversity and physiological types associated with higher impact areas may be related to the increased organic matter of anthropogenic origins (from paint to lint) (Ikner et al., 2007). One of the issues pertaining to the diversity of microbes from human impacted zones is whether or not these microbes have any influence on mineral precipitation or dissolution. These microbes could negatively impact any of the natural cave processes, or even destroy cave paintings by ancient man (e.g., Schabereiter-Gurtner et al., 2004).

The mineralogy and nutrient content of rock has been suggested as the important drivers in the microbial groups that are able to colonize cave surfaces (Barton et al., 2007). Because the geomicrobiology of cave minerals, including carbonates, iron oxides, and nitrates, has been extensively studied and the studies reviewed (e.g., Jones, 2001; Northup and Lavoie, 2001; Barton, 2006; Barton and Northup, 2007), only exemplar investigations are provided here (see Chapter *Microbial Biomineralization*). Carbonate

mineralization is known to be influenced by microbes, as enrichment culturing from carbonate speleothems in Cervo Cave in Italy demonstrated that *Kocuria* spp. were precipitating carbonate at unusually high rates compared to microbes from noncave environments (Cacchio et al., 2004). Similarly, filamentous cells caused calcite to precipitate after colonizing rock surfaces (e.g., Cañaveras et al., 2006). According to Caumartin (1963), most cave sediment contains iron bacteria, and indeed, microscopy and culturing studies revealed that chemolithoautotrophic *Gallionella* spp. and heterotrophic *Leptothrix* and *Crenothrix* spp. are common (see Chapters *Leptothrix* and *Gallionella*). Encrusted sheaths and stalks of bacteria resembling modern iron-oxidizing bacterial cells have been seen from stalactites, sediments, and corrosion crusts from numerous caves (Caumartin, 1963; Peck, 1986; Onac et al., 1997; Northup et al., 2003). Despite the significance of cave nitrates in the early history of caves (i.e., for saltpeter deposits), there has been little work to advance our understanding of the role of microbes in cave nitrate formation (see Chapter *Nitrogen*). Researchers still deliberate over the degree to which bacteria, such as *Nitrosomonas* spp. and *Nitrobacter* spp., participate in the creation of the cave saltpeter deposits, although *Nitrobacter* spp. had been cultured from cave sediments in Mammoth Cave (Fliermans et al., 1974).

Karst ecosystem studies in the future

Like the discovery of life at the deep-sea hydrothermal vents more than 25 years ago (see Chapter *Hydrothermal Environments, Marine*), ongoing research in karst habitats has revealed a rich and diverse microbiota. Some of these cave and karst ecosystems are supported by chemolithoautotrophic primary productivity, thereby changing our perception that all life on Earth is dependent on photosynthesis. Caves and karst are relatively accessible habitats to study from the perspective of carbon and nutrient cycling, and discoveries are being made that relate to rock cycling (from mineral dissolution to precipitation). But, we need to move beyond observational investigations that focus on categorizing microbial communities, and instead to make important advances in understanding the geomicrobiological and biogeochemical roles of microbes in cave and karst to address the rates and mechanisms controlling microbial ecophysiology and ecosystem functioning. This methodology exists and so we should expect that exciting new discoveries will be made with continued technological advances. One outcome from these efforts will be an increase in our knowledge of cave and karst biodiversity to better protect and conserve these novel geological and ecological systems.

Bibliography

Angert, E. R., Northup, D. E., Reysenbach, A. L., Peek, A. S., Goebel, B. M., and Pace, N. R., 1998. Molecular phylogenetic analysis of a bacterial community in Sulphur River, Parker Cave, Kentucky. *American Mineralogist*, **83**, 1583–1592.

- Ashmole, N. P., Orom, P., Ashmole, M. J., and Martin, J. L., 1992. Primary faunal succession in volcanic terrain: lava and cave studies on the Canary Islands. *Biological Journal of the Linnean Society*, **46**, 207–234.
- Barton, H. A., 2006. Introduction to cave microbiology: a review for the non-specialist. *Journal of Cave and Karst Studies*, **68**, 43–54.
- Barton, H. A., and Northup, D. E., 2007. Geomicrobiology in cave environments: past, current and future perspectives. *Journal of Cave and Karst Studies*, **69**(1), 163–178.
- Barton, H. A., Taylor, N. M., Krete, M. P., Springer, A. C., Oehle, S. A., and Bertog, J. L., 2007. The impact of host rock geochemistry on bacterial community structure in oligotrophic cave environments. *International Journal of Speleology*, **36**, 93–104.
- Bates, C. L., Forstner, M. R., Barnes, M. B., Whiteley, M., and McLean, R. J. C., 2006. Heterotrophic limestone-adherent biofilm isolates from the Edwards Aquifer, Texas. *The Southwestern Naturalist*, **51**, 299–309.
- Brigmon, R. L., Martin, H. W., Morris, T. L., Britton, G., and Zam, S. G., 1994. Biogeochemical ecology of *Thiothrix* spp. in underwater limestone caves. *Geomicrobiology Journal*, **12**, 141–159.
- Cacchio, P., Contento, R., Ercole, C., Cappuccio, G., Preit Martinez, M., and Lepidi, A., 2004. Involvement of microorganisms in the formation of carbonate speleothems in the Cervo Cave (L'Aquila-Italy). *Geomicrobiology Journal*, **21**, 497–509.
- Cañaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J. M., and Saiz-Jimenez, C., 2006. On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften*, **93**, 27–32.
- Caumartin, V., 1963. Review of the microbiology of underground environments. *Bulletin of the National Speleological Society*, **25**, 1–14.
- Chelius, M. K., and Moore, J. C., 2004. Molecular phylogenetic analysis of Archaea and bacteria in Wind Cave, South Dakota. *Geomicrobiology Journal*, **21**, 123–134.
- Culver, D. C., 1970. Analysis of simple cave communities I: caves as islands. *Evolution*, **24**, 463–474.
- Culver, D. C., and Sket, B., 2000. Hotspots of subterranean biodiversity in caves and wells. *Journal of Cave and Karst Studies*, **62**, 11–17.
- Eberhard, S. M., Halse, S. A., Scanlon, M. D., Cocking, J. S., and Barron, H. J., 2005. Assessment and conservation of aquatic life in the subsurface of the Pilbara region, Western Australia. *Symposium on World Subterranean Biodiversity Proceedings*, pp. 69–72.
- Egemeier, S., 1981. Cave development by thermal waters. *Bulletin of the National Speleological Society*, **43**, 31–51.
- Engel, A. S., 2007. Observations on the biodiversity of sulfidic karst habitats. *Journal of Cave and Karst Studies*, **69**, 187–206.
- Engel, A. S., Porter, M. L., Kinkle, B. K., and Kane, T. C., 2001. Ecological assessment and geological significance of microbial communities from Cesspool Cave, Virginia. *Geomicrobiology Journal*, **18**, 259–274.
- Engel, A. S., Porter, M. L., Stern, L. A., Quinlan, S., and Bennett, P. C., 2004a. Bacterial diversity and ecosystem function of filamentous microbial mats from aphotic (cave) sulfidic springs dominated by chemolithoautotrophic “*Epsilonproteobacteria*”. *FEMS Microbiology Ecology*, **51**, 31–53.
- Engel, A. S., Stern, L. A., and Bennett, P. C., 2004b. Microbial contributions to cave formation: new insights into sulfuric acid speleogenesis. *Geology*, **32**(5), 369–372.
- Farnleitner, A. H., Wilhartitz, I., Ryzinska, G., Kirschner, A. K. T., Stadler, H., Bartscher, M. M., Hornek, R., Szewzyk, U., Herndl,

- G., and Mach, R. L., 2005. Bacterial dynamics in spring water of alpine karst aquifers indicates the presence of stable autochthonous microbial endokarst communities. *Environmental Microbiology*, **7**, 1248–1259.
- Fliermans, C. B., Bohlool, B. B., and Schmidt, E. L., 1974. Autecological study of the chemoautotroph *Nitrobacter* by immunofluorescence. *Applied Microbiology*, **27**, 124–129.
- Ford, D. C., and Williams, P., 2007. *Karst Hydrogeology and Geomorphology*. Chichester: Wiley, p. 576.
- Gale, S. J., 1992. Long-term landscape evolution in Australia. *Earth Surface Processes and Landforms*, **17**, 323–343.
- Gibert, J., 1986. Ecologie d'un système karstique jurassien. Hydrogéologie, dérive animale, transits de matières, dynamique de la population de *Niphargus* (Crustacé Amphipode). *Mémoires de Biospéologie*, **13**, 1–379.
- Goldscheider, N., Hunkeler, D., and Rossi, P., 2006. Review: microbial biocenoses in pristine aquifers and an assessment of investigative methods. *Hydrogeology Journal*, **14**, 926–941.
- Herbert, R. A., Ranchou-Peyruse, A., Duran, R., Guyoneaud, R., and Schwabe, S., 2005. Characterization of purple sulfur bacteria from the South Andros Black Hole cave system: highlights taxonomic problems for ecological studies among the genera *Allochromatium* and *Thiocapsa*. *Environmental Microbiology*, **7**, 1260–1268.
- Hill, C. A., 1996. *Geology of the Delaware Basin, Guadalupe, Apache, and Glass Mountains*. New Mexico and West Texas: Permian Basin Section (SEPM), Publication no. 96–39, p. 480.
- Hose, L. D., Palmer, A. N., Palmer, M. V., Northup, D. E., Boston, P. J., and DuChene, H. R., 2000. Microbiology and geochemistry in a hydrogen-sulphide rich karst environment. *Chemical Geology*, **169**, 399–423.
- Howarth, F. G., 1973. The cavernicolous fauna of Hawaiian lava tubes. 1. Introduction. *Pacific Insects*, **15**, 139–151.
- Howarth, F. G., 1981. Community structure and niche differentiation in Hawaiian lava tubes. In Mueller-Dombois, D., Bridges, K. W., and Carson, H. L. (eds.), *Island Ecosystems: Biological Organization in Selected Hawaiian Communities*. US/IBP Synthesis Series 15. Stroudsburg, PA: Hutchinson Ross, pp. 318–336.
- Hubbard, D. A., Herman, J. S., and Bell, P. E., 1986. The role of sulfide oxidation in the genesis of Cesspool Cave, Virginia, USA. In *Comissio Organizadora del IX Congrés Internacional d'Espeleologica*, ed. 9th International Congress of Speleology, Barcelona, Spain, Vol. 1, pp. 255–257.
- Humphreys, W. F., 1999a. Physico-chemical profile and energy fixation in Bundera Sinkhole, an anchialine remiped habitat in north-western Australia. *Journal of the Royal Society of Western Australia*, **82**, 89–98.
- Humphreys, W. F., 1999b. Relict stygofaunas living in sea salt, karst and calcrete habitats in arid northwestern Australia contain many ancient lineages. In Ponder, W., and Lunney, D. (eds.), *The Other 99%. The Conservation and Biodiversity of Invertebrates*. Mosman 2088: Transactions of the Royal Zoological Society of New South Wales, pp. 219–227.
- Hutchens, E., Radajewski, S., Dumont, M. G., McDonald, I. R., and Murrell, J. C., 2004. Analysis of methanotrophic bacteria in Movile Cave by stable isotope probing. *Environmental Microbiology*, **6**, 111–120.
- Ikner, L. A., Toomey, R. S., Nolan, G., Neilson, J. W., Pryor, B. M., and Maier, R., 2007. Culturable microbial diversity and the impact of tourism in Kartchner Caverns, Arizona. *Microbial Ecology*, **53**, 30–42.
- Jasinska, E. J., Knott, B., and McComb, A. J., 1996. Root mats in ground water: a fauna-rich cave habitat. *Journal of the North American Benthological Society*, **15**, 508–519.
- Jones, B., 2001. Microbial activity in caves: a geological perspective. *Geomicrobiology Journal*, **18**, 345–358.
- Klimchouk, A. B., 2007. *Hypogene Speleogenesis: Hydrogeological and Morphogenetic Perspective*. Carlsbad, NM: National Cave and Karst Research Institute, (Special Paper no. 1), p. 106.
- Levelle, R. J., Fyfe, W. S., and Longstaffe, F. J., 2000. Geomicrobiology of carbonate-silicate microbialites from Hawaiian basaltic sea caves. *Chemical Geology*, **169**, 339–355.
- Longley, G., 1981. The Edwards Aquifer - Earth's most diverse groundwater ecosystem. *International Journal of Speleology*, **11**, 123–128.
- Macalady, J. L., Lyon, E. H., Koffman, B., Albertson, L. K., Meyer, K., Galdenzi, S., and Mariani, S., 2006. Dominant microbial populations in limestone-corroding stream biofilms, Frasassi Cave system, Italy. *Applied and Environmental Microbiology*, **72**, 5596–5609.
- Macalady, J. L., Jones, D. S., and Lyon, E. H., 2007. Extremely acidic, pendulous cave wall biofilms from the Frasassi cave system, Italy. *Environmental Microbiology*, **13**, 1402–1414.
- Mahler, B. J., Personne, J. C., Lods, G. F., and Drogue, C., 2000. Transport of free and particulate-associated bacteria in karst. *Journal of Hydrology*, **238**, 179–193.
- Northup, D. E., and Lavoie, K., 2001. Geomicrobiology of caves: a review. *Geomicrobiology Journal*, **18**, 199–222.
- Northup, D. E., and Welbourn, W. C., 1997. Life in the twilight zone: lava tube ecology. *New Mexico Bureau of Mines & Mineral Resources Bulletin*, **156**, 69–82.
- Northup, D. E., Dahm, C. N., Melim, L. A., Spilde, M. N., Crossey, L. J., Lavoie, K. H., Mallory, L. M., Boston, P. J., Cunningham, K. I., and Barns, S. M., 2000. Evidence for geomicrobiological interactions in Guadalupe caves. *Journal of Cave and Karst Studies*, **62**, 80–90.
- Northup, D. E., Barns, S. M., Yu, L. E., Spilde, M. N., Schelble, R. T., Dano, K. E., Crossey, L. J., Connolly, C. A., Boston, P. J., Natvig, D. O., and Dahm, C. N., 2003. Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves. *Environmental Microbiology*, **5**, 1071–1086.
- Northup, D. E., Connolly, C. A., Trent, A., Peck, V. M., Spilde, M. N., Welbourn, W. C., and Natvig, D. O., 2008. The nature of bacterial communities in Four Windows Cave, El Malpais National Monument, New Mexico, USA. *AMCS Bulletin*, **19**, 119–125.
- Olson, R. A., and Thompson, D. B., 1988. Scanning electron microscopy and energy dispersive X-ray analysis of artificial and natural substrates from the Phantom Flowstone of Sulphur River in Parker Cave, Kentucky. *National Speleological Society Bulletin*, **50**, 47–53.
- Onac, B. P., Tysseland, M., Bengescu, M., and Hofenpradli, A., 1997. Deposition of black manganese and iron-rich sediments in Vântului Cave (Romania). In Jeannin, P.-Y. (ed.), *Proceedings of the 12th International Congress of Speleology*. International Union of Speleology, pp. 235–238.
- Opsahl, S. P., and Chanton, J. P., 2006. Isotopic evidence for methane-based chemosynthesis in the Upper Floridan aquifer food web. *Oecologia*, **150**, 89–96.
- Palmer, A. N., 1991. Origin and morphology of limestone caves. *Geological Society of America Bulletin*, **103**, 1–21.
- Palmer, A. N., 1995. Geochemical models for the origin of macroscopic solution porosity in carbonate rocks. In Budd, D. A., Saller, A. H., and Harris, P. M. (eds.), *Unconformities and Porosity in Carbonate Strata*. Tulsa: AAPG Memoir 63, pp. 77–101.
- Palmer, A. N., 2007. *Cave Geology*. Dayton, OH: Cave Books, p. 454.
- Peck, S. B., 1986. Bacterial deposition of iron and manganese oxides in North American caves. *Bulletin of the National Speleological Society*, **48**, 26–30.
- Pohlman, J. W., Iliffe, T. M., and Cifuentes, L. A., 1997. A stable isotope study of organic cycling and the ecology of an anchialine cave ecosystem. *Marine Ecology Progress Studies*, **155**, 17–27.

- Polyak, V. J., McIntosh, W. C., Guven, N., and Provencio, P., 1998. Age and origin of Carlsbad Cavern and related caves from $^{40}\text{Ar}/^{39}\text{Ar}$ of alunite. *Science*, **279**, 1919–1922.
- Por, F. D., 2007. Ophel: a groundwater biome based on chemoautotrophic resources. The global significance of the Ayyalon cave finds, Israel. *Hydrobiologia*, **592**, 1–10.
- Porter, M. L., 1999. *Ecosystem Energetics of Sulfidic Karst*. Masters Thesis, University of Cincinnati, Cincinnati, OH, p. 52.
- Pronk, M., Goldscheider, N., and Zopfi, J., 2006. Dynamics and interaction of organic carbon, turbidity and bacteria in a karst aquifer system. *Hydrogeology Journal*, **14**, 473–484.
- Rusterholtz, K., and Mallory, L. M., 1994. Density, activity and diversity of bacteria indigenous to a karstic aquifer. *Microbial Ecology*, **28**, 79–99.
- Sarbu, S. M., Kane, T. C., and Kinkle, B. K., 1996. A chemoautotrophically based cave ecosystem. *Science*, **272**, 1953–1955.
- Schabereiter-Gurtner, C., Saiz-Jimenez, C., Pinar, G., Lubitz, W., and Rölleke, S., 2002a. Phylogenetic 16S rRNA analysis reveals the presence of complex and partly unknown bacterial communities in Tito Bustillo Cave, Spain, and on its Palaeolithic paintings. *Environmental Microbiology*, **4**, 392–400.
- Schabereiter-Gurtner, C., Saiz-Jimenez, C., Piñar, G., Lubitz, W., and Rölleke, S., 2002b. Altamira Cave Paleolithic paintings harbour partly unknown bacterial communities. *FEMS Microbiology Letters*, **211**, 7–11.
- Schabereiter-Gurtner, C., Saiz-Jimenez, C., Piñar, G., Lubitz, W., and Rölleke, S., 2004. Phylogenetic diversity of bacteria associated with Paleolithic paintings and surrounding rock walls in two Spanish caves (Llonin and La Garma). *FEMS Microbiology Ecology*, **47**, 235–247.
- Simon, K. S., 2000. *Organic Matter Dynamics and Trophic Structure in Karst Groundwater*. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, p. 95.
- Simon, K. S., Benfield, E. F., and Macko, S. A., 2003. Food web structure and the role of epilithic biofilms in cave streams. *Ecology*, **84**, 2395–2406.
- Sket, B., 1999. The nature of biodiversity in hypogean waters and how it is endangered. *Biodiversity Conservation*, **8**, 1319–1338.
- Stoessell, R. K., Moore, Y. H., and Coke, J. G., 1993. The occurrence and effect of sulfate reduction and sulfide oxidation on coastal limestone dissolution in Yucatan cenotes. *Ground Water*, **31**, 566–575.
- van Beynen, P., and Townsend, K., 2005. A disturbance index for karst environments. *Environmental Management*, **36**, 101–116.
- Vlasceanu, L., 1999. *Thriving in the Dark: The Microbiology of Two Chemoautotrophically-based Groundwater Ecosystems*. Ph.D. Dissertation, University of Cincinnati, Cincinnati, OH, p. 171.
- Vlasceanu, L., Popa, R., and Kinkle, B., 1997. Characterization of *Thiobacillus thioparus* LV43 and its distribution in a chemoautotrophically based groundwater ecosystem. *Applied and Environmental Microbiology*, **63**, 3123–3127.
- Vlasceanu, L., Sarbu, S. M., Engel, A. S., and Kinkle, B. K., 2000. Acidic cave-wall biofilms located in the Frasassi Gorge, Italy. *Geomicrobiology Journal*, **17**, 125–139.
- White, W. B., 1997. Thermodynamic equilibrium, kinetics, activation barriers, and reaction mechanisms for chemical reactions in Karst Terrains. *Environmental Geology*, **30**, 46–58.
- White, W. B., and Culver, D. C., 2000. Cave, definition of. In Culver, D. C., and White, W. B. (eds.), *Encyclopedia of Caves*. Burlington, MA: Elsevier, pp. 81–85.
- Zimmermann, J., Gonzalez, J. M., Saiz-Jimenez, C., and Ludwig, W., 2005. Detection and phylogenetic relationships of highly diverse uncultured acidobacterial communities in Altamira Cave using 23S rRNA sequence analyses. *Geomicrobiology Journal*, **22**, 379–388.

Cross-references

[Acidophiles](#)
[Carbonate Environments](#)
[Carbonates](#)
[Chemolithotrophy](#)
[Extreme Environments](#)
[Gallionella](#)
[Hydrothermal Environments, Terrestrial](#)
[Leptothrix](#)
[Microbial Biomineralization](#)
[Microbial Ecology of Submarine Caves](#)
[Microbial Mats](#)
[Moonmilk](#)
[Nitrogen](#)
[Speleothems](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)
[Thiotrophic Bacteria](#)

L

LATERAL GENE TRANSFER

Hans-Joachim Fritz
University of Göttingen, Göttingen, Germany

Synonyms

Horizontal gene transfer

Definition

The use of the term in the literature is not entirely unambiguous. For the purpose of this entry, lateral gene transfer (LGT) is defined as the stable transfer of genetic material (DNA, possibly RNA) from one organism to another where the two organisms belong to lineages which are genetically too distant to allow a mutual act of homologous recombination.

Introduction

Vegetative proliferation is characterized by a vertical line of genetic transfer from one cell to two daughter cells. Upon zygote formation, in contrast, sexual reproduction mixes the genetic outfit of two parent organisms. Fertile offspring, however, is only produced if the two parents are genetically close enough to allow for gamete formation (i.e., meiosis with mandatory homologous recombination) in the offspring. This limit defines a breeding pool which, in turn, can serve as a useful approach to the term “species.”

In the bacterial and the archaeal domains of life, the species concept is much more contentious – for reasons partly illustrated by this article. Nevertheless, the homologous recombination criterion can be upheld at least in cases like *Escherichia coli*, in which genetic traits are transferred by conjugation between two pre-existing cells. Conjugation proceeds by transient cell fusion between an Hfr⁺ and an Hfr[−] cell, followed by physical transfer of

DNA from the former to the latter, followed by homologous recombination. Natural transformation in *Streptococcus pneumoniae* provides a similar example.

Against this backdrop, LGT can be viewed as any transfer of genetic material between two organisms that are separated by the homologous recombination barrier. This criterion separates, for example, *E. coli* from the closely related *Salmonella typhimurium*; in cases with no known system of homologous recombination, the criterion has to be replaced by corresponding and somewhat arbitrary measures such as sequence distance. There are two hallmarks of LGT which may be detected simultaneously or alternatively in any particular single case.

1. Presence of genes in a genome that are not present in genomes of otherwise closely related organisms.
2. Topologically different branching patterns obtained in attempts to delineate phylogenetic relationships between a host of lineages, based on sequence comparisons within more than one set of (seemingly) orthologous genes.

Other, less clear-cut indicators of LGT include G/C content and codon usage that deviate from those of the bulk genome and also physical gene linkages that differ from those observed with close relatives.

These statements together make it immediately understandable why the intensity of the debate of LGT developed in parallel with the unfolding of the genome analysis era that followed the publication of the *Haemophilus influenzae* sequence in 1995 (Fleischmann et al., 1995). To date, several hundred genomic sequences have been determined – mostly of bacteria and archaea. By far most of the knowledge we have about LGT concerns the bacterial and archaeal domains (including trans-domain transfer) and it is not entirely clear to which extent this fact reflects (non)availability of the necessary mechanisms in the different domains and to which simple numerical preponderance of sequenced prokaryotic

genomes. The following discussion focuses on LGT in bacteria and archaea (and between bacteria and archaea). Establishment of endosymbiosis can be regarded as an extreme case of LGT; because it is such an exception with respect to mechanism and frequency of occurrence, it is not covered here.

Mechanisms

Mechanistically, LGT can be divided into two consecutive steps:

1. Introduction of foreign DNA into the recipient cell. (In the following, the alternative option of RNA being introduced is no longer explicitly mentioned.)
2. Stable establishment of the transferred DNA as part of the genetic outfit of that cell.

Step 1: DNA can arrive in a recipient cell as the result of conjugation (i.e., as a plasmid or genome fragment) of phage infection (i.e., as a phage genome, in particular of a transducing phage carrying genes of a former host) or by physical uptake (i.e., any piece of naked DNA).

Step 2: The newly arrived DNA can either be degraded or stably established in the cell in parts or *in toto* by any of the following mechanisms: (i) independent replication as an episome (plasmids, certain kinds of lysogenic phage such as *E. coli* P1); (ii) transposition (transposon, IS-elements or the like) from the entering DNA molecule to the recipient's chromosome (or other residing replicon); (iii) site-specific recombination as seen in lysogenic phage such as phage Lambda; (iv) illegitimate recombination (a term collectively used for a number of mechanisms that require no or only very short stretches of high sequence similarity between the two recombining molecules).

Note that in order for any combination of Steps 1 and 2 to qualify as LGT, the criterion of the homologous recombination barrier has to apply for the lineage of the donor and the receptor of the DNA (compare above: Definition).

Plausibly, the acquisition of genetic traits through LGT is limited by incompatibilities of gene expression signals present in the transferred DNA with cognate proteins (RNA polymerases, repressors, gene activators) or other macromolecular components of the gene expression apparatus of the recipient cell (e.g., rRNA).

Some selected cases

Extent of LGT

Porwollik and McClelland (2003) have used comparative genomics and microarray analysis to determine the extent to which LGT participated in shaping the genomes of different *Salmonella enterica* serovars. They come to the conclusion that almost one quarter of the entire *S. enterica* sv. *Typhimurium* genome may have been introduced by LGT.

Trans-domain LGT

In bacteria and eukarya, the repair of DNA-uracil residues (pre-mutagenic DNA lesions resulting from spontaneous

hydrolytic deamination of DNA cytosine residues) is almost invariably initiated by a family 1 UDG enzyme (UDG: Uracil DNA Glycosylase). On the other hand, no family 1 UDG gene has been discovered to date in any archaeal genome. For archaea, UDGs of families 4 and 5 seem to be typical. The genome of *Thermus thermophilus*, a Gram-negative, extremely thermophilic bacterium, almost uniquely for a bacterium, contains no gene for a family 1 UDG, but one gene each for a UDG enzyme of family 4 and 5 (Starkuviene and Fritz, 2002; Henne et al., 2004). This highly unusual finding is most simply explained by lateral transfer of the family 4 and family 5 genes into *T. thermophilus* (or an ancestor thereof) across the domain barrier, followed by loss of the formerly residing family 1 gene whose activity had been made redundant by the LGT event.

Consequences and summary

Lateral gene transfer lies at the heart of some pressing practical problems, among them is the rapid emergence of multiply-resistant pathogens under the selection pressure of widespread (and sometimes inappropriate) use of antibiotics. Naturally spreading promiscuous plasmids of overlapping host range, combined with DNA transposition are arguably the major contributors to that process. The spread of pathogenicity islands is a similar example. More fundamentally, the nature and extent of LGT shakes the entire evolutionary concept of common descent and, along with it, the usefulness of its iconographic representation, the tree of life. Rather, the bacterial and the archaeal domains of life together form a densely woven genetic network along which smaller or larger packages of genes can move with a high enough degree of independence to leave frequent and conspicuous traces of this process in genome structures. Different genes may have come together in a common genome as the result of profoundly different ancestry trajectories. This mosaic nature of the genome potentially frustrates any attempt of deriving stringent phylogenetic relationships at the organismal level from DNA sequence data. As a substitute of low resolution, one may operate with the consensus tree topology one may find for the large majority of genes present in the genomes under consideration.

Bibliography

- Henne, A., et al., 2004. The genome sequence of the extreme thermophile *Thermus thermophilus*. *Nature Biotechnology*, **22**, 547–553.
- Fleischmann, R. D., et al., 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science*, **269**, 496–512.
- Porwollik, S., and McClelland, M., 2003. Lateral gene transfer in *Salmonella*. *Microbes and Infection*, **5**, 977–989.
- Starkuviene, V., and Fritz, H.-J., 2002. A novel type of uracil-DNA glycosylase mediating repair of hydrolytic DNA damage in the extremely thermophilic eubacterium *Thermus thermophilus*. *Nucleic Acids Research*, **30**, 2097–2102.

Cross-references

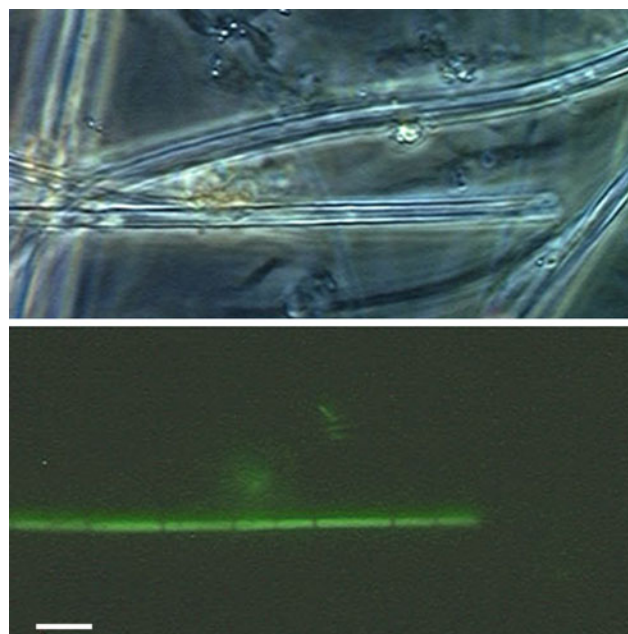
Metagenomics
Microbial Communities, Structure, and Function
Symbiosis

LEPTOTHRIX

David Emerson
Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA

Definition

Leptothrix describes a genus of bacteria that have long been recognized as important in the biological oxidation of iron and manganese (van Veen et al., 1978). It is one of several genera that may be referred to as the “iron bacteria.” At present, there are five recognized species: *L. ochracea*, *L. discophora*, *L. cholodnii*, *L. lopholea*, and *L. mobilis* (Garritty et al., 2004). The genus is in the class Betaproteobacteria. The members of this genus are recognized for the production of an extracellular tubular sheath. The cells typically grow as chains of cells within the sheath, which they excrete as they grow. It is common for the sheaths to be coated in Mn oxides or Fe oxyhydroxides. *L. ochracea* is perhaps the most commonly recognized *Leptothrix* spp. due to copious production of very straight, iron encrusted sheaths. This species has never been cultivated in the laboratory and its exact relationship to other members of the genus is not known. Circumstantial evidence suggests that it is capable of lithotrophic growth using Fe(II) as sole energy source; however, this has not been proven. The evidence stems from its requirement for Fe-rich waters to grow. Typically, these are wetlands, springs, water wells, and like environments where anoxic groundwater enriched in Fe(II) comes into contact with air. Another interesting feature of *L. ochracea*, and also indicative of its use of iron as an energy source, is that only a small percentage (estimated at about 10%) of the sheaths contain cells during active growth (Figure 1). An interesting facet of these metal encrusted sheaths is that they can be preserved in the fossil record (Hofmann and Farmer, 2000). The other species of *Leptothrix* have been cultured in the laboratory and identified using classical microbiological techniques. All of these latter species are heterotrophic organisms utilizing organic carbon sources for growth. To date there is no compelling evidence that they are either lithoautotrophic or mixotrophic. They are typically found in wetlands and other aqueous habitats. Proteins with enzymatic activity for Mn-oxidation have been found in *L. discophora* and *L. cholodnii* (Adams and Ghiorse, 1987; Emerson and Ghiorse, 1992). Both species also accumulate Fe-oxyhydroxides on their sheaths, and a gene has been identified in *L. discophora* that is associated with iron oxidation (Corstjens et al., 1992). These Mn- or Fe-oxidizing



Leptothrix, Figure 1 *Leptothrix ochracea* from a wetland iron seep, note the tubular sheaths that are composed primarily of Fe-oxide. The lower panel shows a filament of cells inside one of the sheaths that has been stained with a DNA-fluorescent dye. The bar = 5 μ m.

proteins appear to be secreted from the cell into the surrounding sheath, the site of metal oxidation. The sheath of *L. cholodnii* is a complex fibrillar polymer consisting primarily of polysaccharides with a high concentration of the amino acid cysteine (Emerson and Ghiorse, 1993a). The fibrils are held together by extensive disulfide bonding, making it a very resilient structure (Emerson and Ghiorse, 1993b). A novel cysteine glycoconjugate is the primary building block of the sheath (Makita et al., 2006).

Bibliography

- Adams, L. F., and Ghiorse, W. C., 1987. Characterization of extracellular Mn^{2+} -oxidizing activity and isolation of an Mn^{2+} -oxidizing protein from *Leptothrix discophora* SS-1. *The Journal of Bacteriology*, **169**, 1279–1285.
- Corstjens, P. L., de Vrind, J. P., Westbroek, P., and de Vrind-de Jong, E. W., 1992. Enzymatic iron oxidation by *Leptothrix discophora*: identification of an iron-oxidizing protein. *Applied and Environmental Microbiology*, **58**, 450–454.
- Emerson, D., and Ghiorse, W. C., 1992. Isolation, cultural maintenance, and taxonomy of a sheath-forming strain of *Leptothrix discophora* and characterization of manganese-oxidizing activity associated with the sheath. *Applied and Environmental Microbiology*, **58**, 4001–4010.
- Emerson, D., and Ghiorse, W. C., 1993a. Ultrastructure and chemical composition of the sheath of *Leptothrix discophora* SP-6. *The Journal of Bacteriology*, **175**, 7808–7818.
- Emerson, D., and Ghiorse, W. C., 1993b. Role of disulfide bonds in maintaining the structural integrity of the sheath of *Leptothrix discophora* SP-6. *The Journal of Bacteriology*, **175**, 7819–7827.

Cross-references

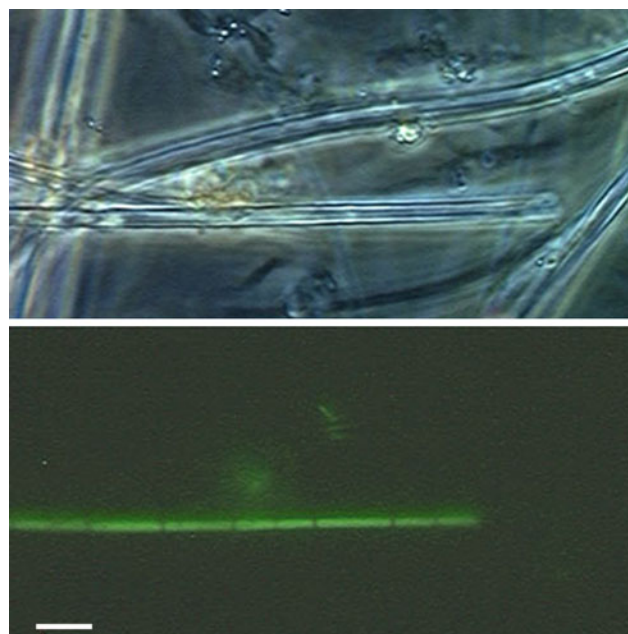
Metagenomics
Microbial Communities, Structure, and Function
Symbiosis

LEPTOTHRIX

David Emerson
Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, ME, USA

Definition

Leptothrix describes a genus of bacteria that have long been recognized as important in the biological oxidation of iron and manganese (van Veen et al., 1978). It is one of several genera that may be referred to as the “iron bacteria.” At present, there are five recognized species: *L. ochracea*, *L. discophora*, *L. cholodnii*, *L. lopholea*, and *L. mobilis* (Garritty et al., 2004). The genus is in the class Betaproteobacteria. The members of this genus are recognized for the production of an extracellular tubular sheath. The cells typically grow as chains of cells within the sheath, which they excrete as they grow. It is common for the sheaths to be coated in Mn oxides or Fe oxyhydroxides. *L. ochracea* is perhaps the most commonly recognized *Leptothrix* spp. due to copious production of very straight, iron encrusted sheaths. This species has never been cultivated in the laboratory and its exact relationship to other members of the genus is not known. Circumstantial evidence suggests that it is capable of lithotrophic growth using Fe(II) as sole energy source; however, this has not been proven. The evidence stems from its requirement for Fe-rich waters to grow. Typically, these are wetlands, springs, water wells, and like environments where anoxic groundwater enriched in Fe(II) comes into contact with air. Another interesting feature of *L. ochracea*, and also indicative of its use of iron as an energy source, is that only a small percentage (estimated at about 10%) of the sheaths contain cells during active growth (Figure 1). An interesting facet of these metal encrusted sheaths is that they can be preserved in the fossil record (Hofmann and Farmer, 2000). The other species of *Leptothrix* have been cultured in the laboratory and identified using classical microbiological techniques. All of these latter species are heterotrophic organisms utilizing organic carbon sources for growth. To date there is no compelling evidence that they are either lithoautotrophic or mixotrophic. They are typically found in wetlands and other aqueous habitats. Proteins with enzymatic activity for Mn-oxidation have been found in *L. discophora* and *L. cholodnii* (Adams and Ghiorse, 1987; Emerson and Ghiorse, 1992). Both species also accumulate Fe-oxyhydroxides on their sheaths, and a gene has been identified in *L. discophora* that is associated with iron oxidation (Corstjens et al., 1992). These Mn- or Fe-oxidizing



Leptothrix, Figure 1 *Leptothrix ochracea* from a wetland iron seep, note the tubular sheaths that are composed primarily of Fe-oxide. The lower panel shows a filament of cells inside one of the sheaths that has been stained with a DNA-fluorescent dye. The bar = 5 μ m.

proteins appear to be secreted from the cell into the surrounding sheath, the site of metal oxidation. The sheath of *L. cholodnii* is a complex fibrillar polymer consisting primarily of polysaccharides with a high concentration of the amino acid cysteine (Emerson and Ghiorse, 1993a). The fibrils are held together by extensive disulfide bonding, making it a very resilient structure (Emerson and Ghiorse, 1993b). A novel cysteine glycoconjugate is the primary building block of the sheath (Makita et al., 2006).

Bibliography

- Adams, L. F., and Ghiorse, W. C., 1987. Characterization of extracellular Mn^{2+} -oxidizing activity and isolation of an Mn^{2+} -oxidizing protein from *Leptothrix discophora* SS-1. *The Journal of Bacteriology*, **169**, 1279–1285.
- Corstjens, P. L., de Vrind, J. P., Westbroek, P., and de Vrind-de Jong, E. W., 1992. Enzymatic iron oxidation by *Leptothrix discophora*: identification of an iron-oxidizing protein. *Applied and Environmental Microbiology*, **58**, 450–454.
- Emerson, D., and Ghiorse, W. C., 1992. Isolation, cultural maintenance, and taxonomy of a sheath-forming strain of *Leptothrix discophora* and characterization of manganese-oxidizing activity associated with the sheath. *Applied and Environmental Microbiology*, **58**, 4001–4010.
- Emerson, D., and Ghiorse, W. C., 1993a. Ultrastructure and chemical composition of the sheath of *Leptothrix discophora* SP-6. *The Journal of Bacteriology*, **175**, 7808–7818.
- Emerson, D., and Ghiorse, W. C., 1993b. Role of disulfide bonds in maintaining the structural integrity of the sheath of *Leptothrix discophora* SP-6. *The Journal of Bacteriology*, **175**, 7819–7827.

- Garrrity, G. M., Bell, J. A., and Lilburn, T. G., 2004. Taxonomic outline of the Prokaryotes. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn. Release 5.0. New York: Springer-Verlag.
- Hofmann, B. A., and Farmer, D. J., 2000. Filamentous fabrics in low-temperature mineral assemblages: are they fossil biomarkers? Implications for the search for a subsurface fossil record on the early Earth and Mars. *Planetary Space Science*, **48**, 1077–1086.
- Makita, H., Nakahara, Y., Fukui, H., Miyanoiri, Y., Katahira, M., Seki, H., Takeda, M., and Koizumi, J., 2006. Identification of 2-(cysteiny)amido-2-deoxy-D-galacturonic acid residue from the sheath of *Leptothrix cholodnii*. *Bioscience, Biotechnology, and Biochemistry*, **70**, 1265–1268.
- van Veen, W. L., Mulder, E. G., and Deinema, M. H., 1978. The *Sphaerotilus-Leptothrix* group of bacteria. *Microbiological Reviews*, **42**, 329–356.

Cross-references

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)

M

MAGNETOTACTIC BACTERIA

Mihály Pósfai
University of Pannonia, Veszprém, Hungary

Definition

Magnetotactic bacteria (MTB) are a diverse group of Gram-negative, motile prokaryotes that align and migrate along the geomagnetic field. This magnetotactic behavior is based on intracellular organelles called magnetosomes, which are inorganic nanocrystals of either magnetite (Fe_3O_4) or greigite (Fe_3S_4), surrounded by a lipid bilayer membrane. The magnetosomes are typically arranged in one or more chains, conveying a magnetic dipole moment to the cell (Figure 1). Magnetotaxis is thought to be beneficial for the cell for finding its optimum position in vertical concentration gradients in aquatic habitats.

History

Bacteria that responded to magnetic fields were discovered independently by Bellini (1963) and Blakemore (1975), who coined the term magnetotaxis and noted the presence of crystals in the cells. Intracellular magnetite was identified and the physics of magnetotaxis was described by Frankel et al. (1979). Iron sulfide-producing MTB were found in 1990 (Mann et al., 1990; Farina et al., 1990). The ecology of MTB and the structures and morphologies of the crystalline components of magnetosomes have been widely studied since the discovery of MTB. In the past decade, significant progress has been made in elucidating the molecular, biochemical, and genetic bases of magnetosome formation (Schüler, 2007).

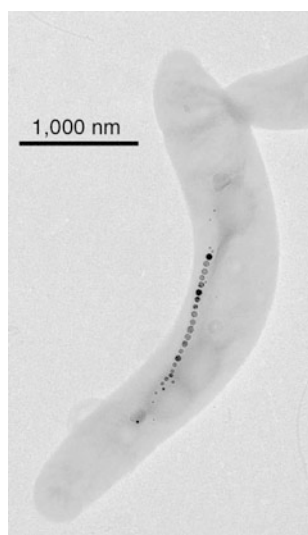
Ecophysiology

MTB are ubiquitous in both marine and freshwater aquatic habitats, where suboxic or anoxic conditions prevail, and

dissolved iron is available. Because of their fastidious nature, only a few strains are available in pure culture. With one exception, all strains in pure culture have a respiratory form of metabolism. All known MTB are microaerophiles (they require a certain amount of oxygen for growth, but exhibit a negative tactic and/or growth response to atmospheric concentrations of oxygen) or anaerobes or facultatively anaerobic microaerophiles (Bazylinski and Frankel, 2004). The physiology of MTB appears to dictate their ecology: since MTB derive energy from the proximity of oxidants and reductants, magnetite-producing organisms occur in largest numbers (up to 10^5 cells/ml) in layers at or near the oxic–anoxic interfaces (OAI) of chemically stratified habitats. Iron sulfide-producing bacteria live below the OAI under anaerobic conditions (Simmons et al., 2004). Whereas magnetite-producing bacteria occur virtually in all types of water bodies, sulfide-producing organisms have been found in marine habitats only. Considering the large amount of iron concentrated in magnetosomes (10^{-15} to 10^{-13} g Fe per cell) and the estimated population density of MTB at and below the OAI, MTB are likely to contribute significantly to the flux of reduced Fe to sediments in chemically stratified marine basins.

Magneto-aerotaxis

Magnetotaxis means active motion guided by the magnetic field. MTB passively orient along the geomagnetic field and actively swim by means of flagella. A key element of magnetotaxis is that the cell should possess a large enough magnetic dipole moment to overcome the disorientating effects of thermal motions and be aligned parallel to the magnetic field. In the geomagnetic field, the magnetic energy of magnetotactic cells is typically ten times larger than thermal energy, which means that the cell can migrate along the field at 90% of its forward speed (Frankel et al., 1979).



Magnetotactic Bacteria, Figure 1 A cell of *Magnetospirillum gryphiswaldense* that contains a single chain of magnetite magnetosomes.

“Polar” and “axial” magnetotaxis are distinguished (Frankel et al., 1997). Polar magnetotaxis is displayed by MTB that swim persistently toward one of the poles of a bar magnet under oxic conditions. Those that swim toward the “south” pole of a magnet have North-seeking polarity, since they would swim northward in the geomagnetic field. MTB from the northern hemisphere are predominantly North-seekers, whereas those from the southern hemisphere are predominantly South-seekers (Bazylinski and Frankel, 2004).

The observation of polar magnetotaxis led to the idea that MTB are guided by the geomagnetic field to less oxygenated regions. Since the geomagnetic field is inclined downward from the horizontal in the northern hemisphere and upward in the southern hemisphere under oxic conditions the cells with polar magnetotaxis swim downward in both hemispheres (Bazylinski and Frankel, 2004). However, if the cell is in a reducing environment, it reverses the direction of flagellar rotation and migrates upward. Since the motion of the cell is determined by both the magnetic field and the oxygen tension, the term “magneto-aerotaxis” was introduced (Frankel et al., 1997).

Axial magnetotaxis means that the axis of motion is determined by the magnetic field but its direction is not. As the cell swims, it samples the oxygen concentration and compares it with that in the recent past. Cells moving toward the optimal habitat have a decreased probability of reversing the sense of flagellar rotation, whereas those that are moving away from the optimal oxygen concentration have an increased probability of reversing the sense of flagellar rotation and thus their swimming direction (Frankel et al., 1997). The magneto-aerotactic response provides an

efficient mechanism for MTB to find the OAI in their chemically stratified habitats.

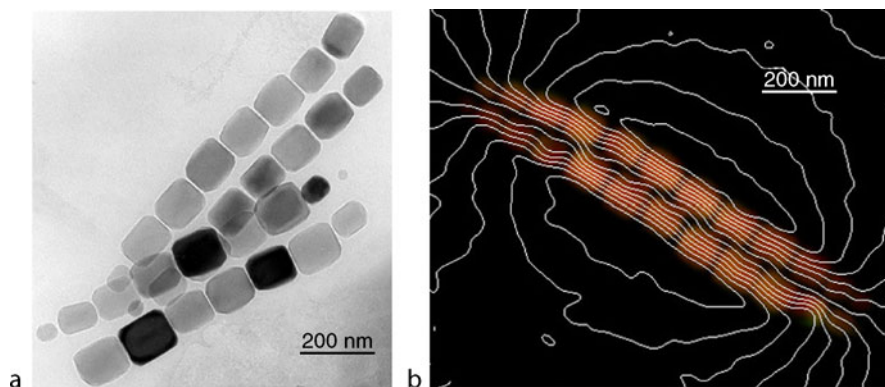
Mineralogy and magnetism

Magnetotaxis relies on intracellular grains of iron oxide or sulfide minerals. Magnetite (Fe_3O_4) is the only iron oxide mineral that has been described to date from magnetotactic cells. The sulfide-producing cells use greigite (Fe_3S_4) for navigation, but they also may contain nonmagnetic mackinawite (FeS) that rapidly converts to greigite through a solid-state phase transition (Pósfai et al., 2007).

Magnetite produced by MTB is chemically pure Fe_3O_4 , in contrast to grains from rocks that usually contain other transition metals besides iron. Structurally, the nanocrystals are either perfect or contain twin boundaries. The morphologies of magnetite magnetosomes are typically highly regulated, strain-specific, and in many cases, unusual for a mineral with cubic symmetry (Pósfai et al., 2007). Whereas magnetotactic spirilla contain cubooctahedral crystals, vibrio and cocci typically produce magnetite with prismatic habits (Figure 2a). Large, rod-shaped cells tend to contain arrowhead- or bullet-shaped magnetite crystals (Bazylinski and Frankel, 2004).

The sizes and shapes of magnetosomes are critical for magnetotaxis. Isolated, isometric magnetite crystals with diameters smaller than ~ 30 nm are superparamagnetic, i.e., they do not have a permanent magnetic moment. Crystals with diameters from ~ 30 to ~ 120 nm are magnetic single domains at room temperature, whereas grains larger than ~ 120 nm contain two or more magnetic domains, reducing the net magnetic moment of the crystal. Most magnetosomes are in the single-domain range, allowing the assembly of a highly efficient internal compass that consists of a chain (or chains) of single-domain magnets. Interactions between neighboring particles in chain configurations ensure that even those magnetite particles that are small enough to be superparamagnetic or large enough to contain two or more magnetic domains if they were isolated are also constrained to be single domains (Dunin-Borkowski et al., 2001). Since elongated particles are arranged with their axis of elongation (which typically coincides with their crystallographic easy magnetization axis) parallel to the chain of magnetosomes, shape anisotropy constrains the magnetic flux lines to be parallel to the axis of motility. Thus, in most magnetite-producing cells the effects of particle size, shape and magnetocrystalline anisotropy, and magnetostatic interactions among particles in chains combine to produce the possible largest magnetic moment (Figure 2b) (Pósfai et al., 2007).

The sizes, shapes, and orientations of iron sulfide magnetosomes are less distinct than those of magnetite magnetosomes. Greigite magnetosomes typically contain planar defects, as a result of an incomplete solid-phase transformation from mackinawite to greigite. The shapes and orientations of the crystals are random,



Magnetotactic Bacteria, Figure 2 (a) Electron micrograph of two double chains of elongated magnetite magnetosomes from a freshwater magnetotactic coccus. (b) Magnetic induction map of a similar double chain of magnetite magnetosomes, obtained from electron holograms.

resulting in poorer confinement and greater variability in the directions of the magnetic induction lines in the cells than in magnetite-bearing bacteria. Remarkably, despite the different crystal structures, orientations, and arrangements of ferrimagnetic crystals, the magnetic moments of different cells are fairly constant (Pósfai et al., 2007), thereby satisfying the requirement for magnetotaxis.

Cell biology and genomic analyses

Magnetic enrichments have shown great morphological and ultrastructural diversity of MTB from a wide variety of aquatic environments. Magnetite-producing morphotypes include spirilla, cocci, vibrios, and rods of various sizes, moving at speeds ranging from 40 to 1,000 $\mu\text{m/s}$. A unique, greigite-producing magnetotactic organism, the “multicellular magnetotactic prokaryote” (MMP) consists of 15–45 cells organized around an internal acellular compartment (Keim et al., 2004). The aggregated cells of the MMP move together as one unit, with the coordinated motion of flagella on the outside of the cells.

The highly controlled, strain-specific properties of magnetite magnetosomes and their chains indicate that biomineralization is under genetic control, executed by specific proteins in the magnetosome membrane. Many details of magnetosome formation have been elucidated by studies of the biochemistry of the magnetosome membrane and genomic analyses of MTB. Workable genetic systems have been developed for two *Magnetospirillum* strains: *M. magnetotacticum* AMB-1 and *M. gryphiswaldense* MSR-1 (Jogler and Schüller, 2007). By genetic manipulation, including the generation of mutants and the expression of magnetobacterial genes in other hosts, the major steps of magnetite biomineralization have been identified in these systems. Magnetite nucleation is preceded by the formation of vesicles, which are invaginations of the cytoplasmic membrane (Komeili et al., 2006). Dissolved ferrous or ferric iron is transported into the cell by an unknown uptake

system. Magnetosome membrane proteins (MamB/MamM) are involved in the transport of ferrous iron from the cytoplasm into the magnetosome vesicle, and then magnetite formation is activated by another protein (MamA). Within the vesicle, iron is oxidized and magnetite nucleation is triggered by the Mms6 protein. The least known aspect of the biomineralization process is the control over the distinct crystal morphology. The vesicles containing fully developed magnetite crystals are anchored to a filamentous cytoskeletal structure by the MamJ protein, resulting in an ordered magnetosome chain (Scheffell et al., 2006). Genomic analyses revealed that the genes that encode the magnetosome proteins are clustered in a 130 kb region, representing a “magnetosome island.” This genomic island appears to be conserved among different *Magnetospirillum* strains and, to a lesser degree, in a magnetotactic coccus (Jogler and Schüller, 2007), raising the question of whether the “magnetosome island” is a universal feature of MTB.

Diversity

The term “magnetotactic bacteria” does not refer to a taxonomical unit. All known MTB belong to various groups within the domain Bacteria. Most magnetotactic microorganisms that are available in pure culture are affiliated with the *Alphaproteobacteria*, including *M. magnetotacticum* and *M. gryphiswaldense*, a marine vibrio (MV-1), and a coccus (MC-1). In addition to the cultivated strains, 16S rRNA analysis of magnetic enrichments from many freshwater and marine samples revealed that a variety of magnetite-producing morphotypes are likely affiliated with the *Alphaproteobacteria* (Amann et al., 2007). Two magnetite-producing candidate species, “*Magnetobacterium bavaricum*” and “*Magnetotacticum bremense*,” are affiliated with the *Nitrospira* phylum.

Sulfide-producing MTB could not have been cultured to date, but a multicellular organism was found to be closely related to the dissimilatory sulfate-reducing

bacteria within the *Deltaproteobacteria*. Another sulfide-producing MTB was assigned to *Gammaproteobacteria*. These findings may suggest that magnetotaxis based on iron oxide and sulfide nanoparticles evolved independently; however, a magnetite-producing, sulfate-reducing organism (*Desulfovibrio magneticus* strain RS-1) is also affiliated with the *Deltaproteobacteria* (Amann et al., 2007).

The diversity of MTB indicates that magnetotaxis evolved independently in several phylogenetic groups. On the other hand, the discovery of a potentially mobil “magnetosome island” in the genome of magnetite-producing MTB could suggest that the trait of magnetotaxis was spread by lateral gene transfer among the various groups of MTB.

Magnetofossils, paleomagnetism

After MTB die, their intracellular magnetite or greigite particles are deposited in the sediment. If diagenetic conditions are favorable, the magnetosome crystals can be preserved as magnetofossils. Magnetofossils have been identified on the basis of the special properties of magnetosome crystals, including a narrow crystal size distribution, characteristic morphologies, chemical purity and, in some cases, chain configurations. Ferrimagnetic nanocrystals presumably originating from MTB have been described from a variety of sedimentary rock types, including clays, carbonates, marls, and evaporites. The magnetofossil record extends from present-day marine and lake sediments to the Cretaceous and with lesser certainty to the late Archean (Kopp and Kirschvink, 2008).

MTB carry a primary remanent magnetization, since they are aligned with the geomagnetic field, presumably even after cell death and burial in the sediment. Because magnetofossils are single magnetic domains, they have an optimized magnetic stability and can be important carriers of paleomagnetic information. Magnetofossils can serve as environmental proxies because MTB live under specific redox conditions. They can also be markers of ancient life, albeit dubious ones, as illustrated by a lengthy debate over whether nanocrystalline magnetite particles in the Martian meteorite ALH84001 represent magnetofossils or had formed inorganically (Winklhofer and Petersen, 2007).

Nanotechnological applications

Magnetic nanoparticles are widely used in technological applications, such as in spintronics, in magnetic inks, in high-density magnetic memory devices, and in medical applications (as contrast agents in magnetic resonance imaging and as magnetic carriers for drug targeting and delivery). Magnetite crystals produced by MTB have uniform sizes and shapes and can be separated from disrupted cells by magnetic methods with their magnetosome membrane intact. The membrane-bound bacterial nanoparticles have a large specific surface area and are well dispersed in aqueous solutions. Antibodies or proteins can be immobilized on the magnetosome membrane, producing

functionalized magnetic nanoparticles (Matsunaga and Arakaki, 2007). Since bound and free analytes can be separated in a magnetic field, bacterial nanoparticles have been used for the rapid and sensitive detection of small molecules, including environmental pollutants, hormones, and toxic detergents. Immunomagnetic particles are also useful for sorting target cells from cell suspensions using a permanent magnet, and for using them in high-performance DNA/mRNA recovery and DNA discrimination analysis (Matsunaga and Arakaki, 2007).

Summary

MTB provide a fascinating example of microbial biomineralization and motivate interdisciplinary research at the junctions of biology, mineralogy, geology, geochemistry, and physics. The molecular and genetic bases of magnetic nanocrystal formation by MTB are now beginning to be understood. The results of research on MTB will be useful for understanding biomineralization processes and magnetic sensing mechanisms in more complex organisms, and for designing nanotechnological applications of bacterial nanocrystals.

Bibliography

- Amann, R., Peplies, J., and Schüller, D., 2007. Diversity and taxonomy of magnetotactic bacteria. In Schüller, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 25–36.
- Bazylinski, D. A., and Frankel, R. B., 2004. Magnetosome formation in prokaryotes. *Nature Reviews Microbiology*, **2**, 217–230.
- Bellini, S., 1963. *Su di un particolare comportamento di batteri d'acqua dolce*. Istituto di Microbiologia dell'Università di Pavia.
- Blakemore, R. P., 1975. Magnetotactic bacteria. *Science*, **190**, 377–379.
- Dunin-Borkowski, R. E., McCartney, M. R., Pósfai, M., Frankel, R. B., Bazylinski, D. A., and Buseck, P. R., 2001. Off-axis electron holography of magnetotactic bacteria: magnetic microstructure of strains MV-1 and MS-1. *European Journal of Mineralogy*, **13**, 671–684.
- Farina, M., Esquivel, D. M. S., and Lins de Barros, H. G. P., 1990. Magnetic iron-sulphur crystals from a magnetotactic microorganism. *Nature*, **343**, 256–258.
- Frankel, R. B., Blakemore, R. P., and Wolfe, R. S., 1979. Magnetite in freshwater magnetotactic bacteria. *Science*, **203**, 1355–1356.
- Frankel, R. B., Bazylinski, D. A., Johnson, M. S., and Taylor, B. L., 1997. Magneto-aerotaxis in marine coccoid bacteria. *Biophysical Journal*, **73**, 994–1000.
- Jogler, C., and Schüller, D., 2007. Genetic analysis of magnetosome biomineralization. In Schüller, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 133–162.
- Keim, C. N., Abreu, F., Lins, U., Lins de Barros, H. G. P., and Farina, M., 2004. Cell organization and ultrastructure of a magnetotactic multicellular organism. *Journal of Structural Biology*, **145**, 254–262.
- Komeili, A., Li, Z., Newman, D. K., and Jensen, G. J., 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science*, **311**, 242–245.
- Kopp, R. E., and Kirschvink, J. L., 2008. The identification and biogeochemical interpretation of fossil magnetotactic bacteria. *Earth-Science Reviews*, **86**, 42–61.

- Mann, S., Sparks, N. H. C., Frankel, R. B., Bazylinski, D. A., and Jannasch, H. W., 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. *Nature*, **343**, 258–261.
- Matsunaga, T., and Arakaki, A., 2007. Molecular bioengineering of bacterial magnetic particles for biotechnological applications. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 227–254.
- Pósfai, M., Kasama, T., and Dunin-Borkowski, R. E., 2007. Characterization of bacterial magnetic nanostructures using high-resolution transmission electron microscopy and off-axis electron holography. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 175–226.
- Scheffel, A., Gruska, M., Faivre, D., Linaroudis, A., Plitzko, J. M., and Schüler, D., 2006. An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature*, **440**, 110–114.
- Schüler, D., 2007. *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, p. 319.
- Simmons, S. L., Sievert, S. M., Frankel, R. B., Bazylinski, D. A., and Edwards, K. J., 2004. Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. *Applied and Environmental Microbiology*, **70**, 6230–6239.
- Winklhofer, M., and Petersen, N., 2007. Paleomagnetism and magnetic bacteria. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 255–274.

Cross-references

Bacteria
 Biosignatures in Rocks
 Iron Sulfide Formation
 Microbial Biomineralization
 Nanocrystals, Microbially Induced

MANGANESE (SEDIMENTARY CARBONATES AND SULFIDES)

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Alabandite; Authigenic manganese minerals; Inca rose; Rambergite; Rhodochrosite

Definition

Rhodochrosite. Manganese(II) carbonate (MnCO_3 ; trigonal, calcite structure), named after the greek word for “rose-colored.” It was named as the state mineral of Colorado (USA) in 2002. Complete solid solution formation with calcite occurs at low temperatures (Böttcher, 1998), the “Ca-rhodochrosites” or “pseudo-kutnahorites” (Mucci, 2004).

Kutnahorite. Manganese(II)-calcium carbonate ($\text{CaMn}(\text{CO}_3)_2$; trigonal; ordered dolomite structure), named after the location KutnoHora (Peacor et al., 1987). Although physicochemically stable at low temperatures

with respect to equally composed solid-solutions (Mucci, 2004), it is not formed in sediments, and only known from high-temperature hydrothermal and metamorphic environments.

Rambergite. Manganese(II) sulfide ($\mu\text{-MnS}$; hexagonal), named after the mineralogist Hans Ramberg, is found as authigenic product in anoxic sediments of the Baltic Sea (Böttcher and Huckriede, 1997; Burke and Kemp, 2002).

Alabandite. Manganese(II) sulfide ($\alpha\text{-MnS}$, cubic), named after the location Alabanda, Turkey, is found in sediments as minor intergrowth with rambergite (Lepland and Stevens, 1998).

Manganese carbonate formation in suboxic/anoxic sediments

Mixed manganese–calcium carbonates, with minor incorporation of magnesium and divalent iron, are formed in (brackish-marine) sediments as authigenic products of suboxic and anoxic early diagenesis, in the Baltic Sea deeps and the Panama Basin. Significant sedimentary Ca-rhodochrosite accumulation requires manganese(IV) dioxide pre-enrichment at the sediment–water interface by episodic water column oxidation, as shown for the temporarily euxinic deeps of the Baltic Sea (Huckriede and Meischner, 1996). The required dissolved carbonate species are formed in the pore water upon mineralization of organic matter, and aqueous Mn^{2+} is derived from microbial or chemical reduction of manganese(III, IV) oxides as electron acceptors (e.g., Banfield and Nealson, 1997). The carbonates occur as kidney-shaped globules, as overgrowth on foraminifers, detrital carbonates, and microbial cells or as idiomorphic crystals, depending on the micro-environment. Experimental studies reveal that the composition of manganese-rich carbonates is controlled by the pore water composition, the abundance of carbonate surfaces, and the presence of inhibitors (Böttcher, 1998; Mucci, 2004). Under equilibrium conditions, natural anoxic pore waters are in the stability field of coexisting rhodochrosite and iron sulfides (Figure 1), and no manganese sulfide should be formed during typically zoned early diagenetic processes.

Manganese sulfide formation in anoxic sediments

The only places worldwide where sedimentary manganese sulfides have been found so far are the Baltic Sea anoxic deeps (e.g., Baron and Debyser, 1957). Idiomorphic MnS (Figure 2) is associated with organic matter, Ca-rhodochrosite, and iron sulfides (Böttcher and Huckriede, 1997; Lepland and Stevens, 1998). Although MnS has been obviously formed under slow diffusion-controlled conditions where rhodochrosite formation was chemically inhibited, the exact process is still under debate. Occurrence of Ca-rhodochrosite replacing hexagonal MnS (Burke and Kemp, 2002) indicates possible readaptation of the metastable sedimentary system toward equilibrium conditions.

- Mann, S., Sparks, N. H. C., Frankel, R. B., Bazylinski, D. A., and Jannasch, H. W., 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. *Nature*, **343**, 258–261.
- Matsunaga, T., and Arakaki, A., 2007. Molecular bioengineering of bacterial magnetic particles for biotechnological applications. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 227–254.
- Pósfai, M., Kasama, T., and Dunin-Borkowski, R. E., 2007. Characterization of bacterial magnetic nanostructures using high-resolution transmission electron microscopy and off-axis electron holography. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 175–226.
- Scheffel, A., Gruska, M., Faivre, D., Linaroudis, A., Plitzko, J. M., and Schüler, D., 2006. An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature*, **440**, 110–114.
- Schüler, D., 2007. *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, p. 319.
- Simmons, S. L., Sievert, S. M., Frankel, R. B., Bazylinski, D. A., and Edwards, K. J., 2004. Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. *Applied and Environmental Microbiology*, **70**, 6230–6239.
- Winklhofer, M., and Petersen, N., 2007. Paleomagnetism and magnetic bacteria. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 255–274.

Cross-references

Bacteria
 Biosignatures in Rocks
 Iron Sulfide Formation
 Microbial Biomineralization
 Nanocrystals, Microbially Induced

MANGANESE (SEDIMENTARY CARBONATES AND SULFIDES)

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Alabandite; Authigenic manganese minerals; Inca rose; Rambergite; Rhodochrosite

Definition

Rhodochrosite. Manganese(II) carbonate (MnCO_3 ; trigonal, calcite structure), named after the greek word for “rose-colored.” It was named as the state mineral of Colorado (USA) in 2002. Complete solid solution formation with calcite occurs at low temperatures (Böttcher, 1998), the “Ca-rhodochrosites” or “pseudo-kutnahorites” (Mucci, 2004).

Kutnahorite. Manganese(II)-calcium carbonate ($\text{CaMn}(\text{CO}_3)_2$; trigonal; ordered dolomite structure), named after the location KutnoHora (Peacor et al., 1987). Although physicochemically stable at low temperatures

with respect to equally composed solid-solutions (Mucci, 2004), it is not formed in sediments, and only known from high-temperature hydrothermal and metamorphic environments.

Rambergite. Manganese(II) sulfide ($\mu\text{-MnS}$; hexagonal), named after the mineralogist Hans Ramberg, is found as authigenic product in anoxic sediments of the Baltic Sea (Böttcher and Huckriede, 1997; Burke and Kemp, 2002).

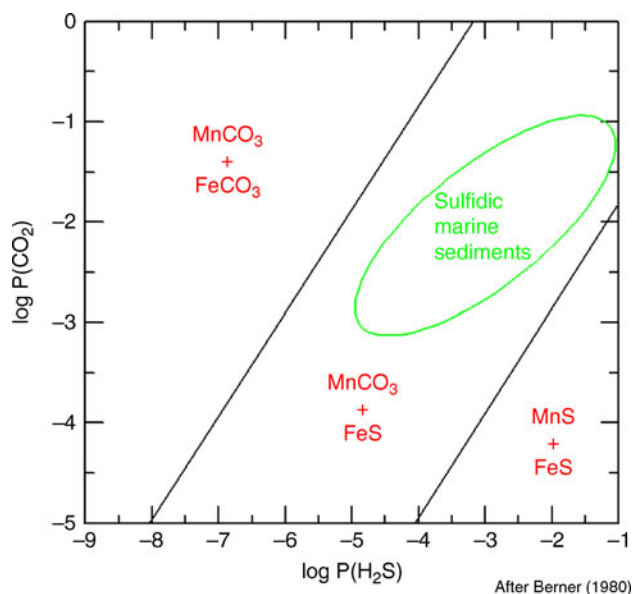
Alabandite. Manganese(II) sulfide ($\alpha\text{-MnS}$, cubic), named after the location Alabanda, Turkey, is found in sediments as minor intergrowth with rambergite (Lepland and Stevens, 1998).

Manganese carbonate formation in suboxic/anoxic sediments

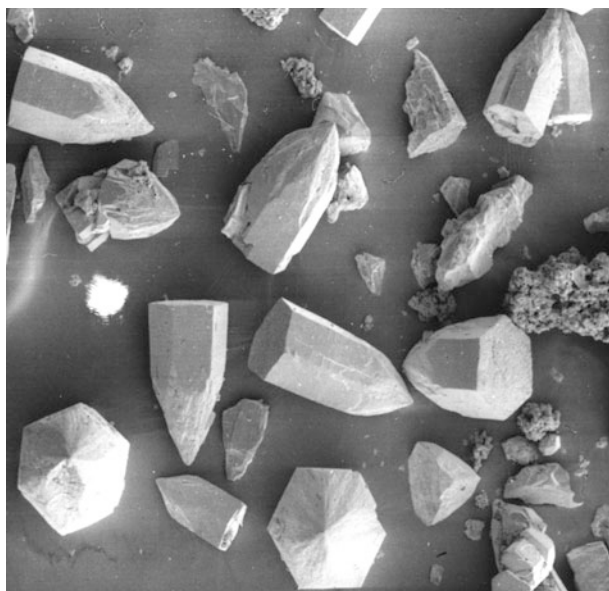
Mixed manganese–calcium carbonates, with minor incorporation of magnesium and divalent iron, are formed in (brackish-marine) sediments as authigenic products of suboxic and anoxic early diagenesis, in the Baltic Sea deeps and the Panama Basin. Significant sedimentary Ca-rhodochrosite accumulation requires manganese(IV) dioxide pre-enrichment at the sediment–water interface by episodic water column oxidation, as shown for the temporarily euxinic deeps of the Baltic Sea (Huckriede and Meischner, 1996). The required dissolved carbonate species are formed in the pore water upon mineralization of organic matter, and aqueous Mn^{2+} is derived from microbial or chemical reduction of manganese(III, IV) oxides as electron acceptors (e.g., Banfield and Nealson, 1997). The carbonates occur as kidney-shaped globules, as overgrowth on foraminifers, detrital carbonates, and microbial cells or as idiomorphic crystals, depending on the micro-environment. Experimental studies reveal that the composition of manganese-rich carbonates is controlled by the pore water composition, the abundance of carbonate surfaces, and the presence of inhibitors (Böttcher, 1998; Mucci, 2004). Under equilibrium conditions, natural anoxic pore waters are in the stability field of coexisting rhodochrosite and iron sulfides (Figure 1), and no manganese sulfide should be formed during typically zoned early diagenetic processes.

Manganese sulfide formation in anoxic sediments

The only places worldwide where sedimentary manganese sulfides have been found so far are the Baltic Sea anoxic deeps (e.g., Baron and Debyser, 1957). Idiomorphic MnS (Figure 2) is associated with organic matter, Ca-rhodochrosite, and iron sulfides (Böttcher and Huckriede, 1997; Lepland and Stevens, 1998). Although MnS has been obviously formed under slow diffusion-controlled conditions where rhodochrosite formation was chemically inhibited, the exact process is still under debate. Occurrence of Ca-rhodochrosite replacing hexagonal MnS (Burke and Kemp, 2002) indicates possible readaptation of the metastable sedimentary system toward equilibrium conditions.



Manganese (Sedimentary Carbonates and Sulfides),
Figure 1 Low temperature equilibrium phase relationships of reduced iron- and manganese-bearing solids as a function of carbon dioxide and hydrogen sulfide partial pressures.



Manganese (Sedimentary Carbonates and Sulfides),
Figure 2 Scanning electron microscope picture of idiomorphic authigenic MnS from the Baltic Sea. Picture width is equivalent to a scale of 950 μm .

Conclusion

Sedimentary authigenic manganese(II) carbonate minerals, especially investigated in the nowadays brackish-marine

Baltic Sea, are indicative of biogeochemical early-diagenetic processes in manganese-rich environments. They have gained a lot of attention in the past 2 decades because of their proxy potential for specific hydrological conditions leading to euxinic water column turnover or glacial-interglacial cycles that are controlled by climate change (e.g., Lepland and Stevens, 1998; Gingele and Kasten, 1994). One of the many open questions within the biogeochemistry and geomicrobiology of manganese in sediments is about the conditions leading to the metastable formation and preservation of manganese(II) sulfides. An interpretation of water profiles in sediments with high manganese load point toward complex interactions between chemical inhibitors, microbial processes, and rates of (temporary) mineral formation processes.

Bibliography

- Banfield, J. F., and Nealson, K. H., 1997. Geomicrobiology: interactions between microbes and minerals. *Reviews in Mineralogy*, **35**, 1.
- Baron, G., and Debyser, J., 1957. Sur la présence dans des vases organiques de la mer Baltique du sulfure manganéux hexagonal. *Comptes Rendues de l'Académie des Sciences Francais*, **245**, 1148.
- Berner, R. A., 1981. Authigenic mineral formation resulting from organic matter decomposition in modern sediments. *Fortschritte Mineralogie*, **59**, 117.
- Böttcher, M. E., 1998. Manganese(II) partitioning during experimental precipitation of rhodochrosite-calcite solid-solutions from aqueous solutions. *Marine Chemistry*, **62**, 287.
- Böttcher, M. E., and Huckriede, H., 1997. First occurrence and stable isotope composition of authigenic γ -MnS in the central Gotland Deep (Baltic Sea). *Marine Geology*, **137**, 201.
- Burke, I. T., and Kemp, A. E. S., 2002. Microfabric analysis of Mn-carbonate laminae deposition and Mn-sulfide formation in the Gotland Deep, Baltic Sea. *Geochimica et Cosmochimica Acta*, **66**, 1589.
- Gingele, F. X., and Kasten, S., 1994. Solid-phase manganese in Southeast Atlantic sediments: implications for the paleoenvironment. *Marine Geology*, **121**, 317.
- Huckriede, H., and Meischner, D., 1996. Origin and environment of manganese-rich sediments within black-shale basins. *Geochimica et Cosmochimica Acta*, **60**, 1399.
- Lepland, A., and Stevens, R. L., 1998. Manganese authigenesis in the Landsort Deep, Baltic Sea. *Marine Geology*, **151**, 1.
- Mucci, A., 2004. The behavior of mixed Ca-Mn carbonates in water and seawater: controls of manganese concentrations in marine porewaters. *Aquatic Geochemistry*, **10**, 139.
- Peacor, D., Essene, E. J., and Gaines, A. M., 1987. Petrologic and crystal-chemical implications of the cation order-disorder in kutnahorite $[\text{CaMn}(\text{CO}_3)_2]$. *American Mineralogist*, **72**, 319.

Cross-references

[Carbonates](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Iron Sulfide Formation](#)
[Microbial Degradation](#)
[Sulfur Cycle](#)

- Rabus, R., Hansen, T., and Widdel, F., 2001. Dissimilatory sulfate- and sulfur-reducing prokaryotes. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.) *The Prokaryotes: An evolving electronic resource for the microbiological community*. Heidelberg, Springer Science Online. Available from: www.prokaryotes.com.
- Rickard, D., and Luther III, G. W., 2007. Chemistry of iron sulfides. *Chemical Reviews*, **107**, 514.
- Rickard, D., and Morse, J. W., 2005. Acid volatile sulfide (AVS). *Marine Chemistry*, **97**, 141.
- Sinninghe Damsté, J. S., and de Leeuw, J. W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. *Organic Geochemistry*, **16**, 1077.
- Skyring, G. W., 1987. Sulfate reduction in coastal ecosystems. *Geomicrobiology Journal*, **5**, 295.
- Smith, J. W., 2000. Isotopic fractionations accompanying sulfur hydrolysis. *Geochemical Journal*, **34**, 95.
- Wortmann, U. G., Chernyavsky, B., Bernasconi, S. M., Brunner, B., Böttcher, M. E., and Swart, P. K., 2007. Oxygen isotope biogeochemistry of pore water sulfate in the deep biosphere: dominance of isotope exchange reactions with ambient water during microbial sulfate reduction (ODP Site 1130). *Geochimica et Cosmochimica Acta*, **71**, 4221.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Biogeochemical Cycles](#)
[Sulfur Isotopes](#)

SULFUR ISOTOPES

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Radioactive sulfur isotopes; Stable sulfur isotopes

Definition

Sulfur. A chemical element that is one of the constituents of living organisms and of the Earth.

Isotopes. Isotopes are different nuclear forms of the same element. For a given element, a constant number of protons but different numbers of neutrons in the nucleus correspond to different isotopes. Sulfur of standard atomic mass 32.065 u has 18 isotopes, most of which are not stable and undergo radioactive decay.

Stable sulfur isotopes. Stable isotopes are isotopes that do not decay within an experimentally observable time frame. Stable isotopes have essentially the same chemical characteristics and, therefore, their behavior is chemically almost identical. The different masses lead to isotope fractionation in chemical, physical, and especially biological reactions. Four stable isotopes occur in nature: ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S (4.21%), and ^{36}S (0.02%) (Hoefs, 2008). Typically, the ratio of the two most

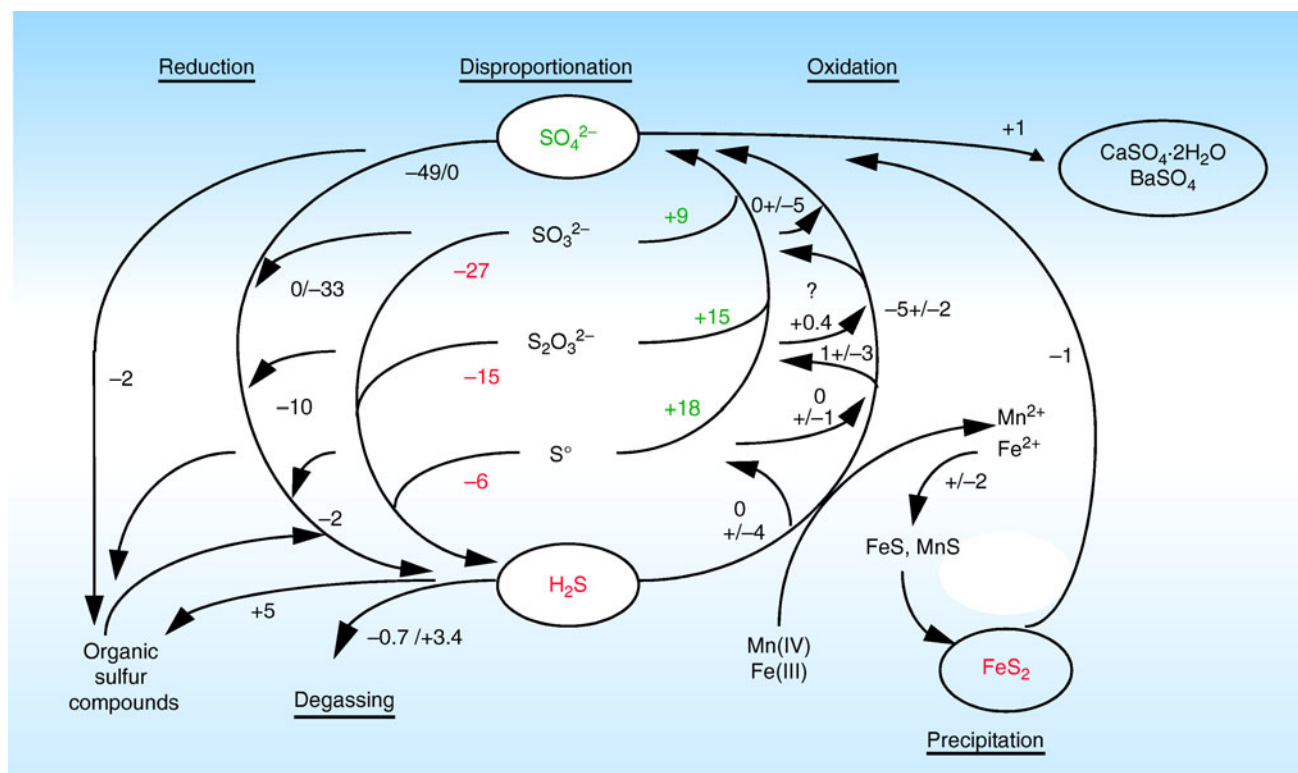
abundant sulfur isotopes is measured and presented in the conventional δ notation as opposed to the Vienna-Canyon-Diablo-Troilite (V-CDT) standard ($\delta^{34}\text{S}$ values per mil versus the V-CDT standard). In aerobic surface environments, the sulfur isotopic composition of sulfate is often used for source identification (e.g., anthropogenic versus geogenic sulfate in rain, river, and ground waters (e.g., Clark and Fritz, 1997). Only recently has the fractionation of the minor isotopes ^{33}S and ^{36}S also been introduced into sulfur isotope biogeochemistry (e.g., Farquhar et al., 2003).

Radioactive sulfur isotopes. Sulfur has 14 unstable isotopes. Radioactive sulfur with mass 35 is formed in the atmosphere from cosmic ray spallation of argon with mass 40 and has a half-life of 87 days. Therefore, it is used in hydrological studies of biologically less active surface environments. ^{35}S -labeled sulfur compounds are used in experiments to determine microbial and chemical sulfur turnover, upon microbial dissimilatory sulfate reduction, for instance (e.g., Kallmeyer et al., 2004). The other radioactive sulfur isotopes are all short-lived.

Fractionation of stable sulfur isotopes

Sulfur isotopes are fractionated upon microbial and abiotic sulfur transformations and the isotope signal may be preserved in sedimentary sulfide- and sulfate-bearing solids, organic matter, and to a minor extent, elemental sulfur (e.g., Canfield, 2001; Amend et al., 2004). The majority of geochemical and microbial studies on sulfur isotopes have so far focused on the fractionation of ^{32}S to ^{34}S . The most important isotope discrimination step occurs during dissimilatory reduction of dissolved sulfate within the bacterial cell. The overall change in the valence state of sulfur by pure cultures leads to hydrogen sulfide that is enriched in the lighter isotope by up to about 50 per mil when compared to sulfate (Figure 1). The magnitude of isotope discrimination depends on conditions such as cellular sulfate reduction rate, substrate and sulfate concentrations, and the organisms (e.g., Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Bolliger et al., 2001; Canfield, 2001; Amend et al., 2004). A maximum fractionation upon sulfate reduction of up to about 70 per mil is obtained from pore water modeling in natural environments (e.g., Wortmann et al., 2001), which is close to the value predicted for thermodynamic equilibrium conditions. Further partial or complete oxidation of hydrogen sulfide is associated with only small isotope discrimination, but may lead to the formation of (metastable) sulfur intermediates. The bacterial disproportionation of these compounds (e.g., elemental sulfur, thiosulfate, sulfite) yields hydrogen sulfide that is further enriched in the lighter isotope compared with the original sulfide (Figure 1).

This may contribute to the magnitude of isotope partitioning found in shallow natural environments and the fossil record (Canfield, 2001).



Sulfur Isotopes, Figure 1 Synthesis of low temperature experimental studies on microbial and abiotic S-34/S-32 fractionations upon sulfur transformations and transport (Amrani and Aizenshtat, 2004; Böttcher and Piel, 2008; Böttcher et al., 1998; Bolliger et al., 2001; Canfield et al., 1998; Chambers and Trudinger, 1979; Cypionka et al., 1998; Fry, 2006; Habicht et al., 1998; Kaplan and Rittenberg, 1964).

Conclusions

Stable sulfur isotope partitioning preserved in the sedimentary record may serve as a proxy for microbial sulfur cycling (e.g., Amend et al., 2004; Canfield, 2001). One of the many open questions within the biogeochemistry of stable sulfur isotopes regards the conditions leading to the sulfur isotope extremes in sediments, which differ from experimental results with modern bacterial cultures. New insights are expected to derive from experiments carried out under boundary conditions that more closely approach natural environments, the consideration of new cultures, and the application of multi-isotope approaches including analysis of the minor sulfur isotopes (e.g., Farquhar et al., 2003) and sulfur-bond oxygen (e.g., Brunner et al., 2005).

Bibliography

- Amend, J. P., Edwards, K. J., and Lyons, T. W. (eds.), 2004. *Sulfur Biogeochemistry - Past and Present*. Boulder: Geological Society of America.
- Amrani, A., and Aizenshtat, Z., 2004. Mechanisms of sulfur introduction chemically controlled: $\delta^{34}\text{S}$ imprint. *Organic Geochemistry*, **35**, 1319.
- Bolliger, C., Schroth, M. H., Bernasconi, S. M., Kleikemper, J., and Zeyer, J., 2001. Sulfur isotope fractionation during microbial

sulfate reduction by toluene-degrading bacteria. *Geochimica et Cosmochimica Acta*, **65**, 3289.

- Böttcher, M. E., and Piel, C., 2008. Kinetic S-34/S-32 fractionation during degassing and hydroxylation of hydrogen sulfide, and protonation of bisulfide. *Geophysical Research Abstracts*, **10**, 232.
- Böttcher, M. E., Smock, A., and Cypionka, H., 1998. Sulfur isotope fractionation during experimental precipitation of iron(II) and manganese(II) sulfide at room temperature. *Chemical Geology*, **146**, 127.
- Brunner, B., Bernasconi, S. M., Kleikemper, J., and Schroth, M. H., 2005. A model for oxygen and sulfur isotope fractionation in sulfate during bacterial sulfate reduction processes. *Geochimica et Cosmochimica Acta*, **69**, 4773.
- Canfield, D. E., 2001. Biogeochemistry of sulfur isotopes. In Valley, J. W., and Cole, D. R. (eds.), *Reviews in Mineralogy and Geochemistry*, The Mineralogical Society of America, 607.
- Canfield, D. E., Thamdrup, B., and Fleischer, S., 1998. Isotope fractionation and sulfur metabolism by pure and enrichment cultures of elemental sulfur disproportionating bacteria. *Limnology and Oceanography*, **43**, 253–264.
- Chambers, L. A., and Trudinger, P. A., 1979. Microbiological fractionation of stable sulfur isotopes: a review and critique. *Geomicrobiology Journal*, **1**, 249.
- Clark, I. D., and Fritz, P., 1997. *Environmental Isotopes in Hydrogeology*. Boca Raton: Lewis.
- Cypionka, H., Smock, A. M., and Böttcher, M. E., 1998. A combined pathway of sulfur compound disproportionation in

- Desulfovibrio desulfuricans*. *FEMS Microbiology Letters*, **166**, 181.
- Farquhar, J., Johnston, D. T., Wing, B. A., Habicht, K. S., Canfield, D. E., Airieau, S., and Thiemens, M. H., 2003. Multiple sulphur isotopic interpretations of biosynthetic pathways: implications for biological signatures in the sulphur isotope record. *Geobiology*, **1**, 27.
- Fry, B., 2006. *Stable Isotope Ecology*. Berlin: Springer.
- Habicht, K. S., Canfield, D. E., and Rethmeier, J., 1998. Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite. *Geochimica et Cosmochimica Acta*, **62**, 2585.
- Hoefs, J., 2008. *Stable Isotope Geochemistry*, 6th edn. Berlin: Springer.
- Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., and Jørgensen, B. B., 2004. A cold chromium distillation procedure for radiolabelled sulfide applied to sulfate reduction measurements. *Limnology and Oceanography: Methods*, **2**, 171.
- Kaplan, I. R., and Rittenberg, S. C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology*, **34**, 195.
- Wortmann, U. G., Bernasconi, S. M., and Böttcher, M. E., 2001. Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step bacterial sulfate reduction. *Geology*, **29**, 647.

Cross-references

[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Sulfur Cycle](#)

SYMBIOSIS

Sharmishtha Dattagupta¹, Frank Zielinski²

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany

Definition

A close, prolonged physical and/or metabolic association between two or more distinct organisms.

Introduction

Most living organisms interact intimately with one or more symbiotic partners that are often vital for their health and survival. Symbiotic organisms are important ecosystem engineers (e.g., corals); they significantly impact biogeochemical cycles (e.g., legumes), and they contribute substantially to geological processes such as rock weathering (e.g., lichens and mycorrhizal fungi). Moreover, symbioses have played a crucial role in the origin and diversification of eukaryotic life.

Important terms and concepts

Symbioses are categorized as being mutualisms, where both organisms involved benefit, commensalisms, where

one organism benefits and the other remains unaffected, or parasitisms, where one organism benefits but the other is harmed. When metabolite exchange occurs between symbiotic organisms, it is called syntrophy. Syntrophic interactions among microorganisms can drive otherwise thermodynamically unfavorable reactions forward due to the removal of metabolic end products.

In symbiosis terminology, the smaller symbiotic partner (often a microbe) is referred to as the “symbiont,” whereas the larger partner is called the “host.” In ectosymbioses, the symbiont is located on the external surface of the body of its host, whereas in endosymbioses, the symbiont lives inside the body of its host, either within its cells or in extracellular spaces. Symbioses vary from being *obligate*, where the interaction is critical for the survival of at least one organism involved, or *facultative*, where the organisms involved can also survive independently of each other. The method by which the host acquires its symbionts is called the mode of transmission. In *vertical transmission*, symbionts are inherited from the previous generation, as they are passed from the parent to the egg or embryo. In *horizontal transmission*, symbionts are acquired anew in each generation either from co-occurring hosts or from the environment.

History

Symbiosis is derived from the Greek words, *syn* (together) and *bio* (life). German biologist Anton de Bary is usually credited with coining the term in the mid-nineteenth century, though an equally important contribution was made by his contemporary, Albert Bernhard Frank (who instead used the term *symbiotism*) (Sapp, 2004). De Bary introduced the term to discuss lichens, which he discovered were composites of algae and fungi. Frank, on the other hand, reported on the intimate association between fungi and roots of forest trees, which he named “mykorrhiza” (fungus root). De Bary defined symbiosis as the phenomenon in which “unlike organisms live together” and explicitly included parasitism in his definition (Kutschera and Niklas, 2005). Therefore, the original definition of symbiosis was meant to include the entire gamut of close and long-term associations between two distinct organisms, irrespective of the benefit or harm caused by the interaction. The historical definition of symbiosis was eventually replaced in many modern textbooks with a more restricted definition that included only mutually beneficial associations, making the meaning of symbiosis synonymous with “mutualism” (Wilkinson, 2001). Unfortunately, research on symbioses has been fraught with confusion and controversies, in part due to the two coexisting definitions of the term (Smith, 2001; Sapp, 2004).

Conceptually, the original definition of symbiosis makes more sense in light of the fact that “benefit” is difficult to quantify in studies of symbioses (Smith, 2001). Cost-benefit ratios of symbioses can change with environmental conditions. For example, symbiotic mycorrhizal

M

MAGNETOTACTIC BACTERIA

Mihály Pósfai
University of Pannonia, Veszprém, Hungary

Definition

Magnetotactic bacteria (MTB) are a diverse group of Gram-negative, motile prokaryotes that align and migrate along the geomagnetic field. This magnetotactic behavior is based on intracellular organelles called magnetosomes, which are inorganic nanocrystals of either magnetite (Fe_3O_4) or greigite (Fe_3S_4), surrounded by a lipid bilayer membrane. The magnetosomes are typically arranged in one or more chains, conveying a magnetic dipole moment to the cell (Figure 1). Magnetotaxis is thought to be beneficial for the cell for finding its optimum position in vertical concentration gradients in aquatic habitats.

History

Bacteria that responded to magnetic fields were discovered independently by Bellini (1963) and Blakemore (1975), who coined the term magnetotaxis and noted the presence of crystals in the cells. Intracellular magnetite was identified and the physics of magnetotaxis was described by Frankel et al. (1979). Iron sulfide-producing MTB were found in 1990 (Mann et al., 1990; Farina et al., 1990). The ecology of MTB and the structures and morphologies of the crystalline components of magnetosomes have been widely studied since the discovery of MTB. In the past decade, significant progress has been made in elucidating the molecular, biochemical, and genetic bases of magnetosome formation (Schüler, 2007).

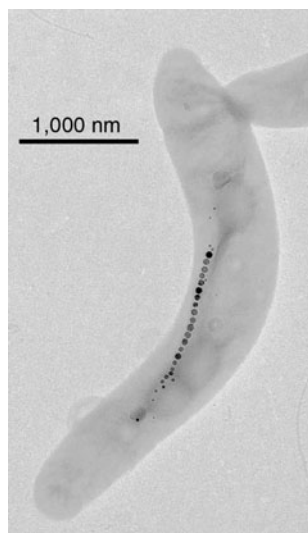
Ecophysiology

MTB are ubiquitous in both marine and freshwater aquatic habitats, where suboxic or anoxic conditions prevail, and

dissolved iron is available. Because of their fastidious nature, only a few strains are available in pure culture. With one exception, all strains in pure culture have a respiratory form of metabolism. All known MTB are microaerophiles (they require a certain amount of oxygen for growth, but exhibit a negative tactic and/or growth response to atmospheric concentrations of oxygen) or anaerobes or facultatively anaerobic microaerophiles (Bazylinski and Frankel, 2004). The physiology of MTB appears to dictate their ecology: since MTB derive energy from the proximity of oxidants and reductants, magnetite-producing organisms occur in largest numbers (up to 10^5 cells/ml) in layers at or near the oxic–anoxic interfaces (OAI) of chemically stratified habitats. Iron sulfide-producing bacteria live below the OAI under anaerobic conditions (Simmons et al., 2004). Whereas magnetite-producing bacteria occur virtually in all types of water bodies, sulfide-producing organisms have been found in marine habitats only. Considering the large amount of iron concentrated in magnetosomes (10^{-15} to 10^{-13} g Fe per cell) and the estimated population density of MTB at and below the OAI, MTB are likely to contribute significantly to the flux of reduced Fe to sediments in chemically stratified marine basins.

Magneto-aerotaxis

Magnetotaxis means active motion guided by the magnetic field. MTB passively orient along the geomagnetic field and actively swim by means of flagella. A key element of magnetotaxis is that the cell should possess a large enough magnetic dipole moment to overcome the disorientating effects of thermal motions and be aligned parallel to the magnetic field. In the geomagnetic field, the magnetic energy of magnetotactic cells is typically ten times larger than thermal energy, which means that the cell can migrate along the field at 90% of its forward speed (Frankel et al., 1979).



Magnetotactic Bacteria, Figure 1 A cell of *Magnetospirillum gryphiswaldense* that contains a single chain of magnetite magnetosomes.

“Polar” and “axial” magnetotaxis are distinguished (Frankel et al., 1997). Polar magnetotaxis is displayed by MTB that swim persistently toward one of the poles of a bar magnet under oxic conditions. Those that swim toward the “south” pole of a magnet have North-seeking polarity, since they would swim northward in the geomagnetic field. MTB from the northern hemisphere are predominantly North-seekers, whereas those from the southern hemisphere are predominantly South-seekers (Bazylinski and Frankel, 2004).

The observation of polar magnetotaxis led to the idea that MTB are guided by the geomagnetic field to less oxygenated regions. Since the geomagnetic field is inclined downward from the horizontal in the northern hemisphere and upward in the southern hemisphere under oxic conditions the cells with polar magnetotaxis swim downward in both hemispheres (Bazylinski and Frankel, 2004). However, if the cell is in a reducing environment, it reverses the direction of flagellar rotation and migrates upward. Since the motion of the cell is determined by both the magnetic field and the oxygen tension, the term “magneto-aerotaxis” was introduced (Frankel et al., 1997).

Axial magnetotaxis means that the axis of motion is determined by the magnetic field but its direction is not. As the cell swims, it samples the oxygen concentration and compares it with that in the recent past. Cells moving toward the optimal habitat have a decreased probability of reversing the sense of flagellar rotation, whereas those that are moving away from the optimal oxygen concentration have an increased probability of reversing the sense of flagellar rotation and thus their swimming direction (Frankel et al., 1997). The magneto-aerotactic response provides an

efficient mechanism for MTB to find the OAI in their chemically stratified habitats.

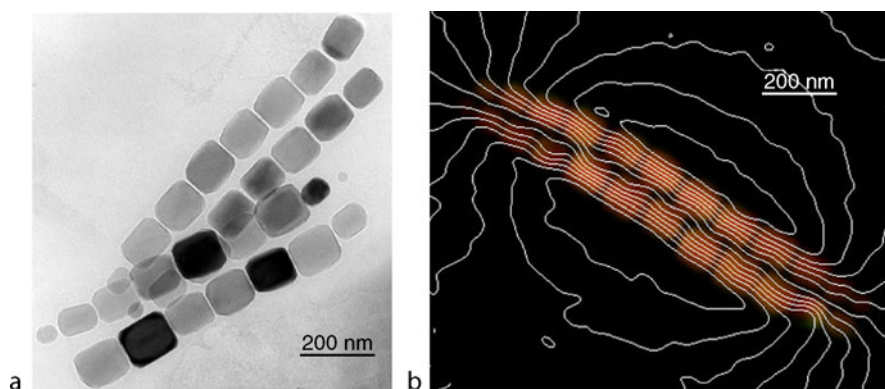
Mineralogy and magnetism

Magnetotaxis relies on intracellular grains of iron oxide or sulfide minerals. Magnetite (Fe_3O_4) is the only iron oxide mineral that has been described to date from magnetotactic cells. The sulfide-producing cells use greigite (Fe_3S_4) for navigation, but they also may contain nonmagnetic mackinawite (FeS) that rapidly converts to greigite through a solid-state phase transition (Pósfai et al., 2007).

Magnetite produced by MTB is chemically pure Fe_3O_4 , in contrast to grains from rocks that usually contain other transition metals besides iron. Structurally, the nanocrystals are either perfect or contain twin boundaries. The morphologies of magnetite magnetosomes are typically highly regulated, strain-specific, and in many cases, unusual for a mineral with cubic symmetry (Pósfai et al., 2007). Whereas magnetotactic spirilla contain cubooctahedral crystals, vibrio and cocci typically produce magnetite with prismatic habits (Figure 2a). Large, rod-shaped cells tend to contain arrowhead- or bullet-shaped magnetite crystals (Bazylinski and Frankel, 2004).

The sizes and shapes of magnetosomes are critical for magnetotaxis. Isolated, isometric magnetite crystals with diameters smaller than ~ 30 nm are superparamagnetic, i.e., they do not have a permanent magnetic moment. Crystals with diameters from ~ 30 to ~ 120 nm are magnetic single domains at room temperature, whereas grains larger than ~ 120 nm contain two or more magnetic domains, reducing the net magnetic moment of the crystal. Most magnetosomes are in the single-domain range, allowing the assembly of a highly efficient internal compass that consists of a chain (or chains) of single-domain magnets. Interactions between neighboring particles in chain configurations ensure that even those magnetite particles that are small enough to be superparamagnetic or large enough to contain two or more magnetic domains if they were isolated are also constrained to be single domains (Dunin-Borkowski et al., 2001). Since elongated particles are arranged with their axis of elongation (which typically coincides with their crystallographic easy magnetization axis) parallel to the chain of magnetosomes, shape anisotropy constrains the magnetic flux lines to be parallel to the axis of motility. Thus, in most magnetite-producing cells the effects of particle size, shape and magnetocrystalline anisotropy, and magnetostatic interactions among particles in chains combine to produce the possible largest magnetic moment (Figure 2b) (Pósfai et al., 2007).

The sizes, shapes, and orientations of iron sulfide magnetosomes are less distinct than those of magnetite magnetosomes. Greigite magnetosomes typically contain planar defects, as a result of an incomplete solid-phase transformation from mackinawite to greigite. The shapes and orientations of the crystals are random,



Magnetotactic Bacteria, Figure 2 (a) Electron micrograph of two double chains of elongated magnetite magnetosomes from a freshwater magnetotactic coccus. (b) Magnetic induction map of a similar double chain of magnetite magnetosomes, obtained from electron holograms.

resulting in poorer confinement and greater variability in the directions of the magnetic induction lines in the cells than in magnetite-bearing bacteria. Remarkably, despite the different crystal structures, orientations, and arrangements of ferrimagnetic crystals, the magnetic moments of different cells are fairly constant (Pósfai et al., 2007), thereby satisfying the requirement for magnetotaxis.

Cell biology and genomic analyses

Magnetic enrichments have shown great morphological and ultrastructural diversity of MTB from a wide variety of aquatic environments. Magnetite-producing morphotypes include spirilla, cocci, vibrios, and rods of various sizes, moving at speeds ranging from 40 to 1,000 $\mu\text{m/s}$. A unique, greigite-producing magnetotactic organism, the “multicellular magnetotactic prokaryote” (MMP) consists of 15–45 cells organized around an internal acellular compartment (Keim et al., 2004). The aggregated cells of the MMP move together as one unit, with the coordinated motion of flagella on the outside of the cells.

The highly controlled, strain-specific properties of magnetite magnetosomes and their chains indicate that biomineralization is under genetic control, executed by specific proteins in the magnetosome membrane. Many details of magnetosome formation have been elucidated by studies of the biochemistry of the magnetosome membrane and genomic analyses of MTB. Workable genetic systems have been developed for two *Magnetospirillum* strains: *M. magnetotacticum* AMB-1 and *M. gryphiswaldense* MSR-1 (Jogler and Schüller, 2007). By genetic manipulation, including the generation of mutants and the expression of magnetobacterial genes in other hosts, the major steps of magnetite biomineralization have been identified in these systems. Magnetite nucleation is preceded by the formation of vesicles, which are invaginations of the cytoplasmic membrane (Komeili et al., 2006). Dissolved ferrous or ferric iron is transported into the cell by an unknown uptake

system. Magnetosome membrane proteins (MamB/MamM) are involved in the transport of ferrous iron from the cytoplasm into the magnetosome vesicle, and then magnetite formation is activated by another protein (MamA). Within the vesicle, iron is oxidized and magnetite nucleation is triggered by the Mms6 protein. The least known aspect of the biomineralization process is the control over the distinct crystal morphology. The vesicles containing fully developed magnetite crystals are anchored to a filamentous cytoskeletal structure by the MamJ protein, resulting in an ordered magnetosome chain (Scheffell et al., 2006). Genomic analyses revealed that the genes that encode the magnetosome proteins are clustered in a 130 kb region, representing a “magnetosome island.” This genomic island appears to be conserved among different *Magnetospirillum* strains and, to a lesser degree, in a magnetotactic coccus (Jogler and Schüller, 2007), raising the question of whether the “magnetosome island” is a universal feature of MTB.

Diversity

The term “magnetotactic bacteria” does not refer to a taxonomical unit. All known MTB belong to various groups within the domain Bacteria. Most magnetotactic microorganisms that are available in pure culture are affiliated with the *Alphaproteobacteria*, including *M. magnetotacticum* and *M. gryphiswaldense*, a marine vibrio (MV-1), and a coccus (MC-1). In addition to the cultivated strains, 16S rRNA analysis of magnetic enrichments from many freshwater and marine samples revealed that a variety of magnetite-producing morphotypes are likely affiliated with the *Alphaproteobacteria* (Amann et al., 2007). Two magnetite-producing candidate species, “*Magnetobacterium bavaricum*” and “*Magnetotacticum bremense*,” are affiliated with the *Nitrospira* phylum.

Sulfide-producing MTB could not have been cultured to date, but a multicellular organism was found to be closely related to the dissimilatory sulfate-reducing

bacteria within the *Deltaproteobacteria*. Another sulfide-producing MTB was assigned to *Gammaproteobacteria*. These findings may suggest that magnetotaxis based on iron oxide and sulfide nanoparticles evolved independently; however, a magnetite-producing, sulfate-reducing organism (*Desulfovibrio magneticus* strain RS-1) is also affiliated with the *Deltaproteobacteria* (Amann et al., 2007).

The diversity of MTB indicates that magnetotaxis evolved independently in several phylogenetic groups. On the other hand, the discovery of a potentially mobil “magnetosome island” in the genome of magnetite-producing MTB could suggest that the trait of magnetotaxis was spread by lateral gene transfer among the various groups of MTB.

Magnetofossils, paleomagnetism

After MTB die, their intracellular magnetite or greigite particles are deposited in the sediment. If diagenetic conditions are favorable, the magnetosome crystals can be preserved as magnetofossils. Magnetofossils have been identified on the basis of the special properties of magnetosome crystals, including a narrow crystal size distribution, characteristic morphologies, chemical purity and, in some cases, chain configurations. Ferrimagnetic nanocrystals presumably originating from MTB have been described from a variety of sedimentary rock types, including clays, carbonates, marls, and evaporites. The magnetofossil record extends from present-day marine and lake sediments to the Cretaceous and with lesser certainty to the late Archean (Kopp and Kirschvink, 2008).

MTB carry a primary remanent magnetization, since they are aligned with the geomagnetic field, presumably even after cell death and burial in the sediment. Because magnetofossils are single magnetic domains, they have an optimized magnetic stability and can be important carriers of paleomagnetic information. Magnetofossils can serve as environmental proxies because MTB live under specific redox conditions. They can also be markers of ancient life, albeit dubious ones, as illustrated by a lengthy debate over whether nanocrystalline magnetite particles in the Martian meteorite ALH84001 represent magnetofossils or had formed inorganically (Winklhofer and Petersen, 2007).

Nanotechnological applications

Magnetic nanoparticles are widely used in technological applications, such as in spintronics, in magnetic inks, in high-density magnetic memory devices, and in medical applications (as contrast agents in magnetic resonance imaging and as magnetic carriers for drug targeting and delivery). Magnetite crystals produced by MTB have uniform sizes and shapes and can be separated from disrupted cells by magnetic methods with their magnetosome membrane intact. The membrane-bound bacterial nanoparticles have a large specific surface area and are well dispersed in aqueous solutions. Antibodies or proteins can be immobilized on the magnetosome membrane, producing

functionalized magnetic nanoparticles (Matsunaga and Arakaki, 2007). Since bound and free analytes can be separated in a magnetic field, bacterial nanoparticles have been used for the rapid and sensitive detection of small molecules, including environmental pollutants, hormones, and toxic detergents. Immunomagnetic particles are also useful for sorting target cells from cell suspensions using a permanent magnet, and for using them in high-performance DNA/mRNA recovery and DNA discrimination analysis (Matsunaga and Arakaki, 2007).

Summary

MTB provide a fascinating example of microbial biomineralization and motivate interdisciplinary research at the junctions of biology, mineralogy, geology, geochemistry, and physics. The molecular and genetic bases of magnetic nanocrystal formation by MTB are now beginning to be understood. The results of research on MTB will be useful for understanding biomineralization processes and magnetic sensing mechanisms in more complex organisms, and for designing nanotechnological applications of bacterial nanocrystals.

Bibliography

- Amann, R., Peplies, J., and Schüller, D., 2007. Diversity and taxonomy of magnetotactic bacteria. In Schüller, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 25–36.
- Bazylinski, D. A., and Frankel, R. B., 2004. Magnetosome formation in prokaryotes. *Nature Reviews Microbiology*, **2**, 217–230.
- Bellini, S., 1963. *Su di un particolare comportamento di batteri d'acqua dolce*. Istituto di Microbiologia dell'Università di Pavia.
- Blakemore, R. P., 1975. Magnetotactic bacteria. *Science*, **190**, 377–379.
- Dunin-Borkowski, R. E., McCartney, M. R., Pósfai, M., Frankel, R. B., Bazylinski, D. A., and Buseck, P. R., 2001. Off-axis electron holography of magnetotactic bacteria: magnetic microstructure of strains MV-1 and MS-1. *European Journal of Mineralogy*, **13**, 671–684.
- Farina, M., Esquivel, D. M. S., and Lins de Barros, H. G. P., 1990. Magnetic iron-sulphur crystals from a magnetotactic microorganism. *Nature*, **343**, 256–258.
- Frankel, R. B., Blakemore, R. P., and Wolfe, R. S., 1979. Magnetite in freshwater magnetotactic bacteria. *Science*, **203**, 1355–1356.
- Frankel, R. B., Bazylinski, D. A., Johnson, M. S., and Taylor, B. L., 1997. Magneto-aerotaxis in marine coccoid bacteria. *Biophysical Journal*, **73**, 994–1000.
- Jogler, C., and Schüller, D., 2007. Genetic analysis of magnetosome biomineralization. In Schüller, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 133–162.
- Keim, C. N., Abreu, F., Lins, U., Lins de Barros, H. G. P., and Farina, M., 2004. Cell organization and ultrastructure of a magnetotactic multicellular organism. *Journal of Structural Biology*, **145**, 254–262.
- Komeili, A., Li, Z., Newman, D. K., and Jensen, G. J., 2006. Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science*, **311**, 242–245.
- Kopp, R. E., and Kirschvink, J. L., 2008. The identification and biogeochemical interpretation of fossil magnetotactic bacteria. *Earth-Science Reviews*, **86**, 42–61.

- Mann, S., Sparks, N. H. C., Frankel, R. B., Bazylinski, D. A., and Jannasch, H. W., 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. *Nature*, **343**, 258–261.
- Matsunaga, T., and Arakaki, A., 2007. Molecular bioengineering of bacterial magnetic particles for biotechnological applications. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 227–254.
- Pósfai, M., Kasama, T., and Dunin-Borkowski, R. E., 2007. Characterization of bacterial magnetic nanostructures using high-resolution transmission electron microscopy and off-axis electron holography. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 175–226.
- Scheffel, A., Gruska, M., Faivre, D., Linaroudis, A., Plitzko, J. M., and Schüler, D., 2006. An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature*, **440**, 110–114.
- Schüler, D., 2007. *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, p. 319.
- Simmons, S. L., Sievert, S. M., Frankel, R. B., Bazylinski, D. A., and Edwards, K. J., 2004. Spatiotemporal distribution of marine magnetotactic bacteria in a seasonally stratified coastal salt pond. *Applied and Environmental Microbiology*, **70**, 6230–6239.
- Winklhofer, M., and Petersen, N., 2007. Paleomagnetism and magnetic bacteria. In Schüler, D. (ed.), *Magnetoreception and Magnetosomes in Bacteria*. Berlin: Springer, pp. 255–274.

Cross-references

Bacteria
 Biosignatures in Rocks
 Iron Sulfide Formation
 Microbial Biomineralization
 Nanocrystals, Microbially Induced

MANGANESE (SEDIMENTARY CARBONATES AND SULFIDES)

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Alabandite; Authigenic manganese minerals; Inca rose; Rambergite; Rhodochrosite

Definition

Rhodochrosite. Manganese(II) carbonate (MnCO_3 ; trigonal, calcite structure), named after the greek word for “rose-colored.” It was named as the state mineral of Colorado (USA) in 2002. Complete solid solution formation with calcite occurs at low temperatures (Böttcher, 1998), the “Ca-rhodochrosites” or “pseudo-kutnahorites” (Mucci, 2004).

Kutnahorite. Manganese(II)-calcium carbonate ($\text{CaMn}(\text{CO}_3)_2$; trigonal; ordered dolomite structure), named after the location KutnoHora (Peacor et al., 1987). Although physicochemically stable at low temperatures

with respect to equally composed solid-solutions (Mucci, 2004), it is not formed in sediments, and only known from high-temperature hydrothermal and metamorphic environments.

Rambergite. Manganese(II) sulfide ($\mu\text{-MnS}$; hexagonal), named after the mineralogist Hans Ramberg, is found as authigenic product in anoxic sediments of the Baltic Sea (Böttcher and Huckriede, 1997; Burke and Kemp, 2002).

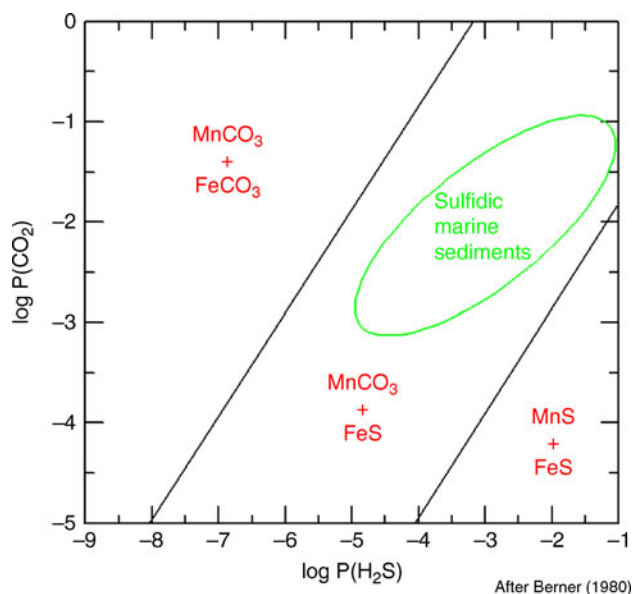
Alabandite. Manganese(II) sulfide ($\alpha\text{-MnS}$, cubic), named after the location Alabanda, Turkey, is found in sediments as minor intergrowth with rambergite (Lepland and Stevens, 1998).

Manganese carbonate formation in suboxic/anoxic sediments

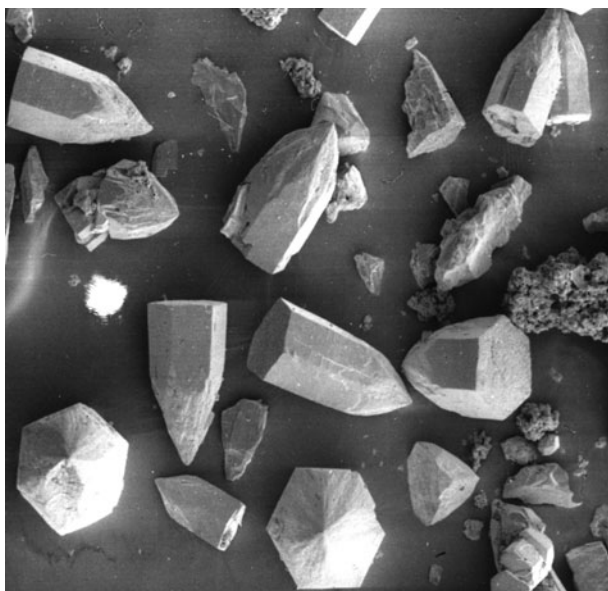
Mixed manganese–calcium carbonates, with minor incorporation of magnesium and divalent iron, are formed in (brackish-marine) sediments as authigenic products of suboxic and anoxic early diagenesis, in the Baltic Sea deeps and the Panama Basin. Significant sedimentary Ca-rhodochrosite accumulation requires manganese(IV) dioxide pre-enrichment at the sediment–water interface by episodic water column oxidation, as shown for the temporarily euxinic deeps of the Baltic Sea (Huckriede and Meischner, 1996). The required dissolved carbonate species are formed in the pore water upon mineralization of organic matter, and aqueous Mn^{2+} is derived from microbial or chemical reduction of manganese(III, IV) oxides as electron acceptors (e.g., Banfield and Nealson, 1997). The carbonates occur as kidney-shaped globules, as overgrowth on foraminifers, detrital carbonates, and microbial cells or as idiomorphic crystals, depending on the micro-environment. Experimental studies reveal that the composition of manganese-rich carbonates is controlled by the pore water composition, the abundance of carbonate surfaces, and the presence of inhibitors (Böttcher, 1998; Mucci, 2004). Under equilibrium conditions, natural anoxic pore waters are in the stability field of coexisting rhodochrosite and iron sulfides (Figure 1), and no manganese sulfide should be formed during typically zoned early diagenetic processes.

Manganese sulfide formation in anoxic sediments

The only places worldwide where sedimentary manganese sulfides have been found so far are the Baltic Sea anoxic deeps (e.g., Baron and Debyser, 1957). Idiomorphic MnS (Figure 2) is associated with organic matter, Ca-rhodochrosite, and iron sulfides (Böttcher and Huckriede, 1997; Lepland and Stevens, 1998). Although MnS has been obviously formed under slow diffusion-controlled conditions where rhodochrosite formation was chemically inhibited, the exact process is still under debate. Occurrence of Ca-rhodochrosite replacing hexagonal MnS (Burke and Kemp, 2002) indicates possible readaptation of the metastable sedimentary system toward equilibrium conditions.



Manganese (Sedimentary Carbonates and Sulfides),
Figure 1 Low temperature equilibrium phase relationships of reduced iron- and manganese-bearing solids as a function of carbon dioxide and hydrogen sulfide partial pressures.



Manganese (Sedimentary Carbonates and Sulfides),
Figure 2 Scanning electron microscope picture of idiomorphic authigenic MnS from the Baltic Sea. Picture width is equivalent to a scale of 950 μm .

Conclusion

Sedimentary authigenic manganese(II) carbonate minerals, especially investigated in the nowadays brackish-marine

Baltic Sea, are indicative of biogeochemical early-diagenetic processes in manganese-rich environments. They have gained a lot of attention in the past 2 decades because of their proxy potential for specific hydrological conditions leading to euxinic water column turnover or glacial-interglacial cycles that are controlled by climate change (e.g., Lepland and Stevens, 1998; Gingele and Kasten, 1994). One of the many open questions within the biogeochemistry and geomicrobiology of manganese in sediments is about the conditions leading to the metastable formation and preservation of manganese(II) sulfides. An interpretation of water profiles in sediments with high manganese load point toward complex interactions between chemical inhibitors, microbial processes, and rates of (temporary) mineral formation processes.

Bibliography

- Banfield, J. F., and Nealson, K. H., 1997. Geomicrobiology: interactions between microbes and minerals. *Reviews in Mineralogy*, **35**, 1.
- Baron, G., and Debyser, J., 1957. Sur la présence dans des vases organiques de la mer Baltique du sulfure manganéux hexagonal. *Comptes Rendues de l'Académie des Sciences Francais*, **245**, 1148.
- Berner, R. A., 1981. Authigenic mineral formation resulting from organic matter decomposition in modern sediments. *Fortschritte Mineralogie*, **59**, 117.
- Böttcher, M. E., 1998. Manganese(II) partitioning during experimental precipitation of rhodochrosite-calcite solid-solutions from aqueous solutions. *Marine Chemistry*, **62**, 287.
- Böttcher, M. E., and Huckriede, H., 1997. First occurrence and stable isotope composition of authigenic γ -MnS in the central Gotland Deep (Baltic Sea). *Marine Geology*, **137**, 201.
- Burke, I. T., and Kemp, A. E. S., 2002. Microfabric analysis of Mn-carbonate laminae deposition and Mn-sulfide formation in the Gotland Deep, Baltic Sea. *Geochimica et Cosmochimica Acta*, **66**, 1589.
- Gingele, F. X., and Kasten, S., 1994. Solid-phase manganese in Southeast Atlantic sediments: implications for the paleoenvironment. *Marine Geology*, **121**, 317.
- Huckriede, H., and Meischner, D., 1996. Origin and environment of manganese-rich sediments within black-shale basins. *Geochimica et Cosmochimica Acta*, **60**, 1399.
- Lepland, A., and Stevens, R. L., 1998. Manganese authigenesis in the Landsort Deep, Baltic Sea. *Marine Geology*, **151**, 1.
- Mucci, A., 2004. The behavior of mixed Ca-Mn carbonates in water and seawater: controls of manganese concentrations in marine porewaters. *Aquatic Geochemistry*, **10**, 139.
- Peacor, D., Essene, E. J., and Gaines, A. M., 1987. Petrologic and crystal-chemical implications of the cation order-disorder in kutnahorite $[\text{CaMn}(\text{CO}_3)_2]$. *American Mineralogist*, **72**, 319.

Cross-references

[Carbonates](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Iron Sulfide Formation](#)
[Microbial Degradation](#)
[Sulfur Cycle](#)

MASS EXTINCTIONS, PHANEROZOIC

Joachim Reitner
University of Göttingen, Göttingen, Germany

Synonyms

Biotic crisis; Extinction event; Extinction-level event

Definition

A *mass extinction* is a sharp decrease in biodiversity, speciation, and diversification rate among micro- and macro-organisms in the fossil record, often also related with a decrease of biomass. The microbial biodiversity, which probably dominates the entire biodiversity, however, is not to be considered under this aspect, because we have no idea about the real microbial biodiversity through time.

Mass extinctions

Mass extinctions are generally important events, which have a large impact on evolutionary processes throughout earth's history. Mass extinctions might have also occurred in the Precambrian, however, these are much more difficult to recognize and interpret, because most of them affected microbial communities. Profound extinction events in the Precambrian are related with the Great Oxidation Event 2.5 Gy ago, during which an unknown number of anaerobic microbial communities disappeared from oxygenated shelf areas and were restricted to the still anaerobic deeper parts of the oceans (Canfield Ocean, see Wiese and Reitner, Chapter *Critical Intervals in Earth History*, this volume). It can be suspected that the Neoproterozoic Cryogenian Snowball Earth situations (730–630 My) damaged early earth's ecosystems as decreasing $\delta^{13}\text{C}$ values prior to the glaciations suggest a decrease in bioproductivity (e.g., Moczyłowska, 2008; Le Guerroué, et al., 2006a, b; Hoffman, this volume). A first macrofossil extinction of eukaryotic multicellular organisms occurred in the Lower Cambrian, when the Ediacara organisms or Vendobionta, respectively (Raup and Sepkoski, 1982; Brasier et al., 1989; Jensen et al., 1998), became extinct.

Within the Phanerozoic, five major mass extinctions are known (Sepkoski, 1984, 1994; Alroy, 2004; Kiessling and Simpson, 2010), in the literature referred to as “the Big Five” (Figure. 1). All of them have potentially different origins (e.g., impact, volcanisms, glaciations a.o.; see below) or are even multicausal. However, in most cases, the triggering mechanisms and feedback loops are often difficult to understand. Apart from cosmic impacts, mass extinctions are most likely no sudden events but have precursory ecosystem perturbations that prelude the phase of the biotic crisis (further reading see Wiese and Reitner, this volume).

End-Ordovician mass extinction (ca. 443 My)

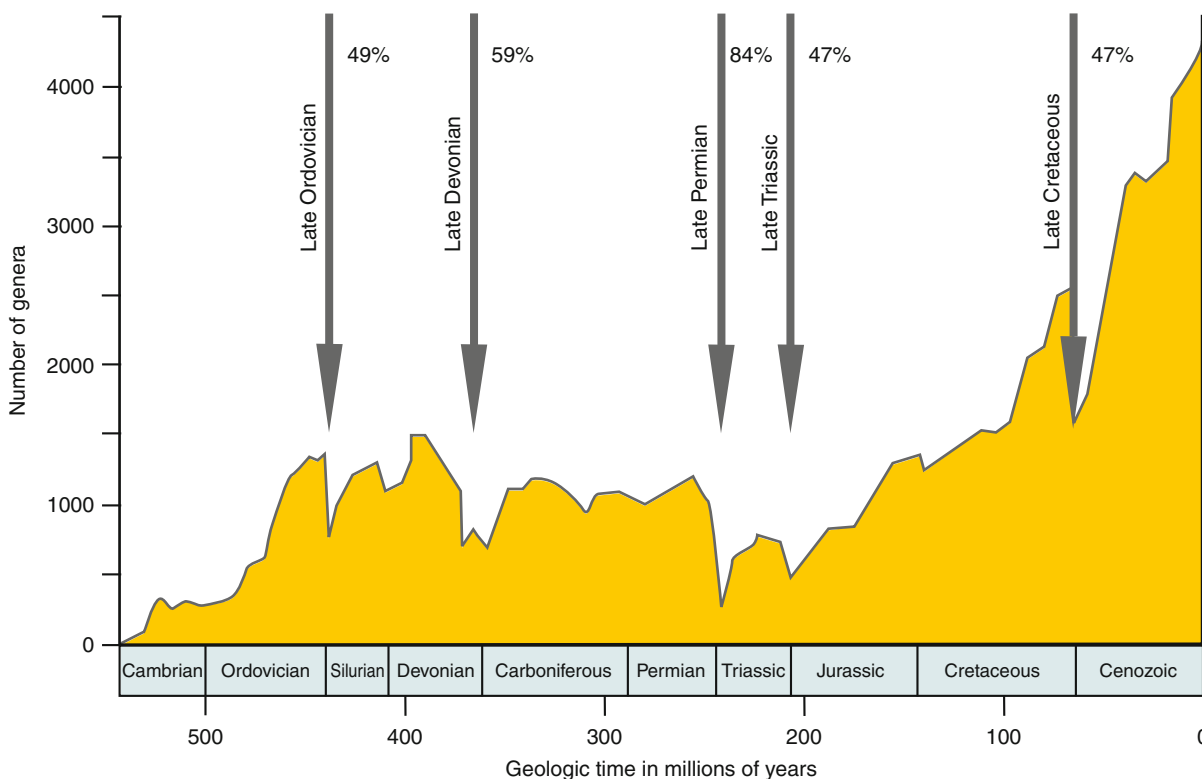
The end-Ordovician mass extinction event is the first major extinction event in the Phanerozoic. Brachiopods, bryozoans, trilobites, graptolites, corals, mollusks, conodonts, etc. were strongly decimated. It is estimated that roughly 50% of known taxa died out (further reading Krug and Patzkowsky, 2004; Sheehan, 2001; Sole and Newman, 2002; Webby and Droser, 2004).

Possible origins

- One reason might be the paleogeographic constellation of the continents at the Ordovician–Silurian boundary, when Gondwana was positioned at the South Pole. The position of large continental areas at the South Pole triggered a severe cooling, which terminated in the Sahara glaciation in the uppermost Ordovician (Hirnantian). Possibly, it was the heaviest glaciation during the entire Phanerozoic. The associated strong regression and a breakdown of oceanic circulation patterns brought up nutrients from the abyssal waters and affected the diversity of organisms negatively (Sheehan, 2001). A further idea is that the glaciation forced an equatorward migration of warmer water organisms, competition in remaining habitats and extinction of a large number of taxa. In a second step, a severe warming, succeeding the Sahara glaciation, forced a poleward migration of the few remaining cold water assemblages, which then also suffered heavy losses (see also Webby and Droser, 2004).
- Melott et al. (2004) argued that the end-Ordovician mass extinction could have been caused by a gamma ray burst from a supernova 6,000 light years distant from the Earth. This burst should have destroyed half of the ozone layer, leading to an immediate kill by the heavy UV radiation. However, any hard data that confirm this model do not exist. As the ozone layer buffers the mutagen UV radiation, an increase of UV radiation should in some kind be visible in the fossil record by an increase of pathologically developed invertebrate macrofossils.
- Young et al. (2009) speculated that CO_2 might play a major role in the end-Ordovician mass extinction. Their idea is that through the late Ordovician massive volcanic CO_2 outgassing was balanced by strong weathering of the uplifting Appalachian Mountains, which sequestered CO_2 . Finally, in the end of the Ordovician, the volcanic activity finished and the continued weathering caused a significant decrease of CO_2 . As a result, the Hirnantian ice age started.

Late Devonian extinction events (ca. 370 My)

Within the late Devonian, several extinction events severely affected biodiversity. The most important ones were the Upper Devonian Kellwasser events in the Frasnian–Famennian boundary interval and the Hangenberg event at the Devonian–Carboniferous boundary (McGhee, 1996; Walliser, 1995; Sandberg et al., 2002). The



Mass Extinctions, Phanerozoic, Figure 1 Compilation of the Big Five Phanerozoic extinction events with the assumed percentage of extinction of known genera. These numbers are highly speculative, because the biodiversity of deep water environments during the extinction events are only poorly known (Figure modified after Sepkoski, 1984, 1994; Alroy, 2004).

extinction seems to have only affected marine life. Reef-building organisms were almost completely wiped out, except some microbial reefs with occasionally few sponges. Roughly 60% of the known genera became extinct. The diversity of brachiopods, cephalopods, trilobites, benthic foraminifera, conodonts, rugose and tabulate corals, jawless fish, stromatoporoid sponges, etc. was strongly influenced by these events. However, the decline of biodiversity is probably a reduction in the formation of new species rather than an extinction of already existing taxa.

Possible origins

Different possibilities have been discussed for the late Devonian mass extinctions such as asteroid impacts, global anoxia, sea-level changes, climatic changes, etc. (Buggisch, 1991; Joachimski, 1997; Joachimski and Buggisch, 1993; Joachimski, 2001). The most intriguing hypothesis is the “Devonian Plant Hypothesis” (Algeo and Scheckler, 1998; Algeo et al., 1995, 2001). Due to the rapid and accelerating evolution of terrestrial plants from the Lower Devonian on, intensified chemical and physical weathering by roots and the increased continental bioturbation resulted in an intensification of pedogenesis. As a result, several feedback loops established. On the

one hand, the increased runoff of nutrients caused eutrophication of the Devonian seas, the genesis of anoxia and black shales, and the extinction of deep water and shelf biota. On the other hand, due to silicate weathering and CO_2 withdrawal from the atmosphere, the alkalinity of the sea increased, enhancing precipitation of carbonates and further CO_2 withdrawal. As a result, a global cooling occurred, and the associated sea-level fall diminished shelf habitat significantly during the Kellwasser and Hangenberg events (e.g., Sandberg et al., 2002).

End-Permian mass extinction (ca. 251.4 My)

The Permian–Triassic extinction was the most severe extinction event within the Phanerozoic. Up to 84% of the known Permian taxa (genera) became extinct. Not only marine organisms were affected, but also terrestrial ecosystems. Many of the characteristic Paleozoic organisms like trilobites, representatives of the cephalopoda and corals, marine and terrestrial vertebrates, and also plants disappeared and died out. However, some of the Permian-style organisms like coralline sponges occurred again in the early–middle Triassic and are interpreted as Lazarus-taxa, a widely, only poorly understood phenomenon. The end-Permian extinction cannot be explained by one event only. The combination of various events is

possibly responsible for this tremendous mass extinction (Benton, 2005; Bowring et al., 1998).

Possible origins

- a. Volcanic events are widely discussed to be the major controlling factor of the Permian–Triassic mass extinction. The most important volcanic events were flood basalt eruptions, which produced the Siberian Traps, some of the largest known volcanic provinces in earth's history. These eruptions have an age of 251.2 ± 0.3 My, and they fit stratigraphically very well with the estimated age of the mass extinction. The Siberian Traps were estimated to exhibit 20% of pyroclastic material, which heavily reduced insolation by backscatter of sunlight. Furthermore, eruptions caused dust clouds, acidic aerosols, and acidic rain, which – in conjunction with the first – disturbed and disrupted photosynthesis on land and in the sea. The Siberian Trap lava intruded into calcareous sediments and large coal beds, and emitted large amounts of CO_2 , causing a slight global warming (White, 2002; Saunders and Reichow, 2009; Reichow et al., 2009).
- b. There is a worldwide negative excursion of $\delta^{13}\text{C}_{\text{carb}}$ to values of ca. -10‰ . There are various possibilities to explain this excursion. However, the release of huge amounts of methane possibly from collapsing marine and permafrost methane hydrates is the most probable reason (Palfy et al., 2001; Twitchett et al., 2001; Payne et al., 2004).
- c. Several pieces of evidence can be brought forward for an impact event in the end of the Permian. There are possible impact craters, which have been proposed as possible causes of the extinction: the Australian Bedout crater and the Wilkes Land crater of East Antarctica. From these sites, shocked quartz and fullerenes, trapping extraterrestrial noble gases, are known. However, this evidence does not really prove the impact event, and the Wilkes Land crater is possibly younger than the Permian–Triassic boundary (Retallack et al., 1998; Becker et al., 2001, 2004). In this respect, the paper by Stanton (2002) is very intriguing, in which he suggests that the Gulf of Mexico is a very huge meteorite crater of end-Permian age – big enough to explain this fatal extinction event.
- d. There is evidence of anoxic seawater conditions around the time of end-Permian extinctions. Major players in anoxic seawater are sulfate-reducing bacteria, which could produce high amounts of H_2S , which is, however, partly re-mineralized in iron sulfides (e.g., pyrite). Dissolved H_2S is a poison and could be responsible for aquatic mass killing. Perhaps, the release of H_2S in to the atmosphere weakened the ozone layer and increased the UV radiation with fatal effects for plants and land animals. There are indications that anaerobic photosynthesis via green sulfur bacteria occurred in the end of the Permian till the early Triassic (Kump et al., 2005; Grice et al., 2005). The triggering mechanism is believed to be the increase of

volcanogenic CO_2 , which lowered the pH value and increased chemical weathering. As a result, the continental runoff of nutrients increased dramatically, causing eutrophication and finally anoxia with the consequences listed above.

- e. Last but not least, sea-level changes are also discussed to explain the mass extinction. Due to the Pangea situation, a very big marine regression is noticed. This reduced the shelfal areas, biodiversity, and the stability of food chains (e.g., Hallam and Wignall, 1999).

End-Triassic mass extinction (ca. 201.4 My)

The end-Triassic extinction defines the boundary between the Triassic and Jurassic, and it is one of the most important Phanerozoic extinction events. Ca. 47% of the known genera got extinct, such as the conodonts, nearly almost all Triassic taxa of ammonites except the stem groups of the Phylloceratina and the Lytoceratina. Many vertebrates such as a high number of large amphibians, Triassic archosaurs, many therapsids, etc. disappeared. The end-Triassic extinction event also destroyed the widespread shallow water coral reef environments, including many types of corals and especially coralline sponges. The recovery of this coral reef environment took a long time and was more or less completed in the Upper Jurassic (Hallam, 1987, 1997; Palfy et al., 2001; Ward et al., 2001).

Possible origins

There is only one seriously discussed reason for the end-Triassic extinction. During this time, a tremendous volcanic activity occurred, the Central Atlantic Magmatic Province (CAMP), which produced huge amounts of flood basalts, linked with a high amount of outgassing CO_2 , sulfur gases, and volcanic ash (Hesselbo et al., 2002). This event heavily influenced the shallow water and terrestrial bioproduction and, probably due to acid rains, also biomineralization. The $\delta^{13}\text{C}$ isotopic composition of organic matter from sediments from this time exhibits an unusual negative excursion (Deenen et al., 2010; Ruhl et al., 2009; Whiteside et al., 2010). Most probable trigger for the extinction is the CAMP-induced climatic change, acidification of the ocean surface waters, and a strong sea-level fall.

End-Cretaceous mass extinction (ca. 65.5 My)

The end-Cretaceous extinction event, also known as the Kreide–Tertiary (K-T) extinction event, was a very short-termed mass extinction event. However, the diversity of some groups of animals like ammonites, belemnites, various Cretaceous bivalves (rudists, inoceramids), dinosaurs, etc. decreased before the K-T event. The most important impact on biodiversity was the extinction of non-feathered dinosaurs, marine large reptiles (mosasaurs, plesiosaurs), pterosaurs, and the decrease of phyto- and zooplankton diversity and biomass in the ocean surface waters. Crocodilian archosaurs, turtles, birds, and small nocturnal mammalian survived the mass extinction event.

Extinction of most taxa of, for example, coccolithophorids and Cretaceous planktonic foraminifera led to a breakdown of primary productivity and calcareous sedimentation rates in the oceans. The K-T boundary layer is worldwide often documented as a black band, which exhibits geochemical anomalies, for example, an enrichment of iridium and in the Pt-group elements in general. Probably, ca. 47% of the known genera became extinct.

Possible origins

- a. The most discussed reason is that huge impacts of cometary parts or asteroids caused the extinction. This assumption is evidenced by the geochemical anomalies (Ir, Pt-group) and the discovery of a very large impact structure in northern Mexico (Yucatan), the Chicxulub crater, which has an age corresponding to that of the K-T boundary. Some scientists assume that more impacts happened; however, no further impact structures are known. The ecological disaster shortly after the huge meteorite impact (estimated size of impactor more than 10 km) caused the extinction of the above-listed organisms.
- b. Beside the bolide impact hypothesis, an increased volcanic activity is also discussed to be responsible for the big extinction event. The Deccan trap basalts have the age of the K-T boundary, and this vast volcanic event might also have influenced the biodiversity and biomass production at that time, which then reflects a comparable scenario like for the Permian-Triassic boundary. It is easily possible that the conjunction of two events – the bolide impact and the Deccan trap volcanism – are responsible for this last large Phanerozoic mega extinction.

Conclusion

All five major extinction events within Phanerozoic are milestones in the evolution of organisms, community structures, and ecosystems. All of them are critical intervals (see contribution Wiese and Reitner this volume) and of fundamental paleobiological importance. All of the major extinction events are multicausal events and cannot be restricted to only one factor. Most of them are also not short-term events, except the K-T boundary event which is proved to be a large meteorite impact. However, also in this case, the extinction of some important taxa has a prelude in the form of successive decrease in diversity, and the K-T event finally wiped them out.

These catastrophic events are probably very important for the entire biotic evolution on earth and obviously one of major evolutionary driving force.

Bibliography

- Algeo, T. J., and Scheckler, S. E., 1998. Terrestrial-marine teleconnections in the Devonian: links between the evolution of land plants, weathering processes, and marine anoxic events. *Philosophical Transactions of the Royal Society of London B*, **353**, 113–130.
- Algeo, T. J., Berner, R. A., Maynard, J. P., and Scheckler, S. E., 1995. Late Devonian oceanic anoxic events and biotic crises: rooted in the evolution of vascular land plants? *Geological Society of America Today*, **45**, 64–66.
- Algeo, T. J., Scheckler, S. E., and Maynard, J. B., 2001. Effects of the Middle to Late Devonian spread of vascular land plants on weathering regimes, marine biota, and global climate. In Gensel, P. G., and Edwards, D. (eds.), *Plants Invade the Land: Evolutionary and Environmental Approaches*. New York: Columbia University Press, pp. 213–236.
- Alroy, J., 2004. Are Sepkoski's evolutionary faunas dynamically coherent? *Evolutionary Ecology Research*, **6**, 1–32.
- Becker, L., Poreda, R. J., Hunt, A. G., Bunch, T. E., Rampino, M., 2001. Impact event at the Permian-Triassic boundary: evidence from extraterrestrial noble gases in fullerenes. *Science*, **291**, 1530–1533.
- Becker, L., Poreda, R. J., Basu, A. R., Pope, K. O., Harrison, T. M., Nicholson, C., and Iasky, R., 2004. Bedout: a possible end-Permian impact crater offshore of northwestern Australia. *Science*, **304**, 1469–1476.
- Benton, M. J., 2005. When life nearly died: the greatest mass extinction of all time. London: Thames and Hudson.
- Bowring, S. A., Erwin, D. H., Jin, Y. G., Martin, M. W., Davidek, K., and Wang, W., 1998. U/Pb Zircon geochronology and tempo of the End-Permian mass extinction. *Science*, **280**, 1039–1045.
- Brasier, M. D., 1989. On mass extinctions and faunal turnover near the end of the Precambrian. In Donovan, S. K. (ed.), *Mass Extinctions, Processes, and Evidence*. New York: Columbia University Press, pp. 73–88.
- Buggisch, W., 1991. The global Frasnian-Famennian Kellwasser event. *Geologische Rundschau*, **80**, 49–72.
- Deenen, M. H. L., Ruhl, M., Bonis, N. R., Krijgsman, W., Kürschner, W. M., Reitsma, M., and Van Bergen, M. J., 2010. A new chronology for the end-Triassic mass extinction. *Earth and Planetary Science and Letters*, **291**, 113–125.
- Grice, K., Cao, C., Love, G. D., Bottcher, M. E., Twitchett, R. J., Grosjean, E., Summons, R. E., Turgeon, S. C., Dunning, W., and Yügan, J., 2005. Photoc zone Euxinia during the Permian-Triassic superanoxic event. *Science*, **307**, 706–709.
- Hallam, A., 1987. Radiations and extinctions in relation to environmental change in the Marine Lower Jurassic of Northwest Europe. *Paleobiology*, **13**(2), 152–168.
- Hallam, A., 1997. Estimates of the amount and rate of sea-level change across the Rhaetian-Hettangian and Pliensbachian-Toarcian boundaries (latest Triassic to early Jurassic). *Journal of the Geological Society*, **154**, 773–779.
- Hallam, A., and Wignall, P. B., 1999. Mass extinctions and sea-level changes. *Earth-Science Reviews*, **48**, 217–250.
- Hesselbo, S. P., Robinson, S. A., Surlyk, F., and Piasecki, S., 2002. Terrestrial and marine extinction at the Triassic-Jurassic boundary synchronized with major carbon-cycle perturbation: a link to initiation of massive volcanism? *Geology*, **30**, 251–254.
- Hillebrandt, V. A., Krystyn, L., and Kürschner, W. M., 2007. A candidate GSSP for the base of the Jurassic in the Northern Calcareous Alps (Kuhjoch section, Karwendel Mountains, Tyrol, Austria). *International Subcommission on Jurassic Stratigraphy Newsletter*, **34**, 2–20.
- Jensen, S., Gehling, J. G., and Droser, M. L., 1998. Ediacara-type fossils in Cambrian sediments. *Nature*, **393**, 567–569.
- Joachimski, M. M., 1997. Comparison of organic and inorganic carbon isotope patterns across the Frasnian-Famennian boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **132**, 133–145.
- Joachimski, M., and Buggisch, W., 1993. Anoxic events in the late Frasnian – causes of the Frasnian-Famennian faunal crisis? *Geology*, **21**, 675–678.

- Joachimski, M., Ostertag-Henning, C., Pancost, R., Strauss, H., Freeman, K., Littke, R., Sinninghe Damste, J. S., and Racki, G., 2001. Water column anoxia, enhanced productivity and concomitant changes in $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ across the Frasnian-Famennian boundary (Kowala-Holt' Cross Mountains/Poland). *Chemical Geology*, **175**, 109–131.
- KieSSLing, W., and Simpson, C., 2010. On the potential for ocean acidification to be a general cause of ancient reef crises. *Global Change Biology*, doi:10.1111/j.1365-2486.2010.02204
- Krug, A. Z., and Patzkowsky, M. E., 2004. Rapid recovery from the Late Ordovician mass extinction. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 17605–17610.
- Kump, L. R., Pavlov, A., and Arthur, M. A., 2005. Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology*, **33**, 397–400.
- Le Guerroué, E., Allen, P. A., and Cozzi, A., 2006a. Chemostratigraphic and sedimentological framework of the largest negative carbon isotopic excursion in Earth history: the Neoproterozoic Shuram formation (Nafun Group, Oman). *Precambrian Research*, **146**(1–2), 68–92.
- Le Guerroué, E., Allen, P. A., Cozzi, A., Etienne, J. L., and Fanning, C. M., 2006b. 50 Myr recovery from the largest negative $\delta^{13}\text{C}$ excursion in the Ediacaran ocean. *Terra Nova*, **18**(2), 147–153.
- McGhee, G. R. Jr., 1996. *The Late Devonian Mass Extinction: the Frasnian/Famennian Crisis*. New York: Columbia University Press.
- Melott, A. L., Lieberman, B. S., Laird, C. M., Martin, L. D., Medvedev, M. V., Thomas, B. C., Cannizzo, J. K., Gehrels, N., and Jackman, C. H., 2004. Did a gamma-ray burst initiate the late Ordovician mass extinction? *International Journal of Astrobiology*, **3**, arXiv:astro-ph/0309415v3.
- Moczydlowska, M., 2008. The Ediacaran microbiota and the survival of Snowball Earth Snowball conditions. *Precambrian Research*, **167**, 1–15.
- Palfy, J., Demeny, A., Haas, J., Htenyi, M., Orchard, M. J., and Veto, I., 2001. Carbon isotope anomaly at the Triassic–Jurassic boundary from a marine section in Hungary. *Geology*, **29**, 1047–1050.
- Payne, J. L., Lehmann, D. J., Wei, J., Orchard, M. J., Schrag, D. P., and Knoll, A. H., 2004. Large perturbations of the carbon cycle during recovery from the End-Permian Extinction. *Science*, **305**, 506.
- Raup, D. M., and Sepkoski, J. J., 1982. Mass extinctions in the marine fossil record. *Science*, **215**(4539), 1501–1503.
- Reichow, M. K., Pringle, M. S., Al'Mukhamedov, A. I., Allen, M. B., Andreichev, V. L., Buslov, M. M., Davies, C. E., Fedoseev, G. S., Fitton, J. G., Inger, S., Medvedev, A. Ya., Mitchell, C., Puchkov, V. N., Safonova, I. Yu., Scott, R. A., and Saunders, A. D., 2009. The timing and extent of the eruption of the Siberian Traps large igneous province: implications for the end-Permian environmental crisis. *Earth and Planetary Science Letters*, **277**, 9–20.
- Retallack, G. J., Seyedolali, A., Krull, E. S., Holser, W. T., Ambers, C. P., and Kyte, F. T., 1998. Search for evidence of impact at the Permian–Triassic boundary in Antarctica and Australia. *Geology*, **26**, 979–982.
- Ruhl, M., Kuerschner, W. M., and Krystyn, L., 2009. Triassic–Jurassic organic carbon isotope stratigraphy of key sections in the western Tethys realm (Austria). *Earth and Planetary Science Letters*, **281**, 169–187.
- Sandberg, C. A., Morrow, J. R., and Ziegler, W., 2002. Late Devonian sea-level changes, catastrophic events, and mass extinctions. In Koeberl, C., and MacLeod, K. G. (eds.), *Catastrophic Events and Mass Extinctions: Impacts and Beyond*. Boulder: Geological Society of America. GSA Special Paper 356, pp. 473–487.
- Saunders, A., and Reichow, M. K., 2009. The Siberian traps and the End-Permian mass extinction: a critical review. *Chinese Science Bulletin*, **54**, 20–37.
- Sepkoski, J. J. Jr., 1984. A kinetic model of Phanerozoic taxonomic diversity. III. post-Paleozoic families and mass extinctions. *Paleobiology*, **10**, 246–267.
- Sepkoski, J. J. Jr., 1994. Extinction and the fossil record. *Geotimes*, **39**, 15–17.
- Sheehan, P., 2001. The late Ordovician mass extinction. *Annual Review of Earth and Planetary Sciences*, **29**, 331–364.
- Sole, R. V., and Newman, M., 2002. Extinctions and Biodiversity in the Fossil Record - Volume Two, The earth system: biological and ecological dimensions of global environment change. *Encyclopedia of Global Environmental Change*, New York: Wiley, pp. 297–391.
- Stanton, M. S., 2002. http://www.aapg.org/explorer/2002/12dec/gom_impact.pdf
- Twitchett, R. J., Looy, C. V., Morante, R., Visscher, H., and Wignall, P. B., 2001. Rapid and synchronous collapse of marine and terrestrial ecosystems during the end-Permian biotic crisis. *Geology*, **29**, 351–354.
- Wallsier, O. H. (ed.), 1995. *Global Events and Event Stratigraphy*. Berlin: Springer.
- Ward, P. D., Haggart, J. W., Carter, E. S., Wilbur, D., Tipper H. W., and Evans, T., 2001. Sudden productivity collapse associated with the Triassic–Jurassic boundary mass extinction. *Science*, **292**, 1148–1151.
- Webby, B. D., and Droser, M. L. (eds.), 2004. *The Great Ordovician Biodiversification Event*. New York: Columbia University Press.
- White, R. V., 2002. Earth's biggest “whodunnit”: unravelling the clues in the case of the end-Permian mass extinction. *Philosophical Transactions of the Royal Society of London*, **360**, 2963–2985.
- Whiteside, J. H., Olsen, P. E., Eglinton, T., Brookfield, M. E., and Sambrotto, R. N., 2010. Compound-specific carbon isotopes from Earth's largest flood basalt eruptions directly linked to the end-Triassic mass extinction. *Proceedings of the National Academy of Sciences*, **107**(15), 6721–6725.
- Young, S. A., Saltzman, M. R., Foland, K. A., Linder, J. J., and Kump, L. R., 2009. A major drop in seawater $87\text{Sr}/86\text{Sr}$ during the Middle Ordovician (Darriwilian): links to volcanism and climate? *Geology*, **37**, 951–954.

Cross-references

Asteroid and Comet Impacts
 Biological Volcanic Rock Weathering
 Comets
 Critical Intervals in Earth History
 Ediacaran Biota
 Snowball Earth

MAT-RELATED SEDIMENTARY STRUCTURES

Hubertus Porada
 University of Göttingen, Göttingen, Germany

Synonyms

Microbially induced sedimentary structures (MISS) (The acronym MISS was introduced by Noffke et al. (2001) for “microbially induced sedimentary structures.” Many of the structures addressed, however, are not truly “induced” by microbes, but rather by physical forces

acting on a biostabilized sediment surface or a microbial mat. Since, in this scenario, the biological component significantly influences the shape of evolving structures, it is suggested to write out the acronym MISS as “microbially influenced sedimentary structures.”).

Definition

Mat-related sedimentary structures are small- to medium-scale sedimentary structures resulting from growth and extension of microbial populations and communities on a sediment surface, stabilization of surface sediments, trapping and binding of sediment particles, and from the impact of environmental factors, such as inundation, sedimentary deposition, subaerial desiccation, wind- or current-induced traction, on epibenthic microbial mats.

Introduction

Originally described from and applied to modern microbial mats (Gerdes et al., 2000; Noffke et al., 2001), mat-related sedimentary structures preferentially serve as proxies of the former existence of microbial mats in ancient siliciclastic sediments. Mat-related structures are in a way analogous to trace fossils (Schieber, 2004), whereby the former presence of mats can be inferred, e.g., from observations suggesting “unusual” sediment properties like original cohesiveness, tensile strength, and erosion-resistance of sand (Eriksson et al., 2007); uncharacteristic grain fabrics; and steep geochemical gradients on a mm-scale. Genetic explanation of the structures relies on an actualistic approach; preservation depends on intrinsic biological, and favorable environmental conditions.

In this chapter, the term “mat-related sedimentary structures” is applied to structures that form on, in, or below microbial mats with distinct properties, e.g., coherence, cohesiveness, minor thickness, and adaptation to periodic subaerial exposure. Further conditions include a fine-grained siliciclastic substrate (sand to silt) and lacking dominance of carbonate precipitation. The term also encompasses primary physical structures that have subsequently been microbially influenced.

Today, well-developed (cyanobacterial) microbial mats that meet the above conditions, are observed mainly in the intertidal to lower supratidal zones of low-gradient tidal flats where they grow in a wide range of climate zones from temperate humid to subtropical/tropical semi-arid and arid, at salinities ranging from brackish to hypersaline. Similar mats may also form in other environments, e.g., lacustrine, fluvial, where similar mat-related structures may partly form. Due to lacking vertical bioturbation in the Precambrian, the distribution of microbial mats may previously have been much wider, extending far into the subtidal zone.

Microbial mats are epibenthic, multilayered microbial communities including different functional groups of microorganisms (Stal, 2000) which act together in a kind of “joint venture” widely balancing organic matter production and destruction (van Gernerden, 1993). Thus, microbial mats in coastal environments normally do not

exceed a thickness of a few centimeters. The “biological stratification” of microorganisms is superimposed by a biosedimentary lamination resulting from the interplay of microbial growth and seasonal, periodic, or episodic depositional events related, e.g., to tides or storms.

The top layer of the mat system, which periodically is exposed to the atmosphere, usually consists of oxygenic, filamentous cyanobacteria, frequently *Microcoleus* sp., which form a “felty” fabric of interwoven filaments (Gerdes et al., 2000), including also sediment grains incorporated by “trapping and binding” processes (Noffke et al., 2003a, with references). The felty layer may be overlain, or in some cases replaced, by a resistant (“leathery”) layer of colloid extracellular polymeric substances (EPS) produced mainly by coccoid cyanobacteria. This top portion of the mat combines high mechanical resistance with low permeability and is the locus at which most of the mat-related sedimentary structures form. Deeper levels of the mat system are much less cohesive. They contain anaerobic bacteria among which sulfate-reducing bacteria produce sulfide leading to precipitation of iron sulfides (FeS) which gradually transform into pyrite. In the ancient record, the stratiform occurrence of pyrite, in some cases siderite or ferroan carbonate, within otherwise well oxidized sediments may serve as a proxy of the former existence of microbial mats.

Formation and types of structures

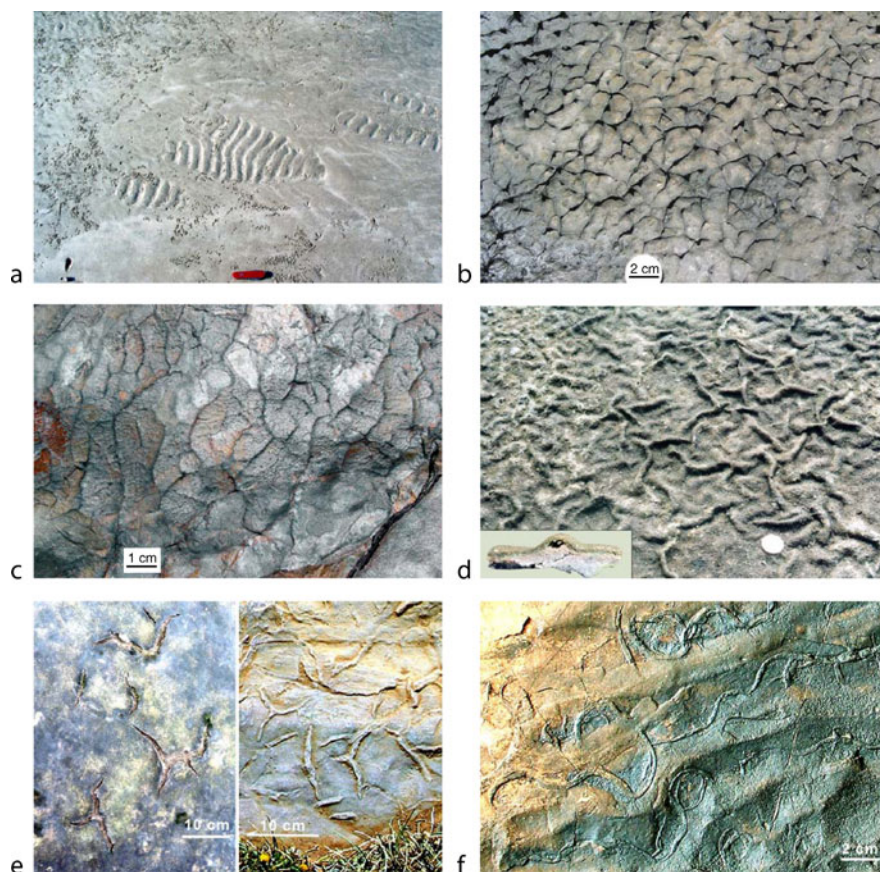
Mat-related sedimentary structures may form during all the stages from first microbial colonization of a sediment surface, through establishment and sustainment of a fully developed mat, to its destruction and final erosion.

Structures related to early microbial colonization

Microbial colonization leads to “biostabilization” (Krumbein et al., 1994) of a sediment surface which then can resist erosion to some degree. This property may lead to “palimpsest ripples” when new sediment is deposited on top a biostabilized rippled surface (Pflüger, 1999); to surfaces with “multi-directional ripple marks” (Noffke, 1998); and to “ripple patches” (Figure 1a) or “erosional pockets” when a biostabilized flat surface or mat is locally eroded (for ancient example, Noffke, 1999; McKenzie, 1972).

Structures related to microbial growth and mat growth

Depending on availability of water for some time, specific filamentous cyanobacteria (e.g., *Lyngbya aestuarii*) start to produce on the mat surface, a characteristic “reticulate growth pattern” of small, sharp-crested ridges stabilized by EPS (Figure 1b). Ancient structures of this type are named “elephant skin” texture (Gehling, 1999) (Figure 1c). There is considerable variability in respect of polygon size (few mm to few cm) and shape varying from nearly circular to narrow rectangular with almost linear crest orientation. A unifying feature, however, are the



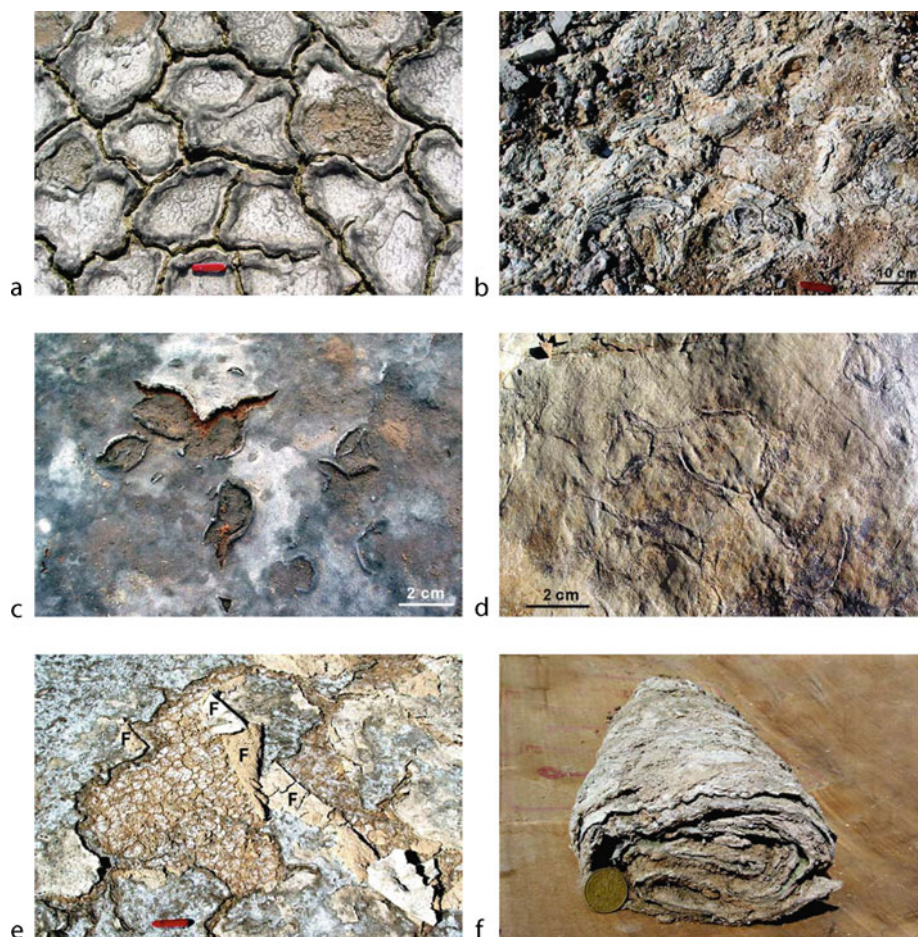
Mat-Related Sedimentary Structures, Figure 1 Structures related to biostabilization, microbial and mat growth, and desiccation. (a) Biostabilized sediment surface with isolated “ripples patches.” Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (b) Reticulate growth pattern produced by *Lyngbya aestuarii*. Locality: Salins du Midi, Réserve Nationale Camargue, southern France. (c) Upper surface of sericitic siltstone with “elephant skin” texture representing “reticulate growth pattern” of a previous microbial mat. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (d) “Petees” originating from lateral mat growth and deforming microbial mat into round-crested bulges: *Inset*: Cross-section showing that upper, cohesive part of the mat is deformed and separated from deeper, less cohesive mat layers. Scale (coin) is 24 mm. Locality: Amrum Island, southern North Sea, Germany. (e) Modern and ancient shrinkage cracks. *Left photograph*: modern microbial mat with isolated, sigmoidal, and tri-radiate cracks. Locality: Amrum Island, southern North Sea, Germany. *Right photograph*: fillings of ancient, spindle-shaped, and tri-radiate cracks. Locality: Neoproterozoic Tizi n-Taghatine Group; Imi n'Tizi area, Anti-Atlas, Morocco. (f) *Manchuriophycus*-type shrinkage cracks meandering in ripple troughs. Locality: Neoproterozoic Tizi n-Taghatine Group; Taghdout area, Anti-Atlas, Morocco.

narrow, sharp-crested ridges preserved on upper bedding surfaces.

A further type of structures related to mat growth are “mat expansion structures,” collectively termed “petees” (Gavish et al., 1985; Bouougri et al., 2007), which deform the upper, cohesive part of the mat into bulges and domes, thus enlarging the mat surface (Figure 1d). Occasionally, the bulges may be arranged in a polygonal pattern. Provided, petees are filled by sediment rising up from below, the structures may be preserved. Ancient examples have been named “petee ridges” (Schieber, 2004).

Structures related to mat desiccation and shrinkage
Subaerial exposure causes dehydration and shrinkage of the EPS and eventually cracking of the mat. Incipient,

isolated “shrinkage cracks” are sigmoidal and spindle-shaped, or tri-radiate starting from triple junctions, or occasionally circular in shape (Figure 1e). Crack propagation at progressive shrinkage leads to more or less complete networks of cracks. In general, shrinkage cracks in microbial mats develop a higher degree of curving unlike normal mud cracks. Ancient shrinkage cracks are also termed “sand cracks” referring to their occurrence in sandy sediment without shrinkable mud present. A specific type of shrinkage cracks, characterized by sinuoidal or sub-circular trends and developed mainly in ripple troughs, is referred to as “*Manchuriophycus*”-type in the ancient record (Porada and Löffler, 2000) (Figure 1f). A characteristic property of many ancient shrinkage cracks is their easy separability from the layers above and below, which is ascribed to soon microbial



Mat-Related Sedimentary Structures, Figure 2 Structures related to mat desiccation and deformation. (a) Polygonal pattern of shrinkage cracks with upturned margins in thick microbial mat. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (b) Surface outcrop of Holocene microbial mat exhibiting relics of polygons with upturned margins. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (c) Irregular to subcircular openings with curled margins, formed in a thin microbial mat due to desiccation and shrinkage. Note newly overgrown margin in lower right of photograph. Locality: Sabkha El Gourine, Mediterranean coast of southern Tunisia. (d) Upper surface of siltstone layer exhibiting irregular to circular cracks. The structures are interpreted as openings in a previous thin mat that underwent desiccation. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (e) Microbial mat, torn and partly removed by current or wind action. Flip-overs of mat margins are indicated by "F." Note small-scale desiccation cracks in exposed mat substratum. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (f) "Roll-up" structure ("jelly-roll") consisting of rolled-up microbial mat and adhering sediment. Scale (coin) is 23 mm. Locality: Bhar Alouane, Mediterranean coast of southern Tunisia.

overgrowth of the crack margins and of the sediment trapped in the crack.

Thick mats, frequently dominated by *Microcoleus chthonoplastes*, tend to form polygonal networks of wide cracks with "upturned" or involute, "curled margins" (Figure 2a); individual polygons may range in size from 10 to 60 cm across, depending likely on the thickness of the mat. Ancient examples exhibit a "chaotic" upper surface with irregularly oriented bedding (Figure 2b) and resemble sedimentary structures ascribed to seismic events (Donaldson and Chiarenzelli, 2007).

Thin mats, frequently dominated by coccoid cyanobacteria and vested with a strong EPS top layer, tend

to form circular openings with curled margins around (Figure 2c). Ancient examples have been described from thin siltstone layers within heterolithic deposits (Bouougri and Porada, 2007) (Figure 2d).

Structures related to mat deformation, destruction, and erosion

Cohesive microbial mats may become torn, detached from the substratum and tightly folded, and eventually eroded by strong currents, e.g., during storm and flooding events. Mats are also destroyed by complete desiccation after cessation of groundwater supply, and dried mat fragments

may be transported by wind over wide distances and be deposited in environments where mats usually do not grow.

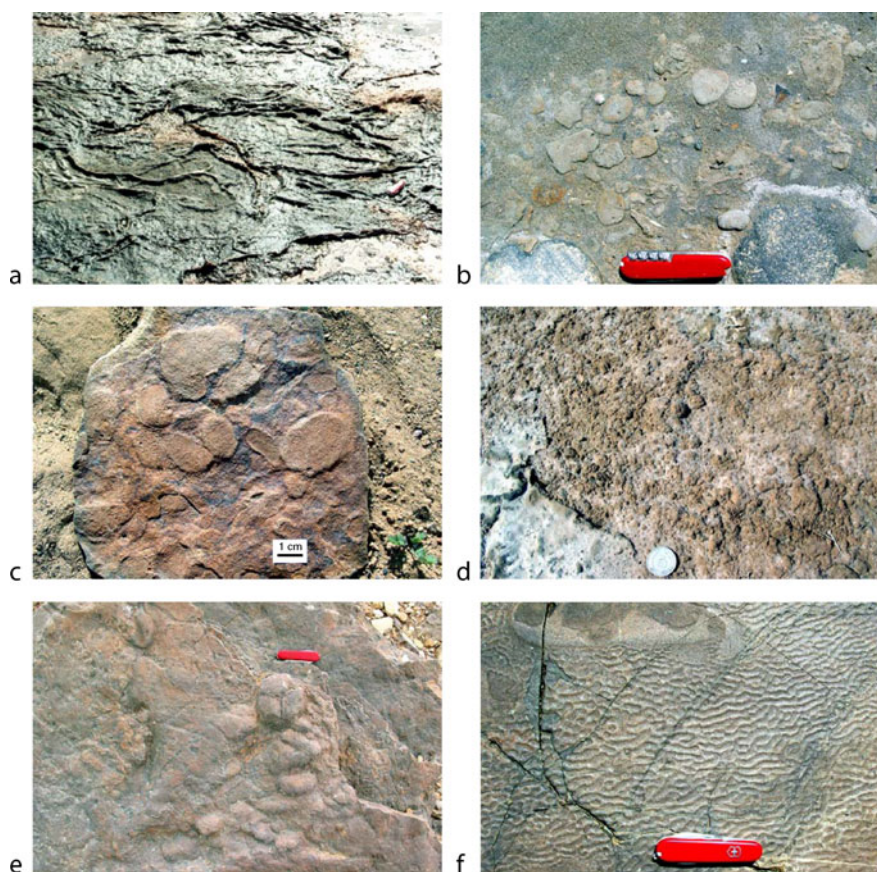
Mat destruction by currents leads to typical structures repeatedly observed in modern environments and in the ancient record: (1) “flip-over” structures (Figure 2e) result when a mat’s edge is flipped over; (2) cigar-shaped “roll-up” structures, also named roll-ups or “jelly-rolls” (Figure 2f), may develop when curled margins or flip-overs undergo additional rolling due to current action; and (3) irregular or arcuate belts of “mat deformation folds” form when a torn and detached mat is crumpled by tractional forces (Figure 3a); similar folds may also result from mat slumping on steep slopes, e.g., along tidal channels. Roll-up structures can withstand transport over some distance and may gather along trash lines, whereas

the other structures largely remain in situ. For ancient examples see Schieber et al. (2007).

“Mat chips” (Gerdes et al., 2000) are small fragments of eroded mats or biostabilized sediment. They primarily are irregular in shape with frayed edges, but may become pebble-shaped with transport (Figure 3b). Ancient examples have been named “microbial sand chips” (Pflüger and Gresse, 1996) or “sand clasts” in contrast to intraformational mud clasts (Figure 3c).

Structures formed beneath microbial mats

From the hydraulic perspective, the sediment beneath cohesive and coherent microbial mats may be addressed and treated as a confined aquifer in which, at sufficient hydraulic head, a potential for liquefaction may develop (Porada et al., 2007). In such case, hydraulic upward



Mat-Related Sedimentary Structures, Figure 3 Structures related to mat deformation and erosion, and subsurface structures. Scale (knife) in a, b, e, and f is 8 cm. (a) Detached, thin microbial mat, torn and strongly deformed by current action. Locality: Coastal sabkha between Gabes and Skhirat, Mediterranean coast of southern Tunisia. (b) Modern sandy-pebbly sediment surface with subrounded to rounded “mat chips”. Locality: Salins du Midi, Réserve Nationale Camargue, southern France. (c) Upper surface of sandstone bed carrying subrounded to rounded “sand clasts,” interpreted as previous “mat chips.”. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (d) Morphological details of a mat subsurface, exposed after removal of the mat. Scale (coin) is 24 mm. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (e) Upper surface of sandstone layer exhibiting irregular bulges and domes. The structure is considered to represent morphological features of a previous mat subsurface. Note tri-radiate crack in well-developed dome just below scale. Locality: Neoproterozoic Tizin-Taghatine Group; Taghdout area, Anti-Atlas, Morocco. (f) Upper surface of sandstone layer with “Kinneyia” structure. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia.

pressure will lead to slow, upward movement of sediment grains. As a result, bulges and domes developed in the mat will gradually be filled from below; “petee ridges” have been quoted as an example above.

Thin microbial mats, particularly those dominated by coccoid cyanobacteria, may develop very irregular surfaces with numerous small domes and buckles which also appear as positive features on the subsurface (Figure 3d). Ancient examples of such “subsurface structures” strongly resemble load structures but are clearly distinguished from these by their occurrence as positive features on *upper* bedding surfaces (Figure 3e).

“Kinneyia” (Figure 3f) is a further structure developed in the sediment beneath a microbial mat. The structure is characterized by millimeter-scale, flat-topped, steeply sided, winding ridges separated by equally sized round-bottomed troughs and pits. It resembles small-scale interference ripples including crest-dominated linear and pit-dominated honeycomb-like patterns. The structure has been described from the Archean (Noffke et al., 2003b) to the Jurassic (Bloos, 1976), but not yet from the Recent. Genetic interpretation is thus difficult. Recent models suggest formation by trapping of gas underneath a sealing mat (Pflüger, 1999) or by reversals of groundwater flow in the liquefied mat substratum (Porada et al., 2007).

Wrinkle structures

The term “wrinkle structure” (Hagadorn and Bottjer, 1997, 1999) is currently used as a collective term for various small-scale irregularities developed on ancient, siliciclastic sediment surfaces. Application of the term implies that a microbial participation in the formation of the structure is suspected, at the least. Within this broad definition, wrinkle structures may originate from very different processes including microbial growth, mat deformation and subsurface processes (Porada and Bouougri, 2007). Included in the term are also structures like elephant skin and Kinneyia which are well defined and for which, if clearly identified, usage of the proper name is recommended.

Preservation of mat-related structures

As most of the organic matter produced by the cyanobacteria is soon decomposed and mineralized so that organic components or even carbon are rarely preserved in the ancient siliciclastic record, preservation of mat-related structures is largely dependent on the volume of inorganic material bound or produced by the mat or trapped by the structure. Microbially supported processes introducing inorganic material into the mat include “trapping and binding” of silt- to sand-sized grains and possibly clay minerals, formation of authigenic clay minerals during bacterial lysis, formation of iron sulfides and pyrite, and precipitation of carbonates. The mat-related structures themselves, being either positive or negative features on the mat surface, may act as sediments traps. Thus, shrinkage cracks may become filled with sediment by currents or

colian action, and involute (curled) margins may trap grains in their windings.

Another mechanism by which some mat-related structures, e.g., domes and bulges developed in the mat, may become preserved is “filling from below.” This process requires comparatively high hydraulic upward pressure enabling liquefaction of the sediment below the sealing mat so that grains are lifted and moved upwards. As a result, a previously flat surface of a sedimentary layer may be transformed into an irregular surface mimicking morphological features of the mat.

Conclusions

Simplistically subsumed under the main groups of “wrinkles,” “unusual cracks,” and mat chips in the fossil record, mat-related sedimentary structures exhibit a wide range of shapes and patterns resulting from various processes and interactions between conflicting biological and physical factors. Due to local microtopographic features and the intrinsic inhomogeneity of microbial mats on a small scale, even well-defined structures, like elephant skin or Kinneyia, may show a high degree of variability. Thus, one structure almost never is like the other and the paradigm of reproducibility is not strictly applicable to them. Nevertheless, the structures are distinctive as exemplified on Figures 1–3.

Mat-related sedimentary structures may serve as tools for detailed facies analysis in siliciclastic peritidal settings. Based on modern analogs, most of the structures form in the intertidal to supratidal zones, but mat extent may have been wider in the Precambrian. Structures requiring (at least temporarily) subaerial exposure for their formation, e.g., types of shrinkage cracks with or without upturned or curled margins, clearly indicate intertidal to supratidal positions, whereas others remain less unequivocal. Subsurface structures including Kinneyia may form down to the shallow subtidal zone, as long as coherent, cohesive mats are present.

In the fossil record, the significance of mat-related sedimentary structures increases greatly if several types are observed in association. Some mat-related structures, e.g., reticulate growth patterns, preferentially occur in heterolithic successions of thinly laminated siltstone and mudstone which, in some cases, may be addressed as “siliciclastic biolaminites” resulting from the interaction of microbial mat growth and sediment supply (Bouougri and Porada, 2007), whereas sand cracks are usually developed on sandstone layers intercalated in the succession. In contrast, Kinneyia structures exclusively occur on upper surfaces of sandstone beds which frequently represent event deposits after storms or floods.

Bibliography

Bloos, G., 1976. Untersuchungen über Bau und Entstehung der feinkörnigen Sandsteine des Schwarzen Jura α (Hettangium u. tiefstes Sinemurium) im schwäbischen Sedimentations-bereich. *Arb Inst Geol Paläont Univ Stuttgart*, **71**, 1–270.

- Bouougri, E. H., and Porada, H., 2007. Siliciclastic biolaminites indicative of widespread microbial mats in the Neoproterozoic Nama Group of Namibia. *Journal of African Earth Sciences*, **48**, 38–48.
- Bouougri, E., Gerdes, G., and Porada, H., 2007. Inherent problems of terminology: definition of terms frequently used in connection with microbial mats. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp.145–151.
- Eriksson, P. G., Schieber, J., Bouougri, E., Gerdes, G., Porada, H., Banerjee, S., Bose, P. K., and Sarkar, S., 2007. Classification of structures left by microbial mats in their host sediments. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp. 39–52.
- Gehling, J. G., 1999. Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. *Palaaios*, **14**, 40–57.
- Gerdes, G., Klenke, Th., and Noffke, N., 2000. Microbial signatures in peritidal siliciclastic sediments: a catalogue. *Sedimentology*, **47**, 279–308.
- Hagadorn, J. W., and Bottjer, D. J., 1997. Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic-Phanerozoic transition. *Geology*, **25**, 1047–1050.
- Hagadorn, J. W., and Bottjer, D. J., 1999. Restriction of a late neoproterozoic biotope: suspect-microbial structures and trace fossils at the Vendian-Cambrian transition. *Palaaios*, **14**, 73–85.
- Krumbein, W. E., Paterson, D. M., and Stal, L. J. (eds.), 1994. *Biostabilization of Sediments*, Oldenburg: BIS Verlag, 526 p.
- McKenzie, D. B., 1972. Tidal sand flat deposits in Lower Cretaceous Dakota Group near Denver, Colorado. *The Mountain Geologist*, **9**, 269–277.
- Noffke, N., 1998. Multidirected ripple marks rising from biological and sedimentological processes in modern lower supratidal deposits (Mellum Island, southern North Sea). *Geology*, **26**, 879–882.
- Noffke, N., 1999. Erosional remnants and pockets evolving from biotic-physical interactions in a recent lower supratidal environment. *Sedimentary Geology*, **123**, 175–181.
- Noffke, N., Gerdes, G., Klenke, T., and Krumbein, W. E., 2001. Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. *Journal of Sedimentary Research*, **71**, 649–656.
- Noffke, N., Gerdes, G., and Klenke, T., 2003a. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty, and evaporitic carbonatic). *Earth-science Reviews*, **62**, 163–176.
- Noffke, N., Hazen, R., and Nhlenko, N., 2003b. Earth's earliest microbial mats in a siliciclastic marine environment (2.9 Ga Mozaan Group, South Africa). *Geology*, **31**, 673–676.
- Pflüger, F., 1999. Matground structures and redox facies. *Palaaios*, **14**, 25–39.
- Porada, H., and Bouougri, E. H., 2007. Wrinkle structures – a critical review. *Earth-science Reviews*, **81**, 199–215.
- Porada, H., and Löffler, T., 2000. Microbial shrinkage cracks in siliciclastic rocks of the Neoproterozoic Nosib Group (Damara Supergroup) of central Namibia. *Communications of the Geological Survey of Namibia*, **12**, 63–72.
- Porada, H., Ghergut, J., and Bouougri, E. H., 2008. Kinneyia-type wrinkle structures –critical review and model of formation. *Palaaios*, **23**, 63–77.
- Schieber, J., 2004. Microbial mats in the siliciclastic rock record: a summary of diagnostic features. In Eriksson, P. G., Altermann, W., Nelson, D., Mueller, W. U., Catuneanu, O., and Strand, K. (eds.), *The Precambrian Earth: Tempos and Events. Developments in Precambrian Geology*. Amsterdam: Elsevier, pp. 663–672.
- Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O., 2007. *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, 311 p.
- Stal, L. J., 2000. Cyanobacterial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Amsterdam: Kluwer, pp. 61–120.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine Geology*, **113**, 3–25.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Iron Sulfide Formation](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Sulfate-Reducing Bacteria](#)
[Tidal Flats](#)

METAGENOMICS

Wolfgang Liebl
 Technische Universität München,
 Freising-Weihenstephan, Germany

Definition

Metagenomics is a research discipline that uses molecular biology methods to acquire, analyze, and interpret sequence data and/or functional data from the metagenomes (the collectivity of genomes) present in the environmental samples, without the need to cultivate the organisms in the samples. Related methodologies are so-called metatranscriptomics and metaproteomics that focus on the collective transcriptomes and proteomes, respectively, rather than on the genomes.

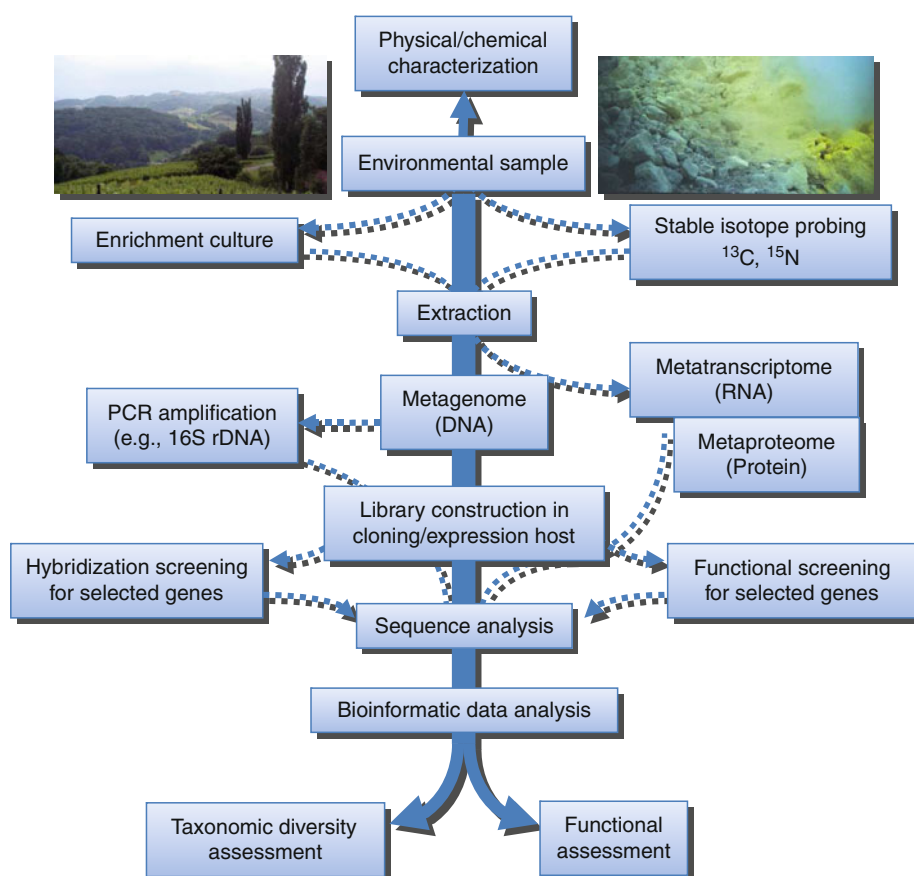
Introduction

Microorganisms constitute major life forms on earth with respect to cell numbers and total biomass, and fulfill essential functions in global element cycles. It has been estimated that the earth's biosphere harbors at least two to three orders of magnitude more prokaryotic cells ($4\text{--}6 \times 10^{30}$) than the number of plant and animal cells combined. The total carbon fixed in prokaryotic cells amounts to $3.5\text{--}5.5 \times 10^{17}$ g of C which is 60–100% of the estimated total carbon in plants (Whitman et al., 1998).

For many decades since the beginnings of microbiology, research in this field has depended on the isolation and cultivation of individual microorganisms (best illustrated for the area of medical microbiology with the work of Robert Koch on the isolation and characterization of pathogenic bacteria in monocultures, leading to the Koch's postulates) or well-defined syntrophic consortia, and the subsequent in-depth analysis of pure cultures. Until today, microbiological research with traditional cultivation and

subsequent chemical, genetic, and physiological characterization has led to the description of roughly 10^4 bacterial and archaeal species deposited as pure cultures in microbial culture collections. However, in the course of the past 2 decades, it has become more and more evident that the microbial diversity in most aquatic habitats, soil, as well as oceanic and terrestrial subsurfaces is enormous (Amann et al., 1995; Hugenholtz et al., 1998; Quince et al., 2008; Sleator et al., 2008) and that at least at present the majority (>99%) of microorganisms in many complex habitats are not cultivated by using standard techniques (Amann et al., 1995). Thus, it must be acknowledged that the number of described species contrasts by orders of magnitude with estimates about the number of prokaryotic taxa on earth which may amount to 10^6 – 10^8 distinct genospecies (Simon and Daniel, 2010). Metagenomics, a relatively young research discipline, can aid in the detection and acquisition of data about this vast majority of uncultured microorganisms. For this methodology, which importantly is independent of cultivation, the collectivity of genomes (the metagenome) present in a given environmental sample is extracted and subjected to completely random or gene-targeted sequence analysis (Figure 1).

Although bacteria and archaea normally are unicellular, they usually occur as highly diverse but often poorly characterized consortia in their natural habitats. Individual environmental samples can contain a breathtaking prokaryotic diversity. For example, the numbers of different molecular species or operational taxonomic units (OTUs) in soil samples could be over 10^6 (Curtis et al., 2002). Statistics-based predictions for a specific Canadian soil sample range from 2×10^4 to 1.4×10^5 taxa (Quince et al., 2008). Highly interesting, based on taxonomy assessment results from deep-sea hydrothermal vent samples and statistical follow-up considerations, even samples from extreme environments – which often are believed to contain only limited biodiversity due to the extreme conditions – have revealed enormous microbial biodiversity, for example, 3×10^5 bacterial taxa at one specific sample site (Quince et al., 2008). These numbers illustrate the problems one faces to reach near-complete metagenomic datasets for complex natural habitats with today's analysis techniques. Thus, even with high-throughput methods, it is difficult if not impossible to generate a complete microbial inventory of a habitat including also most of the rare taxa. However, the methods available do allow the reliable



Metagenomics, Figure 1 Flowchart summarizing steps of environmental meta-analysis.

identification of the abundant taxa that presumably account for the major biogeochemical conversions.

Uses and applications of metagenome analysis

One major advantage of metagenomic analysis is that one can obtain a wealth of genetic information about organisms that live together in more- or less-complex communities, most of which cannot be cultivated with today's standard techniques. It can be used for extremely diverse purposes, such as the analysis of fundamental aspects of microbial community structure, function, and adaptation in environmental microbiology, but also to explore and exploit the microbial genetic, metabolic, and enzymatic diversity space for the development or improvement of biotechnological processes. Examples for the application of metagenomic techniques in geobiology are the analysis of microbial communities in rock material, sediments, aquifers, as well as in biofilms, on rock surfaces or in subsurface caves and mines, etc. It must be stressed that it is of utmost importance for most metagenomic studies to comprehensively record the sample-associated metadata (the data about the data), like date and position of sampling, physical and chemical characteristics, etc., as has been pointed out by Wooley et al. (2010).

Principally, metagenome analysis can be applied to any kind of habitat, irrespective of the complexity of the microbial communities present. For low-diversity habitats, high-throughput metagenome sequencing can yield a comprehensive overview of genetic information present and can even allow the near-complete or complete assembly of whole prokaryotic genomes, which was nicely shown for the organisms present in acid mine drain fluids (Tyson et al., 2004) or community genome sequencing of an anammox bacterium (Strous et al., 2006). However, the more diverse the community, the higher the sequencing and/or screening effort has to be to arrive at results that cover as comprehensively as possible the target genes of interest present in the sample. In some cases, it is possible to reduce the amount of work and costs by choosing a more specific than a completely random metagenome analysis. For example, if the main interest of a study lies in the analysis of genes involved in a certain metabolic trait (like breakdown and utilization of a specific substrate), one can try to enrich for the corresponding genes by carrying out enrichment cultures wherein the growth of organisms which display this metabolic trait is favored (e.g., by supplying the said specific substrate as the sole carbon source). Enrichment cultivation also has the advantages that (i) more cell material becomes available and that (ii) less organic and inorganic contaminating compounds from the original habitat sample that may interfere with DNA isolation yield and quality are present. An alternative is to enrich for genomic DNA or RNA from microorganisms with a certain substrate utilization capability by feeding a natural microbial community with ^{13}C - or ^{15}N -labeled substrate and then to isolate and process only the heavy nucleic acid that stems from

organisms that utilized the added ^{13}C - or ^{15}N -substrate and incorporated the label into their nucleic acids (stable isotope probing) (Friedrich, 2006; Neufeld et al., 2007). Using enrichment cultures to preselect for microorganisms with certain physiological traits has been successfully done in several cases (e.g., Entcheva et al., 2001) and may be of advantage especially for very complex microbial communities, but it has to be kept in mind that any kind of cultivation step before DNA isolation can result in dramatic changes in community composition and diversity.

Assessment of phylogenetic and functional diversity

Sequence analysis of conserved marker genes, usually 16S rRNA genes, is widely used for community diversity assessment. To this end, 16S rRNA gene sequences can be amplified with PCR directly from metagenomic DNA samples, followed by cloning into appropriate vectors and sequence analysis. Also, the sequences of conserved marker genes (rRNA genes, but also protein-encoding marker genes) can be retrieved from large sequence datasets compiled by large-scale random sequencing of metagenome DNA and then used for phylogenetic community composition assessment (von Mering et al., 2007).

Recently, massively parallel sequencing specifically of rRNA gene fragments amplified from metagenomic DNA has become possible by the use of the 454 Life Sciences (Roche) high-throughput tag pyrosequencing technology (Huse et al., 2008), but due to the fact that read-length limitations presently only allow the sequencing of incomplete rRNA gene fragments, this technology is suited for the trustworthy phylogenetic assignment at the phylum level but is not as reliable at the genus or species level. Nonetheless, this method allows interesting insights into the affiliation of microorganisms from complex communities to the large phylogenetic lineages, and technological improvements pertaining to the read lengths will surely allow more precise analyses at the genus or species level in the near future.

Bioinformatic tools are available for the comprehensive phylogenetic tree calculation from alignments of conserved marker sequences, for example, the ARB software package (Ludwig et al., 2004). Also, other sequence information than merely phylogenetically conserved genes available from large metagenomic random DNA sequence datasets can be used for diversity assessment. In the past years, various software tools have been developed that are useful for the so-called taxonomic binning of such sequence information (see Simon and Daniel, 2010). While some algorithms work on the basis of oligonucleotide sequence frequencies (composition-based binning, e.g., PhyloPhytia, TETRA; Teeling et al., 2004; McHardy et al., 2007), which are known to vary between different genomes, others use comparative orthologous ORF analysis for this purpose (CARMA, MEGAN, Sort-ITEMS; Huson et al., 2007; Krause et al., 2008; Monzoorul et al., 2009). Importantly, however, since most of these

bioinformatic tools depend on genome sequences and annotations from the available reference genome databases, which presently cover only a minority of the (readily) culturable species (not to speak of the enormous uncultured species diversity present in nature [see above]), our present possibilities for the assignment of short metagenomic DNA sequences to microbial taxa are quite limited.

Current large-scale efforts to sequence the genomes of the prokaryotic species deposited in major microbiological culture collections (GEBA, a Genomic Encyclopedia of Bacteria and Archaea; <http://www.jgi.doe.gov/programs/GEBA/>; Wu et al., 2009) will surely dramatically improve the reference genome sequence database necessary for a more accurate phylogenetic assignment of metagenomic sequence fragments. Nevertheless, considering that there are still in some cases not a single, in other cases only very few cultured representatives for some deep-rooting phyla, severe limitations in our ability to reliably assign short metagenomic DNA sequences to phylogenetic taxa will remain at least in the near future.

Screening for genes in metagenomes

Metagenomic studies often include the screening of metagenomic libraries or metagenomic sequence datasets for genes of interest, for example, for genes encoding key enzymes characteristic for habitat-specific physiological traits, or for genes for biotechnologically relevant enzymes. Two fundamentally different approaches are used for this: the sequence-based approach and the function-based approach. Both methods have individual advantages and disadvantages and are explained briefly in the following.

The *sequence-based approach* uses in silico sequence mining, PCR amplification or hybridization methods to detect and isolate genes of interest. Metagenomic sequence datasets can be scored for fragments encoding orthologs of known genes deposited in the available reference databases. Various bioinformatic tools such as BLAST, Pfam, SEED, TIGRFAM, MEX, RAMMCAP, or MG-RAST are available which are helpful for the functional annotation of metagenome sequence data (see Simon and Daniel, 2010; Wooley et al., 2010). Another sequence-driven approach is to design degenerate PCR primers directed against conserved sequence motifs in a gene family of interest and to amplify partial ORFs from metagenomic DNA. The fragments can then be used to isolate the complete ORFs, for example, by using them as hybridization probes to identify clones or metagenomic DNA fragments containing the adjacent, missing ORF sequences, or by metagenome walking (see Simon and Daniel, 2010). Alternatively, DNA microarrays carrying oligonucleotides derived from consensus sequences of known genes have been used for the sequence-directed screening of a metagenomic library for a light, oxygen and voltage (LOV) domain blue light photoreceptor gene (Pathak et al., 2009). The major disadvantage of sequence-based gene detection is that this method depends on prior sequence information from known gene families.

Therefore, truly novel genes unrelated to the already known genes encoding for a given function cannot be identified with this approach.

In the *function-based screening approach*, clones from metagenomic libraries are subjected to activity-based assays that indicate the function of interest. The major advantage of this approach is that it allows the recovery of complete genes or gene clusters and of novel types of enzymes without any prior knowledge of the sequence. However, in order to work efficiently, the genes of interest, which often originated from uncultured microorganisms with unknown genetics and physiology, must be compatible with the expression machinery provided by the heterologous screening host with respect to transcription, translation, protein folding, and eventually secretion (Gabor et al., 2004). Therefore, the fraction of the existing enzymatic diversity that can be accessed via functional screening approaches is strongly influenced by the choice of the host organism used. The phylogenetic distance between the gene donor and the cloning/expression host may be crucial for the success of gene expression. Mostly, *Escherichia coli* is used to construct metagenomic libraries for functional screening, but it is clear that the expression of metagenomic genes can be severely biased in this host. Therefore, it is important to establish and improve an alternative host/vector systems in order to enhance the probability of detecting genes from metagenomic samples (Angelov et al., 2009; Steele et al., 2009; Uchiyama and Miyazaki, 2009).

Some restrictions and problems

Despite the power of metagenomic studies to unravel genetic information from microbes in communities that are not amenable to cultivation and in-depth characterization, a number of restrictions and problems remain, only some of which are listed hereafter.

Huge amounts of data are generated, especially in metagenome random sequencing projects. These data need to be generated, processed, and stored in a complex succession of steps. Special care must be taken with respect to library construction and sequencing quality. Exemplarily, the procedure of a metagenomic sequencing project is described by Kunin et al. (2008). A useful compilation of software tools for composition-based and similarity-based taxonomic binning, functional annotation of metagenomic data, and comparative metagenomics can be found in Wooley et al. (2010).

Interpretation of the sequence data and their connection with biochemical functions of biocatalysts remains a challenging task. The tools for ORF extraction from metagenomic sequence data and bioinformatics-driven function prediction are being improved constantly, but it is of course not feasible to check all gene function predictions experimentally, for example, by heterologous expression and biochemical analysis. Also, if only metagenomic DNA sequence data are available, it is unclear if the genes found are expressed at all. Thus, it has to be kept in mind that in many cases sequence data

obtained from metagenomes only indirectly point to (predicted) enzymes/pathways of the organisms in the sample. In future, performing metatranscriptomics and/or metaproteomics experiments in parallel with metagenomics can help to overcome this problem.

Also, linking phylogenetic and functional metagenomic data with the physiology of living microorganisms in the environmental sample is a crucial but not a trivial step for many studies attempting to correlate environmental data with a microbial community. Due to the species complexity in most microbial communities, it is only rarely possible to obtain assembled genomes. Even if conserved marker sequences point to close relatives of certain well-known microorganisms this does not necessarily prove that the organisms from which the metagenomic sequence information was derived have identical physiologies as the reference organisms. Also, some traits believed to be characteristic for certain phylogenetic groups of microorganisms can get lost by simple mutations. Still, it is possible to extract environment-specific gene fingerprints from unassembled random environmental DNA sequencing data that reflect known characteristics of the sampled environments, thus linking metagenomic sequence information with metabolic functions of the organisms present (Tringe et al., 2005).

Finally, since most conclusions reached from metagenome data analysis rely on comparative sequence analysis and thus are indirect, it will be important to develop and improve new microbiological cultivation and characterization techniques that also allow the cultivation and in vivo analysis of physiologically and quantitatively dominant microbial representatives within the studied communities.

Summary

Considering the enormous microbial diversity in most environmental samples on one hand and the current shortcoming of the microbiologists' ability to cultivate the vast majority of these organisms on the other hand, it makes sense that metagenomics has become one of the key methods to study the composition and functions of complex microbial communities. The rapid advance of massively parallel sequencing and high-throughput screening technologies will enable even faster and cheaper access to environmental sequence datasets in the future, but connecting the metagenomic data with the physiology of the microorganisms and the environmental data remains challenging, and it will be crucial that the development of experimental setups and bioinformatic tools keep pace in order to be able to derive meaningful conclusions from metagenomic sequence data.

Bibliography

- Amann, R. I., Ludwig, W., and Schleifer, K. H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Angelov, A., Mientus, M., Liebl, S., and Liebl, W., 2009. A two-host fosmid system for functional screening of (meta)genomic

- libraries from extreme thermophiles. *Systematic and Applied Microbiology*, **32**, 177–185.
- Curtis, T. P., Sloan, W. T., and Scannell J. W., 2002. Estimating prokaryotic diversity and its limits. *Proceedings of the National Academy of Science USA*, **99**, 10494–10499.
- Entcheva, P., Liebl, W., Johann, A., Hartsch, T., and Streit, W., 2001. Direct cloning from enrichment cultures, a reliable strategy for isolation of complete operons and genes from microbial consortia. *Applied and Environmental Microbiology*, **67**, 89–99.
- Friedrich, M. W., 2006. Stable-isotope probing of DNA: insights into the function of uncultivated microorganisms from isotopically labeled metagenomes. *Current Opinion in Biotechnology*, **17**, 59–66.
- Gabor, E. M., Alkema, W. B., and Janssen, D. B., 2004. Quantifying the accessibility of the metagenome by random expression cloning techniques. *Environmental Microbiology*, **6**, 879–886.
- Hugenholtz, P., Goebel, B. M., and Pace, N. R., 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology*, **180**, 4765–4774.
- Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A., and Sogin, M. L., 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, **4**, e1000255.
- Huson, D. H., Auch, A. F., Qi, J., and Schuster, S. C., 2007. MEGAN analysis of metagenomic data. *Genome Research*, **17**, 377–386.
- Krause, L., Diaz, N. N., Goesmann, A., Kelley, S., Nattkemper, T. W., Rohwer, F., Edwards, R. A., and Stoye, J., 2008. Phylogenetic classification of short environmental DNA fragments. *Nucleic Acids Research*, **36**, 2230–2239.
- Kunin, V., Copeland, A., Lapidus, A., Mavromatis, K., and Hugenholtz, P., 2008. A bioinformaticians guide to metagenomics. *Microbiology and Molecular Biology Reviews*, **72**, 557–578.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K.-H., 2004. ARB: a software environment for sequence data. *Nucleic Acids Research*, **32**(4), 1363–1371.
- McHardy, A. C., Martin, H. G., Tsirigos, A., Hugenholtz, P., and Rigoutsos, I., 2007. Accurate phylogenetic classification of variable-length DNA fragments. *Nature Methods*, **4**, 63–72.
- Neufeld, J. D., Vohra, J., Dumont, M. G., Lueders, T., Manefield, M., Friedrich, M. W., and Murrel, J. C., 2007. DNA stable-isotope probing. *Nature Protocols*, **2**, 860–866.
- Pathak, G. P., Ehrenreich, A., Losi, A., Streit, W. R., and Gärtner, W., 2009. Novel blue light-sensitive proteins from a metagenomic approach. *Environmental Microbiology*, **11**, 2388–2399.
- Simon, C., and Daniel, R., 2010. Achievements and new knowledge unraveled by metagenomic approaches. *Applied Microbiology and Biotechnology*, **85**, 265–276.
- Sleator, R. D., Shortall, C., and Hill, C., 2008. Metagenomics. *Letters in Applied Microbiology*, **47**, 361–366.
- Steele, H. L., Jaeger, K.-E., Daniel, R., and Streit, W. R., 2009. Advances in recovery of novel biocatalysts from metagenomes. *Journal of Molecular Microbiology and Biotechnology*, **16**, 25–37.
- Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M. W., Horn, M., Daims, H., Bartol-Mavel, D., Wincker, P., Barbe, V., Fonknechten, N., Vallenet, D., Segurens, B., Schenowitz-Truong, C., Médigue, C., Collingro, A., Snel, B., Dutilh, B. E., Op den Camp, H. J., van der Drift, C., Cirpus, I., van de Pas-Schoonen, K. T., Harhangi, H. R., van Niftrik, L., Schmid, M., Keltjens, J., van de Vossenberg, J., Kartal, B., Meier, H., Frishman, D., Huynen, M. A., Mewes, H. W., Weissenbach, J., Jetten, M. S., Wagner, M., and Le Paslier, D.,

2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature*, **440**, 790–794.
- Teeling, H., Waldmann, J., Lombardot, T., Bauer, M., and Glöckner, F. O., 2004. TETRA: a web-service and a stand-alone program for the analysis and comparison of tetranucleotide usage patterns in DNA sequences. *BMC Bioinformatics*, **5**, 163.
- Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., Detter, J. C., Bork, P., Hugenholtz, P., and Rubin, E. M., 2005. Comparative metagenomics of microbial communities. *Science*, **308**, 554–557.
- Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., and Banfield, J. F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, **428**, 37–43.
- Uchiyama, T., and Miyazaki, K., 2009. Functional metagenomics for enzyme discovery: challenges to efficient screening. *Current Opinion in Biotechnology*, **20**, 616–622.
- von Mering, C., Hugenholtz, P., Raes, J., Tringe, S. G., Doerks, T., Jensen, L. J., Ward, N., and Bork, P., 2007. Quantitative phylogenetic assessment of microbial communities in diverse environments. *Science*, **315**, 1126–1130.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceeding of the National Academy of Science USA*, **95**, 6578–6583.
- Wooley, J. C., Godzik, A., and Friedberg, I., 2010. A primer on metagenomics. *PLoS Computational Biology*, **6**, e1000667.
- Wu, D., Hugenholtz, P., Mavromatis, K., Pukall, R., Dalin, E., Ivanova, N. N., Kunin, V., Goodwin, L., Wu, M., Tindall, B. J., Hooper, S. D., Pati, A., Lykidis, A., Spring, S., Anderson, I. J., D'haeseleer, P., Zemla, A., Singer, M., Lapidus, A., Nolan, M., Copeland, A., Han, C., Chen, F., Cheng, J.-F., Lucas, S., Kerfeld, C., Lang, E., Gronow, S., Chain, P., Bruce, D., Rubin, E. D., Kyrpides, N. C., Klenk, H.-P., and Eisen, J. A., 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. *Nature*, **462**, 1056–1060.

Cross-references

Archaea
Bacteria
Biofilms
Endosymbiosis
Exoenzymes
Symbiosis
Terrestrial Deep Biosphere

METALLOENZYMES

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms

Metalloprotein (broader term)

Definition

Enzyme protein containing one or more metal cofactor(s).

Introduction

Metalloenzymes are enzyme proteins containing metal ions (metal cofactors), which are directly bound to the

protein or to enzyme-bound nonprotein components (prosthetic groups). About one-third of all enzymes known so far are metalloenzymes (see Holm et al., 1996 for a general overview). Besides enzymes, other metalloproteins are involved in non-enzyme electron transfer reactions (cytochromes), may act as storage (e.g., ferritin for iron) or transport proteins (e.g., transferrin for iron). In the latter groups of proteins, the metal storage is reversible and the metal is a temporary component. Also ribozymes, i.e., RNA molecules with enzyme function may contain structurally and/or functionally important metal ions (mostly divalent metal ions such as Mg^{2+}) and may be therefore termed as metalloenzymes in a broader sense (Sigel and Pyle, 2007).

Though primarily focused on enzyme proteins, i.e., proteins acting as biocatalysts in biochemical reactions, also important non-enzyme proteins (without catalytic activity in the strict sense) and their metal centers are discussed in this article.

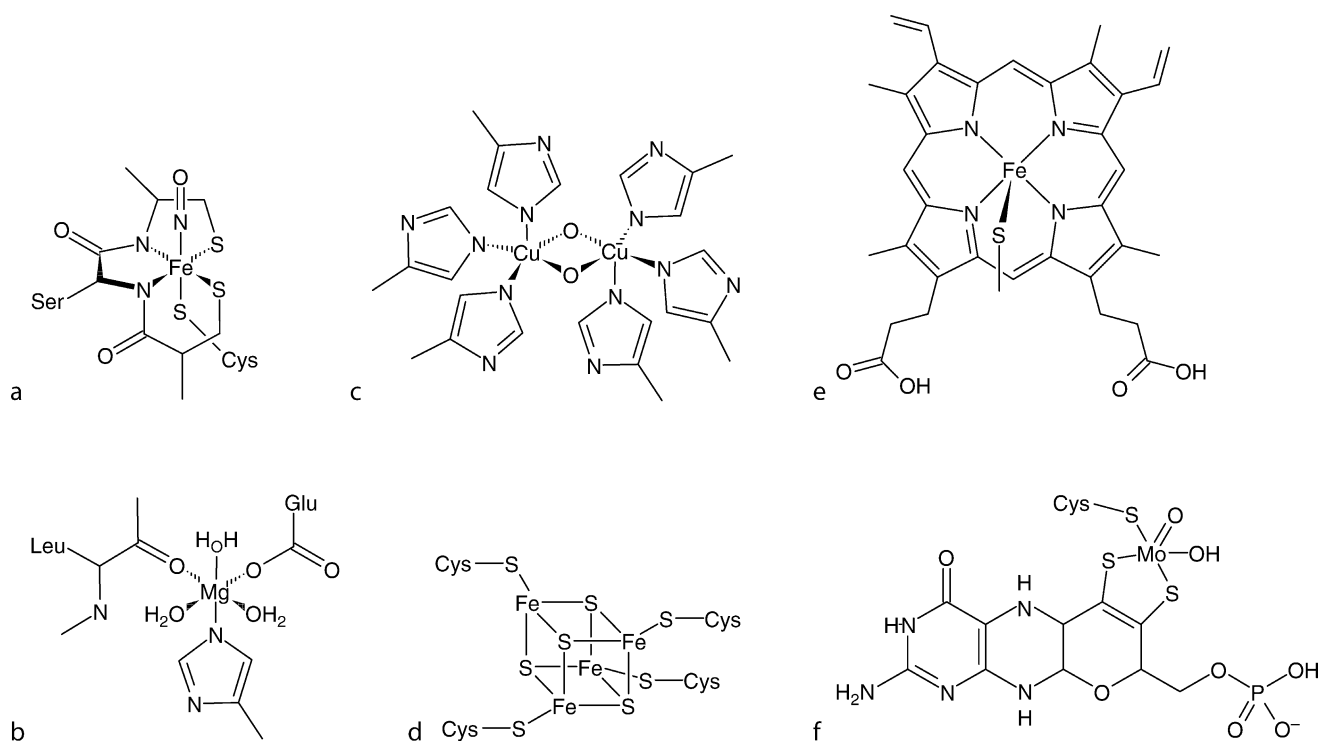
In enzyme catalysis, transition metals are of special relevance due to their redox activity, and/or a high charge density, which allows the polarization of enzyme substrates. Vanadium, molybdenum, tungsten, manganese, iron, cobalt, nickel, and copper metal centers have been characterized so far; the metal atoms can occur in mono-, di-, and multinuclear centers with nitrogen, oxygen, and sulfur atoms of the polypeptide chain as possible ligands (Holm et al., 1996; Degtyarenko, 2000; Wilson et al., 2004; Table 1). For analysis of metal centers in proteins, spectroscopic tools for the investigation of small molecules are of relevance, namely electron paramagnetic resonance (EPR) or the related electron-nuclear double resonance (ENDOR) spectroscopy, electron spin resonance (ESR), resonance Raman or Mössbauer spectroscopy. Data obtained from X-ray protein crystallography are inevitable to locate the metal center and its ligands inside a protein molecule. Combination of proteomic approaches with technologies for elemental trace analysis (inductively coupled plasma mass spectrometry or ICP-MS) introduced tools for determination of the entire set of metalloproteins (the metalloproteome) and/or all trace metals (metallome) in an organism (Szpunar, 2005; Barton et al., 2007).

Metalloenzymes in early evolution

In early evolution, precursors of metal enzymes may have played a key role in catalytic reactions relevant for the synthesis of prebiotic macromolecules. According to the hypotheses originally postulated by Wächtershäuser, surface-bound iron, cobalt, nickel, and other transition metal centers with sulphido, carbonyl, and other ligands were catalysts involved in the synthesis of macromolecular compounds by carbon fixation, thus promoting growth of organic “superstructures” (Wächtershäuser, 1990). The metabolic type of this prebiotic scenario was chemolithoautotrophic, driven by the reducing potential of the outflow from deep-sea hydrothermal vents during

Metalloenzymes, Table 1 Abundance of some metals in bacterial and algal organisms in g/100 g (dry weight) and some important key functions in enzymes (Barton et al., 2007)

Element	Algae	Bacteria	Some specific roles in enzymes
K	1.81	1.7	Protein stabilization
Ca	0.55	0.1	Protein stabilization
Mg	0.35	0.25	Protein stabilization
Fe	0.011	0.015	O ₂ transport, storage and/or activation; electron transport; superoxide breakdown
Mn	0.006	0.005	O ₂ evolution; peroxide and superoxide breakdown
Zn	0.001	0.005	Protein stabilization; hydrolytic cleavage
Cu	0.0007	0.001	O ₂ transport, storage and/or activation; electron transport; superoxide breakdown
Mo	0.0001	0.001	Oxygen transfer; N ₂ activation
Co	0.0003	0.001	Free radical reactions; nucleophil

**Metalloenzymes, Figure 1** Various metal centers (from Degtyarenko, 2000) (a) mononuclear iron center (nitrile hydratase), (b) mononuclear magnesium center (Ni-Fe-hydrogenase from *Desulfovibrio*), (c) dinuclear copper center (oxyhemocyanin), (d) polynuclear iron-sulfur center, (e) heme iron coordination (cytochrome P450), and (f) molybdopterin (sulfite oxidase).

the Hadean period. Namely, the acetyl-CoA biosynthetic pathway may be seen as a model for prebiotic energy metabolism, since in this pathway enzymes and other proteins with FeS and (Fe,Ni)S centers are involved (acetyl CoA-synthetase/carbon monoxide dehydrogenase, hydrogenases, ferredoxin); these metal centers are in principle the cubic Fe₄S₄ as well as (SNiS)(Fe₄S₄)(SFeS) units of greigite (Fe(II)Fe(III)₂S₄). Later on in evolution, the prebiotic metal centers became parts of peptides and, finally, defined proteins (Wächtershäuser, 1990; Russell and Martin, 2004). Among all metalloproteins, iron metalloproteins and, within this class, iron-sulfur proteins

are supposed to be the most important group and ubiquitous in organisms.

Besides FeS-centers, tetrapyrroles as metal-chelating prosthetic groups are interesting from the evolutionary viewpoint. Tetrapyrroles are as cyclic molecules (corrins and porphyrins; e.g., Figure 1e) ideal chelators of cobalt, copper, iron, manganese, and nickel. At the same time, the highly conjugated porphyrin molecules increase the catalytic activities of transition metal ions in electron transfer reactions by several orders of magnitude. Since porphyrins are present in all kingdoms of life, they must have evolved early in evolution, perhaps even in the

prebiotic state. As for FeS centers, the prosthetic groups are found in otherwise completely unrelated metalloproteins, such as methyl-coenzyme M reductase and chlorophylls (see below; Mauzerall, 1998).

Until the Paleozoic era, availability of bioessential transition metals changed by several orders of magnitude. Whereas Cu, Zn, Ni, and Mo concentrations increased in the ocean water, Co, Mn, and particularly iron decreased by several orders of magnitude during the Proterozoic eon (Anbar, 2008). These changes must have had an impact on the biogenic production of greenhouse gases such as nitrous oxide (Cu-dependant) or methane (Ni-dependant) and, hence, respective impact on global climate. The molybdenum cofactor (see below), in particular, plays a key role in the biological nitrogen cycle. It may even be speculated that molybdenum availability in oceans had a tremendous impact on early evolution. Under the reducing atmosphere, molybdenum was depleted in the proterozoic ocean, generating a “bottleneck” for the nitrogen cycle. The low availability of nitrogen must have had a retarding effect of total biomass production of all organisms (especially non-nitrogen-fixing eukaryotes), until the oxidizing atmosphere also provided high molybdenum availability and stopped nitrogen limitation (Zerkle et al., 2006; Anbar, 2008). Limited molybdenum availability may also have been overcome in part by (less effective) molybdenum-independent nitrogenases. The evolutionary history of the enzymes is, however, difficult to reconstruct. There is no phylogenetic evidence that these “alternative” nitrogenases preceeded the molybdenum-dependant enzymes (Raymond et al., 2004).

In recent oceans, though, most metal ions relevant for enzyme metal centers are just present in trace amounts in seawater, and/or form hardly soluble salts. Thus, most organisms utilize specialized chelating compounds, uptake and transport systems for metal ion intake.

Biomimetic metalloenzymes

Metal centers from enzyme proteins may give essential data for the design of biomimetic enzymes in bioorganic synthesis. Biomimetic metal centers from enzymes such as cytochrome oxidases (with Fe-porphyrin), catechol oxidase (based on Mn(IV)-complexes with *o*-aminophenol ligands), or hydrogenase (based, e.g., on six-coordinate Ni-Ru-centers with hydrogen as one of the bridging ligands for NiFe hydrogenase) reveal the biotechnological potential of catalysts derived from biological metal enzymes (Zbaida and Kariv, 1989; Chaudhuri et al., 2005; Song et al., 2005; Ogo et al., 2007).

Classification of metalloenzymes

Since metalloenzymes are found in all enzymes families, simple classification of metal-containing subgroups based on sequence similarities cannot be applied. Reasonable classification schemes are based upon the bioinorganic motifs (Degtyarenko, 2000; Figure 1), i.e., the metal atom and its ligands (Degtyarenko and Contrino, 2004).

Metal centers may be classified by the number of bound metal atoms as mononuclear (for one metal atom, Figure 1a and b) or polynuclear (more than one metal atom, Figure 1c and d) with endogenous ligands (i.e., the polypeptides of the enzyme molecule form a mostly polydentate coordination shell) or exogenous ligands (non-protein component of a conjugated protein such as heme or molybdopterin; Figure 1e and f).

The description given below is, for the sake of simplicity, based on the central atoms. It has to be kept in mind that different metal atoms can be bound to identical or similar bioorganic motifs (e.g., cobalt, copper, iron, magnesium, and nickel by porphyrins or corrins), and different motifs coordinate the same type of metal atom (e.g., either molybdopterin or Fe₇MoS₉ homocitrate cofactor for molybdenum; see below).

Metal centers in metalloenzymes and other metal proteins

Mononuclear iron proteins are oxidoreductases (e.g., aromatic amino acid hydroxylases, aromatic ring cleavage dioxygenases, Fe-superoxide dismutase, lipooxygenases, nitrile hydratase, and Rieske oxygenases), or involved in electron transfer (desulfiredoxins, rubredoxins, and photosynthetic reaction centers). The amino acid ligands are mostly histidine and/or cysteine such as in nitrile hydratase with three cysteine residues and two amide nitrogens (Figure 1a). *Diiron carboxylate proteins* bind a coupled binuclear iron center using, e.g., four carboxylate and two histidine residues. Oxidoreductases (alkene hydroxylase, methane monooxygenase, and phenol hydroxylase), iron storage (bacterioferritin, ferritin), and oxygen storage/transport proteins are members of this group (hemerythrin, myohemerythrin).

Hemoproteins are characterized by an iron porphyrin as prosthetic group (Figure 1e). While four of the iron ligands are occupied by the porphyrin ring, the other two are used for binding of the protein. Hemoproteins are oxidoreductases (catalases, peroxidases), electron transfer proteins (cytochromes), or oxygen transport and storage proteins (globins) (Nordlund and Eklund, 1995; Que and Ho, 1996).

Iron-sulfur proteins are characterized by the presence of iron-sulfur clusters containing sulfide-linked di-, tri-, and tetrairon centers in variable oxidation states (Figure 1d). Iron-sulfur clusters are found in ferredoxins, NADH hydrogenases, dehydrogenases, cytochrome c reductases, nitrogenases, and other proteins. Proteins with iron-sulfur clusters have key roles in nearly all respiratory chains, either in bacteria and archaea or in mitochondria of eukaryotic organisms. Other functions include catalysis of stereo-specific isomerization of citrate to isocitrate (aconitase) in the tricarboxylic acid cycle or the redox-dependent gene regulation. The simplest iron-sulfur cluster type consists of two iron ions bridged by two sulfide ions and is coordinated by four cysteinyl ligands or by two cysteines and two histidins. The oxidized proteins

contain two Fe^{3+} ions, whereas the reduced proteins contain one Fe^{3+} and one Fe^{2+} ion. These species exist in two oxidation states, Fe(III)_2 and Fe(III)Fe(II) . Besides $[\text{2Fe-2S}]$, $[\text{3Fe-4S}]$, $[\text{4Fe-4S}]$, or $[\text{8Fe-7S}]$ clusters are known (Bertini et al., 1995).

Also in *copper-containing proteins*, several metal center types occur. Similar to iron proteins, copper proteins are involved in oxygen transport or activation processes and electron transport. *Type I copper centers* have a single copper atom coordinated by two histidine residues and a cysteine residue in a trigonal planar structure and a variable axial amino acid ligand. Type I proteins are, e.g., ascorbate oxidase, ceruloplasmin, laccase, nitrite reductase, auracyanin, and azurin. *Type II copper centers* are square planar coordinated by N or N/O ligands (e.g., in galactose oxidase). *Type III centers* consist of two copper atoms (Figure 1c), each coordinated by three histidine residues (catechol oxidase, hemocyanins, tyrosinase). The *copper A center* in cytochrome c-oxidase consists of two copper atoms, coordinated by two histidine imidazole residues, methionine, carbonyl-oxygen, and two cysteine-S bridging the copper atoms. In cytochrome oxidase *copper B centers*, the copper atom is coordinated by three histidine imidazole residues. The *four copper atom center* of nitrous oxide oxidase is coordinated by seven histidine residues. The four centers are bridged by one sulfur atom. Besides cytochrome oxidase, also other copper proteins are frequently oxygenases or dioxygenases (monoamine-oxidase, laccase, caeruloplasmin, superoxide-dismutase, hemocyanine) (Adman, 1991).

Zinc proteins represent the third large group of metalloproteins, with one, two, or three zinc ions. Zinc ions in proteins are classified as catalytic, co-catalytic, and structural, where zinc is either directly involved in the opening or closing a chemical bond, or zinc acts in the catalytic reaction in concert with other metal ions, or zinc ions maintain structural integrity of the enzymes. Zinc ions in catalytic sites are generally coordinated to the side chains of three amino acid residues and a water molecule. In structural zinc sites, the ion is coordinated by four amino acid side chains in a tetrahedral symmetry. With a filled *d* orbital, zinc ions do not participate in redox reactions, but rather function as Lewis acids to accept a pair of electrons, which is favorable for reactions like proteolysis or the hydration of carbon dioxide. Important zinc-containing enzymes are the alcohol dehydrogenase (containing both a catalytic and a structural zinc site), metalloproteases, and carbonic anhydrases with catalytic zinc sites. Cocatalytic zinc sites are found in superoxide dismutases, phosphatases aminopeptidases, and β -lactamases. In alcohol dehydrogenases, the zinc catalytic site is responsible for binding of the hydroxyl group oxygen from the alcohol substrate by replacing the zinc-bound water molecule (Lipscomb and Sträter, 1996; McCall et al., 2000).

Nickel in metalloenzymes occurs as Ni-4Fe-5S (e.g., nickel-containing CO dehydrogenases), coordinated in a *tetrapyrrole complex* (e.g., in methyl-coenzyme

M reductase with glutamine as axial ligand) and as *dinuclear NiNi or NiFe complexes* (e.g., in hydrogenases and urease) or a $[\text{4Fe-4S}]^{2+}$ cluster bridged by a cysteine thiolate to a dinuclear NiNi center (acetyl-CoA synthase). Nickel-containing CO-dehydrogenases occur in anaerobic bacteria and archaea (CO-dehydrogenases from aerobic bacteria are molybdopterine enzymes), whereby CO is bound by the nickel atom and hydroxyl oxygen (from water) is transferred to the bound carbon atom. After CO_2 is released, nickel is reduced ($\text{Ni}^{2+} \rightarrow \text{Ni}^+$) and transfers two electrons to ferredoxin. In the methyl-coenzyme M-reductase reaction, methane and a heterodisulfide are generated from the substrate methyl coenzyme M that reacts with coenzyme B (7-thioheptanoylthreoninephosphate). In nickel-iron hydrogenases, nickel of the Ni-Fe center is coordinated by one apical and three equatorial S-cysteins. The Ni-Fe hydrogenases catalyze both H_2 evolution and uptake in vitro, with cytochromes acting as either electron donors or acceptors. Acetyl-CoA synthase catalyzes the reversible formation of acetyl-CoA from carbon monoxide, a methylated corrinoid protein and coenzyme A, and is complexed with a nickel CO dehydrogenase (Ermler et al., 1998).

Manganese in metalloenzymes may also act as a simple Lewis acid catalyst. Manganese (normally Mn(II)) can also be oxidized to the (III)- or (IV)-state in biological systems, which allows redox-cycling reactions. *Manganese metal centers* may be mono-, bi-, tri-, or tetranuclear. Mn-superoxide dismutases (SODs) exhibit *mononuclear centers* and are antioxidant metalloenzymes catalyzing the redox disproportionation of the superoxide radical, O_2^- . SOD decomposes the highly reactive superoxide anion by a two-step disproportionation reaction which generates oxygen hydrogen peroxide. The active site manganese is five-coordinate, with the metal ligands arranged in distorted trigonal bipyramidal geometry. Two known enzymes with *binuclear centers* are arginase and manganese catalase. Arginase catalyzes the conversion of arginine to urea and ornithine. The manganese ions are five- and six-coordinate, respectively, with square-pyramidal and (distorted) octahedral coordination symmetries. The metal centers are interconnected by a solvent-derived hydroxide and carboxylate residues. This metal-bridging hydroxide acts as nucleophile that attacks the arginine guanidinium carbon. Other binuclear manganese enzymes are aminopeptidase P from *Escherichia coli* and the dinitrogenreductase-activating glycohydrolase from *Rhodospirillum rubrum*.

One of the most important biochemical catalytic reactions in our biosphere, the oxygen-generating photolysis of water, is brought about by the *tetranuclear manganese cluster* in oxygenic photosynthesis of cyanobacteria, eukaryotic algae, and higher plants. In oxygenic photosynthesis, water is oxidized by a cluster of Mn ions. Light is captured by antenna chlorophyll and funneled to the primary reaction center, which contains chlorophyll (P680). The oxidized P680 first oxidizes a redox-active tyrosine. Subsequent oxidation of the Mn cluster occurs via this

generated tyrosine radical. The enzyme can exist in five oxidation levels, corresponding to various oxidation states of the cluster (Wiegardt, 1994; Yocum and Pecoraro, 1999; Carrell et al., 2002).

Molybdenum and *tungsten* enzymes catalyze basic metabolic reactions in the nitrogen, sulfur, and carbon cycles. With the exception of the nitrogenase cofactor, molybdenum is associated with the heterocyclic pterin derivative (molybdopterin) that contains a mononucleate center, coordinated to the thiols of the cofactor (Figure 1f). These *molybdenum-cofactor-containing (MoCo) enzymes* catalyze the transfer of an oxygen atom, derived from or incorporated into water, to or from a substrate in a two-electron redox reaction. Three classes of MoCo enzymes are now distinguished, represented by xanthine oxidase and sulfite oxidase families (with single pterin ligands and either ligand-Mo(VI)OS(OH) or ligand-Mo(VI)O₂(S-cys) cores), and DMSO reductase family (with two pterin ligands). The *nitrogenase molybdenum metal center* is not related to the pterin derivatives. In molybdenum nitrogenases, each Mo forms a part of a polynuclear cluster containing 1 Mo, 7 Fe, and 9 S organized into an elongated structure. Within the protein the terminal Fe of the cluster is coordinated by a cysteine residue, while the Mo is coordinated by a histidine residue and a homocitrate ligand.

Tungsten, which lies in the chromium group of the periodic system immediately below molybdenum exhibits similar chemical properties and is complexed in a similar way to the pterin cofactors as described for molybdenum. Due to the higher sensitivity of tungsten toward high redox potential, tungsten enzymes are found in (thermophilic) anaerobic bacterial and archaeal microorganisms (Hille, 2002; Williams and Fausto da Silva, 2002).

Cobalt is the central metal ion in the tertrapyrrol *corrin ring* in the coenzyme B₁₂. Similar to porphyrin rings, four coordination sites are provided by the corrin ring. The B₁₂-bearing enzymes catalyze either the transfer of methyl groups between two molecules or isomerization reactions, i.e., rearrangements of hydrogen atoms with concomitant exchange of a substituent (e.g., an hydroxyl group) between two adjacent carbon atoms in a molecule. In *non-corrin cobalt enzymes*, cobalt is complexed by aminoacids such as in nitrile hydratase (catalyzing the hydration of nitriles to amides; Kobayashi and Shimizu, 1999).

Vanadium occurs as V(III), V(IV), or V(V) in biological systems. The respective enzymes catalyze the two-electron oxidation of a halide by hydrogen peroxide. Besides the vanadium-containing enzymes, also heme-containing haloperoxidases and metal-free enzymes are known. In tunicates (ascidians) vanabin binds 20 V(IV) ions. However, the role of vanabins in vanadium accumulation by the ascidian has not yet been determined. In vanadium nitrogenases, vanadium, instead of molybdenum is present in a FeVCo-center (Michibata et al., 2003; Crans et al., 2004).

Though frequently not classified as metalloenzymes in the strict sense, numerous (enzyme) *proteins bind Na⁺ and*

K⁺ ions. The structures of Na⁺ and K⁺-complexes with proteins have a variety of coordination schemes and functions. Usual coordination numbers are five and six. Though the ions are not directly involved in catalytic steps, they activate numerous enzymes. Prominent examples are Na⁺-activated β -galactosidase or the K⁺-dependent activity of pyruvate-kinase and glycerol dehydratases (Page and Di Cera, 2006).

Specific *binding of calcium ions* frequently leads to conformational changes and increased protein stability. One large family of calcium-binding proteins is denoted *EF-hand proteins*, comprising a calcium-binding structural domain ("helix-turn-helix" motif). In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration, by atoms of the protein backbone and side chains especially of aspartate and glutamate. Examples for EF-hand proteins are calmodulin and parvalbumin. In *non-EF-hand calcium-binding proteins*, the binding motifs are not conserved, such as α -lactalbumin and thermitase (Yang et al., 2002).

Also *magnesium* has a key function in activation and stabilization of enzymes. Isocitrate lyase and glutamine synthetase are two examples of Mg²⁺-activated metabolic enzymes. Enzymes catalyzing phosphorylation of proteins typically use magnesium chelates of ATP as cosubstrate. The bound Mg²⁺ serves to facilitate nucleophilic attack at the γ -phosphate of the ATP substrate. Phosphate and phosphoryl transfer reactions require Mg²⁺ as an essential cofactor. In chlorophyll molecules of photosynthetic reaction centers, magnesium ions are coordinated in a terrapyrrole ring system and to an axial *N*-histidin, *O*-aspartate, *O*-formyl-methionin, *O*-leucin, or water as ligands (Figure 1b; Cowan, 2002).

Conclusion

Since metal centers are present in all enzyme families, and enzymes with metal centers also catalyze a wide variety of different reactions, metal enzymes cannot be assigned to a special group of enzymes or proteins. Thus it is very likely that metal centers developed early in evolution and evolved in several different lineages. Since free transition metal ions are ideal catalysts for numerous reactions in organic chemistry (and biochemistry) at moderate temperatures, they appear to be the natural "precursors" of functional enzyme proteins. Peptides and proteins are, on the other hand, ideal chelators for metal ions. Thus, protein and metal partners may have easily come together frequently during evolution.

Bibliography

- Adman, E. T., 1991. Copper protein structures. *Advances in Protein Chemistry*, **42**, 145–197.
- Anbar, A. D., 2008. Oceans. Elements and evolution. *Science*, **322**, 1481–1483.
- Barton, L. L., Goulhen, F., Bruschi, M., Woodards, N. A., Plunkett, R. M., and Rietmeijer, F. J. M., 2007. The bacterial metallome: composition and stability with specific reference to the anaerobic bacterium *Desulfovibrio desulfuricans*. *BioMetals*, **20**, 291–302.

- Bertini, I., Ciurli, S., and Luchinat, C., 1995. The electronic structure of FeS centers in proteins and models. A contribution to the understanding of their electron transfer properties. *Structure and Bonding*, **83**, 1–53.
- Carrell, T. G., Tyryshkin, A. M., and Dismukes, G. C., 2002. An evaluation of structural models for the photosynthetic water-oxidizing complex derived from spectroscopic and X-ray diffraction structures. *Journal of Biological Inorganic Chemistry*, **7**, 2–22.
- Chaudhuri, P., Wieghardt, K., Weyhermüller, T., Paine, T. K., Mukherjee, S., and Mukherjee, C., 2005. Biomimetic metal-radical reactivity: aerial oxidation of alcohols, amines, aminophenols and catechols catalyzed by transition metal complexes. *Biological Chemistry*, **386**, 1023–1033.
- Cowan, J. A., 2002. Structural and catalytic chemistry of magnesium-dependent enzymes. *BioMetals*, **15**, 225–235.
- Crans, D. C., Smee, J. J., Galdamauskas, E., and Yang, L., 2004. Chemistry and biology of vanadium and the biological activities exerted by vanadium compounds. *Chemistry Reviews*, **104**, 849–902.
- Degtyarenko, K., 2000. Bioorganic motifs: towards functional classification of metalloproteins. *Bioinformatics Review*, **16**, 851–864.
- Degtyarenko, K., and Contrino, S., 2004. COMe: the ontology of bioinorganic proteins. *BMC Structural Biology*, **4**, 3.
- Ermler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer R. K., 1998. Active sites of transition-metal enzymes with a focus on nickel. *Current Opinon Structural Biology*, **8**, 749–758.
- Hille, R., 2002. Molybdenum and tungsten in biology. *Trends in Biochemical Sciences*, **27**, 360–367.
- Holm, R., Kennepohl, P., and Solomon, E. I., 1996. Structural and functional aspects of metal sites in biology. *Chemistry Reviews*, **96**, 2239–2314.
- Kobayashi, M., and Shimizu, S., 1999. Cobalt proteins. *European Journal of Biochemistry*, **261**, 1–9.
- Lipscomb, W. N., and Sträter, N., 1996. Recent advances in zinc enzymology. *Chemistry Reviews*, **96**, 2375–2433.
- Mauzerall, D. C., 1998. Evolution of porphyrins. *Clinics Dermatology*, **16**, 195–201.
- McCall, K. A., Huang, C.-C., and Fierke, C. A., 2000. Function and mechanism of zinc metalloenzymes. *Journal of Nutrition*, **130**, 1437S–1446S.
- Michibata, H., Yamaguchi, N., Uyama, T., and Ueki, T., 2003. Molecular biological approaches to the accumulation and reduction of vanadium by ascidians. *Coordination Chemistry Reviews*, **237**, 41–51.
- Nordlund, P., and Eklund, H., 1995. Diiron-carboxylate proteins. *Current Opinion in Structural Biology*, **5**, 758–766.
- Ogo, S., Kabe, R., Uehara, K., Kure, B., Nishimura, T., Menon, S. C., Harada, R., Fukuzumi, S., Higuchi, Y., Ohhara, T., Tamada, T., and Kuroki, R., 2007. A dinuclear Ni(μ -H)Ru complex derived from H₂. *Science*, **316**, 585–587.
- Page, M. J., and Di Cera, E., 2006. Role of Na⁺ and K⁺ in enzyme function. *Physiological Reviews*, **86**, 1049–1092.
- Que, L. Jr., and Ho, R. Y. N., 1996. Dioxygen activation by enzymes with mononuclear nonheme iron active sites. *Chemistry Reviews*, **96**, 2607–2624.
- Raymond, J. R., Siefert, J. L., Staples, C. R., and Blankenship, R. E., 2004. The natural history of nitrogen fixation. *Molecular Biology and Evolution*, **21**, 541–554.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Sigel, R. K. O., and Pyle, A. M., 2007. Alternative roles for metal ions in enzyme catalysis and the implications for ribozyme chemistry. *Chemistry Reviews*, **107**, 97–113.
- Song, L.-C., Yang, Z.-Y., Bian, H.-Z., Liu, Y., Wang, H.-T., Liu, X.-F., and Hu, Q.-M., 2005. Diiron oxadithiolate type models for the active site of iron-only hydrogenases and biomimetic hydrogen evolution catalyzed by Fe₂(μ -SCH₂OCH₂S- μ)(CO)₂. *Organometallics*, **24**, 6126–6135.
- Szpunar, J., 2005. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst*, **130**, 442–465.
- Wächtershäuser, G., 1990. Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 200–204.
- Wieghardt, K., 1994. A structural model for the water-oxidizing manganese cluster in photosystem II. *Angewandte Chemie International Edition in English*, **33**, 725–728.
- Williams, R. J. P., and Frausto da Silva, J. J. P., 2002. The involvement of molybdenum in life. *Biochemical and Biophysical Research Communications*, **292**, 293–299.
- Wilson, C. J., Apiyo, D., and Wittung-Stafshede, P., 2004. Role of cofactors in metalloprotein folding. *Quarterly Review of Biophysics*, **37**, 285–314.
- Yang, W., Hsiao-Wei, L., Hellings, H., and Yang, J. D., 2002. Structural analysis, identification, and design of calcium-binding sites in proteins. *Proteins Structure, Function, and Genetics*, **47**, 344–356.
- Yocum, C. F., and Pecoraro, V. L., 1999. Recent advances in the understanding of the biological chemistry of manganese. *Current Opinon Structural Biology*, **3**, 182–187.
- Zbaida S., and Kariv, R., 1989. Biomimetic models for monooxygenases. *Biopharmaceutics and Drug Disposition*, **10**, 431–442.
- Zerkle, A. L., House, C. H., Cox, R. P., and Canfield, D. E., 2006. Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle. *Geobiology*, **4**, 285–297.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Methane, Organ](#)
[Methane Oxidation \(Aerobic\)](#)
[Nickel, Biology](#)
[Siderophores](#)
[Zinc](#)

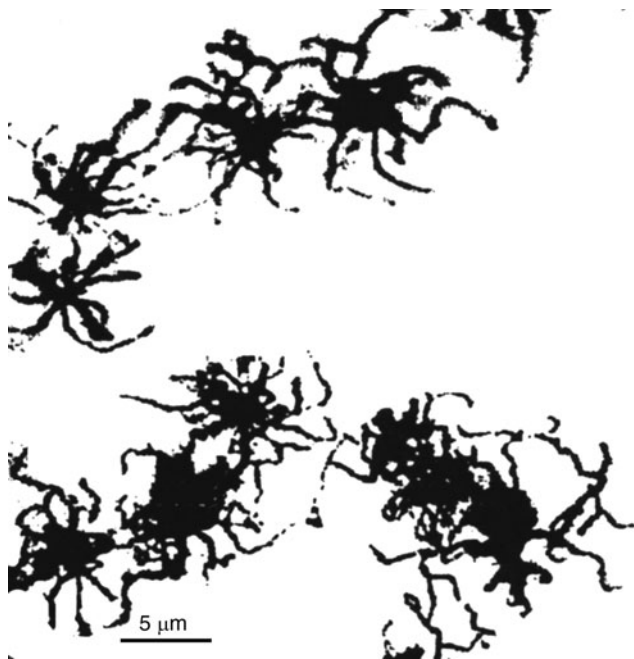
METALLOGENIUM

Joachim Reitner
 University of Göttingen, Göttingen, Germany

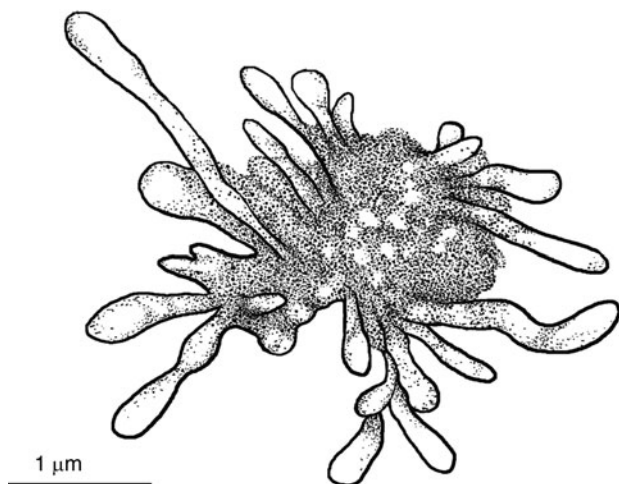
Definition

Metallogenium s.str. is a star-shaped enigmatic bacterium oxidizing Mn and iron. It preferentially lives in limnic environments.

Metallogenium is a relatively large (2–10 μ m) microorganism with bacterial affinities that concentrates manganese and iron oxides in significant amounts. The genus *Metallogenium* has not yet been assigned to any taxonomic group. The cells lack rigid walls and develop flexible, tapering threads from which new cells probably arise. The organism is therefore, star-shaped and is found in various facies, predominantly in limnic environments (Figures 1 and 2). The threads are often heavily encrusted with oxides of iron and/or manganese. The oldest



Metallogenium, Figure 1 *Metallogenium personatum*. (Redrawn from Perfil'ev and Gabe, 1961.)



Metallogenium, Figure 2 *Metallogenium personatum*. (Redrawn from Klaveness, 1999.)

Metallogenium-like fabrics are known from the 1.88 Gy old Paleoproterozoic Gunflint Formation (Barghorn and Tyler, 1965).

The first detailed description of *Metallogenium* was carried out by Perfil'ev and Gabe (1961) who called the type species *Metallogenium personatum*. There is an enormous record of publications about this microorganism (Zavarin, 1964a, b; Dubinia, 1970; Schmidt

and Overbeck, 1984), and many speculations have been made about its systematic position, for example, within the Mycoplasmatales (Zavarin and Hirsch, 1974), Chlamydobacteria (Klaveness, 1977), and Hyphomicrobiales (Perfil'ev and Gabe, 1961). Clasen (1969), Schmidt and Overbeck (1984), and Zavarin (1989), suggested a relationship with the iron bacterium *Leptothrix echinata* (Beger, 1935) which shows a considerable morphological similarity to *Metallogenium*. Unfortunately, valid molecular biological examinations presently do not exist to clarify the phylogenetic position of this organism, although it is common especially in many freshwater lakes (e.g., Klaveness, 1999; Schulte, 1994). Besides *Metallogenium personatum*, which is undoubtedly a prokaryotic organism, "*Metallogenium symbioticum*" was described (Zavarin, 1964b), which is also an Mn-oxidizing prokaryote preferentially growing on fungal microorganisms (Schweisfurth, 1971).

Bibliography

- Barghorn, E. S., and Tyler, S. A., 1965. Microorganisms from the Gunflint Chert. *Science*, **147**, 563–577.
- Beger, H., 1935. *Leptothrix echinata*, ein neues, vorwiegend Mangan fällendes Eisenbakterium. *Zentralbl. Bakteriol. Abt.*, **II** **92**, 401–406.
- Clasen, J., 1969. *Leptothrix echinata* und die Mangankonzentration in der Wahnachtalsperre. *Städtehygiene*, **20**, 171–174.
- Dubinia, G. A., 1970. Untersuchungen über die Morphologie von *Metallogenium* und die Beziehungen zu Mycoplasma. *Zeitschrift für Allgemeine Mikrobiologie*, **10**, 309–320.
- Klaveness, D., 1977. Morphology, distribution and significance of the manganese-accumulating microorganism *metallogenium* in lakes. *Hydrobiologica*, **56**, 25–33.
- Klaveness, D., 1999. *Metallogenium* – a microbial enigma. In Seckbach, J. (ed.), *Enigmatic Microorganisms and Life in Extreme Environments*. Dordrecht: Kluwer, pp. 539–548.
- Perfil'ev, B. V., and Gabe, D. R., 1961. Studies of microlandscape. In *Capillary Methods of Investigating Micro-organisms* (Engl transl. Shewan, J. M., 1969). Toronto: University of Toronto Press, pp. 55–58.
- Schmidt, W. D., and Overbeck, J., 1984. Studies of "iron-bacteria" from Lake Pluss.I. Morphology, finestructure and distribution of *Metallogenium* sp. and *Siderocapsa geminata*. *Zeitschrift für Allgemeine Mikrobiologie*, **24**, 329–339.
- Schulte, N., 1994. Untersuchungen zur Ökologie, Morphologie und Entwicklung von *Metallogenium personatum* in einer Talsperre, Diss., Techn. Univ. Carolo-Wilhelmina Braunschweig.
- Schweisfurth, R., 1971. Manganoxidierende Pilze. *Zeitschrift für Allgemeine Mikrobiologie*, **11**, 415–430.
- Zavarin, G. A., 1964a. Structure of *Metallogenium*, *Mikrobiologiya* (Engl. Transl.), **32**, 864–867.
- Zavarin, G. A., 1964b. *Metallogenium symbioticum*. *Zeitschrift für Allgemeine Mikrobiologie*, **4**, 390–395.
- Zavarin, G. A., 1989. Genus "*Metallogenium*" Perfil'ev and Gabe, 1961. In Staley, J. T., Bryant, M. P., Pfennig, N., and Holt, J. G. (eds.), *Bergey's Manual of Systematic Bacteriology*. Baltimore, MD: Williams & Wilkins, Vol. 3.
- Zavarin, G. A., and Hirsch, P., 1974. Genus *Metallogenium* Perfil'ev and Gabe 1961. In *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD: Williams & Wilkins, pp. 163–165.

Cross-references

Bacteria
 Biofilms
 Leptothrix
 Microbial Biomineralization
 Microbial-Metal Binding
 Ores, Microbial Precipitation and Oxidation
 Subsurface Filamentous Fabrics
 Terrestrial Deep Biosphere

METALS, ACQUISITION BY MARINE BACTERIA

Alison Butler, Vanessa V. Homann
 University of California, Santa Barbara, CA, USA

Definition

Oceanic bacteria produce siderophores to acquire iron. Given that the iron concentration in surface ocean water is very low, siderophores produced by marine bacteria are tailored to the physical and chemical constraints of the marine environment.

Introduction

Microbial iron uptake

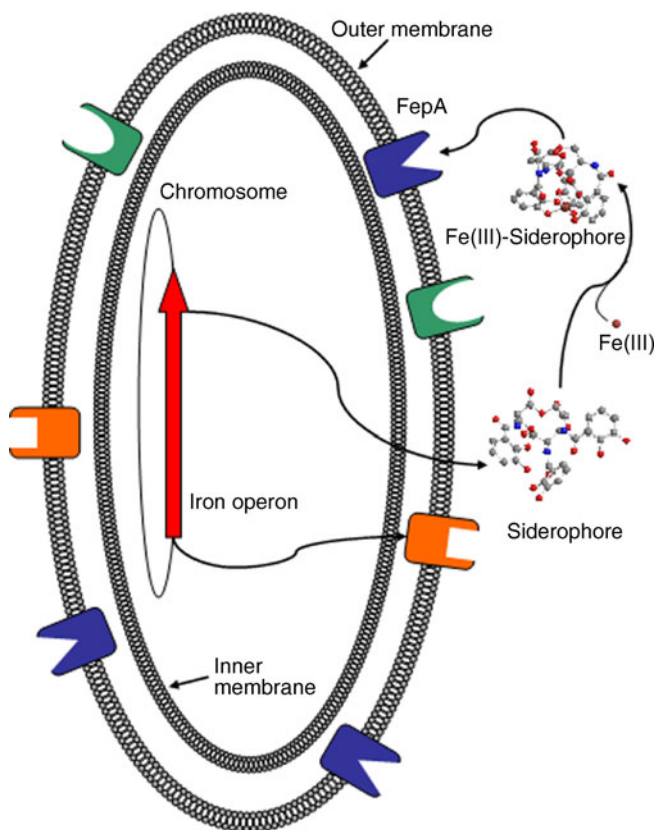
Virtually, all bacteria require iron for growth. Bacteria growing under aerobic conditions utilize a variety of pathways to acquire iron, but under conditions of iron starvation, bacteria often produce siderophores (Crosa et al., 2004). Siderophores are low molecular weight, chelating compounds produced by bacteria to facilitate iron(III) uptake (Crosa et al., 2004; Kraemer et al., 2005; Butler, 2007). The Fe(III)-siderophore complex is recognized by a specific outer membrane protein receptor, which catalyzes the transport of the Fe(III)-siderophore complex across the outer membrane (Figure 1).

The study of marine microbial siderophores is relatively new (Butler and Martin, 2005), yet certain characteristic structural features have already begun to emerge: suites of amphiphilic siderophores and photoreactive Fe(III)-siderophores.

Marine Siderophores

Amphiphilic marine siderophores

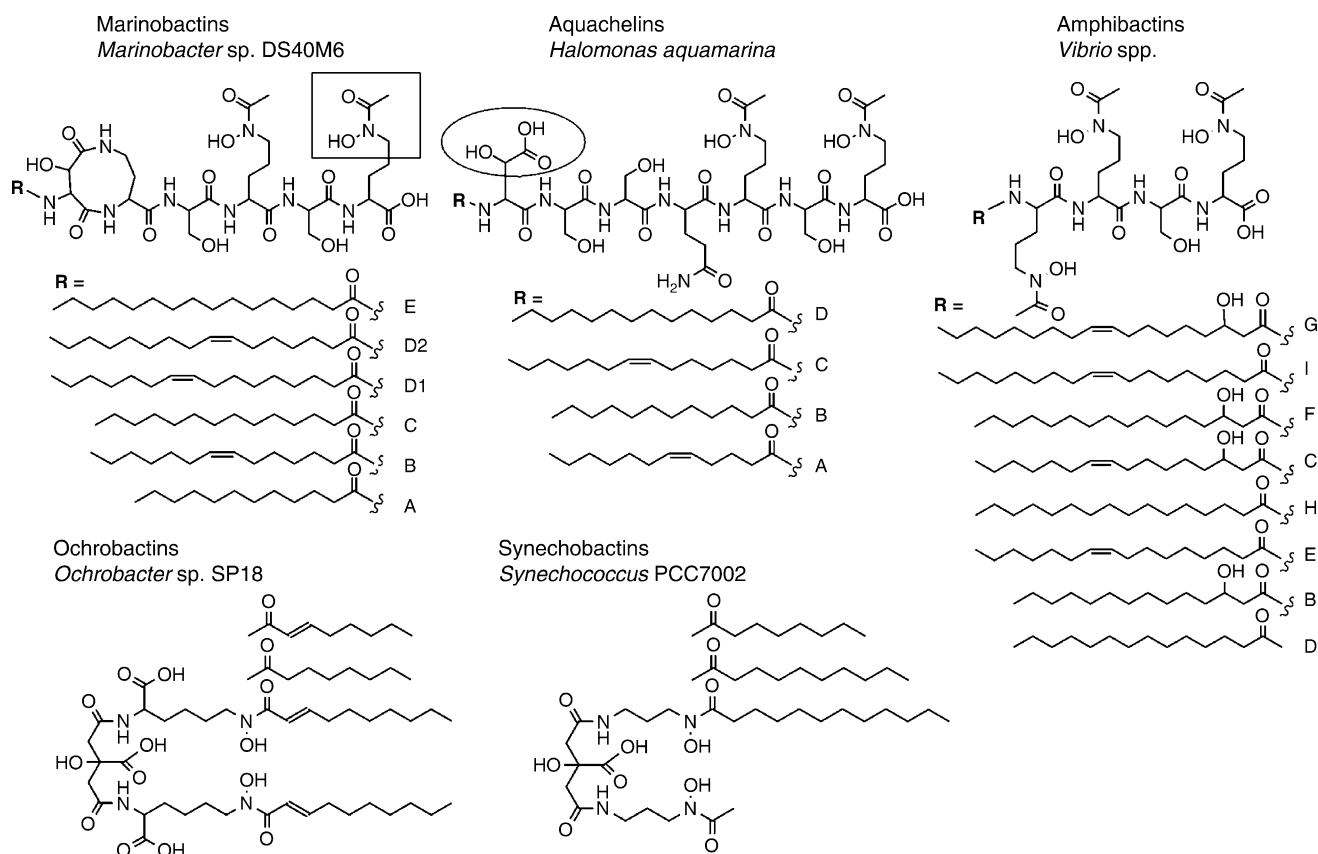
One characteristic of marine siderophores is the predominance of suites of amphiphilic siderophores, comprised of a hydrophilic head group, which coordinates with Fe(III), and one of a series of hydrophobic fatty acid appendages, which confers amphiphilicity (Figure 2). Two types of suites of amphiphilic siderophores are produced by marine bacteria. The aquachelins, marinobactins, and amphibactins are amphiphilic peptidic siderophores produced by distinct genera of marine bacteria (Figure 2) (Martinez et al., 2000, 2003). The relative amphiphilicity of each of these siderophores is



Metals, Acquisition by Marine Bacteria, Figure 1 Siderophore-mediated iron uptake by a bacterium.

defined by both the number of amino acids in the peptide fraction, compared to the fatty acid chain length (e.g., C12–C18). The amphibactins with only four amino acids and relatively long C18 fatty acids are the most hydrophobic of the amphiphilic peptide siderophores. As a result, they remain cell associated and are extracted from the bacterial pellet during the isolation process (Martinez et al., 2003). In contrast, the marinobactins and aquachelins, with five and six amino acids in the head group and a predominance of the shorter fatty acids, are more hydrophilic and are thus isolated from the supernatant following centrifugation of the bacterial culture to pellet the cells (Martinez et al., 2000).

The synechobactins and ochrobactins (Figure 2) are different suites of amphiphilic siderophores, in which the head group is based on citric acid, as opposed to a peptide. The head group is thus smaller than in the peptidic amphiphilic siderophores. The fatty acids are also shorter than observed for the peptidic amphiphilic siderophores. Nevertheless, the ochrobactins are quite hydrophobic as a result of two fatty acid appendages; these siderophores remain associated with the bacterial pellet (Martin et al., 2006). The synechobactins, with



Metals, Acquisition by Marine Bacteria, Figure 2 Amphiphilic marine siderophores. Marinobactins (Martinez et al., 2000); aquachelins (Martinez et al., 2000); amphibactins (Martinez et al., 2003); ochrobactins (Martin et al., 2006); synechobactins (Ito and Butler, 2005). The boxed portion shows the hydroxamic acid group. The circled portion shows the α -hydroxyacid in β -hydroxyaspartic acid.

only one fatty acid appendage, partition between the culture supernatant and the bacterial pellet (Ito and Butler, 2005).

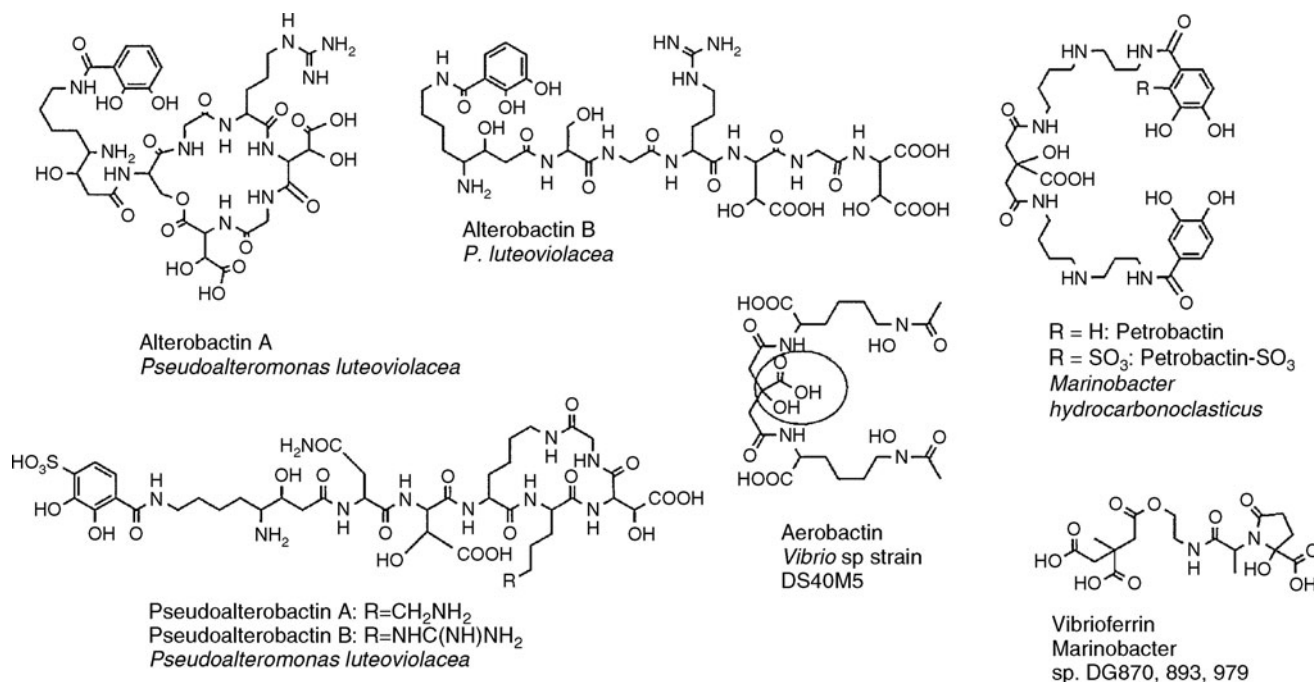
Photoreactive Fe(III)-siderophores

The other characteristic of marine siderophores is the predominance of α -hydroxycarboxylic acid moieties in the form of β -hydroxyaspartic acid or citric acid (Figures 2 and 3).

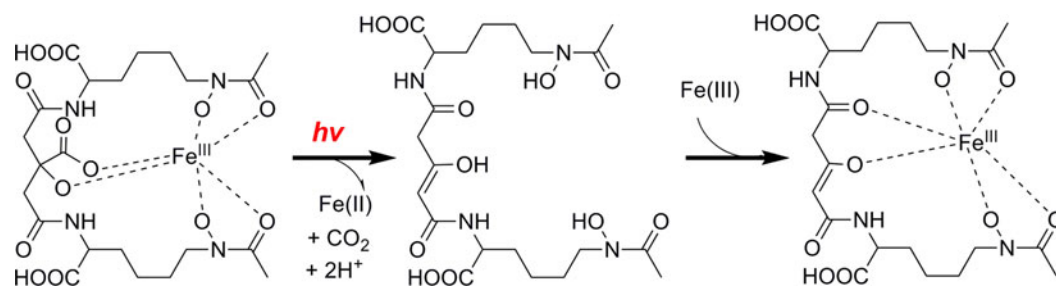
The Fe(III)-complex of an α -hydroxycarboxylic acid moiety is photoreactive in UV light, including natural sunlight, which is a condition experienced by the mixed layer of the upper 30–40 m of the ocean. Upon UV photolysis of the Fe(III)-siderophores containing citric acid, such as the ochrobactins (Martin et al., 2006), the synechobactins (Ito and Butler, 2005), the petrobactins (Bergeron et al., 2003; Hickford et al., 2004), and aerobactin (marine *Vibrio* sp. DS40M5) (Küpper et al., 2006; Haygood et al., 1993), citrate is oxidized to 3-ketoglutarate and Fe(III) is reduced to Fe(II). The resulting ligand photoproduct

retains the ability to coordinate Fe(III) with remarkably high stability constant as a result of the 3-ketoglutarate moiety and the remaining hydroxamic acid groups (Figure 4, for aerobactin (Küpper et al., 2006).

Other marine siderophores such as the aquachelins, the marinobactins, the alterobactins, and the pseudoalterobactins contain β -hydroxyaspartic acid as the α -hydroxycarboxylic acid group. UV photolysis of the Fe(III)-aquachelins results in ligand oxidation, specifically loss of β -hydroxyaspartic acid, as well as reduction of Fe(III) to Fe(II) (Barbeau et al., 2001). This peptide photoproduct retains the two hydroxamate groups and thus the ability to coordinate with Fe(III), although the conditional stability constant is somewhat reduced (Barbeau et al., 2001). Only catalytic amount of Fe(III) is required to effect the complete oxidation of the aquachelins in aerobic conditions, because the Fe(II) that is produced is oxidized back to Fe(III) and thus can coordinate with another apo aquachelin. The ferric complexes of the alterobactins A and B and the marinobactins are also photoreactive (Barbeau et al., 2003), although the



Metals, Acquisition by Marine Bacteria, Figure 3 Marine siderophores with α -hydroxycarboxylic acid groups. The aquachelins, marinobactins, ochrobactins, and synechobactins shown in Figure 2 also contain α -hydroxycarboxylic acid moiety. Alterobactins (Reid et al., 1993); pseudoalterobactins (Kano et al., 2003); aerobactin (Haygood et al., 1993); petrobactin (Bergeron et al., 2003); petrobactin-SO₃ (Hickford et al., 2004); vibrioferriin (Amin et al., 2007). The circled portion shows the α -hydroxyacid in citric acid.



Metals, Acquisition by Marine Bacteria, Figure 4 Reaction products in the photolysis of Fe(III)-aerobactin. (Adapted from Küpper et al., 2006).

structures of the oxidized ligands have not been completed yet. The ferric complexes of pseudoalterobactins A and B, which are chemically related to the alterobactins (Kano et al., 2003), would also be expected to be photoreactive.

Summary

The preponderance of suites of amphiphilic siderophores and of α -hydroxycarboxylic-acid-containing siderophores produced by marine bacteria suggests that these properties have evolved as common iron acquisition strategies for

marine bacteria, although details of the biological advantages conferred by these molecular properties are yet to be elucidated.

Bibliography

- Amin, S. A., Küpper, F. C., Green, D. H., Harris, W. R., and Carrano, C. J., 2007. Boron binding by a siderophore isolated from marine bacteria associated with the toxic dinoflagellate *Gymnodinium catenatum*. *Journal of the American Chemical Society*, **129**, 478–479.
- Barbeau, K., Rue, E. L., Bruland, K. W., and Butler, A., 2001. Photochemical cycling of iron in the surface ocean

- mediated by microbial iron(III)-binding ligands. *Nature*, **413**, 409–413.
- Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., and Butler, A., 2003. The photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria, based on characteristic iron(III)-binding groups. *Limnology and Oceanography*, **48**, 1069–1078.
- Bergeron, R. J., Huang, G., Smith, R. E., Bharti, N., McManis, J. S., and Butler, A., 2003. Total synthesis of petrobactin. *Tetrahedron*, **59**, 2007–2014.
- Butler, A., 2007. Siderophores. In Bertini, I., Gray, H. B., and Valentine, J. S. (eds.), *Biological Inorganic Chemistry: Structure and Reactivity (Metal Transport and Storage Proteins section)*. Sausalito: University Science Books, (Chap. VIII.3), pp. 151–156.
- Butler, A., and Martin, J. D., 2005. The marine biogeochemistry of iron. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *“Biogeochemical Cycles,” Metal Ions in Biological Systems*. New York: M. Dekker, Vol. 43, pp. 21–46.
- Crosa, J. H., Mey, A. R., and Payne, S. M., 2004. *Iron Transport in Bacteria*. Washington, DC: ASM, p. 499.
- Haygood, M. J., Holt, P., and Butler, A., 1993. Aerobactin production by a planktonic marine *Vibrio* sp. *Limnology and Oceanography*, **38**, 1091–1097.
- Hickford, S. J. H., Küpper, F. C., Zhang, G., Carrano, C. J., Blunt, J. W., and Butler, A., 2004. Petrobactin sulfonate, a new siderophore produced by the marine bacterium *Marinobacter hydrocarbonoclasticus*. *Journal of Natural Products*, **67**, 1897–1899.
- Ito, Y., and Butler, A., 2005. Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002. *Limnology and Oceanography*, **50**, 1918–1923.
- Kanoh, K., Kamino, K., Leleo, G., Adachi, K., and Shizuri, Y., 2003. Pseudoalterobactin A and B, new siderophores excreted by marine bacterium *Pseudoalteromonas* sp. KP20–4. *The Journal of Antibiotics*, **56**, 871–875.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Biogenic ligands and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry: Molecular Geomicrobiology*, **59**, 53–76.
- Küpper, F. C., Carrano, C. J., Kuhn, J. U., and Butler, A., 2006. Photoreactivity of iron(III)-aerobactin: Photoproduct structure and iron(III) coordination. *Inorganic Chemistry*, **45**, 6026–6033.
- Martin, J. D., Ito, Y., Homann, V. V., Haygood, M. G., and Butler, A., 2006. Structure and membrane affinity of new amphiphilic siderophores produced by *Ochrobactrum* sp. SP18. *Journal of Biological Inorganic Chemistry*, **11**(5), 633–641.
- Martinez, J. S., Zhang, G. P., Holt, P. D., Jung, H. T., Carrano, C. J., Haygood, M. G., and Butler, A., 2000. Self-assembling amphiphilic siderophores from marine bacteria. *Science*, **287**, 1245–1247.
- Martinez, J. S., Carter-Franklin, J. N., Mann, E. L., Martin, J. D., Haygood, M. G., and Butler, A., 2003. Structure and membrane affinity of a new suite of amphiphilic siderophores produced by a marine bacterium. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(7), 3754–3759.
- Reid, R. T., Live, D. H., Faulkner, D. J., and Butler, A., 1993. A siderophore from a marine bacterium with an exceptional ferric ion stability constant. *Nature*, **366**, 455–457.

Cross-references

Bacteria
 Cyanobacteria
 Fe(II)-Oxidizing Prokaryotes
 Metalloenzymes
 Siderophores

METEORITICS

Mark A. Sephton
 Imperial College London, London, UK

Definition

Meteorites are fragments of extraterrestrial material that fall on the Earth's surface. Most meteorites are parts of asteroids propelled into Earth-crossing orbits by relatively recent collisions in the asteroid belt, initiated by the gravitational effects of Jupiter's orbit. Relatively small numbers of meteorites originate from larger objects such as the Moon and Mars. The Earth acquires 10^2 to 10^3 t of such material each day, but only 1% or less arrives in pieces large enough for identification and recovery (Dodd, 1981). The surface of the meteorite usually melts and emits a glowing tail and a trail of smoke, but the interior of the meteorite is unaffected by passage through the Earth's atmosphere and remains at the temperature of interplanetary space.

Collection of meteorites

“Falls” are meteorites that have been observed to fall and were subsequently collected; “finds” are meteorites that were not seen to fall. Before the 1970s, the total number of known meteorites amounted to less than 1,000 falls and 1,706 authenticated finds. Since then, it has been recognized that certain parts of the Earth's surface preserve and concentrate meteorites. To date, tens of thousands of meteorites have been collected from the Antarctic ice. Similar numbers of meteorite fragments have also been collected from hot deserts. Most recently, telescope and camera network surveys have produced meteorites with photographed fireballs allowing the calculation of their orbits and assignment to a particular source within the solar system (Bland et al., 2009).

Chondrites

About 85% of the meteorites observed to fall on the Earth are chondrites, conglomerate rocks with an overall elemental composition similar to the Sun, if the loss of the most volatile elements is ignored. When their near solar makeup is considered alongside radiometric ages of 4.56×10^9 years, it becomes clear that chondrites are rocks formed during, or shortly after, the birth of the Sun.

The record in chondrites

Their status as some of the earliest formed solids in the solar system combined with a lack of subsequent processing ensures that chondrites represent a valuable record of extraterrestrial environments. Contributing regions include interstellar space, the solar nebula and the asteroidal meteorite parent bodies.

Carbonaceous chondrites

Of all the chondrites, the carbonaceous chondrites are considered the most primitive and these objects represent an enthralling window back in time providing views of the earliest solar system (Figure 1). The name “carbonaceous chondrite” is highly descriptive as these meteorites contain a carbon-rich matrix composed primarily of clay minerals, oxides, and organic matter, and most contain spherules of silicate glass termed chondrules.

Classification of carbonaceous chondrites

The carbonaceous chondrites are classified according to their primary and secondary characteristics (Figure 2). The primary classification (CI, CM, CR, CO, CV, CK)



Meteoritics, Figure 1 The Murchison carbonaceous chondrite.

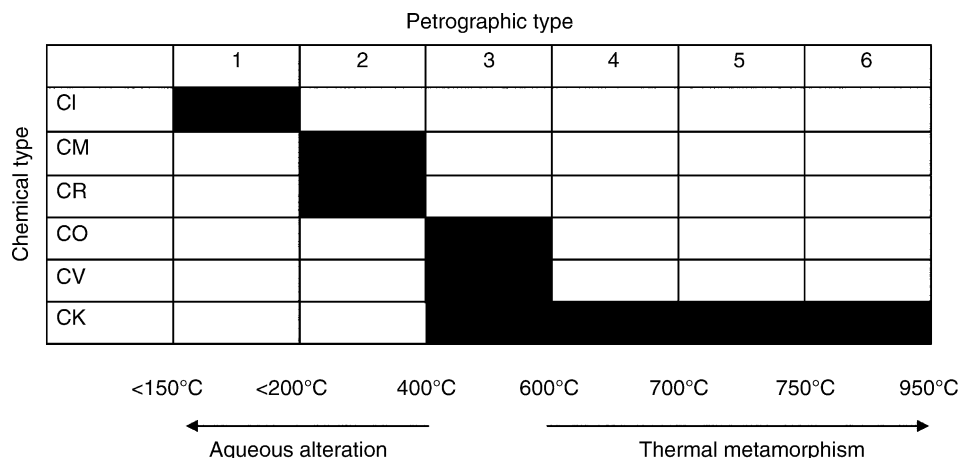
reflects the original properties of the meteorites such as bulk chemistry and mineralogy. The label is derived from the carbonaceous chondrite accepted as the type specimen, for example, CI = Ivuna. The secondary numerical classification reflects petrographic type, which indicates the extent and type of alteration that the meteorite has undergone on its parent body.

Carbon in meteorites

The carbon in carbonaceous chondrites is present in several forms and each has its specific source region(s) (Table 1; Sephton et al., 2003). Materials like silicon carbide and graphite condensed from the atmospheres of stars that existed long before our Sun. Nanometer-sized diamonds also originate as condensation products of stellar outflows as well as from catastrophic supernova explosions. Carbonate minerals were formed in the early solar system as aqueous alteration took place on asteroids. Yet all these components are quantitatively subordinate to the main carbonaceous material in carbonaceous chondrites – organic matter.

Types of organic matter in meteorites

Much of our current understanding of meteoritic organic matter has come from investigations of the Murchison carbonaceous chondrite, approximately 100 kg of which fell in Australia in 1969 (Figure 1). Not all of the organic molecules in meteorites are present in the same form. A minor portion of the organic matter in meteorites is present as small “free” molecules, including various hydrocarbons and the biologically useful amino acids and carboxylic acids (Figure 3), but the majority of organic matter in the carbon-rich meteorites is present as a highly cross-linked organic network or “macromolecular material”.



Meteoritics, Figure 2 The classification system for carbonaceous chondrites.

Free compounds

Amino acids

The amino acids found in meteorites have attracted a great deal of attention owing to their role as building blocks for proteins. The search for amino acids in meteorites accelerated in the 1960s (Degens and Bajor, 1962; Kaplan et al., 1963) following reports of microfossils in certain samples (Claus and Nagy, 1961). Amino acids were discovered, but these initially promising results were later found to be the result of terrestrial contamination. It was only when the organic-rich Murchison meteorite fell and was rapidly analyzed that both protein and, importantly, nonprotein amino acids were found in this fresh sample (Kvenvolden

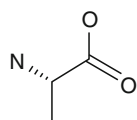
et al., 1970). Today it is known that the amino acids in Murchison are present at around 60 ppm. Compounds containing two through eight carbon atoms exist and they exhibit several features, which suggest that they are not terrestrial contaminants but are extraterrestrial and nonbiological or “abiotic” in origin. The meteoritic amino acids display broad structural diversity, a decline in abundance with increasing molecule size, and the presence of unusual and even unique molecular configurations.

A particular characteristic of amino acids is chirality or handedness. One chiral form rotates the polarized light to the left and is termed levorotatory or “L,” while the other form rotates the light to the right, and is called dextrorotatory or “D.” Chirality is important for theories that link extraterrestrial organic matter with the origin of life, because terrestrial biology preferentially generates L-amino acids whereas abiotic reactions generally have no preference and produce racemic mixtures with equal amounts of L and D forms.

The discussion of whether amino acids in meteorites were racemic (with equal amounts of L and D forms) or nonracemic (with a preference for one or the other) began in the 1950s and continued through the 1960s, (e.g., Mueller, 1953; Nagy et al., 1964) but early results implied that the dominance of L forms was caused by terrestrial contamination and analytical artefacts. Yet following the fall of Murchison in 1969, four protein and seven

Meteoritics, Table 1 The carbon-bearing components in carbonaceous chondrites

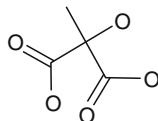
Carbon-bearing component	Abundance (wt. %)	Environment of formation
Organic matter	2.0	Interstellar space, solar nebula, asteroids
Carbonate	0.2	Asteroids
Diamond	0.04	Stars, supernovae
Graphite	0.005	Stars
Silicon carbide	0.009	Stars



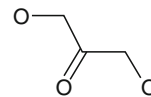
Amino acids
(Alanine)



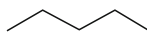
Carboxylic acids
(Ethanoic acid)



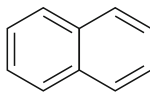
Hydroxyacids
(2-Hydroxy-2-methyl
propanedioic acid)



Polyols
(Dihydroxyacetone)



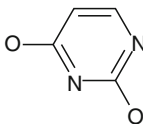
Aliphatic hydrocarbons
(Pentane)



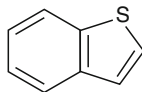
Aromatic hydrocarbons
(Naphthalene)



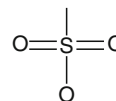
Amines
(Methylamine)



Nitrogen heterocycles
(Uracil)



Sulfur heterocycles
(Benzothiophene)



Sulfonic acids
(Methylsulfonic acid)

Meteoritics, Figure 3 Some free compound classes and examples found in carbonaceous chondrites.

nonprotein amino acids were found to be racemic (Kvenvolden et al., 1970, 1971). More modern analyses have reinvigorated the debate over the racemic nature of meteorite amino acids and a slight L-excess in both protein and nonprotein amino acids has been reported (Engel and Nagy, 1982; Pizzarello and Cronin, 2000).

Proposed mechanisms for producing this L-excess in meteoritic amino acids rely on selective destruction of the right-handed form of the amino acids. Currently, popular hypotheses involve ultraviolet circularly polarized light from a neutron star or reflection nebulae (Bailey, 2001; Bonner and Rubenstein, 1987). It is a necessary consequence of this theory that at least some amino acids were already formed in interstellar space so that preferential destruction of D-amino acids could take place. The recent detection of the simplest amino acid, glycine, in interstellar spectra supports this assertion (Kuan et al., 2003).

Carboxylic acids

Carboxylic acids are another type of biologically useful molecule found in meteorites. In life, they are a convenient and compact store of energy and are common cell membrane molecules. Carboxylic acids in Murchison contain between two and five carbon atoms and display complete structural diversity, a general decrease in abundance with increasing carbon number, and an equal concentration of branched and straight-chain isomers (Lawless and Yuen, 1979; Yuen and Kvenvolden, 1973). The monocarboxylic acids are present in a much higher concentration (332 ppm) than the amino acids and are the most abundant solvent extractable compounds. A structurally diverse suite of dicarboxylic acids containing up to nine carbon atoms are also present in much smaller amounts (26 ppm) (Lawless et al., 1974).

Hydroxyacids

Hydroxycarboxylic acids (or hydroxyacids) in carbonaceous chondrites contain up to nine carbon atoms and are structurally diverse and present in concentrations of 15 ppm (Peltzer and Bada, 1978). Intriguingly, the hydroxyacids correspond in structure to the more abundant amino acids suggesting that some hydroxy and amino acids may have been produced together on the parent asteroid. The relevant chemical reaction is known as the Strecker-cyanohydrin synthesis. Hydrogen cyanide and aldehydes combine in aqueous solution to produce hydroxyacids but if ammonia is also present then the products become amino acids.

Sugar-related compounds

Collectively, sugars and their related compounds are called polyhydroxylated compounds or "polyols." They are compounds containing a number of hydroxyl (OH) groups attached to their carbon skeleton. Sugars are

important biologically as a source of energy for organisms and, when combined to form larger molecules, they can act as food stores and give structural support. Experiments in the 1960s detected polyols in carbonaceous chondrites, but terrestrial contamination was implied (Degens and Bajor, 1962). More recent experiments on Murchison have discovered a number of sugar-related compounds (sugars, sugar alcohols, and sugar acids) in similar abundances to the amino acids (Cooper et al., 2001). The Murchison polyols display many of the features of extraterrestrial compounds. Progressively larger polyols become less abundant, display almost complete structural diversity, and contain some terrestrially rare compounds implying an abiotic source.

Aliphatic and aromatic hydrocarbons

Short aliphatic molecules in Murchison are present in amounts of 1.4 ppm and display many nonbiological features, which imply they are indigenous to the meteorite, such as structural diversity and a decrease in amount with increasing molecule size (Yuen et al., 1984). Aromatic hydrocarbons, in free form are present in carbonaceous chondrites at around 30 ppm (Pering and Ponnamperna, 1971). Compound sizes range from one to seven aromatic rings with the smaller compounds generally being most abundant (Sephton et al., 1998).

Amines and amides

Amines are derivatives of ammonia (NH₃) where hydrogen atoms are replaced by other structures. In Murchison, amine concentration is 8 ppm, amounts of individual amines decrease with increasing carbon number and structural diversity is observed with almost all amine isomers with up to five carbon atoms present (Pizzarello et al., 1994).

Two possible sources of the amines in Murchison have been proposed: they may be directly inherited from the presolar molecular cloud, an assertion supported by the detection of methylamine in interstellar space, or as with the hydroxyl acids, the amines may share a common origin with the amino acids. By simply removing the carboxylic acid group of amino acids, 16 of the 20 amines can be made, a reaction that would take place if the molecules were heated on their parent asteroid.

Amides are structurally analogous to carboxylic acids and, in Murchison, the amide equivalents of monocarboxylic acids, dicarboxylic acids, and hydroxyacids are found in concentrations greater than 70 ppm (Cooper and Cronin, 1995). The amides display complete structural diversity up to and including molecules with eight carbon atoms, a decline in abundance with carbon number, and the presence of many compounds with no terrestrial source. Cyclic amides (lactams) are less abundant (2 ppm) but they are very interesting to

investigators of the origin of life. The chemical structures of cyclic amides permit hydrogen-bonded pair formation and may have acted as parts of a primitive genetic coding apparatus, possible forerunners of the present day nucleic acids.

Nitrogen heterocycles

Another compound class essential for terrestrial life is that of the nitrogen heterocycles. Some of these compounds are building blocks for nucleic acids that store genetic information. The possible presence of nitrogen heterocycles in water extracts of carbonaceous meteorites was first reported in the early 1960s but the initial optimism was dispelled when they turned out to be laboratory contaminants (Oró, 1963). Later work did confirm the presence of nitrogen heterocycles in meteorites such as Murchison and several classes, including purines, pyrimidines, quinolines/isoquinolines, and pyridines were found. All of the purines and pyrimidines found are biologically common and together account for 1.3 ppm in Murchison (Stoks and Schwartz, 1979, 1981). The quinolines, isoquinolines, and pyridines are structurally diverse, contain a large number of isomeric alkyl derivatives, and are present at concentrations of 7 ppm (Pizzarello et al., 2001; Stoks and Schwartz, 1982). Recent work has confirmed the presence and extraterrestrial origin of xanthine and uracil in the Murchison meteorite (Martins et al., 2008).

Sulfur heterocycles

Murchison contains small amounts of sulfur-containing compounds such as thiophenes, benzothiophenes, dibenzothiophenes, and benzonaphthothiophenes. The abundance pattern of thiophenes in Murchison is markedly different from those in terrestrial sediments, indicating an abiotic origin for these molecules (Shimoyama and Katsumata, 2001).

Phosphonic and sulfonic acids

Phosphorus compounds have many biological roles and play a part in cell membranes, energy transactions during metabolism, and the storage and transfer of genetic information. Sulfur compounds are also common in living systems and take part in key biochemical reactions. In Murchison, a series of alkyl phosphonic acids and alkyl sulfonic acids with up to four carbon atoms have been detected (Cooper et al., 1992). As observed for many of the other compound classes in Murchison, the sulfonic and phosphonic acids display an exponential decline in amount with increasing carbon number and exhibit complete structural diversity.

Macromolecular materials

The solvent soluble substances are only trace components, yet they are often the focus of studies on meteorite organic

matter due to their analytical amenability. Essentially, the macromolecular materials can be considered as a cross-linked agglomeration of some of the free compounds but with an overwhelming dominance of aromatic hydrocarbons. The macromolecular materials are generally assumed to be completely indigenous to their meteorite host due to their high molecular weight and immobility.

As the major organic component, the macromolecular material is key to theories of the origin of meteoritic organic matter as a whole. Hence it is interesting to consider that meteoritic organic matter is often compared with that observed in interstellar space. Some scientists believe that the molecular cloud that collapsed to form the solar system bequeathed a significant amount of interstellar organic matter, aliquots of which are preserved in primitive asteroids and the meteorites derived from them. For many years, only one- to four-ring polycyclic aromatic hydrocarbons (PAH) were commonly observed in Murchison macromolecular material breakdown products (Sephton et al., 1998), which contrasted sharply with the greater than 20-ring PAH proposed for the interstellar medium (Pendleton and Allamandola, 2002). Recently, using modern analytical techniques the meteoritic macromolecular material has yielded more information. Up to seven-ring PAH units have been liberated from the macromolecular material in Murchison and it appears that even larger entities are present in the experimental residue (Sephton et al., 2004). These discoveries partly reconcile the apparent disharmony between the meteoritic and interstellar organic inventories and point to a partly presolar origin for this abundant organic component.

Terrestrial contamination

Although some compound classes in meteorites may contain a record that extends back to interstellar environments before the solar system formed others can be much more recent in the form of terrestrial contamination. Unusual and subtle contamination sources exist and an example is provided by data from the Orgueil carbonaceous chondrite, which has a long curation history since its fall in 1864. Terpene-related compounds have been identified in this meteorite (Watson et al., 2003) and these compounds are structurally specific, and are unlikely to be produced by abiotic reactions. Terpenes are common in essential plant oils, used extensively in cleaning products, and may have percolated their way into the porous Orgueil stones.

A more widespread type of terrestrial contaminant is provided by the long-chain aliphatic hydrocarbons (normal alkanes) in meteorites (Sephton et al., 2001). Unlike the short-chain aliphatic hydrocarbons, the normal alkanes are structurally specific with all carbon atoms arranged in a straight chain. Normal alkanes in meteorites were first detected in Orgueil and were attributed to extraterrestrial biology based on similar distributions

for molecules in the meteorite and those found in butter and marine sediments (Nagy et al., 1961). Subsequent analyses on many different meteorites led to the discovery that these compounds were present in several tens of ppm, were concentrated near to the surfaces of samples, and that they were frequently associated with other, obviously terrestrial, compounds (Oró et al., 1966). A subsequent revival in the belief that these compounds were indigenous occurred when normal alkanes were interpreted as products of an abiotic catalytic reaction called the Fischer–Tropsch synthesis (Studier et al., 1968). Modern measurements of individual normal alkanes reveal terrestrial values that are distinct from the majority of meteoritic organic matter (Sephton et al., 2001) and imply that normal alkanes are simply contributions from our terrestrial environment once the meteorite has fallen on the Earth.

The location of organic matter in meteorites

In chondrites, organic matter is present within the fine-grained inorganic meteorite matrix, which contains evidence of alteration by liquid water on the parent asteroid. Hence, a general relationship between extraterrestrial organic matter and inorganic aqueous alteration products in carbonaceous chondrites has been recognized for some time. Recently, however, it has been discovered that the association is highly specific to a particular inorganic phase – clay minerals (Pearson et al., 2002). Clay minerals, therefore, may have trapped and concentrated organic matter in the early solar system and organic–inorganic interactions may have played a role in the assembly of increasingly complex organic entities 4.56×10^9 years ago.

Relevance to the origin of life on Earth

With the recognition that meteorites contain many biologically useful molecules it is enlightening to consider that the amounts of organic matter extraterrestrial objects can deliver to the Earth's surface are considerable. For example, calculations indicate that comet Halley contains organic matter equivalent to approximately 10% of the current biomass of the Earth (Greenberg, 1993). A connection between life and extraterrestrial material was first proposed in the early 1960s by the biochemist Jan Oró (Oró, 1961). It was suggested that extraterrestrial objects may have seeded the early Earth with an extensive list of ingredients for use in the recipe of life. Modern theories imply that the early Earth had a nonreducing atmosphere composed mainly of carbon dioxide and nitrogen in which the in situ generation of organic matter would be rather sluggish and inefficient. Even if only a small fraction of the molecules delivered by extraterrestrial infall proved biologically relevant, they would have had a significant impact on the prebiotic chemistry of the Earth.

Outlook

Until space missions begin to return large quantities of extraterrestrial material back to the Earth for analysis in laboratories, meteorites will remain the most accessible and most frequently studied objects to reveal the origin and evolution of the solar system. In particular, the study of carbonaceous meteorites, which contain bona fide prebiotic organic matter is likely to remain our most important means of determining how chemical evolution led to the origin of life.

Bibliography

- Bailey, J., 2001. Astronomical sources of circularly polarized light and the origin of homochirality. *Origins Life Evolution Biosphere*, **31**, 167–183.
- Bland, P. A., Spurny, P., Towner, M. C., Bevan, A. W., Singleton, A. T., Botke, W. F., Greenwood, R. C., Chesley, S. R., Shrubny, L., Borovicka, J., Ceplecha, Z., McClafferty, T. P., Vaughan, D., Benedix, G. K., Deacon, G., Howard, K. T., Franchi, I. A., and Hough, R. M., 2009. An anomalous basaltic meteorite from the innermost main belt. *Science*, **325**, 1525–1527.
- Bonner, W. A., and Rubenstein, E., 1987. Supernovae, neutron-stars and biomolecular chirality. *Biosystems*, **20**, 99–111.
- Chang, S., Mack, R., and Lennon, K., 1978. Carbon chemistry of separated phases of Murchison and Allende meteorites. *Lunar and Planetary Science*, **9**, 157–159.
- Claus, G., and Nagy, B., 1961. A microbiological examination of some carbonaceous chondrites. *Nature*, **192**, 594–596.
- Cooper, G. W., and Cronin, J. R., 1995. Linear and cyclic aliphatic carboxamides of the Murchison meteorite – hydrolyzable derivatives of amino-acids and other carboxylic-acids. *Geochimica et Cosmochimica Acta*, **59**, 1003–1015.
- Cooper, G., Kimmich, N., Belisle, W., Sarinana, J., Brabham, K., and Garrel, L., 2001. Carbonaceous meteorites as a source of sugar-related organic compounds for the early Earth. *Nature* **414**, 879–883.
- Cooper, G. W., Onwo, W. M., and Cronin, J. R., 1992. Alkyl phosphonic-acids and sulfonic-acids in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **56**, 4109–4115.
- Cooper, G. W., Thieme, M. H., Jackson, T. L., and Chang, S., 1997. Sulfur and hydrogen isotope anomalies in meteorite sulfonic acids. *Science*, **277**, 1072–1074.
- Cronin, J. R., Pizzarello, S., and Cruikshank, D. P., 1988. Organic matter in carbonaceous chondrites, planetary satellites, asteroids and comets. In Kerridge, J. F., and Matthews, M. S. (eds.), *Meteorites and the Early Solar System*. Tucson, AZ: University of Arizona Press, pp. 819–857.
- Degens, E. T., and Bajor, M., 1962. Amino acids and sugars in the Bruderheim and Murray meteorite. *Naturwissenschaften*, **49**, 605–606.
- Dodd, R. T., 1981. *Meteorites: A Chemical and Petrological Synthesis*. London: Cambridge University Press, p. 386.
- Engel, M. H., and Nagy, B., 1982. Distribution and enantiomeric composition of amino-acids in the Murchison meteorite. *Nature*, **296**, 837–840.
- Greenberg, J. M., 1993. Physical and chemical composition of comets – from interstellar space to the Earth. In Greenburg, J. M., and Pirronello, V. (eds.), *Chemistry of Life's Origins*. Dordrecht: Kluwer, pp. 195–207.
- Hayatsu, R., Anders, E., Studier, M. H., and Moore, L. P., 1975. Purines and triazines in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **39**, 471–488.

- Jungclauss, G., Cronin, J. R., Moore, C. B., and Yuen, G. U., 1976a. Aliphatic amines in the Murchison meteorite. *Nature*, **261**, 126–128.
- Jungclauss, G. A., Yuen, G. U., Moore, C. B., and Lawless, J. G., 1976b. Evidence for the presence of low molecular weight alcohols and carbonyl compounds in the Murchison meteorite. *Meteoritics*, **11**, 231–237.
- Kaplan, I. R., Degens, E. T., and Reuter, J. H., 1963. Organic compounds in stony meteorites. *Geochimica et Cosmochimica Acta*, **27**, 805–834.
- Kuan, Y.-J., Charnley, S. B., Huang, H.-C., Tseng, W.-L., and Kisiel, Z., 2003. Interstellar glycine. *The Astrophysical Journal*, **593**, 848–867.
- Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperna, C., Kaplan, I. R., and Moore, C., 1970. Evidence for extra-terrestrial amino acids and hydrocarbons in the Murchison meteorite. *Nature*, **228**, 928–926.
- Kvenvolden, K. A., Lawless, J. G., and Ponnamperna, C., 1971. Nonprotein amino acids in the Murchison meteorite. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 86–490.
- Lawless, J. G., and Yuen, G. U., 1979. Quantification of monocarboxylic acids in the Murchison carbonaceous meteorite. *Nature*, **282**, 396–398.
- Lawless, J. G., Zeitman, B., Pereira, W. E., Summons, R. E., and Duffield, A. M., 1974. Dicarboxylic acids in the Murchison meteorite. *Nature*, **251**, 40–42.
- Martins, Z., Botta, O., Fogel, M. L., Sephton, M. A., Glavin, D. P., Watson, J. S., Dworkin, J. P., Schwartz, A. W., and Ehrenfreund, P., 2008. Extraterrestrial nucleobases in the Murchison meteorite. *Earth and Planetary Science Letters*, **270**, 130–136.
- Meierhenrich, U. J., Munoz Caro, G. M., Bredehoft, J. H., Jessberger, E. K., and Thiemann, W. H. P., 2004. Identification of diamino acids in the Murchison meteorite. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 9182–9186.
- Mueller, G., 1953. The properties and genesis of the carbonaceous complex within the Cold Bokkeveld meteorite. *Geochimica et Cosmochimica Acta*, **4**, 1–10.
- Nagy, B., Meinschein, W. G., and Hennessy, D. J., 1961. Mass spectroscopic analysis of the Orgueil meteorite; evidence for biogenic hydrocarbons. *Annals of the New York Academy of Sciences*, **93**, 25–35.
- Nagy, G., Murphy, T. J., Modzeleski, V. E., Rouser, G. E., Claus, G., Hennessy, D. J., Colombo, U., and Gazzarini, F., 1964. Optical activity in saponified organic matter isolated from the interior of the Orgueil meteorite. *Nature*, **202**, 228–223.
- Oró, J., 1961. Comets and the formation of biochemical compounds on the primitive Earth. *Nature*, **190**, 389–390.
- Oró, J., 1963. Ultraviolet absorbing compounds reported present in the Murray meteorite. *Nature*, **197**, 756–758.
- Oró, J., Nooner, D. W., Zlatkis, A., and Wisktrom, S. A., 1966. Paraffinic hydrocarbons in the Orgueil, Murray, Mokoia and other meteorites. *Life Sciences and Space Research*, **4**, 63–100.
- Pearson, V. K., Sephton, M. A., Kearsley, A. T., Bland, P. A., Franchi, I. A., and Gilmour, I., 2002. Clay mineral-organic matter relationships in the early Solar System. *Meteoritics and Planetary Science*, **37**, 1829–1833.
- Peltzer, E. T., and Bada, J., 1978. α -Hydroxycarboxylic acids in the Murchison meteorite. *Nature*, **272**, 443–444.
- Peltzer, E. T., Bada, J. L., Schlesinger, G., and Miller, S. L., 1984. The chemical conditions on the parent body of the Murchison Meteorite; some conclusions based on amino, hydroxy and dicarboxylic acids. *Advances in Space Research*, **4**, 69–74.
- Pendleton, Y. J., and Allamandola, L. J., 2002. The organic refractory material in the diffuse interstellar medium: mid-infrared spectroscopic constraints. *Astrophysical Journal Supplement Series*, **138**, 75–98.
- Pering, K. L., and Ponnamperna, C., 1971. Aromatic hydrocarbons in the Murchison meteorite. *Science*, **173**, 237–239.
- Pizzarello, S., and Cronin, J. R., 2000. Non-racemic amino acids in the Murray and Murchison meteorites. *Geochimica et Cosmochimica Acta*, **64**, 329–338.
- Pizzarello, S., Feng, X., Epstein, S., and Cronin, J. R., 1994. Isotopic analyses of nitrogenous compounds from the Murchison meteorite – ammonia, amines, amino-acids, and polar hydrocarbons. *Geochimica et Cosmochimica Acta*, **58**, 5579–5587.
- Pizzarello, S., Huang, Y. S., Becker, L., Poreda, R. J., Nieman, R. A., Cooper, G., and Williams, M., 2001. The organic content of the Tagish Lake meteorite. *Science*, **293**, 2236–2239.
- Sephton, M. A., Love, G. D., Watson, J. S., Verchovsky, A. B., Wright, I. P., Snape, C. E., and Gilmour, I., 2004. Hydropyrolysis of insoluble carbonaceous matter in the Murchison meteorite: new insights into its macromolecular structure. *Geochimica et Cosmochimica Acta*, **68**, 1385–1393.
- Sephton, M. A., Pillinger, C. T., and Gilmour, I., 1998. $\delta^{13}\text{C}$ of free and macromolecular aromatic structures in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **62**, 1821–1828.
- Sephton, M. A., Pillinger, C. T., and Gilmour, I., 2001. Normal alkanes in meteorites: molecular $\delta^{13}\text{C}$ values indicate an origin by terrestrial contamination. *Precambrian Research*, **106**, 47–58.
- Sephton, M. A., Verchovsky, A. B., Bland, P. A., Gilmour, I., Grady, M. M., and Wright, I. P., 2003. Investigating the variations in carbon and nitrogen isotopes in carbonaceous chondrites. *Geochimica et Cosmochimica Acta*, **67**, 2093–2108.
- Shimoyama, A., and Katsumata, H., 2001. Polynuclear aromatic thiophenes in the Murchison carbonaceous chondrite. *Chemistry Letters*, **3**, 202–203.
- Stoks, P. G., and Schwartz, A. W., 1979. Uracil in carbonaceous meteorites. *Nature*, **282**, 709–710.
- Stoks, P. G., and Schwartz, A. W., 1981. Nitrogen-heterocyclic compounds in meteorites – significance and mechanisms of formation. *Geochimica et Cosmochimica Acta*, **45**, 563–569.
- Stoks, P. G., and Schwartz, A. W., 1982. Basic nitrogen-heterocyclic compounds in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **46**, 309–315.
- Studier, M. H., Hayatsu, R., and Anders, E., 1968. Origin of organic matter in early solar system; I, Hydrocarbons. *Geochimica et Cosmochimica Acta*, **32**, 151–173.
- Watson, J. S., Pearson, V. K., Gilmour, I., and Sephton, M. A., 2003. Sesquiterpenoid derivatives in the Orgueil carbonaceous chondrite. *Organic Geochemistry*, **34**, 37–47.
- Yuen, G., Blair, N., DesMarais, D. J., and Chang, S., 1984. Carbon isotope composition of low molecular weight hydrocarbons and monocarboxylic acids from the Murchison meteorite. *Nature*, **307**, 252–254.
- Yuen, G. U., and Kvenvolden, K. A., 1973. Monocarboxylic acids in Murray and Murchison carbonaceous meteorites. *Nature*, **251**, 40–42.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Cosmic Molecular Clouds](#)
[Origin of Life](#)

METHANE OXIDATION (AEROBIC)

Helmut Bürgmann

Eawag, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

Synonyms

Methanotrophy

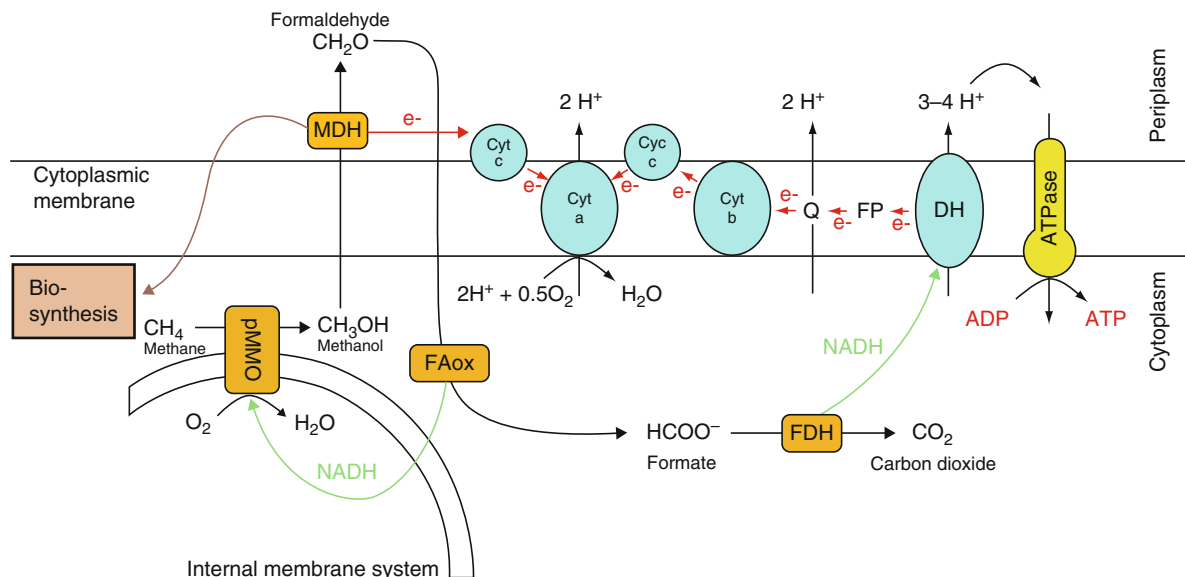
Definition

Methane oxidation is a microbial metabolic process for energy generation and carbon assimilation from methane that is carried out by specific groups of bacteria, the methanotrophs. Methane (CH_4) is oxidized with molecular oxygen (O_2) to carbon dioxide (CO_2).

Biochemical basis

Methanotrophy is the microbially mediated process of the oxidation of CH_4 with O_2 to methanol, formaldehyde, formate, and finally CO_2 (Figure 1) (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). The process is performed by a specialized group of bacteria (qv), the methanotrophs (CH_4 oxidizing bacteria). They are a subgroup of the methylotrophs, bacteria capable of utilizing single-carbon compounds (Bowman, 2006).

Methane is both the energy source (electron donor) and the sole or partial carbon source for methanotrophs. Aerobic oxidation of methane requires specific enzymes, most importantly methane monooxygenase (MMO), which catalyzes the first step in the reaction: the oxidation of methane with molecular oxygen to methanol and water. Two forms of this enzyme are known: particulate methane monooxygenase (pMMO), which is ubiquitous in methanotrophs and contains copper, and the iron-containing soluble methane monooxygenase (sMMO) (Hanson and Hanson, 1996). The reaction mediated by MMO requires additional electrons, which are supplied by cellular redox carriers such as cytochrome C (for pMMO) or NADH (sMMO). Energy is conserved in the subsequent stepwise oxidations of methanol, formaldehyde, and formate. In these reactions, electrons are donated back to a membrane-bound electron transport chain (see Chapter *Aerobic Metabolism*) via a pyrroloquinoline quinone cofactor to cytochrome C (methanol dehydrogenase) or NAD (in formaldehyde oxidation systems and formate dehydrogenase). Electron flow through the membrane ultimately produces a proton motive force that is converted to the cellular energy carrier ATP by the ATPase enzyme complex. O_2 is the terminal electron acceptor (see Chapter *Aerobic Metabolism*) (Figure 1) (Madigan et al., 2003). Thus, aerobic methane oxidation can be considered a special case of aerobic



Methane Oxidation (Aerobic), Figure 1 Pathway of aerobic methane oxidation for energy conservation and carbon assimilation in Type I and II methanotrophs. pMMO, particulate methane monooxygenase; MDH, methanol dehydrogenase; FAox, formaldehyde oxidation system (tetrahydrofolate- or methanopterin-dependent) FDH, formate dehydrogenase. Methane is oxidized with oxygen by pMMO, a step which requires reducing equivalents (e.g., from NADH) supplied by later oxidations in the pathway. Energy is generated by shuttling electrons generated in the subsequent oxidations of methanol (CH_3OH), formaldehyde (CH_2O), and formate (HCOO^-) through a membrane-bound electron transport chain that creates a proton gradient across the cell membrane (proton motive force) that can be converted into ATP by ATPase (oxidative phosphorylation). Formaldehyde (CH_2O) is the starting point for carbon assimilation via the ribulose monophosphate or serine pathways.

respiration. Differences between methanotrophs are thought to exist regarding the enzymes used, especially with regard to formaldehyde oxidation, where different systems are known (Lidstrom, 2006).

Methanotrophs

Most known methanotrophs fall into two well-defined phylogenetic groups belonging to the gamma- (Type I methanotrophs) and alpha-proteobacteria (Type II) that are also distinguished by morphological traits and differences in carbon assimilation (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). Both types share the characteristic extensive intracytoplasmic membrane system (ICM), which is the site of methane oxidation. However, in Type I methanotrophs, the ICM occurs as disk-shaped membrane stacks within the cell, while in Type II methanotrophs, the ICM is located around the periphery of the cell. Both groups are strict aerobes and obligate methylotrophs. The presence of soluble methane monooxygenase (sMMO) seems to be mostly limited to Type II methanotrophs (Hanson and Hanson, 1996; Bowman, 2006). Type I and II methanotrophs employ different carbon assimilation strategies, although both incorporate methane carbon at the level of formaldehyde (Figure 1). Type I methanotrophs use the ribulose monophosphate pathway and obtain all their carbon from methane. Type II methanotrophs use the serine pathway, which in addition to formaldehyde incorporation further involves fixation of one molecule of CO₂ per molecule of formaldehyde assimilated. The former pathway is energetically more favorable, requiring only 1/3 ATP per molecule formaldehyde fixed, instead of 1 ATP and reducing power for the serine pathway. This is in accordance with the observed higher growth rates in Type I methanotrophs (Madigan et al., 2003). Most methanotrophs can also grow using other single-carbon compounds, e.g., methanol, which is the first intermediate of methane oxidation.

Within the Type I methanotrophs, the genera *Methylosphaera*, *Methylobacter*, *Methyломicrobium*, *Methylomonas*, *Methylococcus*, and *Methylocaldum* have been described. The latter two genera are often distinguished as “type X methanotrophs,” as they are phylogenetically and morphologically distinct from the other Type I genera. The cultivated type II methanotrophs belong to the genera *Methylosinus* and *Methylocystis* (Bowman, 2006). Environmental clone libraries suggest the existence of additional, yet uncultured, genera. Recently, acidophilic methanotrophs belonging to the *Verrucomicrobia* phylum have been isolated, showing for the first time that not all methanotrophs belong to the proteobacteria and that methanotrophs are phylogenetically more diverse than previously thought (Dunfield et al., 2007; Pol et al., 2007). The genome sequence of one isolate revealed the presence of pMMO genes (pmoA), but lacked homologs to known methanol

monooxygenases and formaldehyde oxidation genes, indicating a methane oxidation system that deviates from those known from Type I and II methanotrophs (Dunfield et al., 2007).

Habitats

Although current isolates require high methane partial pressures for growth, some methanotrophs can apparently utilize methane with high affinity at low partial pressures, down to atmospheric levels (Hanson and Hanson, 1996). Therefore, they are ubiquitous in soil and water. Increased populations and activity occur wherever increased methane concentrations, whether from biogenic or other sources, meet oxic conditions. Typical habitats are thus closely tied to sites with methanogenesis (see Chapters *Methane, Origin*; *Methanogens*), e.g., rice paddies, swamps, aquatic and terrestrial sediments, land-fill coverings, and the oxycline of stratified water bodies (Hanson and Hanson, 1996). In stratified systems, (e.g., in stratified water bodies or sediments) aerobic methane oxidation will often be located within a relatively narrow band within the oxycline, where methanotrophs can become highly enriched. Methanotrophs also occur as endosymbionts, e.g., of marine mussels found at cold seeps (qv) that use methane released to supply their hosts with carbon and energy (Hanson and Hanson, 1996; Bowman, 2006).

Methods to study aerobic methane oxidation and methanotrophs

Methane oxidation rates in environmental samples are frequently determined by incubation experiments. Samples are placed in a gas-tight container and the consumption of methane over time is determined in the headspace. Alternatively, ¹⁴C labeled methane can be used to quantify the conversion to CO₂ and incorporation into the bacterial biomass. Field methods include the use of push–pull tests (Urmann et al., 2005), or modeling of rates based on methane gradients.

Methanotrophs can be cultured from almost any environment using a nitrate mineral salts medium and a CH₄/air atmosphere (Madigan et al., 2003; Bowman, 2006). Since they are relatively easily cultured, viable count methods can be used but will severely underestimate the actual population, due to the prevalence of viable but nonculturable cells. Since they are phylogenetically well defined, methanotrophs can be detected and quantified using ribosomal RNA as a genetic marker, e.g., using specific oligonucleotide probes (e.g., for fluorescent in-situ hybridization) or primers for polymerase chain reaction based detection (PCR) (McDonald et al., 2008). In addition, the gene sequences of functional genes, most frequently the gene encoding pMMO (pmoA), but also genes for sMMO (mmoX) and methanol dehydrogenase (mxaF), are frequently used as molecular markers for

PCR-based detection (Hanson and Hanson, 1996; Bowman, 2006; McDonald et al., 2008). A hybridization microarray was developed for rapid phylogenetic typing of PCR amplified *pmoA* (Bodrossy et al., 2003). Type I and II methanotrophs also contain biomarkers (see Chapter *Biomarkers (Organic, Compound-Specific Isotopes)*), e.g., specific lipids and phospholipid fatty acids that are useful in tracing community changes (Hanson and Hanson, 1996; Bowman, 2006; Schubert et al., 2006; Blumenberg et al., 2007).

Geochemical impact

Locally, methanotrophs can be an important basis of the food web, if sufficiently large fluxes of methane are converted to biomass and passed on down the food chain. Since biogenic methane (see Chapters *Methane, Origin; Methanogens*) has a strongly ^{13}C depleted carbon isotope signature, carbon flow originating from oxidized methane can be studied using isotope measurements (see Chapter *Isotopes (Methods)*).

The primary importance of aerobic methane oxidation for the global *carbon cycle* (qv) is that it represents (together with *anaerobic methane oxidation* (qv) and photochemical methane oxidation in the atmosphere) a major sink for this radiatively active and therefore, important greenhouse gas. Globally, photochemical methane oxidation is by far the dominant process (94%), but microbial oxidation is an important factor to take into account when projecting global warming effects. Obtaining global estimates of microbial aerobic methane oxidation is not trivial due to high spatial and temporal variability and the difficulty in performing rate measurements in environments where methanogenesis and methanotrophy occur simultaneously. The total sink function of soils has been estimated at 29 Tg year^{-1} , with an uncertainty range of 7–100 Tg year^{-1} (Smith et al., 2000). In rice paddies, methane oxidation can occur in the rhizosphere and at the soil surface, and is estimated to remove 80% of the methane produced in these systems, but there is uncertainty that CH_4 bypass would possibly occur through vascular plants' parenchyma. Methane oxidation rates in soil depend on various factors, e.g., soil bulk density, water content, temperature, and pH (Hanson and Hanson, 1996). The process is also often limited by the rate of methane diffusion into the oxic zone. Further, ammonium is a competing substrate for MMO that is not coupled to energy generation and therefore acts as a poison to methanotrophs at higher concentrations (Madigan et al., 2003). Thus, the methanotroph activity in soil is subject to considerable variability and sensitive to land use changes, and is one factor to be considered for mitigation strategies to reduce atmospheric methane concentrations (Hanson and Hanson, 1996).

Lakes were long considered a minor source of methane, a view that has somewhat changed based on observation of significant methane fluxes from reservoirs (St. Louis

et al., 2000). In permanently or temporally stratified water bodies such as lakes, reservoirs, or the Black Sea, a large proportion of the methane is oxidized by aerobic methanotrophs in the chemocline (Schubert et al., 2006; Blumenberg et al., 2007).

While the open ocean is not considered a major source of methane, and methane oxidation activity is generally low, *cold seeps* (qv) release large amounts of methane from sediments and hydrocarbon deposits (including *gas hydrates* (qv)), creating high local fluxes of methane (see Chapter *Methane, Origin*). This creates a situation where symbiotic as well as free-living methane oxidizers are the basis of large oceanic food webs. However, in anoxic marine sediments *anaerobic methane oxidation* (qv) coupled to sulfate reduction is the most important process.

Summary

Aerobic oxidation of methane is performed by specific bacteria, the methanotrophs, which use a specific metabolic pathway for carbon assimilation and energy generation from methane. Most known methanotrophs belong to the Proteobacteria, but recent discoveries of novel methanotroph *Verrucomicrobia* indicated that the diversity of methanotrophs is not yet fully known. Aerobic methane oxidation is one of the major sinks for the greenhouse gas methane, along with *anaerobic methane oxidation* (qv) and photochemical oxidation in the atmosphere. It is, thus, an important process for the global *carbon cycle* (qv) and an important element in the regulation of the atmospheric concentration of methane.

Bibliography

- Blumenberg, M., Seifert, R., and Michaelis, W., 2007. Aerobic methanotrophy in the oxic-anoxic transition zone of the Black Sea water column. *Organic Geochemistry*, **38**(1), 84–91.
- Bodrossy, L., Stralis-Pavese, N., Murrell, J. C., Radajewski, S., Weilharter, A., and Sessitsch, A., 2003. Development and validation of a diagnostic microbial microarray for methanotrophs. *Environmental Microbiology*, **5**(7), 566–582.
- Bowman, J., 2006. The methanotrophs - the families methylcocccaceae and methylcystaceae. In Dworkin, M. (ed.), *The Prokaryotes*. New York: Springer, Vol. 5, pp. 266–289.
- Dunfield, P. F., Yuryev, A., Senin, P., Smirnova, A. V., Stott, M. B., Hou, S., Ly, B., Saw, J. H., Zhou, Z., Ren, Y., Wang, J., Mountain, B. W., Crowe, M. A., Weatherby, T. M., Bodelier, P. L. E., Liesack, W., Feng, L., Wang, L., and Alam, M., 2007. Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature*, **450**(7171), 879–882.
- Hanson, R. S., and Hanson, T. E., 1996. Methanotrophic bacteria. *Microbiological Reviews*, **60**(2), 439–471.
- Lidstrom, M. E., 2006. Aerobic methylotrophic prokaryotes. In Dworkin, M. (ed.), *The Prokaryotes*. New York: Springer, Vol. 2, pp. 618–634.
- Madigan, M. T., Martinko, J. M., and Parker, J., 2003. *Brock Biology of Microorganisms*. Upper Saddle River, NJ: Pearson Education.

- McDonald, I. R., Bodrossy, L., Chen, Y., and Murrell, C. J., 2008. Molecular ecology techniques for the study of aerobic methanotrophs. *Applied and Environmental Microbiology*, **75**(5), 1305–1315.
- Pol, A., Heijmans, K., Harhangi, H. R., Tedesco, D., Jetten, M. S. M., and Op den Camp, H. J. M., 2007. Methanotrophy below pH1 by a new Verrucomicrobia species. *Nature*, **450**(7171), 874–878.
- Schubert, C. J., Coolen, M. J. L., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E. C., Damste, J. S. S., Wakeham, S., and Kuypers, M. M. M., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**(10), 1844–1856.
- Smith, K. A., Dobbie, K. E., Ball, B. C., Bakken, L. R., Sitaula, B. K., Hansen, S., Brumme, R., Borken, W., Christensen, S., Prieme, A., Fowler, D., Macdonald, J. A., Skiba, U., Klemetsson, L., Kasimir-Klemetsson, A., Degorska, A., and Orlanski, P., 2000. Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Global Change Biology*, **6**(7), 791–803.
- St. Louis, V. L., Kelly, C. A., Duchemin, E., Rudd, J. W. M., and Rosenberg, D. M., 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. *BioScience*, **50**(9), 766–775.
- Urmann, K., Gonzalez-Gil, G., Schroth, M. H., Hofer, M., and Zeyer, J., 2005. New field method: gas push-pull test for the in-situ quantification of microbial activities in the vadose zone. *Environmental Science & Technology*, **39**(1), 304–310.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Carbon Cycle](#)
[Cold Seeps](#)
[Isotopes \(Methods\)](#)
[Methane, Origin](#)
[Methanogens](#)

METHANE, ORIGIN

Carsten J. Schubert
 Eawag, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

Definition

Methane is a colorless and odorless gas, with the chemical formula CH₄. Due to its radiative force, it is a strong greenhouse gas and contributes to the warming of the earth. It is formed in the environment by methanogenesis. The main natural sources are wetlands and termites (30% of total emissions), while anthropogenic sources include rice fields, cattle farming, and energy production (70% of total emissions).

General aspects

Methane is a colorless and odorless gas and the major component (97% vol.) of natural gas. Methane has a boiling point of −161°C at a pressure of one atmosphere. As a gas, it is flammable only over a narrow range of concentrations (5–15%) in air. It is mainly produced by microorganisms (methanogens) under anoxic conditions in a process called methanogenesis. These conditions exist under water-covered soils, such as rice paddies, tundra, swamps, and marshes, but also in freshwater and marine sediments. Other anoxic environments include ruminant stomachs and termite guts (see [Table 1](#)).

Methanogenesis is the final step in the anaerobic degradation of organic carbon, with methane being formed as a waste product. Methanogenesis is performed by strict anaerobes called methanogens, organisms belonging to the Archaea (a domain of life alongside Bacteria and Eukaryota). Methanogens are rather cosmopolitan in respect to environmental conditions (i.e., temperature, pH, salinity; as long as the environment is anoxic) and represent the largest and most diverse group in the Archaea domain (Magonigal et al., 2004; c.f. Boone et al., 1993). Methanogens are able to grow at temperatures between 4°C and 100°C, at pH values from 3 to 9, and at salinities ranging from brines to freshwater. Despite this huge taxonomic diversity, the substrates that can be used by methanogens are rather limited – hydrogen, acetate, formate, some alcohols, and methylated compounds (Zinder, 1993). The most important of these are hydrogen and acetate, which give rise to two different kinds of methanogenesis: (1) the fermentation of acetate to CO₂ and CH₄ and (2) the reduction of CO₂ with H₂.

Garcia et al. (2000) showed that over 70% of methanogens use the latter pathway, which is known as hydrogenotrophic methanogenesis or carbon dioxide/hydrogen reduction. Hydrogen in this reaction is used both as an energy source and electron donor, whereas CO₂ is the electron acceptor and the carbon source. This reaction yields the most energy of all methanogenic processes – much more than acetate fermentation, which yields the least (see [Table 2](#)). Since hydrogen occurs only in very low concentrations in natural environments, it is the limiting substrate in CO₂/H₂ reduction.

Only 10% of the methanogenic genera, i.e., *Methanosarcina* and *Methanosaeta*, are able to use the other pathway, called acetate fermentation or acetoclastic

Methane, Origin, Table 1 Methane properties

Methane properties	
Chemical formula	CH ₄
Mass	16.0425 g/mol
Melting point	−182.5°C
Boiling point	−161.6°C
Solubility in water	3.5 mg/100 ml

methanogenesis (Garcia et al., 2000). Despite its limited taxonomic distribution, most likely related to the small amount of energy gained from this process (see Table 2), acetate fermentation seems to be the dominant pathway for methane production in the environment.

Isotopic composition

One of the diagnostic features of methane often used in microbiological, geological, and biogeochemical investigations is its carbon and, to a lesser extent, hydrogen isotopic composition. Due to its biogenic or thermogenic formation, the ^{13}C composition is strongly depleted (i.e., more negative values, see Equation 1).

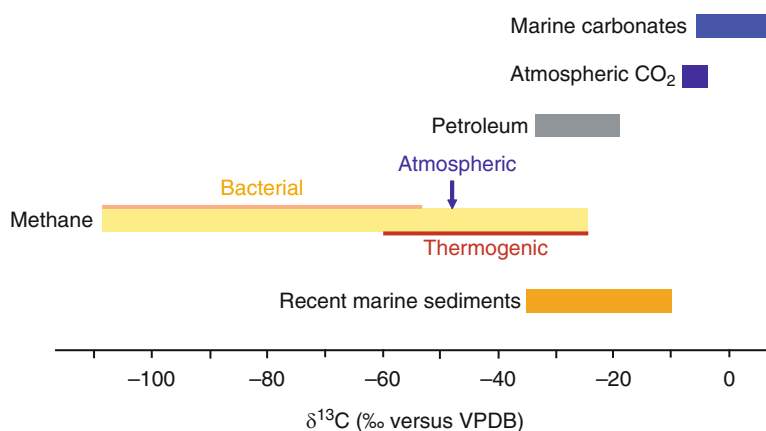
$$\delta^{13}\text{C} = ((^{13}\text{C}/^{12}\text{C} \text{ sample}) / (^{13}\text{C}/^{12}\text{C} \text{ standard}) - 1) \times 1,000 \text{ vs. VPDB} \quad (1)$$

(Vienna Pee Dee Belemnite)

Methane, Origin, Table 2 Reaction and standard changes in Gibbs free energies for methanogenesis (Adapted from Garcia et al., 2000)

Reaction	ΔG° (kJ/mol CH_4)
$4\text{H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	-135.6
$4\text{Formate} \rightarrow \text{CH}_4 + 3\text{CO}_2 + 2\text{H}_2\text{O}$	-130.1
$2\text{Ethanol} + \text{CO}_2 \rightarrow \text{CH}_4 + 2\text{Acetate}$	-116.3
$\text{Methanol} + \text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$	-112.5
$4\text{Methylamine} + 2\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 4\text{NH}_4^+$	-75.0
$4 \text{ 2-Propanol} + \text{CO}_2 \rightarrow \text{CH}_4 + 4\text{Acetone} + 2\text{H}_2\text{O}$	-36.5
$\text{Acetate} \rightarrow \text{CH}_4 + \text{CO}_2$	-31.0

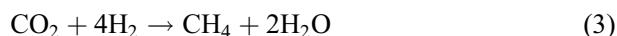
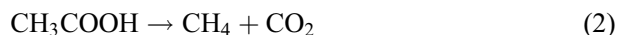
This is related to a kinetic isotope effect where ^{12}C carbon is preferentially used by microorganisms when methane is formed (Whiticar, 1999). This isotopic effect is not as pronounced in the case of methane formed by heating, or with higher pressures during gas formation from a source rock. In Figure 1, mean values of carbon isotopic compositions are shown for various organic and inorganic materials. Whereas carbonates formed in seawater have values around 0‰ VPDB, and atmospheric carbon dioxide shows a global value of around -8‰ VPDB, methane shows highly depleted values between -25‰ and -110‰ VPDB. This significant feature (sometimes in combination with hydrogen isotopes) has been used in numerous studies to reveal, for instance, the origin of methane (Whiticar et al., 1986) or methane oxidation processes in old carbonate rocks, sediments, or water columns of lakes and oceans (Boetius et al., 2000; Peckmann and Thiel, 2004; Schubert et al., 2006). Not only can the formation process (i.e., biological or thermodynamically controlled) be derived, but also changes that occur during its usage and transformation, and therefore the origin/source of methane be determined. Whenever microorganisms use methane as an energy or food source, they preferentially take up the light ^{12}C carbon. This leads to very light carbon isotopic compositions of, for instance, the cellular lipid structure, as can be seen in the process of anaerobic methane oxidation (see Chapter *Anaerobic Oxidation of Methane with Sulfate*). In contrast, the remaining methane will be enriched in ^{13}C , leading to the heavy isotopic values of, for instance, methane in water columns or sediments. Fractionation factors – the relation between the isotopic composition of the original methane and the methane after transformation – provide a tool for quantifying the amount of methane that has reacted.



Methane, Origin, Figure 1 Carbon isotopic composition of various organic and inorganic materials, and recent marine sediments. Note that methane has the widest range of all compounds. Atmospheric methane has a global carbon isotopic value of about -47‰ versus VPDB.

Fractionation factors are also important during methane formation. Whiticar et al. (1986) showed that different microbial formation processes, such as carbon dioxide reduction or fermentation, lead to different isotopic compositions of the methane formed. This is related to the bonds that need to be broken during the methane formation process.

Whereas during acetate fermentation (Equation 2) the methyl group stays intact and therefore only a small overall isotopic fractionation occurs, during hydrogenotrophic methanogenesis (Equation 3) the bond between the oxygen and carbon of the carbon dioxide has to be broken, leading to a much bigger isotope effect.



The $\delta^{13}\text{C}_{\text{CH}_4}$ from different sources/environments covers a fairly wide range due to variations in methanogenic substrates and mechanisms (Tyler, 1991). Additionally, fractionation associated with oxidation leads to a rather wide $\delta^{13}\text{C}$ range. In general, CH_4 from geological or thermogenic sources is isotopically heavier (-30‰ to -50‰ VPDB) than that from biological sources (-40‰ to -80‰ VPDB). Also, the CH_4 produced by decomposition of either C3 or C4 plant material leads to a distinctive isotopic composition, since the original material is formed via different photosynthesis systems known to fractionate carbon in a different way.

Atmospheric methane has a relatively stable carbon isotopic value globally – around -47‰ VPDB (see compilation in Stevens and Wahlen, 2000).

Relevance as a greenhouse gas

Methane is the shortest hydrocarbon and the most abundant organic compound in the atmosphere. Due to its ability to absorb on the infrared band, it is a potent greenhouse gas. Taking into account its residence time in the atmosphere (about 10 years), its concentration, and its specific absorbance wavelength, it is about 23 times more potent as a greenhouse gas than CO_2 (IPCC, 2001).

Methane mixing ratios in the atmosphere have doubled over the last 200 years, increasing by about 1% per year from 1978 and reaching a value of 1.7 ppm in 1990 (Figure 2a). Since then, the yearly increase has slowed down and values of 1.8 ppm are measured today (Figure 2b).

During the past 15 years, the annual growth rate of tropospheric methane has shown striking changes over 2- to 3-year periods, varying from $+1\%$ year $^{-1}$ to slightly negative values (-0.2% year $^{-1}$). These fluctuations are superimposed on an overall slowdown of the CH_4 growth rate since the 1980s. The general reduction in atmospheric methane growth seems to be related not to a change in source strength, but to source stabilization and the methane budget approaching a steady state (Dlugokencky et al., 2003).

Methane sinks

The strongest sink for methane is oxidation by OH radicals in the troposphere (Badr et al., 1992). Approximately, 94% of methane is lost due to oxidation here and in the stratosphere. Due to photochemical reactions, the above-mentioned reaction produces H_2O , CO , and CO_2 , and hence influences concentrations of ozone, hydroxyl radicals, and CO in the troposphere. Another sink term that needs to be taken into account is soils, in which approximately 6% of methane is oxidized by methanotrophic bacteria.

Recently, the focus has been on oxidation processes that take place in aquatic systems. So far, these have not been included in the source/sink budgets, presumably since none of the methane reaches the atmosphere. However, it is mentioned here as it is an important sink that prevents methane from reaching the atmosphere, and therefore from acting as a greenhouse gas. In aquatic systems, two main processes lead to a loss of methane. One is oxidation by methanotrophic bacteria under oxic conditions in the uppermost sediments and overlying water column (see Chapter *Anaerobic Oxidation of Methane with Sulfate*). The other possibility, and probably the major pathway, is oxidation by methanotrophic archaea under anoxic conditions in deeper sediments and water columns lacking oxygen (see Chapter *Anaerobic Oxidation of Methane with Sulfate*). These two main degradation processes largely restrict the emission or ebullition (transport by bubbles) of methane to the atmosphere from aquatic systems. Normally, 95–99% of the methane found in deeper sediments is oxidized either directly in the sediments or later in the water column and does not, therefore, reach the atmosphere.

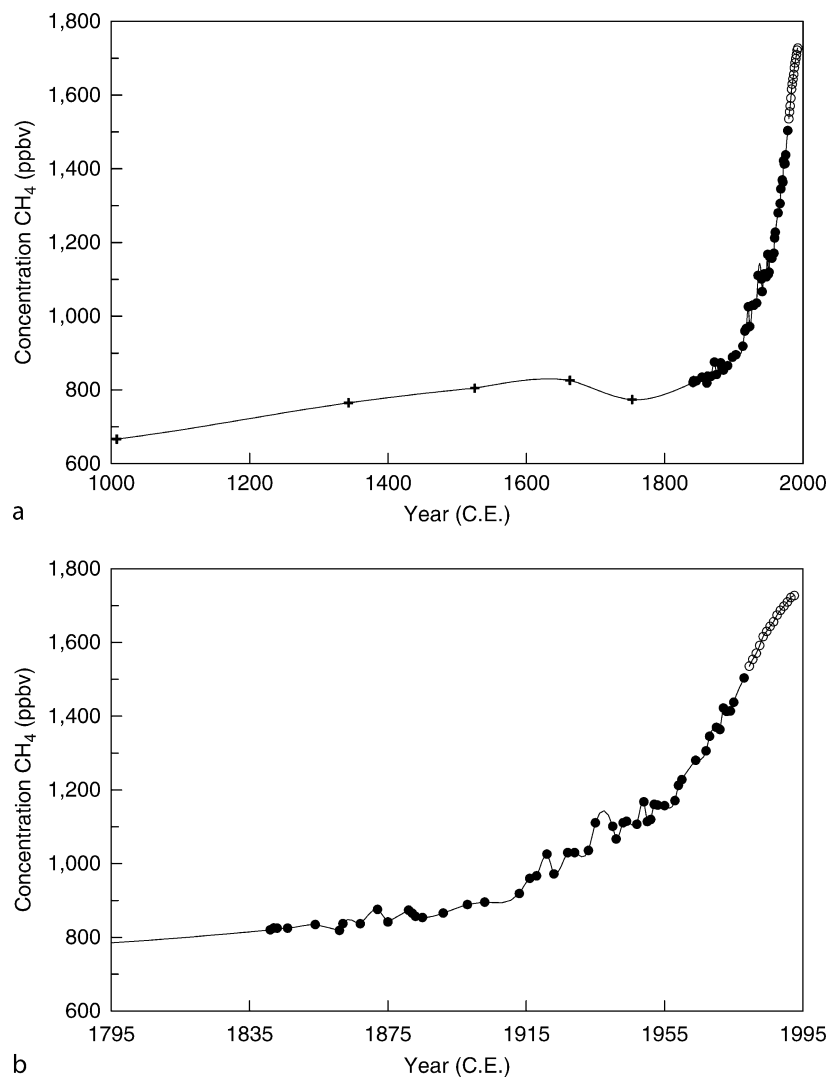
Methane sources

In general, methane sources can be divided into abiotic (20%) and biogenic sources (80%). Abiotic sources include fossil carbon sources, such as coal mining, industrial waste treatment, and combustion processes (e.g., aircraft and automobile exhausts), as well as sea floor and volcanic emissions. Biomass burning during deforestation and for heating purposes also falls into this category. However, most methane is produced by biological processes: the major sources are freshwater wetlands and rice paddies, but ruminants and the guts of termites are also important contributors.

The total methane budget may also be divided into emissions from natural or anthropogenic sources (Figure 3). The major sources are discussed in detail below. In considering the various contributors, it should always be borne in mind that estimates for individual sources still vary widely, although ongoing research is seeking to constrain the budget (c.f. Table 7.6 in Denman et al., 2007).

Wetlands

Wetlands, covering about $5.3 \times 10^{12} \text{ m}^2$ globally (Matthews and Fung, 1987), are one of the most important

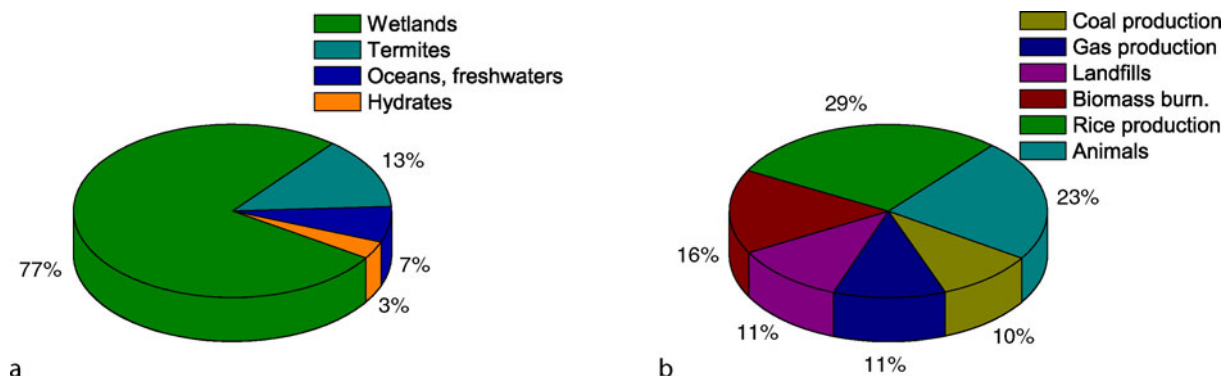


Methane, Origin, Figure 2 Methane mixing ratios over the last 1,000 years (a) and over only the last century (b) (adapted from Khalil, 2000). Data from Rasmussen and Khalil (1984) and Etheridge (1992). It is evident from the data obtained from ice cores that the drastic increase only started 200 years ago. During the last 20 years, a slowdown in methane concentrations in the atmosphere is visible.

natural sources of atmospheric methane, with an estimated contribution of 115 Tg year^{-1} (Cicerone and Oremland, 1988). Peat-rich bogs in the northern hemisphere ($50\text{--}70^\circ\text{N}$) were mentioned in particular, accounting for 60% of total wetland methane emissions, with tropical and subtropical swamps releasing only 25% of the total (Matthews and Fung, 1987; Fung et al., 1991). The rest was attributed to fens, marshes, and floodplains. However, the high level of emissions from northern wetlands (based on only a few methane emission measurements) was later scaled down, using a process-based methane emission model (Cao et al., 1996). In northern wetlands, according to this publication, CH_4 flux rates per square meter are low ($40 \text{ mg CH}_4 \text{ m}^{-2}\text{day}^{-1}$), but the area of wetland is

large. Therefore, northern wetlands and moist/dry tundra together contribute 25% to global emissions. The second term includes temperate wetlands with a higher mean flux rate ($150 \text{ mg CH}_4 \text{ m}^{-2}\text{day}^{-1}$) but a much smaller area, leading to a contribution of 19%. Since tropical wetlands – mainly swamps – have an even higher mean daily flux ($199 \text{ mg CH}_4 \text{ m}^{-2}$), they account for 56% of total wetland emissions despite covering a much smaller area than northern wetlands.

The carbon isotopic composition of methane emitted from wetlands varies from -86‰ to -31‰ VBDP (compilation by Bréas et al., 2001). This rather wide range is, of course, a function of the formation process (methanogenesis by acetate fermentation or CO_2/H_2



Methane, Origin, Figure 3 Net emissions of methane stemming from natural (a) and anthropogenic sources (b). Numbers have been taken from Scenario 7 of Fung et al. (1991). The drastic variations from one study to another are attributable to the wide ranges of emission estimates (for instance, wetland emission estimates vary from 100 to 230 Tg CH₄ year⁻¹), and freshwater emissions (see below) have yet to be accounted for.

reduction), the original degradation material forming the substrate for methanogenesis (C3 or C4 plants), and more or less strong methane oxidation, leaving behind isotopically heavy methane.

Termites

Termites feed on all kinds of food, including wood, grass, leaf litter, and agricultural crops. It is very difficult to estimate the amount of methane produced anaerobically in the guts of termites, since it is highly variable between species and colonies. The total amount, mainly produced in tropical savannahs and forests, is estimated at 20 Tg CH₄ year⁻¹.

The carbon isotopic composition of methane emitted from termites varies from −73‰ to −44‰ VBDP (Tyler et al., 1988). Interestingly, the $\delta^{13}\text{CH}_4$ of termite-emitted methane is independent of diet.

Rice fields

Rice is cultivated either on dry land, from which no methane is emitted, or – in the case of about 80% of global cultivation – on irrigated fields, leading to high methane emissions. Rice is cultivated between 50°N and 50°S on an area of 1.3×10^6 km², mostly situated in Asia (90%) (Bréas et al., 2001).

Globally, methane emissions from rice fields are second to those from wetlands and represent the highest anthropogenic budget term (100 Tg CH₄ year⁻¹). On rice fields, a methane release rate between 0.4 and 0.8 g m⁻² day⁻¹ has been observed (Holzapfel-Pschorn and Seiler, 1986). Methane in rice fields is produced by methanogens which find an ideal environment in flooded soils, i.e., high organic carbon loads and anoxic conditions. The amount of methane produced has been shown to be influenced by several factors, such as soil properties, amount and type of fertilizer, irrigation, etc. The carbon

isotopic composition of methane emitted from rice fields also varies widely due to the same variables as found in wetlands and lies between −50‰ and −68‰ VBDP (compilation in Bréas et al., 2001).

Enteric fermentation in animals

Methane is formed in the rumen of animals as a by-product of carbohydrate chain (e.g., cellulose) degradation. During digestion, ingested macromolecules are broken down into smaller molecules by enzymes. Subsequently, the fermentation of, for example, glucose leads to even smaller molecules, such as acetate, butyrate, H₂ and CO₂, etc. The H₂ produced, together with CO₂, is then immediately used by methanogens to form methane.

Global methane emissions mainly from cattle, buffalo, and sheep range between 65 and 100 Tg CH₄ year⁻¹ (Cicerone and Oremland, 1988) and are therefore comparable to rice cultivation and wetland emissions.

Biomass burning

Whereas the burning of biomass mainly produces CO₂, the subsequent smoldering phase – involving compounds with low combustion efficiency – mainly produces methane. About 85% of methane emissions related to biomass burning stem from tropical areas due to deforestation and fuel wood use. It is estimated that biomass burning accounts for 8–10% (i.e., 40–55 Tg CH₄ year⁻¹) of total methane emissions (Cicerone and Oremland, 1988; Hein et al., 1997).

Mining, gas drilling, natural geological sources

During coal mining and gas drilling, methane is produced not by biological processes but from catagenesis of coal or maturation of petroleum and subsequently released into the atmosphere. Cicerone and Oremland (1988) and

Fung et al. (1991) estimated these emissions to be in the order of $75 \text{ Tg CH}_4 \text{ year}^{-1}$.

Recently, Kvenvolden and Rogers (2005) estimated the amount of methane emitted from natural geological sources, i.e., not related to petroleum production or mining, to be in the order of $45 \text{ Tg CH}_4 \text{ year}^{-1}$. Those geological sources include natural macro- and micro-seeps, mud volcanoes, and other miscellaneous sources such as gas hydrates, magmatic volcanoes, geothermal regions, and mid-ocean ridges.

Landfills

Globally, methane emitted from landfills following the breakdown of organic material via methanogenesis is estimated to contribute about $43 \text{ Tg CH}_4 \text{ year}^{-1}$ (Hein et al., 1997).

Oceanic emissions

Methane production in the ocean is found basically in two different environments: (1) in anoxic microenvironments of surface waters, e.g., in fish intestines, plankton samples, marine snow, and fecal pellets and (2) in anoxic sediments due to the breakdown of organic material. The first of these is also known as the “Ocean Methane Paradox,” since methane production would not be possible normally in oxic ocean water and is therefore restricted to these microenvironments. Although only relatively small amounts of methane are formed by this mechanism, leading to supersaturation of surface waters, the huge area of the oceans is responsible for making this term globally significant. Methane produced in sediments might be released into the water column by diffusion or by methane seeps and vents, or it might be locked up in gas hydrates (see below).

Methane emissions from the various kinds of seeps that occur in the oceans – in shelf regions, slopes, or deep ocean locations – are estimated to be between 1 and $65 \text{ Tg CH}_4 \text{ year}^{-1}$ (see compilation in Judd et al., 2002). The best investigated regions in this respect are the Santa Barbara Basin, where at Coal Oil Point an emission of $28 \text{ g CH}_4 \text{ m}^{-2} \text{ year}^{-1}$ was estimated (Hornafius et al., 1999), and the Black Sea, where more than 2,000 seeps were detected in the area west of the Crimean Peninsula alone. The yearly atmospheric methane contribution from seeps in the Black Sea is estimated to range between 0.03 and 0.15 Tg (Dimitrov, 2002).

Since most of the methane from seeps or sediments is oxidized by either aerobic or anaerobic processes in the sediments or in the water column, total oceanic emissions today are estimated to be in the order of 10 Tg year^{-1} (Cicerone and Oremland, 1988; Fung et al., 1991). It has to be taken into account that for methane released into the water column in the form of bubbles, the contribution to the atmosphere may be much higher, depending on the water depth. However, when bubbles travel through the water column, they exchange methane with the ambient

water; consequently, most of the gas in the bubbles at the water surface is composed of nitrogen rather than methane (McGinnis et al., 2005).

Gas hydrates

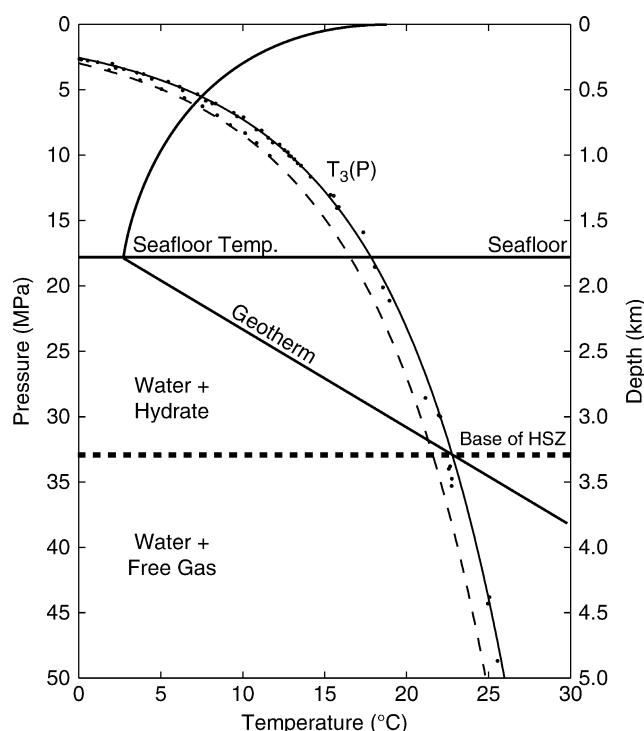
Gas hydrates are nonstoichiometric solid structures composed of ice-like minerals that form at low temperatures and high pressures in the deep sea or at low temperatures in permafrost regions. They can be thought of as a hydrogen-bonded water framework called a host lattice that traps guest molecules (typically gases). In general, three types of gas hydrates can be distinguished based on the structure in which they crystallize. These are structure I, structure II, and structure H (which occurs more rarely). Hydrates contain gases such as hydrocarbons (including methane, hydrogen sulfide, and carbon dioxide) that glue themselves inside symmetrical cages of water molecules to form hydrate crystals (mainly found in type I structures). Smaller molecules, such as argon, krypton, and nitrogen, are found in smaller cages related to structure II.

Gas hydrates or clathrates are known to occur mainly on continental margins all over the world at depths of around 600 – 3,000 m, depending on the prevailing temperature gradient (see Figure 4). Since pipelines of offshore drilling platforms are also located in these depths, clogging of pipes due to the formation of gas hydrates is a well-recognized problem.

Gas hydrates are confined to the top few hundred meters of marine sediments. They are stable when the temperature is at or below the value $T_3(P)$ for a three-phase equilibrium between clathrate, liquid water, and methane gas (Buffett and Archer, 2004). Experimental determinations of $T_3(P)$ are shown in Figure 4 as a function of depth, assuming a hydrostatic increase in pressure. Superimposed on this figure is a schematic illustration of the temperature profile through the ocean and sediments. The zone of stability is limited by the intersection of the local temperature profile with $T_3(P)$, although clathrate is confined to the sediments because the concentration of methane in the ocean is too low to form clathrate and, even if it did form, the buoyancy of clathrate in seawater would carry it out of the stability zone. Clathrate is unlikely to form below water depths of less than 600 m (except in the cold Arctic Ocean) because the bottom water is too warm for stability. As the depth of the water increases, and the temperature of the seafloor decreases, the zone of stability becomes thicker and capable of accommodating larger volumes of clathrate.

It must be understood that gas hydrates are by no means stable: these structures dynamically trap methane from, or release methane into, the ocean. This feature led Hovland and Judd (1988) to conclude that slides and craters on ocean shelves might be related to rapid hydrate decomposition.

Going back in time, gas hydrates have even been claimed to be responsible for larger environmental



Methane, Origin, Figure 4 Schematic illustration of temperature through the ocean and uppermost marine sediments (Buffett and Archer, 2004). The temperature for clathrate stability, $T_3(P)$, increases with pressure (or depth). Experimental data for $T_3(P)$ in pure water (dashed line) and seawater (solid line) are extrapolated using a thermodynamic model (Sloan, 1998). The base of the stability zone is defined by the intersection of the geotherm with $T_3(P)$.

catastrophes. Based on light carbon isotopic excursions in the late Paleocene (50 million years ago), Dickens et al. (1995) suggested that methane from gas hydrates might have been released into the atmosphere, leading to significant warming (4°C) during that time.

One of the main features used to find gas hydrates on ocean margins today is the bottom-simulating reflector, which shows up using seismological methods. This is formed by the base of solid gas hydrate and free gas accumulating below in the sediments.

It has been estimated that the amount of carbon (and therefore, energy) stored in gas hydrates (10,000 Gt C) is at least twice as great as in all known oil, gas, and coal fields combined (Kvenvolden and Lorenson, 2001). This would be equal to 2,000 times the atmospheric methane inventory. However, recent estimates have brought the abundance of gas hydrates down from 1×10^{17} – 10^{18} m^3 to $(1\text{--}5) \times 10^{15} \text{ m}^3$, also reducing the energy stored in gas hydrates globally to 500–2,500 Gt C (Milkov et al., 2004). Buffett and Archer (2004) estimated that about 3,000 Gt C occurs in clathrate and 1,800 Gt C in methane bubbles. Estimates of the amount

of gas hydrate-related methane actually emitted into the atmosphere reach $5 \text{ Tg CH}_4 \text{ year}^{-1}$ (Wuebbles and Hayhoe, 2002).

Freshwater emissions

Recently, freshwater environments, including lakes and reservoirs for hydropower production, have become the focus of attention not only for CO_2 , but also for methane emissions. Lakes kept in a natural state or with only minor influence by humans undergo normal aerobic degradation of organic material and mainly emit CO_2 . However, when organic material production is high, natural lakes also turn anoxic either in the water column or in the sediments and release methane due to anaerobic degradation, i.e., methanogenesis. This occurs even more rapidly in systems that are highly eutrophicated, e.g., due to intensive agriculture or effluents from sewage treatment plants.

In contrast, reservoirs are normally man-made. By damming rivers, huge areas are flooded and the water is stored for energy production. If the flooded area is a forest, peatland, or grassland, then – instead of being a sink for CO_2 due to photosynthesis – this area will become a source of greenhouse gases due to anaerobic decomposition of the submerged organic material. For example, in the boreal region of Canada, flooded peatlands represent a worst-case scenario, since they possess a large store of organic carbon held in peat, which can decompose and return to the atmosphere as greenhouse gases over a long period (Kelly et al., 1997). It makes a difference to the emission rate whether an area has only been flooded recently or was flooded decades ago. A newly formed reservoir will emit methane at a much higher rate, since fresh organic material is rapidly degraded. In contrast, reservoirs which are already tens of years old show lower methane emissions, since the older and more refractory organic material is harder to degrade.

Due to recent plans to trade or tax carbon dioxide emissions, “green” hydropower is under debate and it is therefore necessary to define the related CO_2 and methane emissions.

In a compilation (Table 1 in St. Louis et al., 2000), methane fluxes from temperate reservoirs varied from 1 to $260 \text{ mg m}^{-2} \text{ day}^{-1}$. Tropical reservoirs showed even higher fluxes, evolving from higher productivities, of $2\text{--}3,800 \text{ mg m}^{-2} \text{ day}^{-1}$ (St. Louis et al., 2000). Average fluxes were $20 \text{ mg m}^{-2} \text{ day}^{-1} \text{ CH}_4$ for temperate and $300 \text{ mg m}^{-2} \text{ day}^{-1} \text{ CH}_4$ for tropical reservoirs. Taking an area of $0.9 \times 10^6 \text{ km}^2$ for temperate and $0.6 \times 10^6 \text{ km}^2$ for tropical reservoirs, a global flux of 70 Tg can be estimated (St. Louis et al., 2000).

Emissions from natural lake systems have been recently evaluated by Bastviken et al. (2004). In these systems, three different pathways for methane emission are described: (1) ebullition, i.e., emission through bubbles, (2) diffusive flux, which mainly depends on surface methane concentrations and wind speed over surface waters, and (3) storage, i.e., methane released during lake

turnover. On average, these various pathways contribute 62%, 31%, and 7% to total emissions, respectively.

Using different regression equations for surface CH₄ concentrations, ebullition, diffusive flux, and storage, together with data on lake area and lake numbers (Kalf, 2002), Bastviken et al. (2004) estimated methane emissions from lakes on a global basis. When compared to global nonanthropogenic emission values from Wuebbles and Hayhoe (2002), the estimated lake emissions of 8–48 Tg CH₄ year⁻¹ represent 6–16% or 2–10% of total emissions.

Taking the two systems (natural lakes and reservoirs) together results in global methane emissions between 78 and 118 Tg year⁻¹. These figures are similar to emissions from wetlands, the largest natural contributor, and much higher than estimated oceanic emissions. Although these estimates might be on the high side, they clearly show that emissions from freshwaters have to be included in global methane budgets.

Summary

Methane is the end product of organic matter degradation under anoxic conditions. In freshwater environments methanogenesis is the main pathway due to the low sulfate concentrations, whereas in the marine environment methanogenesis becomes important only after sulfate, as an electron acceptor, has been used up completely. Methane is formed by microorganisms belonging to the Archaea known as methanogens. Methanogens are able to grow at temperatures between 4°C and 100°C, at pH values from 3 to 9, and at salinities ranging from brines to freshwater. Despite this huge taxonomic diversity, the substrates that can be used by methanogens are rather limited – hydrogen, acetate, formate, some alcohols, and methylated compounds. The two main pathways described in nature are hydrogenotrophic methanogenesis (also known as carbon dioxide/hydrogen reduction) which is mainly found in marine environments and acetate fermentation (acetoclastic methanogenesis) which is more dominant in freshwater environments. These two processes can be distinguished from each other by measurements of the carbon or hydrogen isotopic composition of the formed methane, since the fractionation factors between substrate (CO₂ or acetate) and methane vary.

Methane is a potent greenhouse gas, ranking second after carbon dioxide as a contributor to global warming. Its concentration has doubled over the past 200 years, although the increase has slowed down over the past 20 years which is thought to be related to source stabilization and the methane budget approaching a steady state.

The main sources of natural emissions are wetlands, termites, and aquatic systems, making up 30% of total global emissions, while the main anthropogenic sources, such as rice paddies, ruminants, energy production, and landfills, account for 70%. Gas hydrates occurring on continental slopes and permafrost regions also store high

amounts of methane; however, most of this methane is oxidized to carbon dioxide before it reaches the atmosphere. Recently, lakes and especially reservoirs have been identified to be major contributors to the global methane budget questioning of hydropower as a “green energy” source.

The main sinks for methane are oxidation with OH in the troposphere (accounting for more than 90%), loss in the stratosphere, and oxidation by methanotrophs in soils.

Bibliography

- Badr, O., Probert, S. D., and Ocallaghan, P. W., 1992. Sinks for atmospheric methane. *Applied Energy*, **41**(2), 137–147.
- Bastviken, D., Cole, J., Pace, M., and Tranvik, L., 2004. Methane emissions from lakes: dependence of lake characteristics, two regional assessments, and a global estimate. *Global Biogeochemical Cycles*, **18**(4), 1–12.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Boone, D. R., Whitmann, W. B., and Rouvière, P., 1993. Diversity and taxonomy of methanogens. In Jerry, J. G. (ed.), *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*. New York: Chapman Hall, pp. 35–80.
- Bréas, O., Guillou, C., Reniero, F., and Wada, E., 2001. Review, the global methane cycle: isotopes and mixing ratios, sources and sinks. *Isotopes in Environmental and Health Studies*, **37**, 257–379.
- Buffett, B., and Archer, D., 2004. Global inventory of methane clathrate: sensitivity to changes in the deep ocean. *Earth and Planetary Science Letters*, **227**(3–4), 185–199.
- Cao, M. K., Marshall, S., and Gregson, K., 1996. Global carbon exchange and methane emissions from natural wetlands: application of a process-based model. *Journal of Geophysical Research-Atmospheres*, **101**(D9), 14399–14414.
- Cicerone, R. J., and Oremland, R. S., 1988. Biogeochemical aspects of atmospheric methane. *Global Biogeochemical Cycles*, **2**(4), 299–327.
- Denman, K. L. et al., 2007. Couplings between changes in the climate system and biogeochemistry. In Solomon, S. et al. (eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press.
- Dickens, G. R., Oneil, J. R., Rea, D. K., and Owen, R. M., 1995. Dissociation of oceanic methane hydrate as a cause of the carbon-isotope excursion at the end of the paleocene. *Paleoceanography*, **10**(6), 965–971.
- Dimitrov, L., 2002. Contribution to atmospheric methane by natural seepages on the Bulgarian continental shelf. *Continental Shelf Research*, **22**(16), 2429–2442.
- Dlugokencky, E. J. et al., 2003. Atmospheric methane levels off: temporary pause or a new steady-state? *Geophysical Research Letters*, **30**(19).
- Etheridge, D. M., Pearman, G. I., and Fraser, P. J., 1992. Changes in tropospheric methane between 1841 and 1978 from a high accumulation-rate Antarctic ice core. *Tellus Series B*, **44**, 282–294.
- Fung, I. et al., 1991. 3-dimensional model synthesis of the global methane cycle. *Journal of Geophysical Research-Atmospheres*, **96**(D7), 13033–13065.
- Garcia, J. L., Patel, B. K. C., and Ollivier, B., 2000. Taxonomic phylogenetic and ecological diversity of methanogenic Archaea. *Anaerobe*, **6**(4), 205–226.

- Hein, R., Crutzen, P. J., and Heimann, M., 1997. An inverse modeling approach to investigate the global atmospheric methane cycle. *Global Biogeochemical Cycles*, **11**(1), 43–76.
- Holzapfel-Pschorn, A., and Seiler, W., 1986. Methane emission during a cultivation period from an Italian rice paddy. *Journal of Geophysical Research-Atmospheres*, **91**(D11), 1803–1814.
- Hornafius, J. S., Quigley, D., and Luyendyk, B. P., 1999. The world's most spectacular marine hydrocarbon seeps (Coal Oil Point, Santa Barbara Channel, California): quantification of emissions. *Journal of Geophysical Research-Oceans*, **104**(C9), 20703–20711.
- Hovland, M., and Judd, A. G., 1988. *Seabed Pockmarks and Seepages: Impact on Geology, Biology and the Marine Environment*. London: Graham and Trotman.
- IPCC, 2001. Climate change 2001: the scientific basis. In Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C.A. (eds.), *Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 881 p.
- Judd, A. G., Hovland, M., Dimitrov, L. I., Garcia Gil, S., and Jukes, V., 2002. The geological methane budget at continental margins and its influence on climate change. *Geofluids*, **2**(2), 109–126.
- Kalff, J., 2001. *Limnology: Inland water ecosystems*. Prentice Hall, 592 p.
- Kelly, C. A. et al., 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. *Environmental Science & Technology*, **31**(5), 1334–1344.
- Khalil, M. A. K., 2000. Atmospheric methane: an introduction. In Khalil, M.A.K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 1–8.
- Kvenvolden, K. A., and Lorensen, T. D., 2001. The global occurrence of natural gas hydrate. In Paull, C. K., and Dillon, W. D. (eds), *Natural Gas Hydrates: Occurrence, Distribution and Detection*. Geophysical Monograph. Washington, DC: American Geophysical Union.
- Kvenvolden, K. A., and Rogers, B. W., 2005. Gaia's breath - global methane exhalations. *Marine and Petroleum Geology*, **22**(4), 579–590.
- McGinnis, D. F., Wüest, A., Schubert, C. J., Klauser, L., Lorke, A., and Kipfer, R., 2005. Upward flux of methane in the Black Sea: does it reach the atmosphere? In Lee, J. H. W., and Lam, K. M. (eds.), *Environmental Hydraulics and Sustainable Water Management*. London: Taylor & Francis Group, pp. 423–429.
- Matthews, E., and Fung, I., 1987. Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles*, **1**(1), 61–86.
- Megonigal, J. P., Hines, M. E., and Visscher, P. T., 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In Schlesinger, W. H. (ed.), *Biogeochemistry*. Oxford: Elsevier-Pergamon, pp. 317–424.
- Milkov, A. V., 2004. Global estimates of hydrate-bound gas in marine sediments: how much is really out there? *Earth-Science Reviews*, **66**(3–4), 183–97.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**(3–4), 443–467.
- Rasmussen, R. A., and Khalil, M. A. K., 1984. Atmospheric methane in the recent and ancient atmospheres: concentrations, trends, and interhemispheric gradient. *Journal of Geophysical Research*, **89**, 11599–11605.
- Schubert, C. J. et al., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**(10), 1844–1856.
- St. Louis, V. L., Kelly, C. A., Duchemin, E., Rudd, J. W. M., and Rosenberg, D. M., 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. *Bioscience*, **50**(9), 766–775.
- Sloan, E. D., 1998. *Clathrate Hydrates of Natural Gas*, New York: Marcel Dekker.
- Stevens, C. M., and Wahlen, M., 2000. The isotopic composition of atmospheric methane and its sources. In Khalil, M. A. K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 25–41.
- Tyler, S. C. et al., 1988. Measurements and interpretation of $\delta^{13}\text{C}$ of methane from termites, rice paddies, and wetlands in Kenya. *Global Biogeochemical Cycles*, **2**(4), 341–355.
- Tyler, S. C., 1991. The global methane budget. In Rogers, J. E., and Whitman, W. B. (eds.), *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*. Washington, DC: American Society for Microbiology, pp. 7–38.
- Whiticar, M. J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 219–314.
- Whiticar, M. J., Faber, E., and Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation - Isotope evidence. *Geochimica et Cosmochimica Acta*, **50**, 693–709.
- Wuebbles, D. J., and Hayhoe, K., 2002. Atmospheric methane and global change. *Earth-Science Reviews*, **57**(3–4), 177–210.
- Zinder, S. H., 1993. Physiological ecology of methanogens. In Jerry, J. G., (ed.), *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*, New York: Chapman Hall, pp. 128–206.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Cap Carbonates](#)
[Carbonates](#)
[Cold Seeps](#)
[Methane Oxidation \(Aerobic\)](#)

METHANOGENS

Please refer to “*Methane, Origin*” and “*Archaea*.”

MICROBIAL BIOMINERALIZATION

Christine Heim
 University of Göttingen, Göttingen, Germany

Synonyms

Microbial biomineral formation

Definition

Microbial biomineralization describes the formation and deposition of minerals directly mediated or indirectly influenced by microorganisms (Mann, 2001; Weiner and Dove, 2003; Ehrlich, 1999). A huge variety of minerals results from individual biomineralization pathways linked to the phylogeny and metabolic activity of the microorganisms involved (Weiner and Dove, 2003;

Minsky et al., 2002). Moreover, microbial biominerals may differ distinctly from their inorganically formed equivalents in shape, size, crystallinity, isotopic, and trace element composition (Bazylinski et al., 2007; Haferburg and Kothe, 2007; Takahashi et al., 2007; Weiner and Dove, 2003). A compilation of microbial biominerals and their source organisms is given in Table 1.

Two principal modes of microbial biomineralization processes occur, namely biologically induced mineralization (BIM) and biologically controlled mineralization

(BCM, Lowenstam, 1981; Lowenstam and Weiner, 1989). These modes are introduced in the following.

Biologically induced mineralization (BIM)

In BIM, the nucleation and growth of biominerals are extracellular processes triggered by the metabolic activity of the microorganism. Biomineralization takes place because of the changes in the chemical equilibrium of the surrounding environment and may also be linked to

Microbial Biomineralization, Table 1 Overview of minerals formed by microbial biomineralization and the organism(s) involved

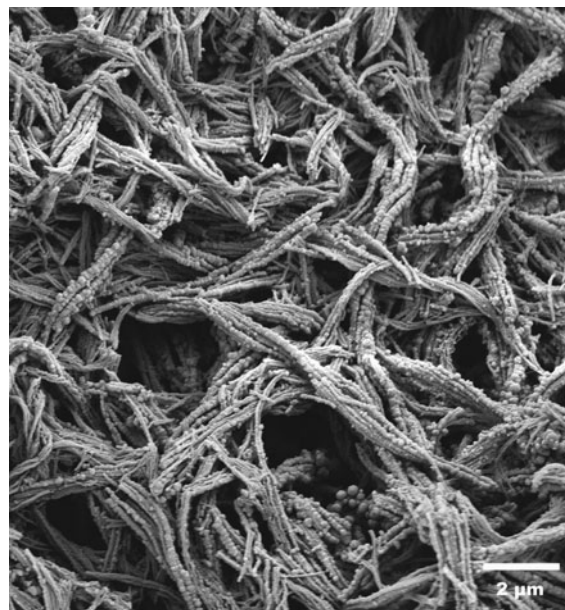
Chemical formula	Mineral name	Involved microorganism	References
Fe(OH) ₃ (approx.)	Ferric/iron oxyhydroxide	Fe-oxidizing bacteria	Chan et al. (2009) and Yoshida et al. (2008)
2Fe(OH) ₃ • Fe(OH) ₂ (approx.)	Green rust	<i>Shewanella putrefaciens</i>	Kukkadapu et al. (2004) and O'Loughlin (2008)
α-FeO(OH)	Goethite	<i>Gallionella ferruginea</i>	Hallberg and Ferris (2004)
γ-FeO(OH)	Lepidocrocite	Marine bacteriophage, <i>Bacillus subtilis</i>	Daughney et al. (2004) and Châtellier et al. (2001)
5Fe ₂ O ₃ • 9H ₂ O	Ferrihydrite	<i>Gallionella ferruginea</i> , <i>Leptothrix ochracea</i> , <i>Bacillus subtilis</i>	Hallberg and Ferris (2004) and Kennedy et al. (2004)
Fe ₂ O ₃	Hematite	<i>Gallionella ferruginea</i>	Hallberg and Ferris (2004)
Fe ₃ O ₄	Magnetite	<i>Shewanella putrefaciens</i> , (Fe(III)-reducing bacteria), magnetotactic bacteria (e.g., <i>Magnetospirillum magnetotacticum</i>), SRB (e.g., <i>Desulfovibrio magneticus</i>), thermophilic iron-reducing bacteria	Frankel et al. (1983), Lovley et al. (1987), Lovley (1991), Bazylinski et al. (1993), Zhang et al. (1997), Zhang et al. (1998), Kukkadapu et al. (2004), Bazylinski et al. (2007) and Faivre and Schüler (2008)
γ-Fe ₂ O ₃	Maghemite	<i>Actinobacter</i> sp., thermophilic iron-reducing bacteria	Zhang et al. (1997) and Bharde et al. (2008)
FeCO ₃	Siderite	<i>Shewanella alga</i> , thermophilic iron-reducing bacteria	Zhang et al. (1997) and Parmar et al. (2000)
FePO ₄ • nH ₂ O	Hydrous ferric phosphate	<i>Acidovorax</i> sp.	Miot et al. (2009)
Fe ₃ (PO ₄) ₂ • 2H ₂ O	Vivianite	<i>Shewanella putrefaciens</i> , <i>Desulfovibrio alaskensis</i> (SRB), <i>Alkaliphilus metalliredigens</i>	Kukkadapu et al. (2004), Zegeye et al. (2007), and Roh et al. (2007)
FeS	Cubic FeS (Sphalerite-type)	Magnetotactic bacteria	Pósfai et al. (1998a, b)
FeS	Mackinawite (tetragonal FeS)	Magnetotactic bacteria, <i>Desulfovibrio desulfuricans</i>	Pósfai et al. (1998a, b) and Ivarson and Hallberg (1976)
Fe ₃ S ₄	Greigite	Magnetotactic bacteria, <i>Actinobacter</i> sp., SRB	Bharde et al. (2008), Farina et al. (1990), Mann et al. (1990), Heywood et al. (1990), Reiter et al. (2005), and Faivre and Schüler (2008)
Fe _{1-x} S	Pyrrhotite	Magnetotactic bacteria	Farina et al. (1990)
FeS ₂	Pyrite	Magnetotactic bacteria, SRB	Mann et al. (1990), Bazylinski (1996), Folk (2005), Wilkin and Barnes (1997), and Donald and Southam (1999)
KFe ₃ (SO ₄) ₂ (OH) ₆	Jarosite	<i>Sulfobacillus thermosulfidooxidans</i> , <i>Acidithiobacillus ferrooxidans</i> , <i>Thiobacillus ferrooxidans</i>	Ding et al. (2007), Daoud and Karamanev (2006), and Ivarson and Hallberg (1976)
Fe ₈ O ₈ SO ₄ (OH) ₆	Schwertmanite	<i>Acidithiobacillus ferrooxidans</i>	Egal et al. (2009)
MnCO ₃	Rhodochrosite	<i>Leptothrix discophora</i>	Zhang et al. (2002)
MnO ₂	Manganese oxides	<i>Pseudomonas putida</i> , <i>Leptothrix discophora</i> , <i>Bacillus</i> sp.	Tebo et al. (2004), Villalobos et al. (2003), and Brouwers et al. (2000)
Na ₄ Mn ₁₄ O ₂₇ • 9H ₂ O	Birnessite	<i>Pseudomonas putida</i>	Villalobos et al. (2003)
S ⁰	Elemental sulfur	<i>Chromatiaceae</i> , <i>Beggiatoa</i> spp., <i>Thiothrix</i> , <i>Thiovulum</i> , <i>Thioploca</i>	Brune (1989), Brune (1995), Smith and Strohl (1991), Strohl et al. (1981) and Pasteris et al. (2001)

Microbial Biomineralization, Table 1 (Continued)

Chemical formula	Mineral name	Involved microorganism	References
Au ⁰	Elemental gold	<i>Bacillus</i> sp., <i>Rhodopseudomonas capsulate</i> , <i>Shewanella algae</i> , SRB	Reith et al. (2009), He et al. (2007), Konishi et al. (2007), Konishi et al. (2006), and Lengke and Southam (2006)
CaCO ₃	Calcite	Communities of SRB and archaea, cyanobacteria, soil bacteria (<i>Bacillus megaterium</i>), Algae (e.g., <i>Halimeda</i> , <i>Emiliana huxleyi</i>)	Boetius et al. (2000), Reitner et al. (2005), Thompson and Ferris (1990), Lian et al. (2006), and de Vrind-de Jong and de Vrind (1997)
	Aragonite	Cyanobacteria (<i>Synechococcus leopoliensis</i>), <i>Nesterenkonia halobia</i> , <i>Halomonas eurihalina</i>	Obst et al. (2009) and Rivadeneyra et al. (1998, 2000)
	Vaterite	<i>Kocuria</i> , <i>Myxococcus Xanthus</i> , <i>Bacillus sphaericus</i>	Zamarreño et al. (2009), and Rodriguez-Navarro et al. (2007)
CaMg(CO ₃) ₂	Dolomite	<i>Nesterenkonia halobia</i>	Rivadeneyra et al. (2000)
SiO ₂ • nH ₂ O	Amorphous silica	<i>Calothrix</i> , <i>Fischerella</i> sp., <i>Shewanella oneidensis</i>	Benning et al. (2004), Konhauser et al. (2001), and Furukawa and O'Reilly (2007)
SiO ₂	Silica	Diatoms, radiolarians, <i>Thiobacillus</i> , <i>Bacillus subtilis</i>	de Vrind-de Jong and de Vrind (1997), Fortin and Beveridge (1997), and Urrutia and Beveridge (1993)
Ca ₅ (PO ₄) ₃ (OH)	Hydroxyapatite/ calcium phosphate	<i>Ramlibacter tataouinensis</i> , <i>Corynebacterium matruchotii</i> , <i>Streptococcus mutans</i> , <i>Streptococcus sanguis</i>	Benzerara et al. (2004), Van Dijk et al. (1998), and Streckfuss et al. (1974)
MgNH ₄ PO ₄ • 6H ₂ O	Struvite	<i>Myxococcus xanthus</i> , <i>Pseudomonas</i> , <i>Flavobacterium</i> , <i>Acinetobacter</i> , <i>Yersinia</i> , <i>Corynebacterium</i> , <i>Azotobacter</i>	Da Silva et al. (2000) and Rivadeneyra et al. (1983)

For comprehensive reading, the following books are recommended: Sigel et al. (2008), Baeuerlein (2000), Banfield and Nealson (1997), Dove et al. (2003), Driessens and Verbeeck (1990), Lovely (2000), Lowenstam and Weiner (1989).

particular metabolic products. The resulting biominerals typically show a poor crystallinity, are chemically heterogeneous, and often closely associated with the cell wall (Frankel and Bazylinski, 2003). An active and a passive mineralization process can be distinguished (Fortin and Beveridge, 2000; Southam, 2000). Active mineralization refers to mineralization by (a) the direct redox conversion of specific metal ions bound to the bacterial surface or (b) the excretion of metabolically produced ions and thereby forming minerals. The term passive mineralization is used when *nonspecific* binding of cations and the involvement of surrounding anions causes nucleation and growth of minerals. Passive mineralization can even be mediated by dead cells, due to the exposure of negatively charged surfaces acting as nucleation sites for metal cations (Urrutia and Beveridge, 1993). Particularly in BIM, extracellular polymeric substances (EPS) are involved in the mineralization process (Chan et al., 2009; Ercole et al., 2007). As an example of BIM, the precipitation of iron oxyhydroxides by the iron oxidizing bacterium *Gallionella ferruginea* is displayed in Figure 1.



Biologically controlled mineralization (BCM)

BCM implies that the organism actively controls the nucleation site, growth, morphology, and final location of the mineral (Banfield and Nealson 1997; Bazylinski

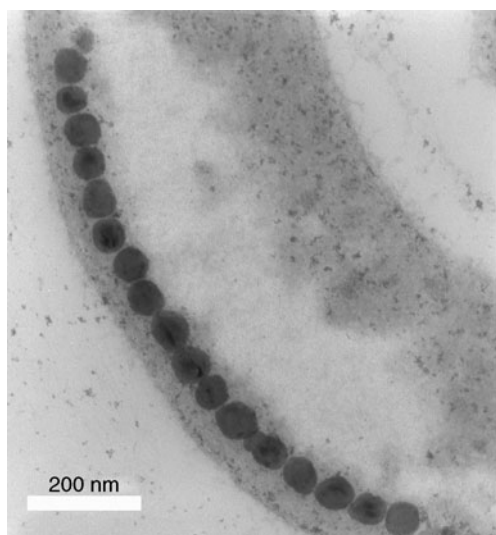
Microbial Biomineralization, Figure 1 Stalk-shaped extracellular polymeric substances (EPS) produced by the iron oxidizing bacterium *Gallionella ferruginea* are heavily encrusted with iron oxyhydroxide minerals formed by biologically induced mineralization (BIM).

and Frankel, 2003). Although the modes of exerting crystallochemical control over the mineralization process may greatly vary across species, the common characteristic of BCM is that mineral formation takes place in a closed, isolated environment. While Weiner and Dove (2003) categorized BCM in *extra*-, *inter*- or *intracellular* mineralization, Mann (2001) differentiated between two key modes of BCM, namely *matrix-mediated* mineralization and *boundary-organized* mineralization. Generally speaking, extracellular mineralization corresponds to matrix-mediated mineralization, whereas inter- and intracellular mineral formation are equivalent to boundary-organized mineralization.

Extracellular BCM implies the production of a macromolecular matrix outside the cell. This matrix is typically composed of proteins, polysaccharides, or glycoproteins, forming a three dimensional framework, and the cell actively supplies cations to the matrix for an “on-site” nucleation and growth of the biomineral (Weiner and Dove, 2003).

Intercellular BCM occurs mostly in single-celled organisms, existing in a community. This type of mineral formation is not common in bacteria, but is commonly found in calcareous algae, for example, *Halimeda* (de Vrind-de Jong and de Vrind, 1997; Borowitzka, 1982).

Intracellular BCM takes place inside specific compartments within the cell, for instance vesicles or vacuoles. Thus, the organism is able to exactly regulate the chemical composition, morphological structure, and particle size of the mineral. The only bacteria known to perform intracellular BCM are magnetotactic bacteria, which use a vacuole-based system for the crystallization of magnetic biominerals (Table 1). Similar systems of intracellular



Microbial Biomineralization, Figure 2 Intracellular iron sulfide (greigite, Fe_3S_4) crystals formed by biologically controlled mineralization (BCM) within a magnetotactic bacterium. (Image courtesy of Joachim Reitner.)

BCM are only known from higher eukaryotic organisms, controlling for example, the bone and teeth formation in mammals (Kirschvink and Hagadorn, 2000). An example of microbial biominerals formed by intracellular BCM is shown in Figure 2.

Bibliography

- Baeuerlein, E., 2000. *Biomineralization: From Biology to Biotechnology and Medical Application*. Weinheim: Wiley-VCH Verlag GmbH, 316 p.
- Banfield, J. F., and Nealson, K. H., (eds.), 1997. *Geomicrobiology: Interactions Between Microbes and Minerals. Reviews in Mineralogy*, Ribbe, P. H. (series ed.). Washington, DC: Mineralogical Society of America. Vol. 35, 448 p.
- Bazylinski, D. A., 1996. Controlled biomineralization of magnetic minerals by Magnetotactic bacteria. *Chemical Geology*, **132**, 191–198.
- Bazylinski, D. A., and Frankel, R. B., 2003. Biologically controlled mineralization in prokaryotes. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), Rosso, J. J. (series ed.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 217–247.
- Bazylinski, D. A., Frankel, R. B., and Konhauser, K. O., 2007. Modes of biomineralization of magnetite by microbes. *Geomicrobiology Journal*, **24**, 465–475.
- Bazylinski, D. A., Heywood, B. R., Mann, S., and Frankel, R. B., 1993. Fe_3O_4 and Fe_3S_4 in a bacterium. *Nature*, **366**, 218.
- Benning, L. G., Phoenix, V. R., Yee, N., and Tobin, M. J., 2004. Molecular characterization of cyanobacterial silification using synchrotron infrared micro-spectroscopy. *Geochimica et Cosmochimica Acta*, **68**(4), 729–741.
- Benzerara, K., Menguy, N., Guyot, F., Skouri, F., de Luca, G., Barakat, M., and Heulin, T., 2004. Biologically controlled precipitation of calcium phosphate by *Ramlibacter tataouinensis*. *Earth and Planetary Science Letters*, **228**, 439–449.
- Bharde, A. A., Paraikh, R. Y., Baidakova, M., Jouen, S., Hannoyer, B., Enoki, T., Prasad, B. L. V., Shouche, Y. S., Ogale, S., and Sastry, M., 2008. Bacteria-mediated precursor-dependent biosynthesis of superparamagnetic iron oxide and iron sulfide nanoparticles. *Langmuir*, **24**, 5787–5794.
- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Borowitzka, M. A., 1982. Morphological and cytological aspects of algal calcification. *International Review of Cytology*, **74**, 127–162.
- Brouwers, G. J., Vijgenboom, E., Corstjens, P. L. A. M., De Vrind, J. P. M., and De Vrind-De Jong, E. W., 2000. Bacterial Mn^{2+} oxidizing systems and multicopper oxidases: an overview of mechanisms and functions. *Geomicrobiology Journal*, **17**, 1–24.
- Brune, D. C., 1989. Sulfur oxidation by phototrophic bacteria. *Biochimica et Biophysica Acta*, **975**, 189–221.
- Brune, D. C., 1995. Isolation and characterization of sulfur globule proteins from *Chromatium vinosum* and *Thiocapsa roseopersicina*. *Archives of Microbiology*, **163**, 391–399.
- Chan, C. S., Fakra, S. C., Edwards, D. C., Emerson, D., and Banfield, J. F., 2009. Iron oxyhydroxide mineralization of microbial extracellular polysaccharides. *Geochimica et Cosmochimica Acta*, **73**(13), 3807–3818.
- Châtellier, X., Fortin, D., West, M. M., Leppard, G. G., and Ferris, F. G., 2001. Effect of the presence of bacterial surfaces during the synthesis of Fe oxides by oxidation of ferrous ions. *European Journal of Mineralogy*, **13**(4), 705–714.

- Da Silva, S., Bernet, N., Delgenès, J. P., and Moletta, R., 2000. Effect of culture conditions on the formation of struvite by *Myxococcus Xanthus*. *Chemosphere*, **40**, 1289–1296.
- Daoud, J., and Karamanev, D., 2006. Formation of jarosite during Fe^{2+} oxidation by *Acidithiobacillus ferrooxidans*. *Minerals Engineering*, **19**(9), 960–967.
- Daughney, C. D., Châtellier, X., Chan, A., Kenward, P., Fortin, D., Suttle, C. A., and Fowl, D., 2004. Adsorption and precipitation of iron from seawater by a marine bacteriophage (PWH3a-P1). *Marine Chemistry*, **91**, 101–115.
- de Vrind-de Jong, E. W., and de Vrind, J. P. M., 1997. Algal deposition of carbonates and silicates. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 267–307.
- Ding, J.-N., Gao, J., Wu, X.-l., Zhang, C.-G., and Qiu, G.-Z., 2007. Jarosite-type precipitates mediated by YN22, *Sulfobacillus thermosulfidooxidans*, and their influences on strain. *Transactions of Nonferrous Metals. Society of China*, **17**(5), 1038–1044.
- Donald, R., and Southam, G., 1999. Low temperature anaerobic bacterial diagenesis of ferrous monosulfide to pyrite. *Geochimica et Cosmochimica Acta*, **63**(13/14), 2019–2023.
- Dove, P. M., De Yoreo, J. J., and Weiner, S., (eds.), 2003. *Biomineralization. Reviews in Mineralogy and Geochemistry*, Rosso, J. J. (series ed.). Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, 381 p.
- Driessens, F. C. M., and Verbeeck, R. K., 1990. *Biominerals*. Boca Raton, Florida: CRC Press, 440 p.
- Egal, M., Casiot, C., Morin, G., Parmentier, M., Bruneel, O., Lebrun, S., and Elbaz-Poulichet, F., 2009. Kinetic control on the formation of tooeleite, schwertmannite and jarosite by *Acidithiobacillus ferrooxidans* stains in an As(III)-rich acid mine water. *Chemical Geology*, **265**(3–4), 432–441.
- Ehrlich, H. L., 1999. Microbes as geologic agents: their role in mineral formation. *Geomicrobiology Journal*, **16**, 135–153.
- Ercole, C., Cacchio, P., Botta, A. L., Centi, V., and Lepidi, A., 2007. Bacterially induced mineralization of calcium carbonate: the role of exopolysaccharides and capsular polysaccharides. *Microscopy and Microanalysis*, **13**, 42–50.
- Faivre, D., and Schüller, D., 2008. Magnetotactic bacteria and magnetosomes. *Chemical Reviews*, **108**, 4875–4898.
- Farina, M., Esquivel, D. M. S., and Lins de Barros, H. G. P., 1990. Magnetic iron-sulphur crystals from magnetotactic microorganism. *Nature*, **343**, 256–258.
- Folk, R. L., 2005. Nannobacteria and the formation of framboidal pyrite: textural evidence. *Journal of Earth System Science*, **114**(3), 369–374.
- Fortin, D., and Beveridge, T. J., 1997. Role of the bacterium *Thiobacillus* in the formation of silicates in acidic mine tailings. *Chemical Geology*, **141**, 235–250.
- Fortin, D., and Beveridge, T. J., 2000. Mechanistic routes to biomineral surface development. In Bäuerlein, E. (ed.), *Biomineralization: From Biology to Biotechnology and Medical Application*. Weinheim: Wiley-VCH GmbH, pp. 7–24.
- Frankel, R. B., and Bazylinski, D. A., 2003. Biologically induced mineralization by bacteria. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), Rosso, J. J. (series ed.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington, DC: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 95–114.
- Frankel, R. B., Papaefthymiou, G. C., Blakemore, R. P., and O'Brien, W., 1983. Fe_3O_4 precipitation in Magnetotactic bacteria. *Biochimica et Biophysica Acta*, **763**, 147–159.
- Furukawa, Y., and O'Reilly, S. E., 2007. Rapid precipitation of amorphous silica in experimental systems with nontronite (Nau-1) and *Shewanella oneidensis* MR-1. *Geochimica et Cosmochimica Acta*, **71**(2), 363–377.
- Haferburg, G., and Kothe, E., 2007. Microbes and metals: interactions in the environment. *Journal of Basic Microbiology*, **47**, 453–467.
- Hallberg, R., and Ferris, F. G., 2004. Biomineralization by Gallionella. *Geomicrobiology Journal*, **21**, 325–330.
- He, S., Gui, Z., Zhang, Y., Zhang, S., Wang, J., and Gu, N., 2007. Biosynthesis of gold nanoparticles using the bacteria *Rhodospseudomonas capsulata*. *Material Letters*, **61**(18), 3984–3987.
- Heywood, B. R., Bazylinski, D. A., Garratt-Reed, A., Mann, S., and Frankel, R. B., 1990. Controlled biosynthesis of greigite (Fe_3S_4) in magnetotactic bacteria. *Naturwissenschaften*, **77**, 536–538.
- Ivarson, K. C., and Hallberg, R. O., 1976. Formation of mackinawite by the microbial reduction of jarosite and its application to tidal sediments. *Geoderma*, **16**, 1–7.
- Kennedy, C. B., Scott, S. D., and Ferris, F. G., 2004. Hydrothermal phase stabilization of 2-line ferrihydrite by bacteria. *Chemical Geology*, **212**, 269–277.
- Kirschvink, J. L., and Hagadorn, J. W., 2000. 10 a Grand unified theory of Biomineralization. In Bäuerlein, E. (ed.), *The Biomineralisation of Nano- and Microstructures*. Weinheim: Wiley-VCH GmbH, pp. 139–150.
- Konhäuser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial-silica interactions in Icelandic hot spring sinter: possible analogues for some Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–433.
- Konishi, Y., Tsukiyama, T., Ohno, K., Saitoh, N., Nomura, T., and Nagamine, S., 2006. Intracellular recovery of gold by microbial reduction of AuCl_4^- ions using the anaerobic bacterium *Shewanella algae*. *Hydrometallurgy*, **81**(1), 24–29.
- Konishi, Y., Tsukiyama, T., Tachimi, T., Saitoh, N., Nomura, T., and Nagamine, S., 2007. Microbial deposition of gold nanoparticles by the metal-reducing bacterium *Shewanella algae*. *Electrochimica Acta*, **53**(1), 186–192.
- Kukkadapu, R. K., Zachara, J. M., Fredrickson, J. K., and Kennedy, D. W., 2004. Biotransformation of two-line silica-ferrihydrite by dissimilatory Fe(III)-reducing bacterium: formation of carbonate green rust in the presence of phosphate. *Geochimica et Cosmochimica Acta*, **68**(13), 2799–2814.
- Lengke, M., and Southam, G., 2006. Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold(I)-thiosulfate complex. *Geochimica et Cosmochimica Acta*, **70**, 3646–3661.
- Lian, B., Hu, Q., Chen, J., Ji, J., and Teng, H. H., 2006. Carbonate biomineralization induced by soil bacterium *Bacillus megaterium*. *Geochimica et Cosmochimica Acta*, **70**, 5522–5535.
- Lovley, D. (ed.), 2000. *Environmental Microbe-Metal Interactions*. Washington, DC: ASM Press, 395 p.
- Lovley, D. R., 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiology Reviews*, **55**, 259–287.
- Lovley, D. R., Stolz, J. F., Nord, Jr. G. L., and Phillips, E. J. P., 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. *Nature*, **330**, 252–254.
- Lowenstam, H. A., 1981. Minerals formed by organisms. *Science*, **211**, 1126–1131.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press, 324 p.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. In Compton, R. G., Davies, S. G., and Evans, J. (eds.), *Oxford Chemistry Masters Vol. 5*. New York: Oxford University Press, 216 p.
- Mann, S., Sparks, N. H. C., Frankel, R. B., Bazylinski, D. A., and Jannasch, H. W., 1990. Biomineralization of ferrimagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a Magnetotactic bacterium. *Nature*, **343**, 258–261.

- Minsky, A., Shimoni, E., and Frenkel-Krispin, D., 2002. Stress, order and survival. *Nature Reviews Molecular Cell Biology*, **3**, 50–60.
- Miot, J., Benzerara, K., Morin, G., Kappler, A., Bernard, S., Obst, M., Férard, C., Skouri-Panet, F., Guigner, J.-M., Posth, N., Galvez, M., Brown, Jr. G. E., and Guyot, F., 2009. Iron biomineralization by anaerobic neutrophilic iron-oxidizing bacteria. *Geochimica et Cosmochimica Acta*, **73**(3), 696–711.
- Obst, M., Dynes, J. J., Lawrence, J. R., Swerhone, G. D. W., Benterara, K., Kaznatcheev, K., Tyliszczak, T., and Hitchcock, A. P., 2009. Precipitation of amorphous CaCO₃ (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. *Geochimica et Cosmochimica Acta*, **73**(14), 4180–4198.
- O'Loughlin, E. J., 2008. Effects of electron transfer mediators on the bioreduction of Lepidocrocite (γ -FeOOH) by *Shewanella putrefaciens* CN32. *Environmental Science and Technology*, **42**(18), 6876–6882.
- Parmar, N., Warren, L. A., Roden, E. E., and Ferris, F. G., 2000. Soil phase capture of strontium by the iron reducing bacteria *Shewanella alga* strain BR-2. *Chemical Geology*, **169**, 281–288.
- Pasteris, J. D., Freeman, J. J., Goffredi, S. K., and Buck, K. R., 2001. *Chemical Geology*, **180**, 3–18.
- Pósfai, M., Buseck, P. R., Bazylinski, D. A., and Frankel, R. B., 1998a. Reaction sequence of iron sulfide minerals in bacteria and their use as biomarkers. *Science*, **280**, 880–883.
- Pósfai, M., Buseck, P. R., Bazylinski, D. A., and Frankel, R. B., 1998b. Iron sulfides from magnetotactic bacteria: structure, compositions, and phase transitions. *American Mineralogist*, **83**, 1469–1481.
- Reith, F., Wakelin, S. A., Gregg, A. L., and Schmidt-Mumm, A., 2009. A microbial pathway for the formation of gold-anomalous calcrite. *Chemical Geology*, **258**(3–4), 315–326.
- Reitner, J., Peckmann, J., Blumenberg, M., Michaelis, W., Reimer, A., and Thiel, V., 2005. Concretionary methane-seep carbonates and associated microbial communities in Black Sea sediments. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 18–30.
- Rivadeneira, M. A., Delgado, G., Ramos-Cormenzana, A., and Delgado, R., 1998. Biomineralization of carbonates by *Halomonas eurihalina* in solid and liquid media with different salinities: crystal formation sequence. *Research in Microbiology*, **149**, 277–287.
- Rivadeneira, M. A., Delgado, G., Soriano, M., Ramos-Cormenzana, A., and Delgado, R., 2000. Precipitation of carbonates by *Nesterenkonia halobia* in liquid media. *Chemosphere*, **41**, 617–624.
- Rivadeneira, M. A., Ramos-Cormenzana, A., and García-Cervigón, A., 1983. Bacterial formation of struvite. *Geomicrobiology Journal*, **3**, 151–163.
- Rodríguez-Navarro, C., Jiménez-López, C., Rodríguez-Navarro, A., González-Muñoz, M. T., and Rodríguez-Gallego, M., 2007. Bacterially mediated mineralization of vaterite. *Geochimica et Cosmochimica Acta*, **71**(5), 1197–1213.
- Roh, Y., Chon, C.-M., and Moon, J.-W., 2007. Metal reduction and biomineralization by alkaliphilic metal reducing bacterium, *Alkaliphilus metalliredigens* (QYMF). *Geosciences Journal*, **11**(4), 415–423.
- Sigel, A., Sigel, H., and Sigel, R. O. (eds.), 2008. *Biomineralization: from Nature to Application. Metal Ions in Life Sciences*. West Sussex: Wiley and Sons, 4 Vols, 700 p.
- Smith, D. W., and Strohl, W. R., 1991. Sulfur oxidizing bacteria. In Shively, J. M., and Barton, L. L., (eds.), *Variations in Autotrophic Life*. London: Academic Press, pp. 121–146.
- Southam, G., 2000. Bacterial surface-mediated mineral formation. In Lovley, D. R. (ed.), *Environmental Microbe-Metal Interactions*. Washington DC: ASM Press, pp. 257–276.
- Streckfuss, J. L., Smith, W. N., Brown, L. R., and Campbell, M. M., 1974. Calcification of selected strains of *Streptococcus mutans* and *Streptococcus sanguis*. *Journal of Bacteriology*, **120**, 502–506.
- Strohl, W. R., Geffers, I., and Larkin, J. M., 1981. Structure of the sulfur inclusions envelopes from four beggiatoas. *Current Microbiology*, **6**, 75–79.
- Takahashi, Y., Hirata, T., Shimizu, H., Ozaki, T., and Fortin, D., 2007. A rare earth element signature of bacteria in natural waters. *Chemical Geology*, **244**, 569–583.
- Tebo, B. M., Bargar, J. R., Clement, B. G., Dick, G. J., Murray, K. J., Parker, D., Verity, R., and Webb, S. M., 2004. Biogenic manganese oxides: properties and mechanisms of formation. *Annual Review of Earth and Planetary Sciences*, **32**, 287–328.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Urrutia, M. M., and Beveridge, T. J., 1993. Mechanism of silicate binding to the bacteria cell wall in *Bacillus subtilis*. *Journal of Bacteriology*, **175**, 1936–1945.
- Van Dijk, S., Dean, D. D., Zhao, Y., Cirgwin, J. M., Schwartz, Z., and Boyan, B. D., 1998. Purification, amino acid sequence, and cDNA sequence of novel calcium-precipitating proteolipids involved in calcification of *Corynebacterium matruchotii*. *Calcified Tissue International*, **62**, 350–358.
- Villalobos, M., Toner, B., Bargar, J., and Sposito, G., 2003. Characterization of manganese oxide produced by *Pseudomonas putida*. *Geochimica et Cosmochimica Acta*, **67**(14), 2649–2662.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy and Geochemistry*. Washington DC, USA: Mineralogical Society of America and Geochemical Society, Vol. 54, pp. 1–29.
- Wilkin, R. T., and Barnes, H. K., 1997. Formation processes of framboidal pyrite. *Geochimica et Cosmochimica Acta*, **61**(2), 323–339.
- Yoshida, H., Yamamoto, K., Murakami, Y., Katsuta, N., Hayashi, T., and Naganuma, T., 2008. The development of Fe-nodules surrounding biological material mediated by microorganisms. *Environmental Geology*, **55**, 1363–1374.
- Zamarreño, D. V., Inkpen, R., and May, E., 2009. Studies on carbonate crystals precipitated by freshwater bacteria and their use as a limestone consolidant. *Applied and Environmental Microbiology*, **75**, 5981–5990.
- Zegeye, A., Huhuet, L., Abdelmoula, M., Carteret, C., Mullet, M., and Jorand, F., 2007. Biogenic hydroxysulfate green rust, a potential electron acceptor of SRB activity. *Geochimica et Cosmochimica Acta*, **71**, 5450–5462.
- Zhang, C., Liu, S., Phelps, T. J., Cole, D. R., Horita, J., Fortier, S. M., Elless, M., and Valley, J., 1997. Physicochemical, mineralogical, and isotopic characterization of magnetite-rich iron oxides formed by thermophilic iron-reducing bacteria. *Geochimica et Cosmochimica Acta*, **61**(21), 4621–4632.
- Zhang, C., Vali, H., Romanek, C. S., Phelps, T. J., and Liu, S. V., 1998. Formation of single-domain magnetite by a thermophilic bacterium. *American Mineralogist*, **83**, 1409–1418.
- Zhang, J., Lion, L. W., Nelson, Y. M., Shuler, M. L., and Ghiorse, W. C., 2002. Kinetics of Mn (II) oxidation by *Leptothrix discophora* SS1. *Geochimica et Cosmochimica Acta*, **65**(5), 773–781.

Cross-references

Banded Iron Formations
Biofilms and Fossilization

Biomining (Mineral Bioleaching, Mineral Biooxidation)
 Biosignatures in Rocks
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Extracellular Polymeric Substances (EPS)
 Fe(II)-Oxidizing Prokaryotes
 Fe(III)-Reducing Prokaryotes
 Gallionella
 Gold
 Iron Sulfide Formation
 Leptothrix
 Magnetotactic Bacteria
 Manganese (Sedimentary Carbonates and Sulfides)
 Metallogenium
 Metals, Acquisition by Marine Bacteria
 Microbial-Metal Binding
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Nanocrystals, Microbially Induced
 Ores, Microbial Precipitation and Oxidation
 Organomineralization
 Shewanella
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Tufa, Freshwater

MICROBIAL COMMUNITIES, STRUCTURE, AND FUNCTION

Michael W. Friedrich
 University of Bremen, Bremen, Germany

Definition

Microbial species. Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization (Stackebrandt and Goebel, 1994); thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy.

Microbial population. Individual microorganisms (of the same “species”) form a population.

Guild. Metabolically related populations, e.g., sulfate-reducing microorganisms.

Microbial community. All microbial populations in a habitat.

Microbial community structure. The composition of a microbial community and the abundance of its members.

Microbial diversity. The number of different species in a habitat; sometimes (falsely) used as a synonym for community structure.

Habitat. The physical location or dwelling place of a particular organism, where an individual microbial ecotype can be found or isolated from.

Ecosystem. A community of microorganisms and their natural environment.

Ecological niche. Different from the habitat (spatial), the niche defines the function of an organism.

Introduction

Microorganisms are an indispensable part of the biogeochemical cycles on Earth, have the longest history in evolution, and represent the largest genetic reservoir. Mostly invisible to the naked eye, microbes represent the largest source of biomass on Earth, with an estimated number of $4\text{--}6 \times 10^{30}$ cells, and 350–550 Pg of C (1 Pg = 10^{15} g), and have colonized almost any niche available on Earth (Whitman et al., 1998). In their habitats, microorganisms usually do not exist as single, genetically identical population, but live together with other microbial populations as microbial communities carrying out important functions of an ecosystem such as primary production and remineralization of biomass.

A hallmark of microbial life is its diversity. For a long time, only little was known about the magnitude of microbial diversity, as this was based on cultivation. Only about ~7,000 noneukaryotic species have been formally described (Euzéby, 1997) (<http://www.bacterio.cict.fr/number.html>), and insects were regarded as much more diverse than microorganisms. In microbiology, the term “diversity” is often used to refer to the number of different species in a given habitat, or, when based on molecular data, refers to the number of different sequences present. Diversity reflects the types of microbes present in a habitat, but “community structure” encompasses quantitative information on the different taxa and guilds (Liesack et al., 1997). Eventually, the analysis of community structure will allow for assessing the role and importance of microbes in their habitats and ecosystems. Presently, it is still methodologically difficult to obtain a complete view of microbial diversity and community structure.

Methods of community structure analysis

Cultivation: Traditionally, the diversity of microbial communities has been studied by cultivation, i.e., by viable plate counts or most-probable-number (MPN) dilution techniques. However, the morphology of microorganisms is too simple for reliable identification and classification. Identification of microorganisms by cultivation requires pure cultures for testing physiological, biochemical, and cell biological characterization and suitable media for their growth, which is a major bottle neck. There are no cultivation media available that allow for capturing growth requirements of all microorganisms in a sample, and we often do not even know of their existence and presence. Direct microscopic counts can be of orders of magnitude higher than the counts of bacteria obtained on growth media (“plate counts”). Typically, only a small

fraction of microorganisms grows on standard media, and in some environments this has been estimated to be as low as 0.001–0.1%, e.g., in seawater (Amann et al., 1995).

Molecular approaches: The limitations of cultivation-based analysis to describe the diversity of microorganisms adequately were overcome by applying methods of molecular biology. Today, microorganisms are routinely classified by using the RNA of the small ribosomal subunit (small subunit ribosomal RNA – SSU rRNA), or its coding gene (SSU rRNA gene) as a molecular marker. In the 1970s, Carl Woese and coworkers used the sequences of the SSU rRNA to establish a phylogenetic framework for all life, from which the concept of the three domains of life – prokaryotes, archaea, and eukaryotes – emerged (Woese and Fox, 1977; Woese, 1987). Molecular classification of microorganisms was further developed by Norman Pace and his group to cultivation independent analysis of microbial communities in environmental samples by cloning the 16S rRNA gene directly from biomass (Pace et al., 1986). The basic idea is simple: rRNA sequences can be used as identifiers of microorganisms even if they were previously unknown; the sequence allows to establish an evolutionary relationship to other sequences of microorganisms in the databases. Directly extracting DNA from biomass, followed by cloning and sequencing of the (SSU rRNA) sequences of interest, facilitates to examine any environmental habitat. In August 2010, 418,497 16S rRNA sequences were held in the dedicated database Ribosomal database project II (RDP; <http://rdp.cme.msu.edu/>), indicating how successful this approach has been. As the number of different microbial species can be very high in certain habitats, computational approaches have been developed to estimate diversity coverage of clone libraries (e.g., DOTUR) (Schloss and Handelsman, 2005). Cloning and sequencing approaches give an overview of microbial species diversity, but this approach is PCR based and therefore, may not adequately reflect the abundance of community members (von Wintzingerode et al., 1997). Analysis of community structure requires quantification of individual groups of microorganisms. Quantitative dot blot hybridization and fluorescent in situ hybridization (see Chapter *Fluorescence In Situ Hybridization (FISH)*), techniques are being used to determine abundance of uncultured prokaryotes using oligonucleotide probes directed against the SSU rRNA (Amann et al., 1995). Specific probes have been designed using SSU rRNA sequences obtained from the habitat studied and public databases; probeBase is an extensive repository of published rRNA-targeting oligonucleotide probe sequences available to the public (<http://www.microbial-ecology.de/probebase/>).

How diverse is diversity?

Microbial diversity in natural environments: By applying molecular techniques such as DNA reassociation kinetics, it was estimated that 30 g of soil might contain approximately 4,000 different microbial genomes, possibly

representing as many as 13,000 different microbial species (Torsvik et al., 1990). Ever since, soils have been regarded as one of the habitats with the most diverse microbial communities; however, a recent comparative study suggests a higher phylogenetic diversity in sediments possibly linked to salinity as major determinant (Lozupone and Knight, 2007). Estimates from PCR-based 16S rRNA gene sequencing suggested up to 100,000 species per gram of soil (Schloss and Handelsman, 2006). However, these estimates were based on relatively low numbers of sequences (up to 1,000). A new DNA sequencing technology, massively parallel pyrosequencing (Margulies et al., 2005), has allowed to sample as many as 53,000 16S rRNA sequences from one soil sample (Roesch et al., 2007); estimates of diversity based on these sequences suggested that the number of unique sequences never exceeded 52,000.

Function of microbial communities

Ecosystems are individually functioning units including primary producers, consumers, and mineralizing organisms. Microbial communities are the most numerous organisms in any ecosystem, and they control the annual primary production including the recycling of carbon, sulfur, nitrogen, and iron. In a lake ecosystem for instance, photic zone, water column, and sediment harbor functionally different microbial communities. Light energy drives the primary production in the photic zone by a community of phototrophic microorganisms. Photosynthetically fixed carbon and carbon from other sources move through the ecosystem and fuel the community of pelagic chemoorganotrophic aerobes (or anaerobes) in the water column. Eventually, part of the carbon sinks down to the sediment, where a specifically adapted community consisting of several guilds of microorganisms degrades the organic matter in a concerted interdependent manner, resulting in the flow of carbon through the anaerobic microbial food chain (see Chapter *Carbon (Organic, Degradation)*). Several different guilds are involved in the degradation of polymers including polymer hydrolyzing, fermenting bacteria, fatty acid fermenting syntrophs, homoacetogens, and methanogens (Schink and Stams, 2006). As oxygen diffusion into the sediment is severely limited, often microoxic to anoxic conditions occur within a few millimeters depth of the sediment surface because of microbial respiration activity in the very top layer. Concomitantly, anaerobically respiring microorganisms oxidize carbon compounds and transfer electrons to inorganic electron acceptors such as nitrogen oxides, manganese oxides, ferric iron, and sulfate in a sequence that reflects the thermodynamic theory in many environments (Conrad, 1996). Once these electron acceptors are depleted, methanogenesis becomes the terminal electron accepting process, which is typically found in freshwater sediments rich in organic matter, in swamps, or in flooded soils such as rice paddies (Schink and Stams, 2006). The function of the major guilds involved in these processes is known in

principle, mainly because representative microorganisms have been identified by enrichment and isolation techniques. The numerical importance as well as the actual physiological function of such guild representatives in the vast diversity of habitats are still elusive. Although 16S rRNA-based surveys with varying degrees of diversity sampling depths exist for a large number of habitats, mostly, sequences and derived phylogenetic identity alone do not reveal the physiological role these microorganisms have. The SSU rRNA is a very valuable phylogenetic marker, however, most guilds are not monophyletic, i.e., not all microorganisms falling into a coherent phylogenetic cluster have the same physiology. For example, within the Proteobacteria phototrophy is widespread, and thus, a close relative of a phototroph can be a nonphototrophic microorganism. Assignment of physiological characteristics based on close SSU rRNA relationship requires careful evaluation and often is not possible at all. The use of marker genes coding for key enzymes has allowed to target phylogenetically diverse but physiologically coherent guilds of microorganisms (Teske et al., 2003). For example, anaerobic respiratory processes carried out by methanogenic archaea (Friedrich, 2005) or sulphate-reducing (Wagner et al., 2005) prokaryotes can be traced in the environment by targeting the respective key genes.

Linking identity and function to microbial populations

Our understanding of microbial community functioning, mostly, is of principal nature for many ecosystems. Even for numerically abundant microbial species identified by molecular techniques, their ecophysiology and role in ecosystem functioning remain elusive. A major goal in current microbial ecology research is therefore to understand what uncultured microorganisms do in their habitats. What are their metabolic properties, and which microbial species are responsible for defined processes in their habitat?

An elegant concept for the elucidation of the ecophysiology of uncultivated microorganisms relies on the incorporation of isotopically labeled substrates into biomass and the subsequent identification of the microorganisms actively incorporating the label. This approach enables to link metabolic activity to active microorganisms by measuring label incorporation into biomarkers or even whole cells.

Microautoradiography in combination with FISH (MAR-FISH) can visualize cells actively incorporating radioactively labeled substrates in their natural habitats permitted that samples are suitable for FISH (Lee et al., 1999). Alternatively, oligonucleotide probe array technology detects radiolabel incorporation into rRNA extracted from environmental samples (Adamczyk et al., 2003).

The use of stable isotopes (^{13}C , ^{15}N , ^{18}O) for labeling active microbial populations has led to a whole suite of approaches that rely on tracing the incorporation of

labeled substrates into cell components such as lipids (Boschker et al., 1998), DNA (Radajewski et al., 2000), RNA (Manefield et al., 2002), or even whole cells (Lechene et al., 2006). Tracing ^{13}C incorporation into lipids is highly sensitive; however, phospholipids lack phylogenetic resolution and require pure cultures for assigning individual lipids to species. Stable isotope probing (SIP) of nucleic acids represents a direct way to identify microbial populations active in a defined metabolic process. SIP is based on the incorporation of labeled substrate into the cellular biomarker nucleic acids, separation of labeled nucleic acids from unlabeled nucleic acids by density gradient centrifugation, and molecular identification of active populations carrying labeled DNA or RNA. By now, SIP has been used to identify the active players involved in carbon and nitrogen cycling, as well in bioremediation in a large variety of different habitats and has been extended even to metagenomic analysis of labeled DNA (for a recent overview see (Neufeld et al., 2007)).

Whereas the original SIP is based on physical separation of labeled nucleic acid from unlabeled nucleic acid, newer approaches target single cells. Raman confocal microscopy detects shifts in vibrational energy of chemical bonds induced by ^{13}C labeling of cellular of single microbial cells. In combination with FISH, Raman microscopy enables identification of ^{13}C label incorporating active cells (Huang et al., 2007). A conceptually similar approach uses secondary ion mass spectrometry (SIMS) operating at lateral resolution of now down to 33 nm. NanoSIMS has high sensitivity allowing to detect shifts in isotopic signals at per mill rather than the atomic percent range (standard SIP and Raman SIP) and high isotope measurement precision (Lechene et al., 2006). Recently, it has been demonstrated that NanoSIMS analyses can be combined with 16S rRNA targeting oligonucleotide probes by employing halogen atoms as element label allowing in parallel phylogenetic identification and detection of metabolic activity of individual cells (Li et al., 2008).

Conclusions

Molecular biology based approaches have advanced our understanding of microbial diversity enormously over the last 20 years. However, we are just at the beginning of a new era in surveying microbial diversity: we will be witnessing the rapid accumulation of staggering amounts of sequence data not only for the single phylogenetic marker 16S rRNA, but also for whole metagenomes fueled by parallel DNA sequencing technology. New developments in linking phylogenetic identity to function, including renewed efforts in cultivation, will boost our understanding of the ecophysiology of the uncultivated vast majority of microorganisms. Eventually, this ongoing revolution of microbial ecology will allow to test general ecological theory using microbial systems (Prosser et al., 2007).

Bibliography

- Adamczyk, J., Hesselsoe, M., Iversen, N., Horn, M., Lehner, A., Nielsen, P. H., Schloter, M., Roslev, P., and Wagner, M., 2003. The isotope array, a new tool that employs substrate-mediated labeling of rRNA for determination of microbial community structure and function. *Applied Environmental Microbiology*, **69**, 6875–6887.
- Amann, R. I., Ludwig, W., and Schleifer, K. H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiology Reviews*, **59**, 143–169.
- Boschker, H. T. S., Nold, S. C., Wellsbury, P., Bos, D., de Graaf, W., Pel, R., Parkes, R. J., and Capenberg, T. E., 1998. Direct linking of microbial populations to specific biogeochemical processes by ^{13}C -labelling of biomarkers. *Nature*, **392**, 801–805.
- Conrad, R., 1996. Soil microorganisms as controllers of atmospheric trace gases (H_2 , CO , CH_4 , OCS , N_2O , and NO). *Microbiology Reviews*, **60**, 609–640.
- Euzéby, J. P., 1997. List of bacterial names with standing in nomenclature: a folder available on the Internet. *International Journal of Systematic Bacteriology*, **47**, 590–592.
- Friedrich, M. W., 2005. Methyl-coenzyme M reductase genes – unique functional markers for methanogenic and anaerobic methane-oxidizing *Archaea*. *Methods Enzymology*, **397**, 428–442.
- Huang, W. E., Stoecker, K., Griffiths, R., Newbold, L., Daims, H., Whiteley, A. S., and Wagner, M., 2007. Raman-FISH: combining stable-isotope Raman spectroscopy and fluorescence in situ hybridization for the single cell analysis of identity and function. *Environmental Microbiology*, **9**, 1878–1889.
- Lechene, C., Hillion, F., McMahon, G., et al., 2006. High-resolution quantitative imaging of mammalian and bacterial cells using stable isotope mass spectrometry. *Journal of Biology*, **5**, 20.
- Lee, N., Nielsen, P. H., Andreasen, K. H., Juretschko, S., Nielsen, J. L., Schleifer, K. H., and Wagner, M., 1999. Combination of fluorescent in situ hybridization and micro autoradiography – a new tool for structure-function analyses in microbial ecology. *Applied Environmental Microbiology*, **65**, 1289–1297.
- Li, T., Wu, T. D., Mazeas, L., Toffin, L., Guerquin-Kern, J. L., Leblon, G., and Bouchez, T., 2008. Simultaneous analysis of microbial identity and function using NanoSIMS. *Environmental Microbiology*, **10**, 580–588.
- Liesack, W., Janssen, P. H., Rainey, F. A., Ward-Rainey, N. L., and Stackebrandt, E., 1997. Microbial diversity in soil: the need for a combined approach using molecular and cultivation techniques. In van Elsas, J. D., Trevors, J. T., and Wellington, E. M. H. (eds.), *Modern Soil Microbiology*. New York: Marcel Dekker, pp. 375–439.
- Lozupone, C. A., and Knight, R., 2007. Global patterns in bacterial diversity. *Proceedings of National Academy of Sciences United States of America*, **104**, 11436–11440.
- Manefield, M., Whiteley, A. S., Griffiths, R. I., and Bailey, M. J., 2002. RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Applied Environmental Microbiology*, **68**, 5367–5373.
- Margulies, M., Egholm, M., Altman, W. E., et al., 2005. Genome sequencing in microfabricated high-density picolitre reactors. *Nature*, **437**, 376–380.
- Neufeld, J. D., Wagner, M., and Murrell, J. C., 2007. Who eats what, where and when? Isotope-labelling experiments are coming of age. *ISME J*, **1**, 103–110.
- Pace, N. R., Stahl, D. A., Lane, D. J., and Olsen, G. J., 1986. The analysis of natural microbial populations by ribosomal RNA sequences. *Advances in Microbial Ecology*, **9**, 1–55.
- Prosser, J. I., Bohannan, B. J. M., Curtis, T. P., et al., 2007. The role of ecological theory in microbial ecology. *Nature Reviews Microbiology*, **5**, 384–392.
- Radajewski, S., Ineson, P., Parekh, N. R., and Murrell, J. C., 2000. Stable-isotope probing as a tool in microbial ecology. *Nature*, **403**, 646–649.
- Roesch, L. F., Fulthorpe, R. R., Riva, A., et al., 2007. Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME Journal*, **1**, 283–290.
- Schink, B., and Stams, A. J. M., 2006. Syntrophism among prokaryotes. In Dworkin, M. (ed.), *The Prokaryotes*, 3rd edn. New York: Springer, Vol. 2, pp. 309–335.
- Schloss, P. D., and Handelsman, J., 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied Environmental Microbiology*, **71**, 1501–1506.
- Schloss, P. D., and Handelsman, J., 2006. Toward a census of bacteria in soil. *Plos Computational Biology*, **2**, 786–793.
- Stackebrandt, E., and Goebel, B. M., 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *International Journal of Systematic Bacteriology*, **44**, 846–849.
- Teske, A., Dhillon, A., and Sogin, M. L., 2003. Genomic markers of ancient anaerobic microbial pathways: sulfate reduction, methanogenesis, and methane oxidation. *Biological Bulletin*, **204**, 186.
- Torsvik, V., Goksoyr, J., and Daae, F. L., 1990. High diversity in DNA of soil bacteria. *Applied Environmental Microbiology*, **56**, 782–787.
- von Wintzingerode, F., Goebel, U. B., and Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples – pitfalls of PCR-based rRNA analysis. *FEMS Microbiology Review*, **21**, 213–229.
- Wagner, M., Loy, A., Klein, M., Lee, N., Ramsing, N. B., Stahl, D. A., and Friedrich, M. W., 2005. Functional marker genes for identification of sulphate-reducing prokaryotes. *Methods Enzymology*, **397**, 469–489.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of National Academy of Sciences United States of America*, **95**, 6578–6583.
- Woese, C. R., 1987. Bacterial evolution. *Microbiology Reviews*, **51**, 221–271.
- Woese, C. R., and Fox, G. E., 1977. Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of National Academy of Sciences United States of America*, **74**, 5088–5090.

Cross-references

[Acetogens](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Cycling\)](#)
[Carbon \(Organic, Degradation\)](#)
[Chemolithotrophy](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fluorescence In Situ Hybridization \(FISH\)](#)
[Metagenomics](#)
[Methanogens](#)
[Photosynthesis](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

MICROBIAL DEGRADATION

Erika Kothe
Friedrich Schiller University Jena, Jena, Germany

Synonyms

Mineralization; Nutrient cycling

Definition

Microbial. Processes driven by microorganisms.

Degradation. Use of chemical substances as carbon and energy source for metabolism, thereby breaking down larger molecules to smaller ones.

Introduction

Microorganisms are the basis for the process of mineralization by utilizing organic molecules for their catabolism, thereby returning the elements to the geobiological cycles. In catabolism, or energy conservation, energy-rich adenosine triphosphate (ATP) is formed (for general reading on microbial physiology: Madigan and Martinko, 2006). This is the basis for metabolic degradation performed by microorganisms in a great diversity. The degradation of organic material in terrestrial, limnological, and marine environments is thus based on the growth of microorganisms on organic compounds, which is termed chemoheterotrophic growth style, depending on the availability of organic nutrients for growth. The processes can be dependent on aerobic respiration, but many degradative processes, e.g., in sediments, are performed in the absence of oxygen under anaerobic conditions (Schink, 2002).

Aerobic and anaerobic respiration

The respiration with oxygen as the terminal electron acceptor is the process yielding the most ATP per mol substance oxidized and thus, aerobic respiration is the preferred route for degradation whenever enough oxygen is available. Under aerobic conditions, the degradation of organic matter is performed following general and ubiquitous pathways such as glycolysis; the pyruvate produced in the glycolytic chain enters the citrate cycle; and the reduction equivalents produced are then reoxidized by the respiratory chain coupled to the reduction of oxygen to water. All building blocks of living cells can be degraded that way, allowing growth of microorganisms. The carbon moieties are released as CO₂ into the atmosphere.

Under anaerobic conditions, anaerobic respiratory chains using alternative electron acceptors can ensue. For example, nitrate, nitrite, sulfate, sulfite, sulfur, iron, manganese or fumarate, or carbon dioxide in methanogenesis is then used to accept the electrons, thereby reducing these substances. Halogenated compounds can serve as electron acceptors in dehalorespiration.

All these respiratory chains depend on membrane-bound electron transport, and the reactions of electron transport are coupled to proton export across the cellular membrane to form a proton motive force. The proton motive force is then used to drive ATP formation *via* the enzyme ATP synthase, which can form 1 mol of ATP per 3 mol of protons carried back into the cytoplasm. The electrons for electron transport chains are derived from oxidation of the organic molecules of the nutrient source. If possible, the organic matter is fully oxidized to carbon dioxide. In this way, the organic matter is fully decomposed.

Fermentation

Another route, independent of respiratory chains, is possible for the fermentative degradation of organic matter. The fermentations performed by microorganisms can use oxidation of substrates to produce ATP and then reduce organic molecules for reoxidation of the reducing equivalents. While ATP yield in these systems is limited when compared to respiration, it still allows growth under conditions when no inorganic electron acceptor is available in sufficient amounts, or when iron as part of the electron carriers in the respiratory chain is not available and therefore, an electron transport chain cannot be synthesized.

In contrast to respiration, both aerobic and anaerobic, during fermentation the organic substrate is not fully oxidized to CO₂. Rather, energy conservation is dependent on the oxidation of an organic molecule in which an aldehyde may be formed as intermediate, which subsequently can be oxidized to form an acid. This exergonic reaction is coupled to the activation of an inorganic phosphate moiety which then can be transferred to ADP, thereby forming ATP. This process is called substrate-level phosphorylation in contrast to the electron transport phosphorylation occurring in respiratory chains. The oxidative reaction releases reduction equivalents, which cannot be transferred to an inorganic electron acceptor due to the lack of respiratory chains in fermenting microbes. Thus, the reduction of an organic compound is necessary to release the carrier for a new cycle of oxidation. The reduced organic substance is released into the environment. An alternative is to release the reduction equivalents as H₂.

The fermentation of sugars, which are present in large amounts due to photosynthesis products and constituents of plant cell wall material, can be performed using the Embden–Meyerhof pathway of glycolysis, the Entner–Doudoroff pathway, or modifications or parts thereof. Sugars with 5 C moieties, sugar acids, or carbonic acids can be converted by different pathways. Examples for sugar fermentation are homo- or hetero-fermentative lactic acid fermentation, alcoholic fermentation, propionate fermentation, butyrate or butanole fermentation, mixed acid fermentation, solvent fermentation, and many others. The names of the pathways indicate the products released in these different fermentative pathways.

Many bacteria can switch between fermentative and respiratory metabolism, depending on the availability of

electron acceptors for respiratory chains. If they can switch between anaerobic and aerobic metabolism, they are called facultative aerobes, while anaerobes are usually oxygen sensitive and grow exclusively under anaerobic conditions. Within this group, the Gram-positive *Clostridia* have evolved many fermentation pathways including those to degrade fatty acids, lipids, amino acids and proteins, or nucleic acids under anaerobic conditions. Thus, degradation of all constituents of the cytosol of living organisms under anaerobic conditions can be achieved.

As no oxygen is necessary for the fermentative metabolism, most fermentations will proceed under anoxic conditions. However, aerotolerant microorganisms such as some lactic acid bacteria that are active in decomposition of plant litter can perform this reaction also under oxic conditions, although these anaerobes do not use oxygen as electron acceptor.

Syntrophic degradation

The reduced compounds released by fermenting microorganisms constitute good nutritional sources for organisms that can use respiratory systems. In turn, the use of the product will allow for the continuous production of this compound, as no restrictions for the fermenting bacteria will inhibit their metabolism. The concomitant occurrence of bacteria in an ecological niche in which both partners are dependent on each other and degradation of the substrate is only possible when both partners are present is termed syntrophy.

The basis is the metabolic activity of one organism which forms a substrate for the other. This constant removal of a metabolic end product is exemplified by the release of H_2 which is often a link in such syntrophic systems. Use of hydrogen by a second organism allows the first organism to constantly produce this compound. In pure culture, the partial pressure of H_2 reaches levels inhibitory to the metabolism of the producing organism which subsequently ceases growth. Since the syntrophic partner constantly removes hydrogen, no cessation of growth is observed due to product inhibition in this coculture. This syntrophy, depending on hydrogen transfer between two microbes present in the same environment, is of special importance, and the term interspecies hydrogen transfer has been coined for this syntrophic relationship. However, many different examples can be given for such syntrophic associations of microorganisms (e.g., Ueda and Beppu, 2007). Degradation of recalcitrant substrates, including, e.g., halogenated compounds, is often seen in syntrophic associations.

Cometabolic degradation

Recalcitrant substrates are not, or very slowly, degraded because their conversion is not easily or not at all coupled with energy conservation. Thus, the degradation of these compounds is costly for the cell, and the microorganisms do not profit directly from degradation. Instead, the

bacteria have to use energy from a different, catabolic reaction to drive the unfavorable degradation process.

An example of immediate importance is lignin degradation, which is performed by white rot fungi. The brown rot fungi degrade lignin mainly in order to get access to the cellulose of plant cell wall which is a good nutrient source but not directly accessible in wood and timber. The recalcitrant nature of lignin is seen under anoxic conditions, where the degradation is not possible and lignin of plant origin is thus the basis for coal formation.

As cometabolic systems are costly, they are mostly linked to respiration. Degradation of plant alkaloids, which are also recalcitrant substrates, often occurs at higher rates in oxic conditions.

Degradation of plant litter and soil formation

Plant material is composed of the contents of the living cells and the cell wall materials. Within the cell, secondary plant metabolites are stored in the vacuole. Upon leaf fall or death of a plant, especially the secondary metabolites and the wall materials persist, while proteins and lipids are easily degradable in the unsaturated oxygen containing upper soil layers.

Plant cell walls are composed of cellulose, hemicellulose, pectin, polygalacturonans, and lignin. Even in herbaceous plants, a quarter of the secondary cell wall is lignin. This macromolecule can be degraded aerobically, while anoxic conditions lead to the formation of coal, since under these conditions lignin cannot be degraded. Wood rotting fungi, both brown rot and white rot fungi, are able to degrade lignin (Crawford, 1981). The enzymes involved in lignin degradation, peroxidases and laccases, are able to attack the bonds in the macromolecule linking the phenolic monomers to form the high molecular weight lignin. The monomers can then be degraded further and are metabolized to carbon dioxide. These processes have been studied in some detail at the molecular level (Gold and Alic, 1993). Whether bacteria are also able to degrade wood is still under discussion. Streptomycetes are able to perform some partial degradation. However, complete decomposition has yet to be shown with bacterial systems.

An interesting question in wood and lignin degradation is regarding the possible symbiosis of wood degrading fungi and bacterial populations at an oxic/anoxic border. Anaerobic bacteria able to convert lignin decomposition products and partially degrade these compounds have been described. The fermentation products of these organisms may serve as growth substrates for the lignin degrading fungi. Secondary metabolites of plants and microorganisms are often recalcitrant because they contain aromatic rings, heterocycles, and polyketides or are halogenated. Such compounds are not easily degraded, especially under anoxic conditions. If degradation does not lead to complete oxidation of carbon moieties to CO_2 , the formation of humic acids and fulvic acids and thus the production of humus ensues. This process is the driving force for soil genesis. Under aerobic conditions,

enzymatic reactions known from lignin oxidation are often involved (Bumpus and Aust, 1987; Hammel, 1989).

Degradation of aromatic and halogenated compounds

Under aerobic conditions, the chemically very stable aromatic ring can be broken by activities of mono- and di-oxygenases. While monooxygenases introduce one of the oxygen atoms of molecular oxygen into the organic molecule forming a hydroxyl group at one site of the ring, dioxygenases incorporate both oxygen atoms which leads to decyclization and ring opening. The linear compound can then be further oxidized after activation and fueled into general metabolic pathways. The aerobic degradation of aromatic compounds can be used to clean oil spills or other anthropogenic contaminations, especially applicable for the residual contamination after a first mechanical cleansing has been performed. Inoculation of a beach after an oil spill could show visible removal of the remaining oil within 1 year.

Under anoxic conditions, the ring opening seems more difficult and it had long been argued that this would not be possible. However, bacterial enrichment cultures could show that an anaerobic degradation of aromatic compounds is possible usually via activation and conversion to benzoyl CoA followed by ring reduction. These energetically unfavorable reactions are feasible with a thermodynamically favorable electron acceptor, e.g., when nitrate is available in an anaerobic respiratory chain. Thus, bacteria related to the nitrogen fixing genus *Azoarcus* link the degradation of aromatic compounds with denitrification. Many gene clusters with different substrate specificities have been identified to perform anaerobic degradation of different organic molecules including aromatic amino acids, benzene and its derivatives, phenols, and heterocyclic compounds. The linearized structures again can be fueled into metabolic pathways via acetyl CoA or propionyl CoA. Anthropogenic aromatic compounds can be degraded by these same processes, thus allowing complete decomposition and natural attenuation. The introduction of bacteria able to anaerobically degrade aromatic compounds into aquifers with (anthropogenic) contaminations opens the road to bioremediation of contaminated groundwater resources. In tertiary oil recovery, introduction of bacteria into the well to partially degrade the high molecular crude oil fraction uses bacterial enzymatic activities to form molecules of lower molecular weight from the tar fraction that then can be recovered.

Many anthropogenic contaminations contain halogen atoms which generally decrease metabolic degradation of the compounds. However, halogenated compounds are also known from natural sources. These include antibiotics with chloride or bromide atoms, plant-derived alkyl halides, or deterrents incorporated into, e.g., marine slugs or tropical frogs. Since nature already has learned to cope with these halogenated compounds, anthropogenic

halogenated substances can also be degraded by bacterial consortia, both aerobically and anaerobically (Abraham et al., 2002; Smidt and de Vos, 2004). Not only bacteria are known to be able to perform dehalogenation reactions, but also basidiomycete fungi can also degrade organohalogens (de Jong and Field, 1997). While aerobic dehalogenation is a simple enzymatic reaction, anaerobic dehalorespiration is an interesting field of research. In this case, the dehalogenation is coupled with electron transfer and directly coupled to ATP formation in an anaerobic respiratory process.

Use of microbes in bioremediation

The properties discussed above make the use of microbia or microbial consortia useful for bioremediation of different contamination scenarios. These include aerobic conditions such as in soil, anoxic condition such as in aquifers or sediments, and all imaginable sources of contamination from oil spill to xenobiotics such as plant protection chemicals or heavy metals. Some recent reviews are included to facilitate further reading on microbial degradation (Holtze et al., 2008; Johnsen and Karlson, 2007; Demnerova et al., 2005; Pieper, 2005; Kim and Rhee, 2003) and bioremediation (Watanabe et al., 2002; R  ling and van Versefeld, 2002; Schwitzguebel et al., 2002).

Summary

The biogeochemical cycles of the macronutrients carbon, nitrogen, sulfur, and phosphorus are dependent on microbial degradation of organic matter. Organic matter originating from carbon dioxide fixation, which is in terrestrial system usually performed by photosynthetically active bacteria and plant, is degraded to form carbon dioxide, either directly in one metabolic reaction or by cocultures in which the intermediate is released and taken up by another microbe. In addition, the formation of methane from CO₂ or acetate is possible. Thus, the carbon cycling involves CO₂, CH₄, and organic molecules. Nitrogen fixation of N₂ to NH₃ followed by fixation in organic amines constitutes one side of the cycle, while nitrification/denitrification releases the bound nitrogen into the atmosphere. The latter reaction sequences, i.e., oxidation of NH₃ to NO₂[−] or NO₃[−] and nitrate or nitrite respiration leading to N₂ formation, are dominant processes in the cycling of nitrogen. For the sulfur cycle, assimilation of sulfates and fixation into organic biomass, the oxidation of sulfur and reduction of sulfate or sulfite to elemental sulfur, or reduction of sulfur to H₂S are the main processes. Sulfur is a constituent of the amino acids cysteine and methionine and of Fe/S clusters in redox enzymes. Phosphate is generally not altered; however, there have recently been reports on the use of phosphite in an unusual energy fixation process.

These elemental cycles in the biogeosphere all involve steps of fixation within living biomass, and the degradation of dead biomass is an essential part in releasing the mineralized elements back into the cycle. Higher

organisms are not capable of using plant litter, e.g., directly and thus entirely dependent on microbial degradation processes to have sufficient minerals available for their nutrition. Properties of microbes for degradation can be useful in bioremediation approaches.

Bibliography

- Abraham, W. R., Nogales, B., Golyshin, P. N., Pieper, D. H., and Timmis, K. N., 2002. Polychlorinated biphenyl-degrading microbial communities in soils and sediments. *Current Opinion in Microbiology*, **5**, 246–253.
- Bumpus, J. A., and Aust, D., 1987. Biodegradation of environmental pollutants by the white rot fungus *Phaenorachete chrysosporium*: involvement of the lignin-degrading system. *Bioessays*, **6**, 166–170.
- Crawford, R. L., 1981. *Lignin Biodegradation and Transformation*. New York: Wiley.
- de Jong, F., and Field, D. A., 1997. Sulfur tuft and turkey tail: biosynthesis and biodegradation of organohalogenes by basidiomycetes. *Annual Review of Microbiology*, **51**, 375–414.
- Demnerova, K., Mackova, M., Specakova, V., Beranova, K., Kochankova, L., Lovecka, P., and Rylslava, E., 2005. Two approaches to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial populations. *International Microbiology*, **8**, 205–211.
- Gold, M. H., and Alic, M., 1993. Molecular biology of the lignin-degrading basidiomycete *Phaenorachete chrysosporium*. *Microbiology Reviews*, **57**, 605–622.
- Hammel, K. E., 1989. Organopollutant degradation by ligninolytic fungi. *Enzyme and Microbial Technology*, **11**, 776–777.
- Holtze, M. S., Sorensen, S. R., Sorensen, J., and Aamand, J., 2008. Microbial degradation of the benzonitrile herbicides dichlobenil, bromoxynil and ioxynil in soil and subsurface environments – insights into degradation pathways, persistent metabolites and involved degrader organisms. *Environmental Pollution*, **154**, 155–168.
- Johnsen, A. R., and Karlson, U., 2007. Diffuse PAH contamination of surface soils: environmental occurrence, bioavailability, and microbial degradation. *Applied Microbiology and Biotechnology*, **76**, 533–543.
- Kim, D. Y., and Rhee, Y. H., 2003. Biodegradation of microbial and synthetic polyesters by fungi. *Applied Microbiology and Biotechnology*, **61**, 300–308.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock: Biology of Microorganisms*. Upper Saddle River: Prentice-Hall.
- Pieper, D. H., 2005. Aerobic degradation of polychlorinated biphenyls. *Applied Microbiology and Biotechnology*, **67**, 170–191.
- Röling, W. F., and van Verseveld, H. W., 2002. Natural attenuation: what does the subsurface have in store? *Biodegradation*, **13**, 53–64.
- Schink, B., 2002. Anaerobic digestion: concepts, limits and perspectives. *Water Science and Technology*, **45**, 1–8.
- Schwitzguebel, J. P., Aubert, S., Grosse, W., and Laturnus, F., 2002. Sulphonated aromatic pollutants. Limits of microbial degradability and potential of phytoremediation. *Environmental Science and Pollution Research International*, **9**, 62–72.
- Smidt, H., and de Vos, W. M., 2004. Anaerobic microbial dehalogenation. *Annual Review of Microbiology*, **58**, 43–73.
- Ueda, K., and Beppu, T., 2007. Lessons from studies of *Symbiobacterium thermophilum*, a unique syntrophic bacterium. *Bioscience Biotechnology and Biochemistry*, **71**, 1115–1121.
- Watanabe, K., Futamata, H., and Harayama, S., 2002. Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. *Antonie van Leeuwenhoek*, **81**, 655–663.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Phosphorus, Phosphorites](#)
[Soils](#)

MICROBIAL ECOLOGY OF SUBMARINE CAVES

Francesco Canganella, Giovanna Bianconi
 University of Tuscia, Viterbo, Italy

Definition

Essential concepts, current investigations, and discussion.

In recent years, the search for the limit of biodiversity in extreme and/or uncommon environments led to the investigation of peculiar ecosystems such as deep-sea, subterranean niches, Antarctic cores, hydrothermal vents, and extraterrestrial sites.

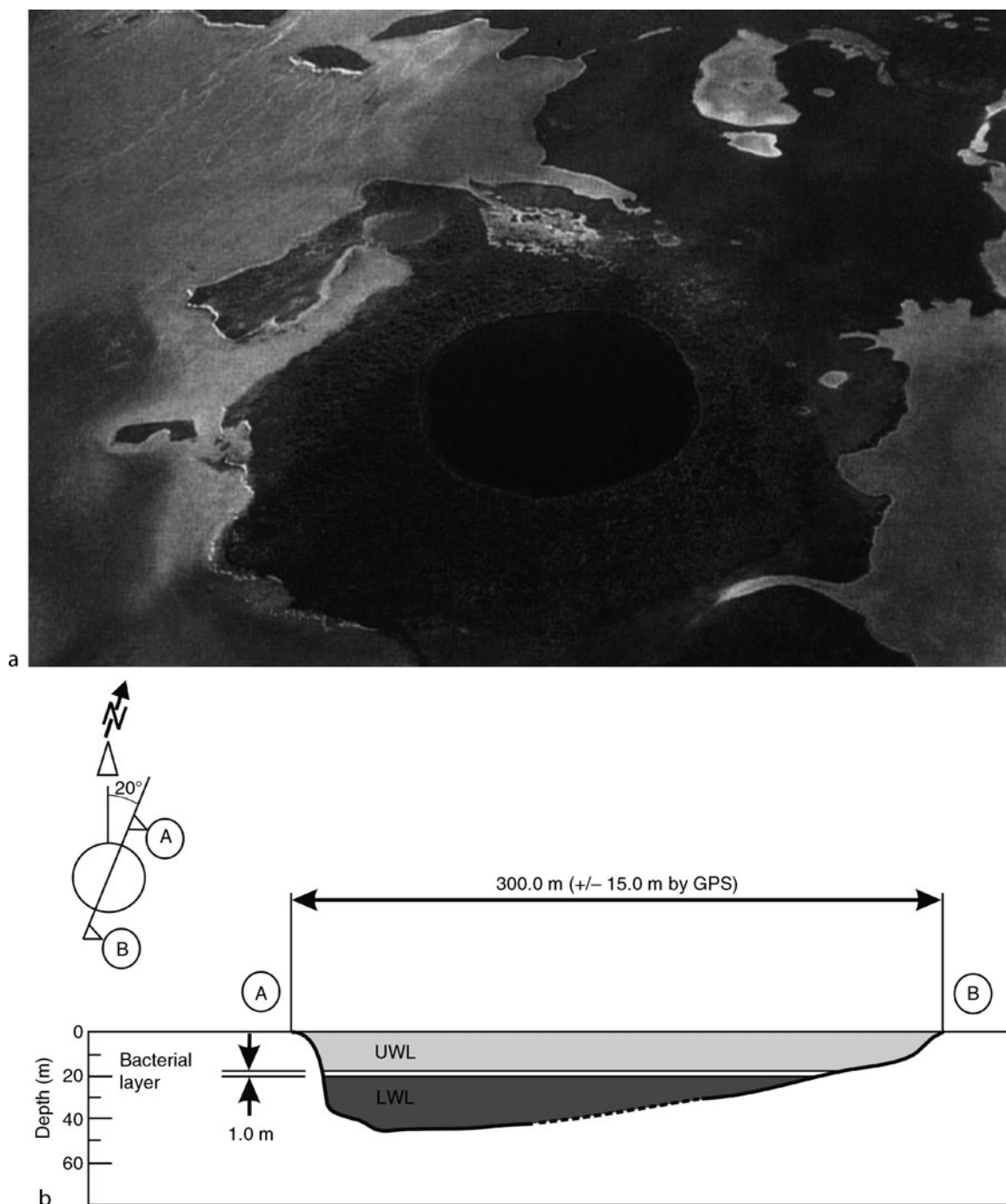
Submarine caves and cavities have also been explored, some were mainly exploited as popular attractions for scuba divers, but some were shown to be of great interest for geologists, biologists, and microbiologists.

Available scientific data on these ecosystems are not abundant, particularly as far as biological disciplines are concerned. Most of the published works have been related to specific submarine cavities known as Blue Holes and Black Holes, as well as to submarine caves showing inner sulfidic emissions. A larger number of data are currently available regarding the microbiology of terrestrial caves, particularly on the microbial ecology of carbonate layers, sediments, bat guano, and flooding waters (Vlasceanu et al., 1997, 2000; Holmes et al., 2001; Engel et al., 2003; Northup et al., 2003; Sugita et al., 2005; Zhou et al., 2007), but these latter ecosystems are not part of the current review.

Blue holes and black holes

The so-called blue holes are underwater karst systems which can develop horizontally in a very extensive way and appear blue due to a combination of blue sky reflection with the white carbonate sand deposited in the cave (Smart et al., 1988; Mylroie et al., 1995; Schwabe et al., 1997; Marano-Briggs, 2000; Colantoni et al., 2003; Canganella et al., 2004). Most of these sites were found and described in Bahamas and Hawaii.

Other cavern systems are known as “black holes,” which are cave systems without known lateral passages and located in the interior regions of the larger Bahamian islands. Most black holes are located in the central to western side of the island of South Andros in the Bahamas (Figure 1), although one has been found on the northern transitional shore of the Grand Bahama Island. They are vertical cave systems which



Microbial Ecology of Submarine Caves, Figure 1 A schematic drawing of the South Andros black hole, (a) aerial view and (b) bathymetry. UWL, upper water layer; LWL, lower water layer; —, floor area not surveyed. (Reprinted from Schwabe and Herbert, 2004 *Black Holes of the Bahamas: what they are and why they are black*, *Quaternary International* 121: 3–11 with permission from Elsevier.)

develop from the surface downward and appear to have no direct link to the sea, except through rock fissures and local porosity (Schwabe, 1998; Schwabe and Herbert, 2004).

In both cave systems, as water exchange is severely restricted, physicochemical gradients are highly stable and even strict anaerobic conditions can develop with remarkable hydrogen sulfide concentrations (from 5 μM to 6 mM).

Submarine caves with sulfidic water springs

A unique marine ecosystem is represented by the submarine caves of Capo Palinuro (Salerno, Italy), of which 13 have inside sulfidic springs exhibiting temperatures up to 25°C that remarkably affect the surrounding ecosystem.

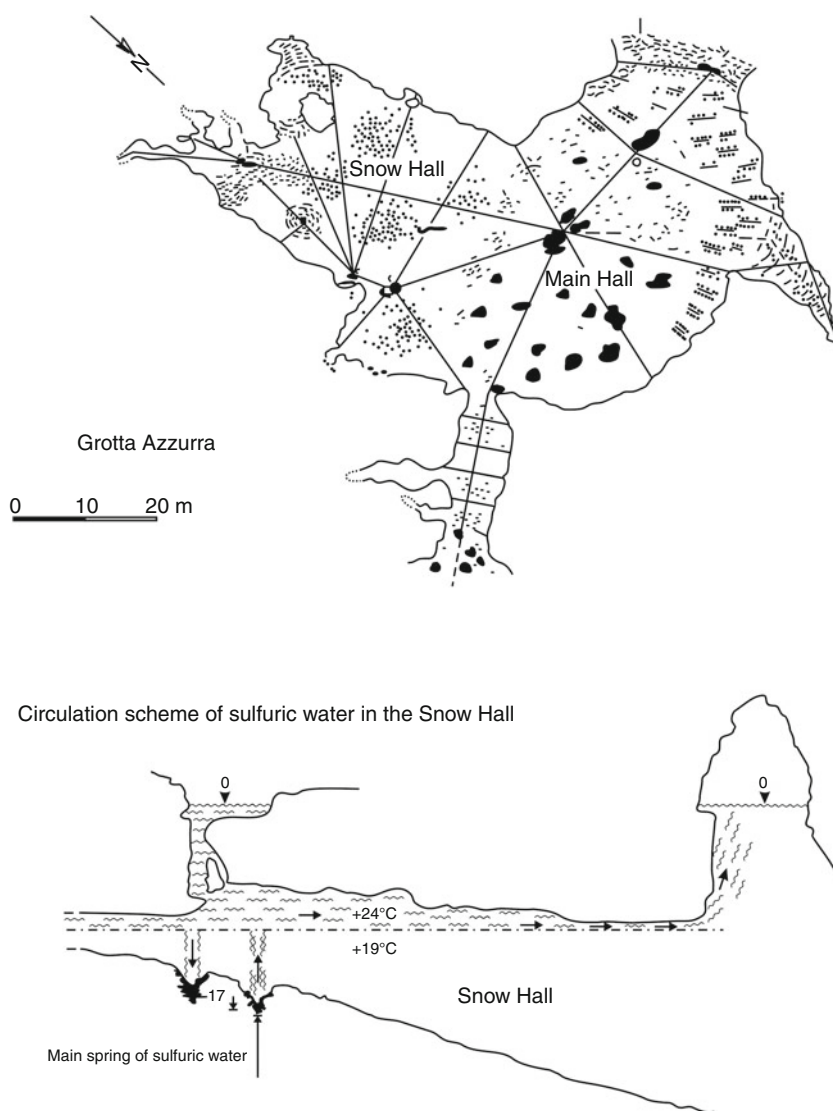
Preliminary investigations concerning the submarine speleology of the site have been carried out since the mid 1980s, but only after 1990 an extensive biological knowledge was achieved. Several ecological and geological studies were performed (Alvisi et al., 1994a, b; Mattison et al., 1998), particularly on the “Grotta Azzurra” (Figure 2). The cave may be separated into two topographically distinct regions: a weakly illuminated outer region and an inmost dark region (Snow Hall). The latter is

characterized by the presence of sulfur springs that arise from fissures in the cave floor. Currently, no other submarine caves with sulfide springs have been reported.

Microbiological studies

The blue holes

Abundant microbial development has been observed in these cave systems, usually linked to temperature anomalies in the range of 36–41°C as recorded by investigators. Marano-Briggs (2000) reported temperatures up to 41°C in the Tarpon Blue Hole, supporting the theory that the mass population of anoxygenic phototrophic bacteria present at a specific water layer may dissipate excess light energy by heat. A dense layer of purple sulfur bacteria



Microbial Ecology of Submarine Caves, Figure 2 A drawing map of the “Grotta Azzurra” showing both entrances, halls, and freshwater circulation.

was indeed found at 4.5–5.5 m depth in the estuarine Tarpon Blue Hole and the dominant population was represented by *Chromatiaceae* species. These bacteria are capable either of photolithoautotrophic growth with sulfide or elemental sulfur under anoxic conditions in the light or of chemo-organotrophic growth under micro-oxygenic conditions in the dark. Their abundance in the site was consistent with their physiological traits, and among them a phototrophic bacterium in particular was identified as a novel strain of *Marichromatium purpuratum* that contained okenone instead of spirilloxanthin as its major carotenoid.

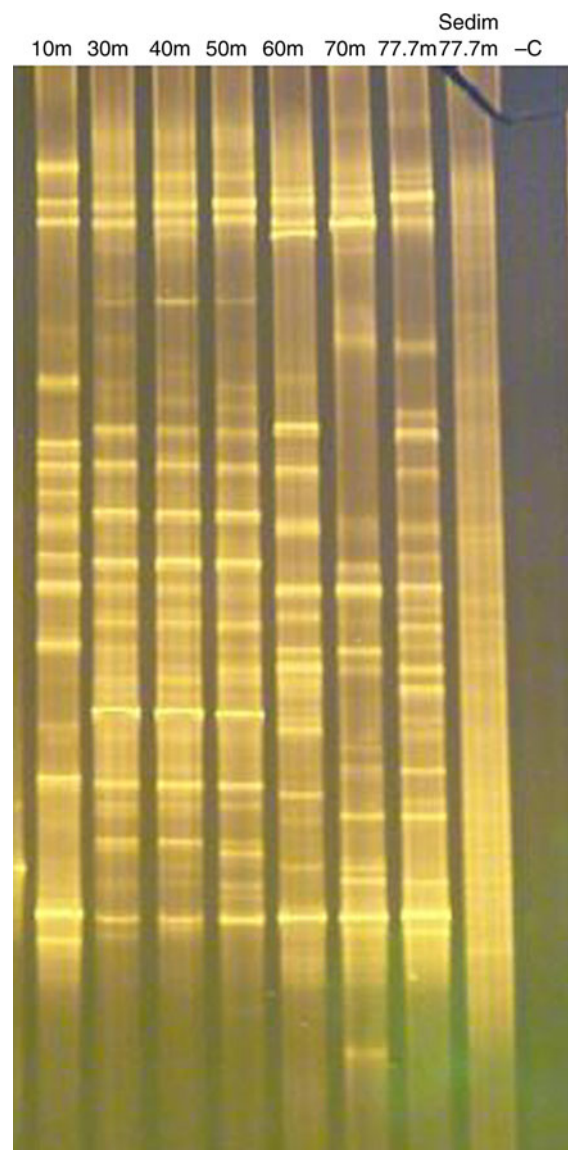
Recently, microbiological studies on a blue hole discovered in the Indian Ocean (Canganella et al., 2004) were preliminarily performed in order to investigate the distribution of microbial populations along the water column and in the bottom sediment of the cave. In Figure 3, the DGGE analysis carried out shows how bacterial populations are distributed among surface layers, mid- and deep-layers, indicating in particular a similar biodiversity between 30 and 50 m depth. The same was investigated by the Biolog system, particularly for anaerobic populations.

The molecular investigation showed that at surface there was a complex microbial community showing several peculiarities with respect to standard pelagic seawater. Characteristic species were *Thioalkalivibrio* and *Thioploca* within the Gamma-Proteobacteria and *Desulfosarcina* and other Delta-Proteobacteria; sequences within the Delta-Proteobacteria related to sponge symbionts were also found. Subsurface bacteria present in the water column appeared dominated by Alpha-Proteobacteria represented by sequences related to photoorganotrophs (*Rhodovibrio*-related), methanotrophs, and coral surface-related bacteria.

Near the bottom, although the Alpha-Proteobacteria were still well represented, the proportion of Delta-Proteobacteria increased. These Delta-Proteobacteria represent sulfate-reducing bacteria mainly belonging to the Desulfobacteriaceae, or related to *Desulfonanticus*, *Syntrophus*, and others related to marine and salt-marsh uncultured bacteria.

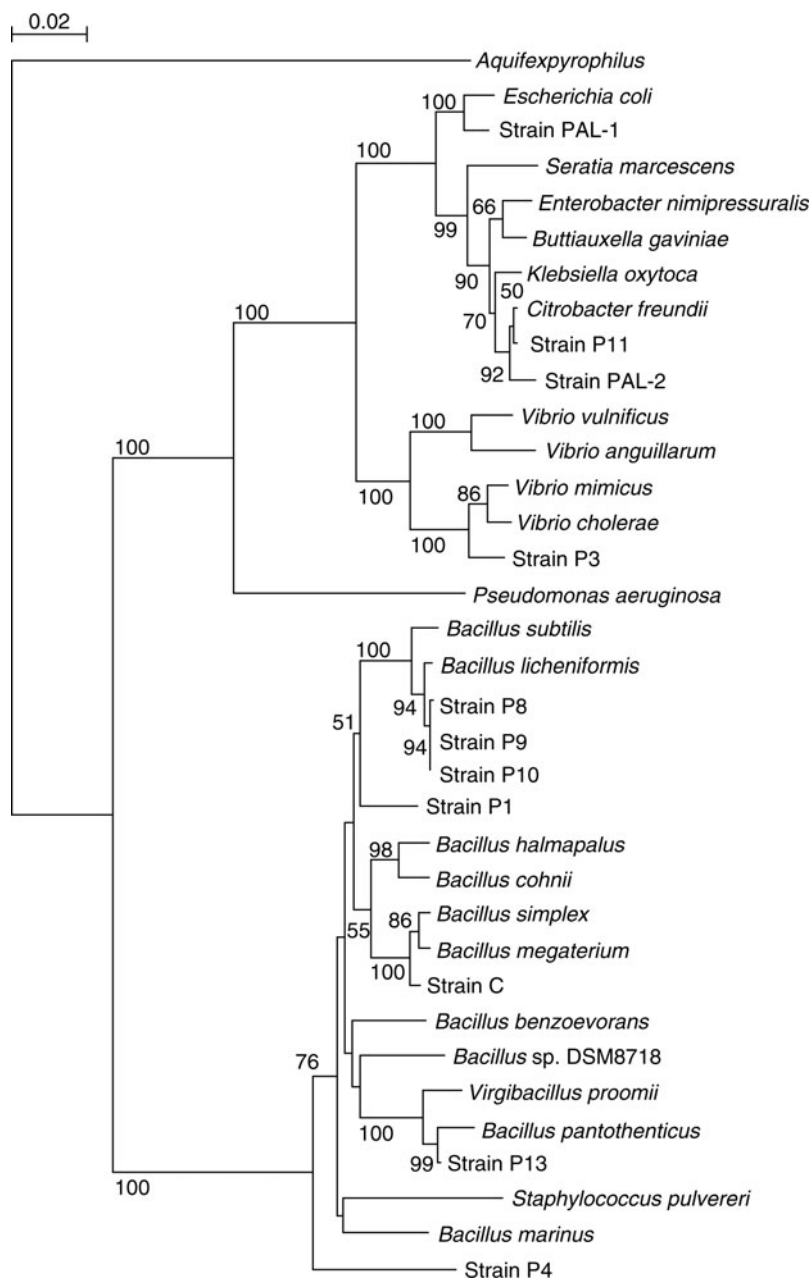
The black holes of the Bahamas

The water in these holes appears black in color due to the presence of a 1 m thick white microbial veil located within the upper third of the water column (18–19 m), at the boundary between the oxygenic low salinity upper water mass and the denser anoxic saline water layer. The boundary between the two water masses is characterized by sharp discontinuities in physicochemical gradients: salinity increased from 12 to 35 psu and temperature from 29°C to 36°C; pH decreased from 8.6 to 6.45 as well as dissolved O₂ from 6 mg/l to <1 mg/l. Morphological microscopic observations of collected samples showed mainly nonmotile spherical and motile rod-shaped purple sulfur bacteria. The dominant members of this warm (36°C), saline, and sulfide-rich layer have been identified



Microbial Ecology of Submarine Caves, Figure 3 DGGE analysis of the water column and bottom sediment of the blue hole in the Indian Ocean.

as anoxygenic phototrophic bacteria belonging to the genera *Allochromatium* and *Thiocapsa* and showed population densities $>10^7$ viable cells/ml. These bacteria grow well in the presence of sulfide and carbon dioxide in the light. During photoautotrophic growth sulfur globules are stored intracellularly as intermediate oxidation products. Moreover, the intracellular photosynthetic membranes are of the vesicular type, and bacteriochlorophyll *a* and carotenoids of the normal spirilloxanthin series are present (Herbert et al., 2005). Calculations made by these authors revealed that the layer of anoxygenic phototrophic bacteria in the South Andros black hole may have a biomass content of approximately 5.06 ton dry weight.

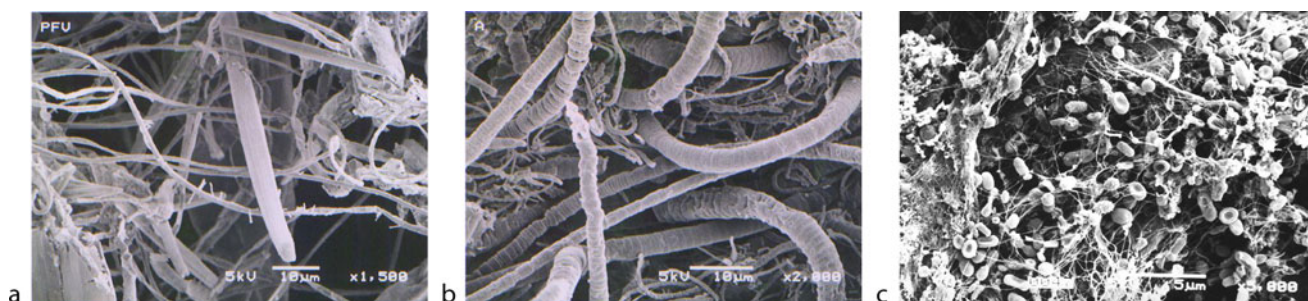


Microbial Ecology of Submarine Caves, Figure 4 A phylogenetic tree based on 16S rRNA gene sequence comparison, including representative heterotrophic isolates from the "Grotta Azzurra."

The marine caves of Capo Palinuro

The emergence of sulfidic water in some of the caves (particularly in the "Grotta Azzurra," "Grotta Sulphurea," and "Grotta di Cala Fetente") gives rise to an unusual situation, characterized by abiotic as well as biotic fluctuations, represented by emissions from sulfidic springs and higher concentration of sulfur-utilizing bacteria following each outflowing event, respectively. Warm sulfidic water of reduced salinity enters the Snow Hall (Grotta Azzurra),

from fissures in the bottom rocks, and rises above the more dense seawater to form a thermocline and chemocline as a visible boundary at a depth of about 9.5 m. The geochemistry of water samples from the Grotta Azzurra was extensively reported by Mattison et al. (1998): above and below the chemocline temperatures were 24.0 ± 0.2 and 22.8 ± 0.3 , respectively; NaCl concentrations (%) were 2.6 ± 0.1 and 3.0 ± 0.1 , respectively; pH values were 7.22 ± 0.02 and 8.15 ± 0.25 , respectively.



Microbial Ecology of Submarine Caves, Figure 5 (a) A SEM picture of a sample collected in the bacterial mat on the vault of the “Grotta Azzurra”; (b) a SEM picture of a sample collected in the bacterial mat on the vault of the “Grotta Azzurra”; (c) A SEM picture of a sediment sample collected nearby a sulfataric spring along the main entrance of the “Grotta Azzurra.”

The present ecosystem is biologically unique particularly because of the following points: (1) the peculiar emissions represent the only available model in shallow waters resembling the deep-sea hydrothermal vents (Canganella, 2001); (2) alike what happens in usual submarine caves, the development of micro- and macro-fauna increases with the decrease of irradiation; (3) the freshwater flow is placed above the seawater and, inside the cave, an interface between the warm/anaerobic upper zone (H_2S -enriched) and the cold, sulfurless lower zone can be observed; (4) giant forms, particularly among *Cnidaria* and *Porifera*, occur mainly along the aerobic/anaerobic interface (Alvisi et al., 1994a, b).

During the last decade both, “Grotta Azzurra” and “Grotta Sulfurea” have been investigated. Some preliminary data on the lithotrophic microflora were published (Mattison et al., 1998), and other studies were performed in order to (1) investigate the microscopic structure of bacterial mat and (2) to understand both the taxonomy and physiology of heterotrophic bacteria inside the “Grotta Azzurra” (Canganella and Bianconi, 1999, 2003). The 16S rRNA genes analysis (Figure 4) showed that heterotrophic isolates were closely related to the genera *Escherichia*, *Citrobacter*, *Vibrio*, and *Bacillus*.

In addition to the analysis of the heterotrophic microflora, recently a preliminary molecular investigation was performed to evaluate the biodiversity of both eubacterial and archaeal populations (Canganella F. and Bianconi G. 2009).

Some bacterial sequences were closely related to the sulfur-oxidizing bacterial group, involving the genus *Beggiatoa* and *Thiothrix*. Showing that these microorganisms may well represent a major microbial community in the bacterial mat of the cave. Other bacterial sequences were phylogenetically linked to the Delta-proteobacterial group, particularly to sulfate-reducing bacteria. Archaeal sequences were partially related to methanogen archaeal group, but the phylogenetic distance was still remarkable; so the involvement of a novel archaeal group can be considered. Other archaeal sequences were related to MG-1,

which is a common uncultivable archaeal group in marine environment.

As regards the microscopic structure of bacterial mat inside the cave and sediments collected nearby a sulfidic spring along the main entrance of the “Grotta Azzurra,” the distribution of various morphological forms is shown in Figures 5a–c. Filamentous microbial forms were abundant and morphologically similar to *Beggiatoa*, *Desulfonema*, *Leucothrix*, or *Thiothrix* species; morphology was, however, not enough to identify such organisms. Several cells showing either coccoid or rod morphologies were also observed in samples collected inside as well as outside the mat.

Conclusions

As regards the blue and black holes, it has been shown that the dominant anoxygenic phototrophic bacteria in the microbial layers are member of the *Chromatiaceae* (Imhoff et al., 1998). The presence of dense phototrophic populations of purple sulfur bacteria particularly explains why the South Andros black hole appears black and not blue. Moreover, the sulfide profile data obtained in this site showed that sulfide concentration was low (30 μM) suggesting that biomass production by the purple sulfur bacteria was sulfide rather than light limited.

Another important point to consider is that the dense microbial phototrophic population probably plays a significant role in enhancing carbonate CO_2 -mediated dissolution operated by heterotrophic bacteria. The effects of microbiological activity on degradation of limestone deposits have been described by several authors (Ehrlich, 1996; Golubic and Schneider, 1979; Paine et al., 1993) and similar observations were reported for the Bahamas cave systems from Smart et al. (1988).

Recent studies have shown that biotic oxidation of sulfide into sulfuric acid can occur in anoxic marine ecosystems due to chemoautotrophic sulfur-oxidizing bacteria such as *Beggiatoa* (Mattison et al., 1998; Vlasceanu et al., 1997, 2000). This biological process may also be involved in carbonate dissolution for the South Andros black hole but the role of sulfur-oxidizing bacteria needs

to be evaluated by further investigations. The same consideration may be valid for the blue hole in the Indian Ocean. In this site the occurrence of highly toxic sulfide and low oxygen content caused the death of the macrobenthos in deep waters, where a chemolithoautotrophic microbial community settled on the cave walls as mucilaginous and hardened short filaments of bacterial origin whose study is still in progress.

In the "Grotta Azzurra" cave of Capo Palinuro, fluids emitted from active springs with maximum temperatures of 25°C and enriched in dissolved sulfur species (H_2S , $\text{S}_2\text{O}_3^{2-}$) allowed the development of a significant micro- and macro-fauna, resembling in some way what occurs around hydrothermal vent sites (Jeanthon, 2000; Canganella, 2001).

Morphological/physiological observations were previously carried out by Mattison et al. (1998) on microbial mats in the "Grotta Azzurra" of Capo Palinuro. In recent studies both sampling and isolation were carried out regardless of the chemocline. Future investigations on the distribution of microbial populations will eventually lead to discrimination between more thermotolerant and more mesophilic species living above or below the chemocline; the same can be true for noncultivable species.

The characterized heterotrophic isolates partially represent the microbial ecology of the site, showing its biological complexity at a phylogenetic level. Moreover, the wide resistance of isolates to temperature, salinity and pH may be associated with the specific ecological system, an hypothesis that certainly deserves further investigations.

In general terms, similar environmental traits can be described for the blue hole in the Indian Ocean and the submarine caves of Palinuro Cape; first of all, the presence of elevated concentrations of hydrogen sulfide, ranging from 5 μM to 200 mM. A chemocline in the water column may be clearly observed in the blue hole of the Indian Ocean or in the black hole of the Bahamas, whereas in the submarine caves of Palinuro Cape the environmental borderline inside the habitat is represented by the threshold between upper anoxic, sulfide-rich freshwater and lower seawater.

As regards the autoctonous microflora, the blue hole of the Indian Ocean showed the presence of filamentous forms as described in the Palinuro caves but mucilaginous forms on the rocky side walls were not found in the latter; moreover sulfate-reducers and sulfide-oxidizers were also detected in both systems.

Some microbiological features of both systems are also shared with other sulfide-rich environments, such as the presence of anaerobic methane oxidizers, filamentous bacteria, anaerobic phototrophs, and archaeal strains. However, the incomplete data set on the inhabitant microflora, the geological diversity of the systems described earlier, and their different geographical locations make a microbiological comparative study difficult. With no

doubts the combination of different approaches and techniques may be a powerful tool to investigate further such astonishing ecosystems by a multiphasic approach.

As a matter of fact, further investigations on the microbial diversity in sulfide-rich, either marine or freshwater, caves will be based on traditional techniques of isolation coupled to simultaneous molecular investigations.

Bibliography

- Alvisi, M., Barbieri, F., and Colantoni, P., 1994a. Le grotte marine di Capo Palinuro. In Alvisi, M., Colantoni, P., Forti, P. (eds.), *Grotte Marine d'Italia*. Bologna: Memorie dell'Istituto Italiano di Speleologia, (pp 143–181).
- Alvisi, M., Barbieri, F., Bruni, R., Cinelli, F., Colantoni, P., Grandi, F., and Maltoni, P., 1994b. La Grotta Azzurra di Capo Palinuro (Salerno). In Alvisi, M., Colantoni, P., Forti, P. (eds.), *Grotte Marine d'Italia*. Bologna: Memorie dell'Istituto Italiano di Speleologia, (pp 51–56).
- Canganella, F., 2001. Hydrothermal vent communities. In *Encyclopedia of Life Sciences*. London: Macmillan.
- Canganella, F., and Bianconi, G., 1999. Microbial investigations on submarine caves in the area of Cape Palinuro (Salerno, ITALY) in Brazilian Microbiology Society (Ed). In *Proceedings of the XX Meeting of the Microbiological Brazilian Society*, Oct 21–25, 2001, p. 223, Salvador, BA.
- Canganella, F., and Bianconi, G., 2003. Microscopic investigations on the cell structure of autoctonous microflora in a sulphidic submarine cave (transl.). In *Fifth Congress FISV*, Oct 10–13, 2003, p 154, Rimini, Italy.
- Canganella, F., Bianconi, G., and Maugeri, T., 2004. A multiphasic approach to investigate the microbial ecology of a Blue Hole in the Indian Ocean. In *Extremophiles Meeting*, September 19–23, 2004, p 83, Cambridge, MA.
- Colantoni, P., Baldelli, G., Bianchi, C. N., Capaccioni, B., Morri, C., Mandrini, M., and Tassi, F., 2003. A cave flooded by marine water with hydrogen sulphide highlights the recent evolution of the Maldives (Indian Ocean): preliminary notes. *Le grotte d'Italia*, 4, 29–37.
- Ehrlich, H. L., 1996. *Geomicrobiology*, 3rd edn. New York: Marcel Dekker, pp 172–215.
- Engel, A. S., Lee, N., Porter, M. L., Stern, L. A., Bennett, P. C., and Wagner, M., 2003. Filamentous "Epsilonproteobacteria" dominate microbial mats from sulfidic cave springs. *Applied and Environmental Microbiology*, 69, 5503–5511.
- Golubic, S. I., and Schneider, J., (1979). Carbonate dissolution. In Trudinger, P. A., Staine, D. J. (eds.), *Biogeochemical Cycling of Mineral-Forming Elements*. Amsterdam: Elsevier, pp. 107–129.
- Herbert, R. A., Ranchou-Peyruse, A., Duran, R., Guyoneaud, R., and Swabe, S., 2005. Characterization of purple sulphur bacteria from the South Andros Black Hole cave system: highlights taxonomic problems for ecological studies among the genera *Allochromatium* and *Thiocapsa*. *Environmental Microbiology*, 7, 1260–1268.
- Holmes, A. J., Tujula, N. A., Holley, M., Contos, A., James, J. M., Rogers, P., and Gillings, M. R., 2001. Phylogenetic structure of unusual aquatic microbial formations in Nullarbor caves, Australia. *Environmental Microbiology*, 3, 256–264.
- Imhoff, J. F., Söling, J., and Petri, R., 1998. Phylogenetic relationships among the *Chromatiaceae*, their taxonomic reclassification and description of the new genera *Allochromatium*, *Halochromatium*, *Isochromatium*, *Marichromatium*, *Thiococcus*, *Thiohalocapsa* and *Thermochromatium*. *International Journal of Systematic Bacteriology*, 48, 1129–1143.

- Jeanthon, C., 2000. Molecular ecology of hydrothermal vent microbial communities. *Antonie van Leeuwen.*, **77**, 117–133.
- Marano-Briggs, K., 2000. *Water Chemistry, Bacterial Abundance and Anoxygenic Photosynthetic Bacteria in an Estuarine Blue Hole, Andros Island, The Bahamas*. PhD Thesis, George Mason University, Fairfax, VA.
- Mattison, R. G., Abbiati, M., Dando, P. R., Fitzsimons, M. F., Pratt, S. M., Southward, A. J., and Southward, E. C., 1998. Chemoautotrophic microbial mats in submarine caves with hydrothermal sulphidic springs at Cape Palinuro, Italy. *Microbial Ecology*, **35**, 58–71.
- Myloie, J. E., Carew, J. L., and Moore, A. I., 1995. Blue holes: definition and genesis. *Carbonate and Evaporites*, **10**, 225–233.
- Northup, D. E., Barns, S. M., Yu, L. E., Spilde, M. N., Schelble, R. T., Dano, K. E., Crossey, L. J., Connolly, C. A., Boston, P. J., Natvig, D. O., and Dahm, C. N., 2003. Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves. *Environmental Microbiology*, **5**, 1071–1086.
- Paine, S. G., Lingood, F. V., Schimmer, F., and Thrupp, T. C., 1993. *The Relationship of Microorganisms to the Decay of Stone*. London: Philosophical Transactions of the Royal Society, pp. 97–127.
- Schwabe, S. J., 1998. The Black Hole. Caves and caving. *British Cave Research Association*, **80**, 1–22.
- Schwabe, S. J., and Herbert, R. A., 2004. Black Holes of the Bahamas: what they are and why they are black. *Quaternary International*, **121**, 3–11.
- Schwabe, S. J., Parkes, R. J., and Smart, P. L., 1997. Biogeochemical investigation of submerged cave systems (Blue Holes) within Bahamian carbonate platforms. In *97th Applied and Environmental Microbiology General Meeting*, May 4–7, 1997, p. 398. Miami, FL.
- Smart, P. L., Dawans, J. M., Whitaker, F., 1988. Carbonate dissolution in a modern mixing zone. *Nature*, **335**, 811–813.
- Sugita, T., Kikuchi, K., Makimura, K., Urata, K., Someya, T., Kamei, K., Niimi, M., and Uehara, Y., 2005. Trichosporon species isolated from guano samples obtained from bat-inhabited caves in Japan. *Applied and Environmental Microbiology*, **71**, 7626–7629.
- Vlasceanu, L., Popa, R., and Kinkle, B., 1997. Characterization of *Thiobacillus thioparus* LV43 and its distribution in a chemoautotrophically based ecosystem. *Applied and Environmental Microbiology*, **63**, 33123–33127.
- Vlasceanu, L., Sarbu, S., Engel, A. S., and Kinkle, B., 2000. Acidic, cave wall biofilms located in the Frasassi George, Italy. *Geomicrobiology*, **17**, 125–139.
- Zhou, J., Gu, Y., Zou, C., and Mo, M., 2007. Phylogenetic diversity of bacteria in an earth-cave in Guizhou province, southwest of China. *Journal of Microbiology*, **45**, 105–112.

Cross-references

Bacteria
 Beggiatoa
 Biofilms
 Hydrothermal Environments, Marine
 Karst Ecosystems
 Metals, Acquisition by Marine Bacteria
 Microbial Communities, Structure, and Function
 Microbial Mats
 Nanocrystals, Microbially Induced
 Siderophores
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Sulfur Cycle

MICROBIAL MATS

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Algal mats; Bacterial mats; Biomats; Thick biofilms

Definition

Microbial mats are often centimeter-thick multilayered structures of microorganisms, mainly bacteria, archaea, fungi, and sometimes these mats are enriched with protozoans. Microbial biofilms are in contrast much thinner (10–100 µm) than microbial mats and have a different architecture (Characklis and Wilderer, 1989). Microbial mats grow at interfaces between different types of material, mostly on moist surfaces, but some of them are also found in dry environments like deserts (e.g., Stal and Caumette, 1994; Characklis and Wilderer, 1989). They colonize environments located in high altitude, they are common in the Deep Biosphere, in subterranean environments with temperatures from –40°C to +120°C. There are also common as endosymbiotic structures, for example, in animals. They are very common in extreme environments like hydrothermal vents, cold seeps, and alkaline and hypersaline lakes (Van Dover, 2000; Pederesen, 2006; Arp et al., 2003; Riding and Awramik, 2000).

Geobiological implications

A microbial mat consists of several layers dominated by specific types of microorganism (Figures 1 and 2). Although the composition of individual mats varies depending on the environment, as a rule the by-products of each group of microorganisms serve as energy source for other groups. Different types of microorganisms dominate different layers based on their comparative advantage (Canfield and Des Marais, 1993; Allison et al., 2000). Ecological relationships between different groups are a combination of competition and cooperation. The metabolic capabilities of bacteria and archaea are generally depend on their phylogeny. In a wet environment where sunlight is available, the upper layers are generally dominated by aerobic consortia like cyanobacteria (Figure 1), while the lowest layers are normally dominated by anaerobic microorganisms such as sulfate-reducing bacteria, methanogenic and/or methanotrophic archaea, or general archaea. Marine mats may grow to a few centimeters in thickness, of which only the top few millimeters are oxygenated. Microbial mats are held together and bound to their substrates by sugar- and protein-rich extracellular polymeric substances (EPS), which they secrete (Decho et al., 2005; Wingender et al., 1999). In many cases, some of these microorganisms form filaments (threads), which tangle and thus increase the microbial



Microbial Mats, Figure 1 Phototrophic 5-cm-thick microbial mat from a hypersaline lake (Lake 21) on Kiritimati Island (Kiribati, Line Islands). 1 – Orange layer with cyanobacteria; 2 – Green layer with green bacteria, zone with the highest diversity of prokaryotes; 3 – Purple layer, zone with photosynthetic non-sulfur purple bacteria (alpha-, and beta proteobacteria); 4 – Gray layer with sulfate-reducing bacteria and archaea, zone of heavy carbonate and gypsum mineralization within degrading extracellular polymeric substances (EPS). The entire mat is interspersed with authigenic, spherulitic aragonite, and gypsum crystals that are growing within EPS.

colonies structural strength, especially if the filaments have sheaths.

Microbial mats are the earliest complex form of life on Earth. There is a fossil record from 3,500 million years ago, and they have always been the most important members and maintainers of earth ecosystems (Allwood et al., 2006; van Kranendonk, 2007). Early Precambrian microbial mats are the first evolved highly complexly organized multicellular organisms. Microbial mats occasionally mineralize if the ionic strength in the aquatic environment allows it. The mineralized structures are known as stromatolites and thrombolites and they represent the most important organo-sedimentary fabrics since the early beginning of microbial life on earth (Burne and Moore, 1987; Riding, 2000). Originally, microbial mats first occurred at hydrothermal vents for energy. Since the late Precambrian microbial mats have also played an important role at cold hydrocarbon seeps, so-called Cold Seeps. Most of the Cold Seeps are methane vents coupled with hydrogen sulfur microbial metabolism (Reitner et al., 2005) (Figure 2). However, phototrophic microorganisms such as cyanobacteria, unicellular algae (e.g., diatoms), and related consortia are the most abundant microorganisms in the formation of microbial mats (e.g., Arp et al., 2010). Within subterranean environments iron-oxidizing microbial consortia show a strong ability to form thick microbial mats (Kurz et al., 2010).

Microbial mats are the context within which the first multicellular organisms evolved. Sponges are, for



Microbial Mats, Figure 2 Anaerobic microbial mat from a cold seep environment of the Black Sea. Black mat represents the outer margin of the microbial mat. This mat is dominated by ANME 2 archaea and greigite-bearing sulfate reducing bacteria (GHOSTDABS field, for details see Reitner et al., 2005; Reitner, 2010). Orange-pink mat represents an archaea-(ANME1)-dominated mat portion. The green mat represents the inner most area close to the methane vent. This mat is also dominated by ANME archaea, Crenarcheota, and anaerobic bacteria. The entire mat is stabilized by authigenic methane-related aragonite and calcite crystal aggregates, which are also growing within EPS.

example, highly evolved microbial mats with a mutual relationship with eukaryote choanoflagellates. May be the Vendobionta, large Ediacaran organisms with characteristic quilt bauplan are part of well-developed microbial mats and may themselves be large colonies of various microbial communities (Seilacher, 1999; Schieber et al., 2007; Steiner and Reitner, 2001).

Conclusion

Microbial mats are normally cm-thick microbial communities often related with microbially induced mineral precipitates (calcite, aragonite, iron-oxides etc.). Characteristic for these mats is a multilayered structure which represents various microbial communities with different metabolic activities. In phototrophic microbial mats (e.g., cyanobacteria) dominate the uppermost layers whereas within the deep layers of the mats anaerobic microbes dominate like sulphate reducing bacteria and archaea. Microbial mats are complex multicellular systems which exhibit some accordance to basic metazoans like sponges. It is speculated that microbial mats are a model for the basic metazoan bauplan.

Bibliography

- Allison, D. G., Gilbert, P., Lappin-Scott, H. M., and Wilson, M., 2000. *Community Structure and Co-operation in Biofilms*. Cambridge: Cambridge University Press, 349 p.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–717.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.

- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., de Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. Tufa-forming biofilms of German karstwater streams: microorganisms, exopolymers, hydrochemistry and calcification. In Rogerson, M., and Pedley, M. M. (eds.), *Tufas and Speleothems: Unravelling the Microbial and Physical Controls*. London: Geological Society Special Publication 336, pp. 83–118.
- Burne, R. V., and Moore, L., 1987. Microbialites; organosedimentary deposits of benthic microbial communities. *Palaios*, **2**, 241–254.
- Canfield, D. E., and Des Marais, D. J., 1993. Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. *Geochimica et Cosmochimica Acta*, **57**, 3971–3984.
- Characklis, W. G., and Wilderer, P. A., 1989. Structure and function of Biofilms. *Life Sciences Research Report*, **46**, 1–387 (Wiley).
- Decho, A. W., Visscher, P. T., and Reid, P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 71–86.
- Kurz, J., Simon, K., Heim, C., Reitner, J., Nadia-Valérie Quéric, N.-V., and Volker Thiel, V., 2010. Trace element and biomarker signatures in iron-precipitating microbial mats from the tunnel of Åspö (Sweden). In Reitner, J., Quéric, N.-V., and Arp, G. (eds.), *Advances in Geobiology of Stromatolite Formation, Proceedings of the Kalkowsky Symposium held in Göttingen 2008*. Lecture Notes of Earth Sciences (Springer).
- Pedersen, K., 2006. Microbial life in deep granitic rock. *FEMS Microbiology Reviews*, **20**, 399–414.
- Reitner, J., 2010. Architecture of archaeal-dominated microbial mats from Cold Seeps in the Black Sea (Dnjepr Canyon, Lower Crimean Shelf). In Seckbach, J., and Oren, A. (eds.), *Microbial Mats, Modern and Ancient Microorganisms in Stratified Systems. - Cellular Origin, Life in Extreme Habitats and Astrobiology*, 14 (Springer).
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 66–79.
- Riding, R., and Awramik, S. M. (eds.), 2000. *Microbial Sediments*. Berlin: Springer, 331 pp.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**(Supplement 1), 179–214.
- Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu, O., 2007. *An Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record*. Amsterdam, The Netherlands: Elsevier.
- Seilacher, A., 1999. Biomat-related lifestyles in the Precambrian. *Palaios*, **14**, 86–93.
- Stal, L., and Caumette, P., 1994. Microbial mats. NATO ASI Series, Series G. *Ecological Sciences*—. Berlin: Springer, Vol. 35, pp. 1–463.
- Steiner, M., and Reitner, J., 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. *Geology*, **29**, 1119–1122.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton: Princeton University Press, 424 p.
- Van Kranendonk, M. J., 2007. A review of the evidence for putative Paleoarchean life in the Pilbara Craton. In Van Kranendonk, M. J., Smithies, R. H., and Bennet, V. (eds.), *Earth's Oldest Rocks – Developments in Precambrian Geology*. Amsterdam, The Netherlands: Elsevier, pp. 855–896.
- Wingender, J., Neu, T. R., and Flemming, H.-C., 1999. Microbial Extracellular Polymeric Substances; Characterization, Structure, and Function. Berlin: Springer, 258 p.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biofilms and Fossilization](#)
[Calcite Precipitation, Microbially Induced](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Cyanobacteria](#)
[Diatoms](#)
[Ediacaran Biota](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Microbial-Metal Binding](#)
[Microbialites, Modern](#)
[Mud Mounds](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Protozoa \(Heterotroph, Eukaryotic\)](#)

MICROBIAL SILICIFICATION – BACTERIA (OR PASSIVE)

Kurt O. Konhauser, Brian Jones
 University of Alberta, Edmonton, Alberta, Canada

Definition

The encrustation, impregnation, and/or replacement of microbial tissue by silica minerals, usually opal-A.

Overview

Silica precipitation is an important geological process in many modern geothermal systems where venting of super-saturated solutions leads to the formation of finely laminated siliceous sinters around geyser and hot spring vents and on their discharge aprons. The spring waters, which typically originate from deep, hot reservoirs, at equilibrium with quartz, commonly contain dissolved silica concentrations significantly higher than the solubility of amorphous silica at 100°C (Fournier, 1985). Therefore, when these fluids are discharged at the surface, decompressional degassing, rapid cooling to ambient temperatures, evaporation, mixing, and changes in solution pH collectively cause the solution to suddenly become supersaturated with respect to amorphous silica (also known as opal-A). In such supersaturated solutions, the discharged monomeric silica, Si(OH)₄, polymerizes, initially to oligomers (e.g., dimers, trimers, and tetramers), and eventually to polymeric species with spherical diameters of 1–5 nm, as the silanol groups (–Si–OH–) of each oligomer condense and dehydrate to produce the siloxane (–Si–O–Si–) cores of larger polymers. The polymers grow in size through Ostwald ripening such that a bimodal

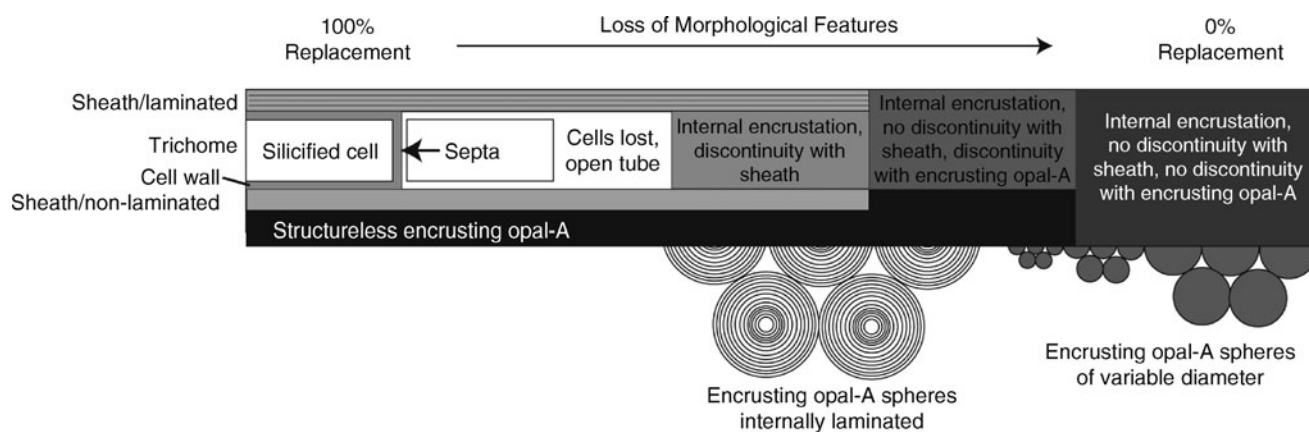
composition of monomers and particles of colloidal dimensions (>5 nm) are generated. The latter stay in suspension due to the external silanol groups exhibiting a residual negative surface charge, they coagulate via cation bridging and nucleate homogeneously, or they precipitate heterogeneously on a solid substratum (Ihler, 1979).

Siliceous sinters commonly contain numerous mineralized microorganisms (e.g., bacteria, algae, fungi) in varying stages of preservation (Jones et al., 1997; Konhauser et al., 2001). Examination under the scanning or transmission electron microscopes (SEM/TEM) shows that their preservation typically involved the precipitation of spheroidal, amorphous silica grains on (i.e., encrustation) or within (i.e., impregnation, replacement) the walls and/or sheaths of the microbes. The manner in which a particular microbe is preserved depends upon the balance between the timing of opal-A precipitation/impregnation and decay of the soft tissues of the microbe (Figure 1). In many cases, a microbe merely acts as a template as opal-A is precipitated around its outer surface. Such encrustation begins with the attachment of preformed silica colloids, on the order of only 10 s of nanometers in diameter, to the cell surfaces (Figure 2a). Those silica particles then grow in size, and may reach micrometer diameters in size. If opal-A precipitation is sustained, those particles invariably coalesce until the individual precipitates are no longer distinguishable, resulting at times in entire colonies being cemented together in a siliceous matrix (Figures 2b and 3a–d).

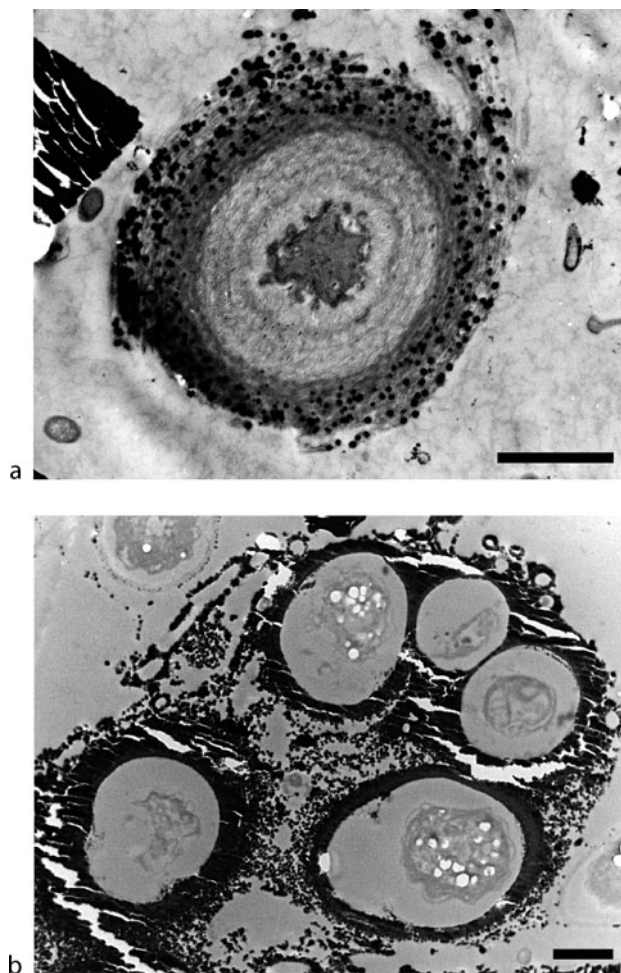
Replacement, which involves impregnation of the organic tissue by opal-A, can lead to superb preservation of the microbes (Figure 3e). Jones et al. (2001, their Fig. 8), for instance, illustrated silicified specimens of

Calothrix in which the sheath and outer splayed lamina are still evident. Septa may also be preserved in this way (Figure 3h). If encrustation is the sole process, then many silicified filamentous microbes will be evident only as hollow tubes (Figure 3g and i). Subsequent filling of that tube with opal-A will produce a solid opal-A rod, with the size of the original lumen/filamentous microbe only being apparent if there is a discontinuity preserved (e.g., Figure 3f). Irrespective of the nuances, the silicification of microbes must take place quickly if they are to be preserved, and certainly before the microbes undergo any degree of desiccation and degradation (Jones et al., 2001). This notion is supported by the fact that the three-dimensional form of most microbes is still apparent following silicification (Figure 3j–l). Indeed, some microbes are coated with silica while they are still viable (Phoenix et al., 2000) and it seems that silicification must take place in a matter of days in order for the three-dimensional morphology to be preserved.

Plant material bathed in the polymerizing solutions may be encrusted, impregnated, and/or replaced with opal-A similar to microbes. In many of the hot spring systems of New Zealand, for example, grass stems, pieces of wood, and leaves are heavily encrusted with opal-A but their tissues have usually not been replaced. In fact, the grass is commonly still growing even though encased by opal-A. In contrast, wood and leaves on the discharge apron around Geysir, Iceland that have been superbly preserved through replacement have not been encrusted with opal-A. Similar comparisons apply to some of the microbes. The factors that dictate encrustation as opposed to replacement are currently not understood as there are no obvious correlations between preservation style and the chemical attributes of the associated waters.



Microbial Silicification – Bacteria (or Passive), Figure 1 Schematic longitudinal cross-section of a sheathed filamentous microbe showing morphological variations that can develop as a result of silicification. Modified form Jones et al. (2001, their Fig. 10).



Microbial Silicification – Bacteria (or Passive),

Figure 2 Transmission electron micrographs (TEM) of cyanobacteria from a geyser outflow channel at Strokkur, Iceland. (a) Cell showing abundant silica spheres on, and within the surrounding sheath material. (b) Colony of unidentified cyanobacteria that are completely encrusted by silica matrix. Remnants of cytoplasm are still evident inside some cells. Scale bars = 3 μm .

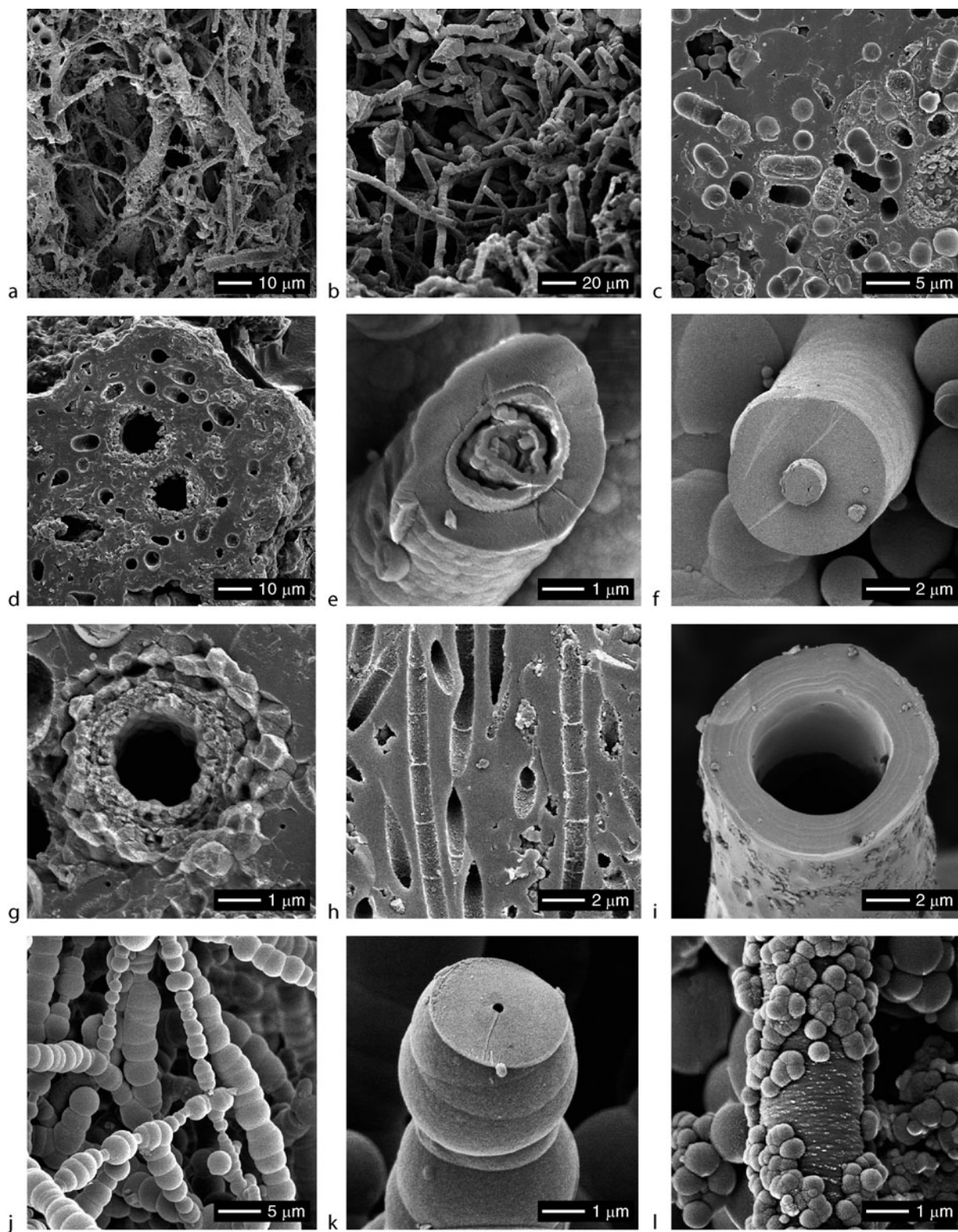
Despite the fact that many silicified microbes appear to be superbly preserved, only a limited number of features are usually apparent. Even in the best-preserved specimens, features are generally limited to overall morphology, filament diameter, presence/absence of branching, presence/absence of a sheath, and/or size of cells. Identification in terms of extant taxa is usually difficult because the limited numbers of morphological features apparent in the silicified forms are insufficient unless they are particularly distinctive in some way. This issue is compounded by the fact that many extant taxa are defined by their DNA characteristics and/or the conditions under which they were cultured in a laboratory setting. In most cases,

therefore, the identification of silicified microbes must be done with caution.

The role that the microbes (and some plant material) play in their own silicification has been a matter of debate. Being soft-bodied organisms they do not “actively” mediate amorphous silica formation in the manner of diatoms, radiolarians, and sponges. In contrast, bacteria, algae, and fungi facilitate silica precipitation by creating a suitable microenvironment and/or template for opal-A precipitation. For example, they may produce organic ligands that promote the mineralization process (Lalonde et al., 2005), or they may change the pH immediately around the cell through their metabolic activity, and in this regard, induce supersaturation with respect to opal-A (Amores and Warren, 2007). At other times, the microbes may simply act as solid surfaces for silica precipitation and exert no direct influence over the process (Walter et al., 1972).

Experimental evidence seems to indicate that the microbial role in the silicification processes is incidental and not limited to any particular taxa. First, microbes have little affinity for monomeric silica, even at high bacterial densities and low pH conditions where most organic functional groups are fully protonated and neutrally charged (Fein et al., 2002). Second, at supersaturated conditions, there appears to be very little difference in the rates or magnitude of silica precipitated between microbial and microbial-free systems (Yee et al., 2003; Benning et al., 2004). Third, silicification occurs on dead cells, and continues autocatalytically and abiogenically for some time after their death due to the high reactivity of the newly formed silica. This notion is supported by microscopic examination of thermal spring deposits where it has been shown that the silica precipitated in the porous spaces between filaments has the same basic motif as the silica precipitated on the original filaments (Jones et al., 1998). From these observations, it can be inferred that at high silica levels there is such a strong chemical driving force for silica polymerization, homogeneous nucleation, and ultimately silica precipitation that there is no obvious need for microbial catalysis.

Despite the observations above, the fact that there are species-specific patterns of silicification infers that some microbes may influence silicification in some way. The initial coating of opal-A spheres around microbes in Iodine Pool in the Waimangu geothermal area, for example, are consistent in size and highly organized in terms of their distribution (Jones et al., 2004, their Fig. 9). In contrast, the outer layer of opal-A beads is larger, variable in diameter, and follows no set distribution pattern. Thus, it seems that some aspect of the microbes may have influenced the initial phase of opal-A precipitation but not later phases. This seems feasible given that the initial layer of opal-A precipitates would have effectively encased the microbes and thereby isolated their surfaces from the water. Furthermore, different microbes exhibit different degrees of silicification. This is not surprising



Microbial Silicification – Bacteria (or Passive), Figure 3 (Continued)

given that the actual mechanisms of silicification rely, in part, on the microbes providing reactive surface ligands that adsorb silica from solution, and accordingly, reduce the activation energy barriers to heterogeneous nucleation (Konhauser et al., 2004). This means that cell surface charge may have a fundamental control on the initial silica sorption process.

At present there appears to be three different mechanisms by which microbes bind dissolved/colloidal silica; (1) hydrogen bonding, (2) cation bridging, and (3) electrostatic interactions (Figure 4). In the first instance, Phoenix et al. (2002) showed that the polysaccharide-rich sheath of *Calothrix* is electrically neutral at pH 7, and opal-A precipitation occurs through hydrogen bonding between the hydroxy groups associated with the sugars and the hydroxyl ions of the dissolved silica. Second, the highly anionic nature of most bacteria, such as *Bacillus subtilis*, limits opal-A from occurring on the cell wall, likely as a result of electrostatic charge repulsion between the anionic ligands of the organic functional groups and the negatively-charged silica species (Phoenix et al., 2003). Therefore, in order for silica to sorb, some form of cation bridge (e.g., Fe^{3+} , Al^{3+}) is necessitated (Fein et al., 2002). Third, some species, such as *Aquificales*, produce protein-rich biofilms that contain sufficient cationic amino groups (NH_4^+) to electrostatically react with dissolved silica (Lalonde et al., 2005).

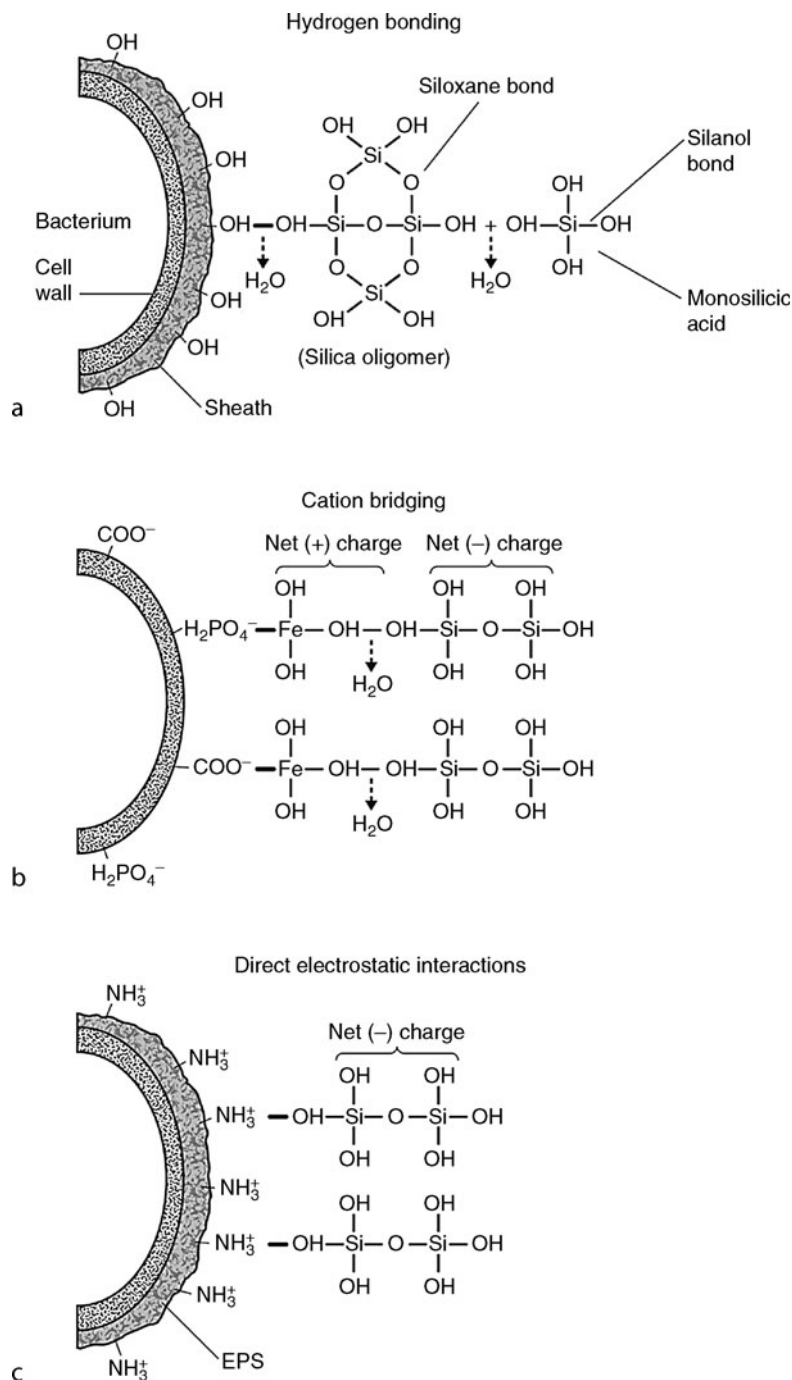
Given that a wide assortment of microbes live in environments where opal-A is being precipitated, it is important to determine if those species survive the mineralization process. In this regard, Phoenix et al. (2000) tested cell viability during biomineralization, and observed that experimental encrustation of *Calothrix* by silica was not notably detrimental to the organisms. Even after 12 days of incubation in a 600 mg l^{-1} silica solution, during which many of the filaments developed extensive mineral crusts up to $5 \text{ }\mu\text{m}$ thick, the cells still fluoresced,

they continued to generate oxygen and the mineralized colonies exhibited comparable rates of photosynthesis to the non-mineralized colonies. Interestingly, silica precipitation only occurred upon the outer surface of sheath material from viable cyanobacterial cells – no viable cells exhibited internal or cell wall mineralization. This contrasts with dead and lysed cells where mineralization of the cell wall and cytoplasm had occurred. In a more recent study with the thermophilic chemolithoautotroph *Sulfurihydrogenibium azorense*, Lalonde et al. (2005) showed that the bacterium, when grown as a H_2 -oxidizer, reacted to increasing silica concentrations by producing excess protein that appears linked to biofilm production. Since silicification was not observed directly on the cell surface, they proposed that *S. azorense* may prevent cellular silicification to some degree by providing abundant reactive sites in the biofilm matrix and regulating biofilm production appropriately, with potential contributions from metabolic effects. Thus, it appears that some microbes can thrive in silica-rich environments because they form protective layers that isolate the cells from the potentially damaging effects of silicification.

Summary

Microbial silicification includes the encrustation, impregnation, and replacement of cells by amorphous silica. Most microbes play a passive role in the mineralization process by providing organic surfaces that facilitate the binding of silica polymers and colloids from solution. These sorption reactions can occur by either (1) hydrogen bonding between dissolved silica and cell hydroxy functional groups, (2) cation bridging between anionic cell functional groups and the silica, or (3) direct electrostatic interaction of silica with cell surface ammonium groups.

Microbial Silicification – Bacteria (or Passive), Figure 3 Examples of silicified microbes from various hot-spring sinters in the Taupō Volcanic Zone of the North Island of New Zealand. (a) Silicified microbial mat formed of small-diameter filamentous microbes wrapped around erect, large-diameter filamentous microbes. Tokaanu geothermal area. (b) Silicified microbial mat formed of interwoven small-diameter filaments. Waiotapu geothermal area. (c) Group of silicified bacteriform microbes embedded in opal-A matrix. Tokaanu geothermal area. (d) Two large-large diameter filamentous microbes (*Calothrix*?) with small-diameter filamentous microbes (*Phormidium*?) wrapped around them, all encased by opal-A. Tokaanu geothermal area. (e) Transverse cross-section through silicified microbe showing what appears to be opal-A encrusting a sheath; note possible silicified microbial material inside the sheath. Tokaanu geothermal area. (f) Transverse cross section through a silicified filamentous microbe showing opal-A encrusted around a solid opal-A core. Iodine Pool, Waimangu. (g) Transverse cross-section through a laminated sheath, probably from *Calothrix*, with open lumen. Tokaanu geothermal area. (h) Longitudinal cross-section through small-diameter filamentous microbes showing septa, and hence cell-size. Tokaanu geothermal area. (i) Transverse cross-section through a silicified filamentous microbe. Note growth lines in encrusting silica that record progressive precipitation of opal-A around the original filament. Waikite Geyser, Whakarewarewa. (j) General view of group of filamentous microbes that have been encrusted by opal-A spheres of variable diameter. Waikite Geyser, Whakarewarewa. (k) Cross-section through filamentous microbe showing small open lumen and variable diameter of encrusting opal-A. Waikite Geyser, Whakarewarewa. (l) Filamentous microbe showing silicified cell wall or sheath encrusted by small opal-A spheres. Iodine Pool, Waimangu.



Microbial Silicification – Bacteria (or Passive), Figure 4 Three mechanisms by which microorganisms silicify: (a) hydrogen bonding between silica polymers with hydroxy groups in sheaths of *Calothrix*; (b) cation bridging between negatively-charged cell walls in *Bacillus subtilis* and silica; and (c) electrostatic interactions between silica and positively-charged amino groups in the biofilm of *Sulfurihydrogenibium azorense*. Reproduced from Konhauser (2007) with permission of Wiley-Blackwell.

Bibliography

Amores, D. A., and Warren, L. A., 2007. Identifying when microbes biosilicify: the interconnected requirements of acidic pH, colloidal SiO_2 and exposed microbial surface. *Chemical Geology*, **240**, 298–312.

Benning, L. G., Phoenix, V. R., Yee, N., and Konhauser, K. O., 2004. The dynamics of cyanobacterial silicification: an infrared micro-spectroscopic investigation. *Geochimica et Cosmochimica Acta*, **68**, 743–757.

- Fein, J. B., Scott, S., and Rivera, N., 2002. The effect of Fe and Si adsorption by *Bacillus subtilis* cell walls: insights into non-metabolic bacterial precipitation of silicate minerals. *Chemical Geology*, **182**, 265–273.
- Fournier, R. O., 1985. The behaviour of silica in hydrothermal solutions. *Reviews in Economic Geology*, **2**, 45–61.
- Ihler, R. K., 1979. *The Chemistry of Silica*. New York: Wiley-Interscience.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1997. Biogenicity of silica precipitation around geysers and hot-spring vents, North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1998. Microbial biofacies in hot-spring sinters: a model based on Ohaaki Pool, North Island, New Zealand. *Journal of Sedimentary Research*, **68**, 413–434.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2001. Taphonomy of silicified filamentous microbes in modern geothermal sinters – implications for identification. *Palaios*, **16**, 580–592.
- Jones, B., Konhauser, K. O., Renaut, R., and Wheeler, R., 2004. Microbe silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial-silica interactions in modern hot spring sinter: possible analogues for Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–435.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A.-L., and Ferris, F. G., 2005. Thermophilic silicification: the role of Aquificales in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Phoenix, V. R., Adams, D. G., and Konhauser, K. O., 2000. Cyanobacterial viability during hydrothermal biomineralization. *Chemical Geology*, **169**, 329–338.
- Phoenix, V. R., Martinez, R. E., Konhauser, K. O., and Ferris, F. G., 2002. Characterization and implications of the cell surface reactivity of the cyanobacteria *Calothrix* sp. *Applied and Environmental Microbiology*, **68**, 4827–4834.
- Phoenix, V. R., Konhauser, K. O., and Ferris, F. G., 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe-Si systems: implications for microbial silicification in hot-springs. *Canadian Journal of Earth Sciences*, **40**, 1669–1678.
- Walter, M. R., Bauld, J., and Brock, T. D., 1972. Siliceous algal and bacterial stromatolites in hot spring and geyser effluents of Yellowstone National Park. *Science*, **178**, 402–405.
- Yee, N., Phoenix, V. R., Konhauser, K. O., Benning, L. G., and Ferris, F. G., 2003. The effect of cyanobacteria on silica precipitation at neutral pH: implications for bacterial silicification in geothermal hot springs. *Chemical Geology*, **199**, 83–90.

Cross-references

[Cherts](#)
[Cyanobacteria](#)
[Diatoms](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Biomineralization](#)
[Microbial Surface Reactivity](#)
[Radiolaria](#)

MICROBIAL SURFACE REACTIVITY

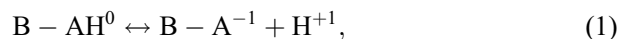
David A. Fowle, Kurt O. Konhauser
 University of Alberta, Edmonton, Alberta, Canada

Definition

The chemical charge on a cell surface caused by the deprotonation/protonation of organic functional groups.

Overview

Similar to an aqueous organic acid, bacterial cell surfaces can either bind or release protons (H^+) into solution depending upon the solution pH. In the case of the latter, this deprotonation process, which accurately mimics a number of the functional groups associated with cell surfaces, leads to the formation of an organic anion, or ligand. Unlike an aqueous organic acid, which exhibits proton buffering over a narrow pH range (± 1 –2 units), bacterial cell walls have continuous buffering capacity over a wide range of pH values which span most geologic settings (pH 2–10). Ultimately, most surface reactivity of bacteria with its external environment results from the enhanced electronegativity of the cell wall as these functional groups deprotonate. In its most simplistic form, deprotonation can be expressed by the following equilibrium reaction:



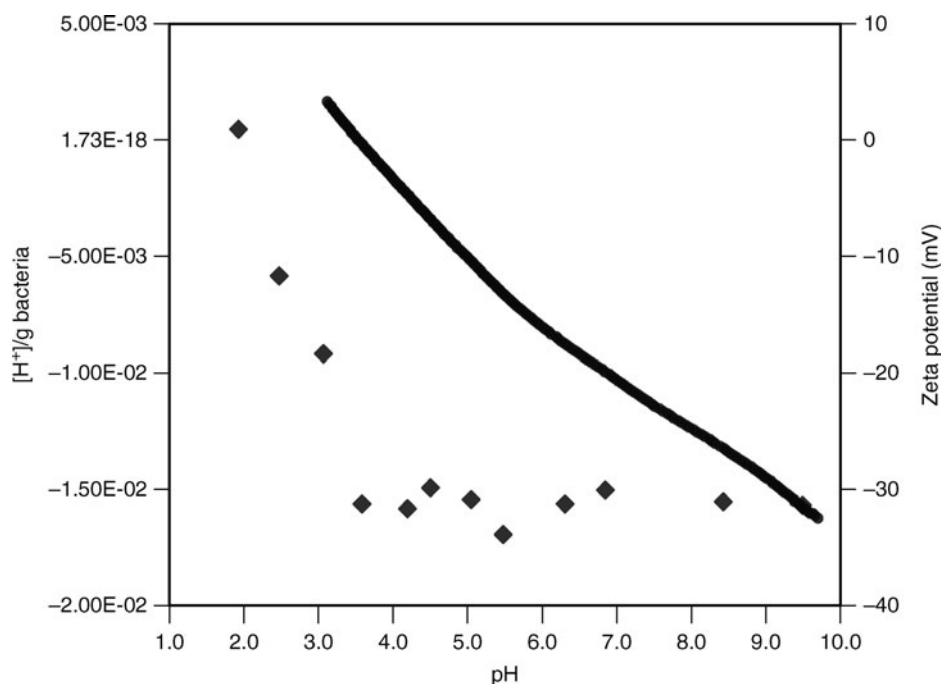
where B denotes a bacterium to which each protonated ligand type, A, is attached. The distribution of protonated and deprotonated sites can be quantified with the corresponding mass action equation:

$$K_a = \frac{[B - A^{-1}][H^+]}{[B - AH^0]}, \quad (2)$$

where K_a is the dissociation constant; $[B - A^{-1}]$ and $[B - AH^0]$ represent the concentration of exposed deprotonated and protonated ligands on the bacterium, respectively (in mol l^{-1}); and $[H^+]$ represents the activity of protons in solution. Each functional group has its own K_a , and based on equation (2), the pH at which $[B - A^{-1}]$ and $[B - AH]$ are equivalent is known as the pK_a value, where $pK_a = -\log_{10} K_a$. At $pH < pK_a$ surfaces are protonated and at $pH > pK_a$ they are deprotonated.

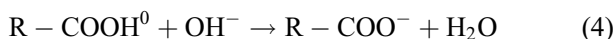
The ionization of functional groups provides an electrical charge ($[L]_T$ represented in mol mg^{-1}), which in the laboratory can be calculated as a function of pH from the difference between total base or acid added to a suspension of organic material and the equilibrium H^+ and OH^- ion activities (Reaction 3):

$$[L]_T = \frac{C_a - C_b \pm [OH^-] - [H^+]}{S} \quad (3)$$



Microbial Surface Reactivity, Figure 1 Experimental data demonstrating the buffering capacity of *Bacillus subtilis* during acid–base titration (circles; molality of protons added normalized with respect to the wet weight of bacteria present) and zeta potential (diamonds) gathered from titrations of *Bacillus subtilis* in 0.01 M NaNO_3 that were cultured in tryptocase soy broth.

The components, C_a and C_b , are the concentrations of acid and base added, respectively; n_{OH^-} and n_{H^+} are the number of moles of OH^- and H^+ in the solution at the measured pH, respectively; while “S” is the quantity of the solid (usually expressed in dry weight, i.e., mg). When such calculations are done over an entire pH range, an acid–base titration curve is created that shows the pH range over which some functional groups are chemically active and how the net surface charge of the organic surface varies with pH (Figure 1). A large difference in the total base or acid added and the free H^+ ion activity indicates significant pH buffering and a high concentration of surface functional groups. On the titration curve this is shown by a steep slope. A small difference in the total base or acid added and the free H^+ ion activity indicates weak pH buffering and a low concentration of surface functional groups. This translates into a gentle slope on a titration curve. In terms of microbial biomass, carboxyl groups account for the buffering capacity from pH 2–6 (Reaction 4), phosphate groups from pH 5–8 (Reaction 5), amino groups at pH > 8 (Reaction 6), and hydroxy groups deprotonate at pH values above 10 (Reaction 7) (see Konhauser, 2007).



As a consequence of the deprotonation reactions, the microbial surface develops an electrical charge. Therefore, when subjected to an electric field, microorganisms move in the direction, and at a rate, commensurate with the polarity and density of the overall surface charge. This process is termed electrophoresis, and the electrophoretic mobility of a microbial suspension can be quantified by measuring their velocity in an electric field. This measurement can, in turn, be used to calculate the zeta potential (ζ), using the Smoluchowski equation below (Hunter, 2001):

$$\zeta = \frac{\eta\mu}{\epsilon_0\epsilon} \quad (8)$$

The component “ μ ” is the electrophoretic mobility of a particle; ϵ_0 and ϵ are the relative dielectric constants of the vacuum and solution, respectively; and η is the viscosity of the solution. The zeta potential reflects the electrical potential at the interfacial region (viewed as a shear plane) separating an inner layer, where cations are held tightly in place and move with the bacterium, and an outer layer where ions are mobile (Wilson et al., 2001). The greater the absolute value of the ζ potential, the greater the charge

density on the surface (Blake et al., 1994). The electrophoretic mobility (and zeta potential) is pH dependent because the activity of protons in solution controls the ionization reactions of functional groups at the microbial surface (Ahimou et al., 2001) (Figure 1). This leads us to the concept of isoelectric point, which is defined as the pH value where the net surface charge of a solid is zero. The isoelectric point can be estimated from acid–base titrations, but it can also be directly measured with electrophoretic mobility experiments because at the isoelectric point microbes do not exhibit motion in an electric field. The isoelectric point of bacterial cell walls is typically between pH 2–4 (Harden and Harris, 1953). This means that at low pH, when the surface functional groups are fully protonated, bacteria are either neutral or positively-charged, the latter being the result of a cell possessing abundant amino groups. Meanwhile, at the growth pH of most bacteria, cells inherently display a net negative charge and the magnitude of negativity increases with higher pH values. As a result, under low pH conditions, most bacterial surfaces behave hydrophobically, but with an increasingly hydrophilic nature with increasing pH.

One of the major challenges faced by the researchers today is how to translate acid–base titration data in terms of cell wall biochemistry. Ascribing pK_a values from a titration curve to specific functional groups can be problematic because there is often significant variation in pK_a values for the same functional group. This occurs because the magnitude of the dissociation constant is controlled by the structure of the molecule to which it is attached (see Martell and Smith, 1977 for details). Consequently, a single carboxyl group in two different organic acids will have different pK_a values, as will an organic acid with multiple carboxylic groups. As might then be expected, the pK_a for a carboxyl group on one microbial species versus another could yield widely different values simply because of subtle conformational variations within the wall macromolecules. Furthermore, titration experiments are only able to resolve those groups that contribute significant amounts of protons to solution. Minor groups are simply undetectable with the resolution of current techniques. It is thus important to keep in mind that the model-derived binding sites do not directly represent the functional groups of the cell surface; their identity can only be inferred by comparison of the functional group pK_a values with pK_a values of model compounds. Unequivocal identification of the types of functional groups responsible for acid–base buffering may be obtained by spectroscopic techniques, such as Fourier transform infrared spectroscopy (FTIR), calorimetry or gas/liquid chromatography of cell wall extracts. For instance, Yee et al. (2004) and Jiang et al. (2004) used infrared spectroscopy to examine the pH dependence of deprotonation of bacterial cell-wall functional groups. Although not all pH conditions were studied, it was clear that the vibrational frequencies of carboxyl type groups increased with increasing pH. Calorimetric measurements of protonation reactions on bacterial surfaces indicate

these reactions are exothermic with relatively small site-specific entropies (Gorman-Lewis et al., 2006). This provides more evidence that (1) hydrogen bonding between protonated and deprotonated sites likely stabilizes the cell wall, and (2) bacterial cell wall functionalities are similar to multifunctional organic acids rather than simple organic acids.

Summary

Microbial surface reactivity results from the protonation and deprotonation of the organic moieties of microbial cell surfaces including carboxyl, phosphate, amino, and potentially hydroxyl groups. These groups behave similarly to multifunctional organic acids – they hydrogen bond with each other which leads to negative charge on the cell wall as pH increases. At most environmentally relevant pH values, microbial cell surfaces will possess a net negative charge, and therefore, will interact with positively charged cations or mineral surfaces.

Bibliography

- Ahimou, F., Paquot, M., Jacques, P., Thonart, P., and Rouxhet, P. G., 2001. Influence of electrical properties on the evaluation of the surface hydrophobicity of *Bacillus subtilis*. *Journal of Microbiological Methods*, **45**, 119–126.
- Blake, R. C., Shute, E. A., and Howard, G. T., 1994. Solubilization of minerals by bacteria: electrophoretic mobility of *Thiobacillus ferrooxidans* in the presence of iron, pyrite, and sulfur. *Applied and Environmental Microbiology*, **60**, 3349–3357.
- Gorman-Lewis, D., Fein, J. B., and Jensen, M. P., 2006. Enthalpies and entropies of proton and cadmium adsorption onto *Bacillus subtilis* bacterial cells from calorimetric measurements. *Geochimica et Cosmochimica Acta*, **70**, 4862–4873.
- Harden, V. P., and Harris, J. O., 1953. The isoelectric point of bacterial cells. *Journal of Bacteriology*, **65**, 198–202.
- Hunter, R. J., 2001. *Foundations of Colloid Science*. New York: Oxford University Press.
- Jiang, W., Saxena, A., Song, B., Ward, B. B., Beveridge, T. J., and Myneni, S. C. B., 2004. Elucidation of functional groups on Gram-positive and Gram-negative bacterial surfaces using infrared spectroscopy. *Langmuir*, **26**, 11433–11442.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Publishing.
- Martell, A. E., and Smith, R. M., 1977. *Critical Stability Constants. III. Other Organic Ligands*. New York: Plenum Press.
- Wilson, W. W., Wade, M. M., Holman, S. C., and Champlin, F. R., 2001. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. *Journal of Microbiological Methods*, **43**, 153–164.
- Yee, N., Benning, L. G., Phoenix, V. R., and Ferris, F. G., 2004. Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environmental Science and Technology*, **38**, 775–782.

Cross-references

[Metalloenzymes](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Biomineralization](#)
[Microbial-Metal Binding](#)
[Organomineralization](#)
[Siderophores](#)

MICROBIALITES, MODERN

Christophe Dupraz¹, R. Pamela Reid², Pieter T. Visscher¹

¹University of Connecticut, Storrs, CT, USA

²University of Miami, Miami, FL, USA

Synonyms

“Cryptalgal sedimentary rock” (Aitken, 1967); Microbolite (Riding, 1991)

Definition

Microbialite: “Organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987), or “Benthic microbial deposits” (Riding, 1991).

Introduction

In this encyclopedia, microbialite is discussed under two chapters: (1) modern and (2) fossil. This part discusses modern microbialites, with emphasis on processes of formation. The fossil extension and classification of microbial deposits (fossil forms being more diverse) are treated in the “fossil microbialite” section of the encyclopedia (see Chapter *Microbialites, Stromatolites, and Thrombolites*). Microbialites are composed of trapped, bound, and/or precipitated sediment, and exhibit a range of mineralogies (Figure 1). This chapter focuses on carbonate microbialites, which are the most widespread and the most studied.

Microbialites are “rocks” that are produced, induced, or influenced by benthic microbial communities, primarily bacteria and sometimes also microeukaryotes, such as diatoms. These microbial communities are organized in mats or biofilms (e.g., Stolz, 2000), consisting of microscopic organisms enveloped in an organic matrix of extracellular polymeric substances (EPS; Decho, 1990; Bhaskar and Bhosle, 2005; see Chapter *Extracellular Polymeric Substances (EPS)*). Because the microbial mat is dominantly organic, it is rarely preserved in the fossil record. In some unusual cases, exceptional taphonomic conditions can preserve intact cells and even the EPS structure (e.g., silicification or preserved remnants of EPS matrices, Golubic and Hofmann, 1976; Barbieri et al., 2004; Barbieri and Cavalazzi, 2005; Altermann et al., 2006; Benzerara et al., 2006; Kumar and Pandey, 2008; Lepot et al., 2008). More commonly, the organic matter decomposes to carbon dioxide (CO₂) or methane (CH₄), although in some cases incomplete degradation can lead to organic matter enrichment of the sediment. Evidence of the original microbial mat or biofilm is generally indirect (e.g., specific sedimentary (bio)structure, micromorphology, organic biomarkers, and stable isotopic fractionation). Microbial mats can help preserve sedimentary structures in the fossil record (“microbially-induced sedimentary structures” – MISS; Noffke et al., 2003). The most

spectacular examples are carbonate microbialites. Examples of modern microbialites in the following section are followed by a discussion of processes of formation.

Types of modern microbialite

Microbialite descriptions incorporate a range of scales, from macrometers to micro/nanometers (e.g., Shapiro, 2000). Megastructure relates to the geographical extension of a microbial deposit and overall deposit morphology (e.g., ridges, biostrome, and bioherm). Macrostructure characterizes the morphology of an individual buildup (e.g., domal, branching, knobby). Mesostructure refers to structures visible with the naked eye, such as lamination. Microstructure is observed with a microscope, such as light, confocal, scanning electron (SEM), or transmission electron microscope (TEM).

Microbialite classification is based on mesostructure with four common types (Figure 2): laminated microbialites classified as stromatolites, thrombolites are clotted, leiolites are structureless, and dendrolites are dendritic (Burne and Moore, 1987; Riding, 1991, 2000; Dupraz and Strasser, 1999, 2002). All of these structures exhibit a wide range of microstructures including micropeloidal, densely micritic, or agglutinated microfabrics (Riding, 1991; Dupraz and Strasser, 1999). Three types of modern microbialites are described in the following section; dendrolites have not yet been found in modern environments. For further description of microbialite classification and microstructure see Chapter *Microbialites, Stromatolites, and Thrombolites*.

Stromatolite

The term stromatolite is derived from the Greek “stroma,” meaning mattress or stratum and “lithos,” meaning rock. The term was first introduced by Kalkowsky in 1908 to refer to “laminated benthic microbial deposits” (see also Riding, 1999; Chapter *Microbialites, Stromatolites, and Thrombolites*). The first modern stromatolites were found in the Shark Bay, Western Australia in 1954 in the hypersaline water of Hamelin Pool. Until this date, scientists believed that stromatolites were extinct, found only in the fossil record. Although the biogenicity of 3.5 Ga stromatolites has been questioned (Buick et al., 1981; Lowe, 1994; Grotzinger and Knoll, 1999; Lindsay et al., 2003), all modern microbialites called stromatolites are associated with microbial communities that induce, or serve as organic substrate for, precipitation (Riding, 2000). Modern stromatolites are found in many different locations including marine, hypersaline, freshwater, and even continental environments.

Open marine stromatolites

The Bahamas hosts the only known examples of open marine stromatolites. These structures are found at a variety of locations on the margins of Exuma Sound including Schooner Cays, Lee Stocking Island, Stocking Island Highborne Cay, and Darby Island (Dravis, 1983;

Carbonates

Calcite - CaCO_3
 Mg-Calcite - $(\text{Mg}_x\text{Ca}_{1-x})\text{CO}_3$
 Aragonite - CaCO_3
 Vaterite - CaCO_3
 Monohydrocalcite - $\text{CaCO}_3 \cdot \text{H}_2\text{O}$
 Protodolomite - $\text{MgCa}(\text{CO}_3)$
 Hydrocerussite - $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$
 Amorphous forms - $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ or CaCO_3

Phosphates

Octacalcium phosphate - $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$
 Brushite - $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$
 Francolite - $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$
 Carbonated-hydroxylapatite (dahllite) - $\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3(\text{OH})$
 Whitlockite - $\text{Ca}_8\text{H}_2(\text{Mg, Fe})_2 + 2(\text{PO}_4)_4\text{I}_4$
 Struvite - $\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$
 Vivianite - $\text{Fe}_3 + 2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
 Amorphous Calcium Phosphate (at least 6 forms) variable
 Amorphous Calcium Pyrophosphate - $\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$

Sulfates

Gypsum - $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
 Barite - BaSO_4
 Celestite - SrSO_4
 Jarosite - $\text{KFe}_3^{+3}(\text{SO}_4)_2(\text{OH})_6$

Sulfides

Pyrite - FeS_2
 Hydrotroilite - $\text{FeS} \cdot \text{nH}_2\text{O}$
 Sphalerite - ZnS
 Galena - PbS
 Hydrotroilite $\text{FeS} \cdot \text{nH}_2\text{O}$
 Sphalerite ZnS
 Wurtzite ZnS
 Greigite Fe_3S_4
 Mackinawite $(\text{Fe, Ni})_9\text{S}_8$
 Amorphous Pyrrhotite Fe_{1-x}S ($x = 0-0.17$)
 Acanthite Ag_2S

Arsenates

Orpiment - As_2S_3

Hydrated Silica

Amorphous Silica - $\text{SiO}_2 \cdot \text{nH}_2\text{O}$

Chlorides

Atacamite - $\text{Cu}_2\text{Cl}(\text{OH})_3$

Fluorides

Fluorite - CaF_2
 Hieratite - K_2SiF_6

Oxides

Magnetite - Fe_3O_4
 Amorphous Iron Oxide - Fe_2O_3
 Amorphous Mn Oxide - Mn_3O_4
 Amorphous Ilmenite $\text{Fe}^{+2}\text{TiO}_3$

Metals

Sulfur - S

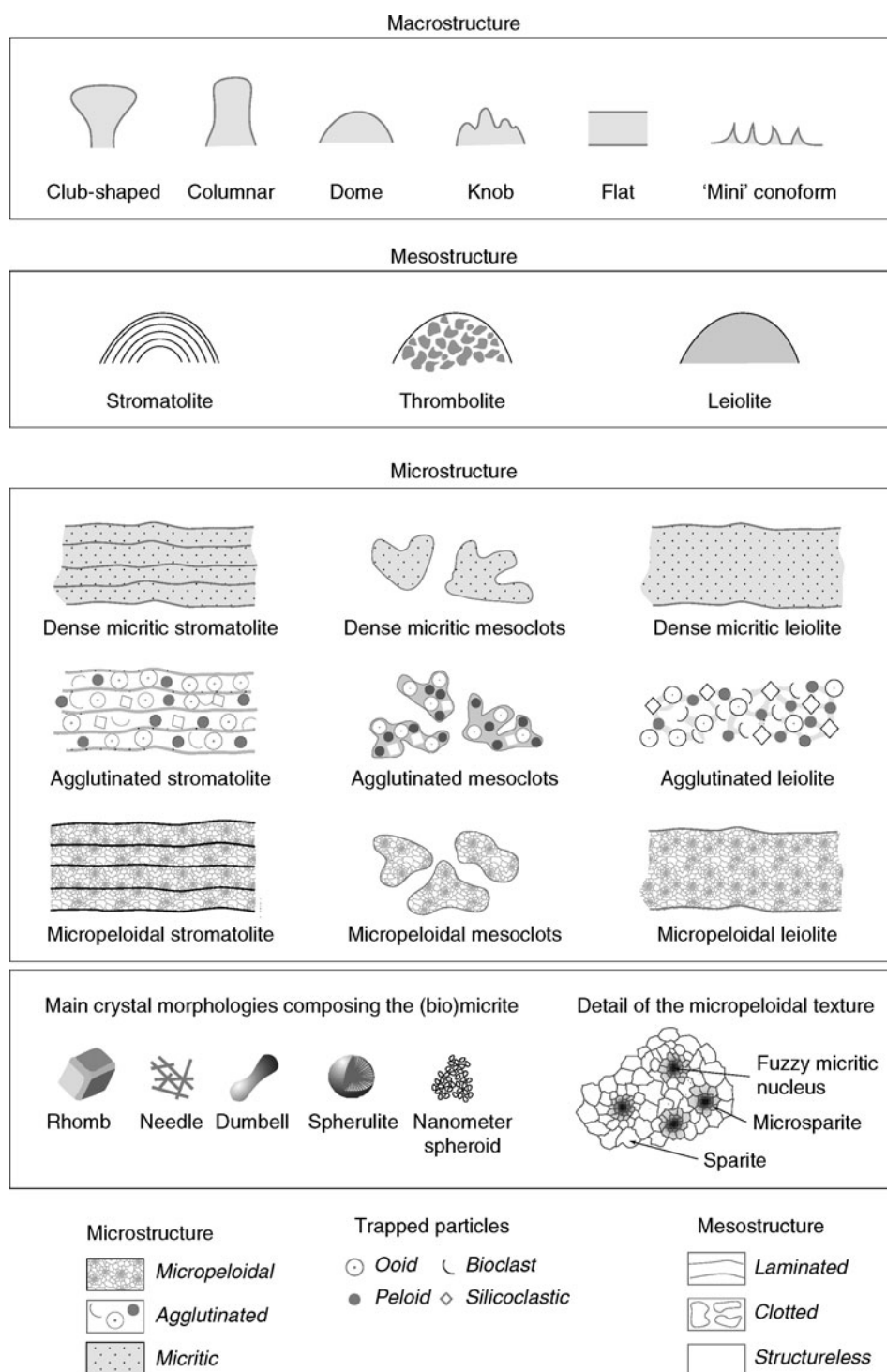
Hydroxides and Hydrous Oxides

Goethite - $\alpha\text{-FeOOH}$
 Ferrihydrite - $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
 Lepidocrocite - $\gamma\text{-FeOOH}$
 Ferrihydrite - $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
 Todorokite - $(\text{Mn}^{+2}\text{CaMg})\text{Mn}_3^{+4}\text{O}_7 \cdot \text{H}_2\text{O}$
 Birnessite - $\text{Na}_4\text{Mn}_{14}\text{O}_{27} \cdot 9\text{H}_2\text{O}$

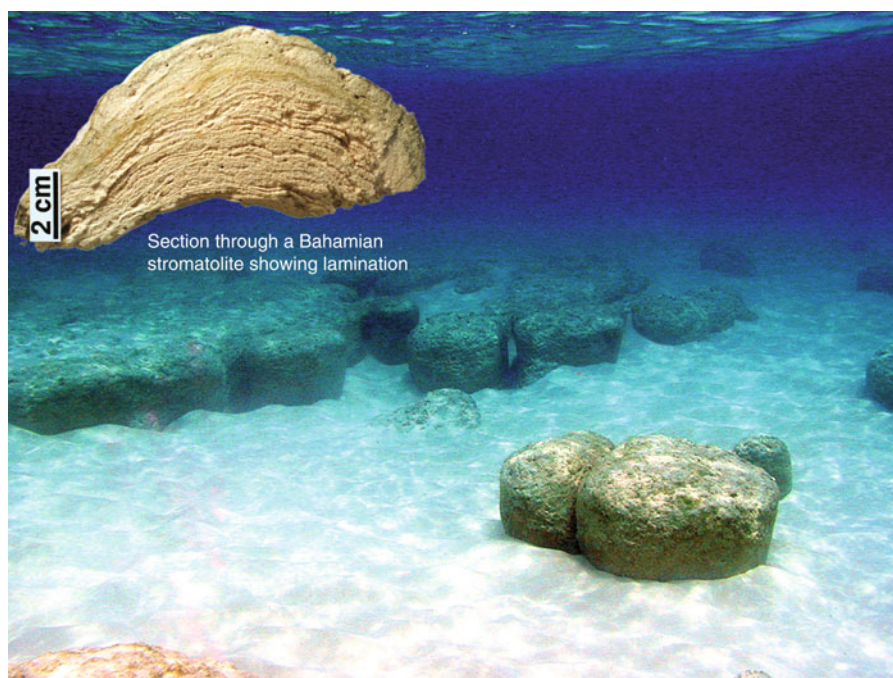
Organic Crystals following Lowenstam & Weiner (1989)

Whewellite - $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$
 Weddellite - $\text{CaC}_2\text{O}_4 \cdot (2+x)\text{H}_2\text{O}$
 Mn Oxalate - $\text{Mn}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
 Earlandite - $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$
 Glushinskite - $\text{MgC}_2\text{O}_4 \cdot 4\text{H}_2\text{O}$
 Manganese Oxalate - $\text{Mn}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
 Sodium urate - $\text{C}_5\text{H}_3\text{N}_4\text{NaO}_3$
 Uric Acid - $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$
 Ca tartrate - $\text{C}_4\text{H}_4\text{CaO}_6$
 Ca malate - $\text{C}_4\text{H}_4\text{CaO}_5$
 Paraffin Hydrocarbon
 Guanine - $\text{C}_5\text{H}_3(\text{NH}_2)\text{N}_4\text{O}$

Microbialites, Modern, Figure 1 Nomenclature and chemical compositions of minerals produced by biologically-related mineralization processes. (Modified from Weiner and Dove, 2003; data from Lowenstam and Weiner, 1989; Simkiss and Wilbur, 1989; Mann, 2001; Weiner and Addadi, 2002.)



Microbialites, Modern, Figure 2 Descriptive terminology for microbialite macro-, meso, and microfabrics. (From Kennard and James, 1986; Riding, 1991; Schmid, 1996; Dupraz and Strasser, 1999; Shapiro, 2000.)



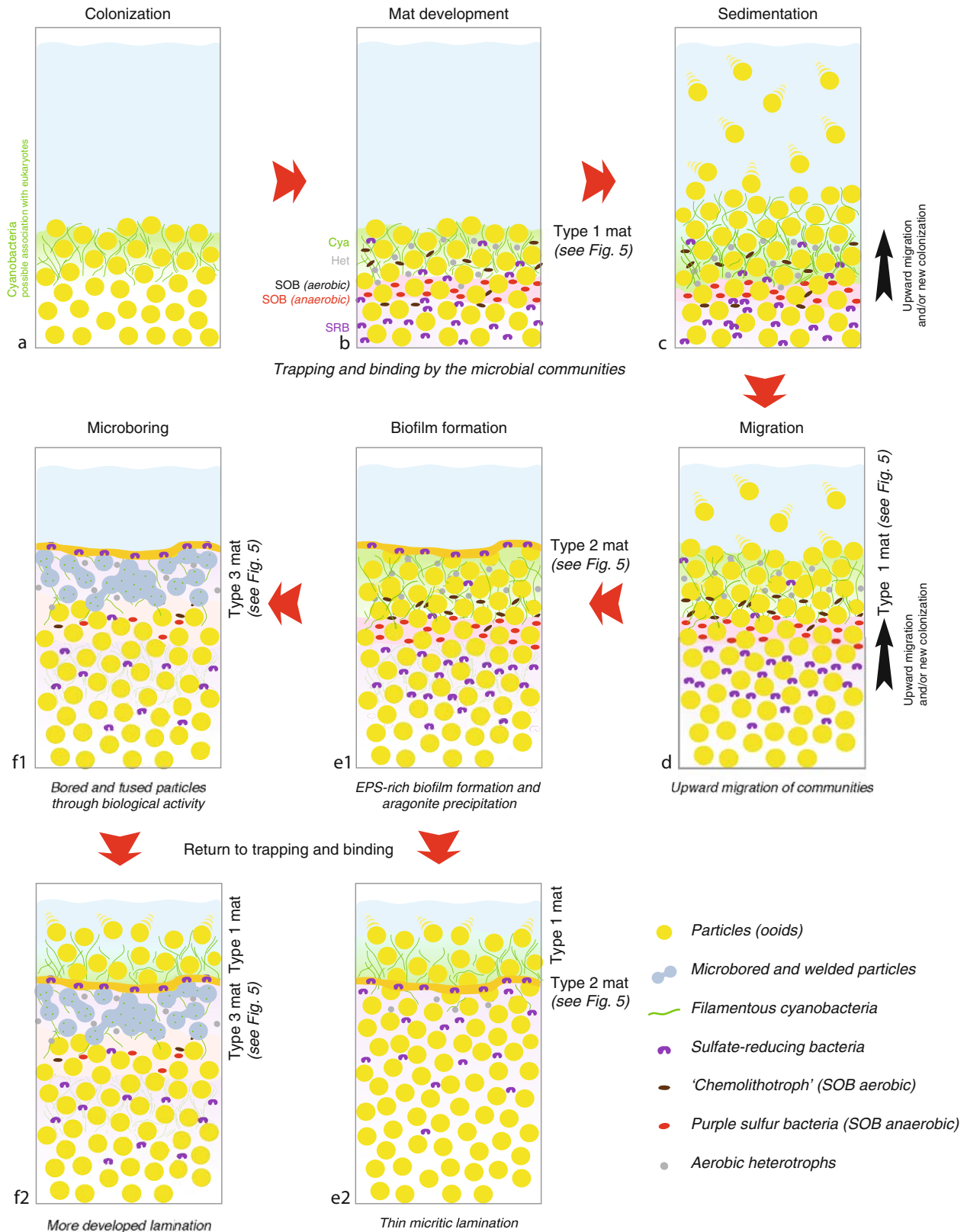
Microbialites, Modern, Figure 3 Underwater photograph showing shallow subtidal stromatolites (Darby Island, Bahamas) with a vertical section (upper left hand insert) showing well-developed lamination.

Dill et al., 1986; Reid et al., 1995). These stromatolites are coarse grained structures, composed of ooid sand; they form in intertidal and subtidal environments, exposed to high wave energy and strong tidal currents (e.g., Gebelein, 1976; Reid et al., 2000). Many of the stromatolites exhibit well-developed laminae (Figure 3; e.g., Reid et al., 2000). The lamination results from iterative growth of different types of microbial mats on the top of the build-ups (Figure 4). Each microbial mat forms a distinct mineral product, which can be recognized in thin section and SEM (Figure 5; Reid et al., 2000). The first mat type (1) is dominated by the filamentous cyanobacterium *Schizothrix*, which traps and binds ooids. The stickiness of the mat EPS results in the trapping of particles, which are subsequently bound by the upward growth of the cyanobacterial filaments. Other microorganisms (e.g., diatoms that produce copious amounts of EPS) can also participate in trapping grains. The second mat community (2) consists of a thin biofilm with abundant heterotrophic bacteria. Precipitation of aragonite within this biofilm forms a micritic lamina, which caps the underlying ooids. The third mat type (3) is characterized by an abundance of the endolithic coccoid cyanobacterium *Solentia* spp. *Solentia* tunnels through grains, leaving bore holes filled with EPS. Aragonite precipitation within the EPS results in micritization of the grains and, when tunnels cross between grains, welds the grains together. This process forms a well-cemented layer (Macintyre et al., 2000; Reid and Macintyre, 2000; Reid et al., 2000). The in situ precipitation of carbonate, which leads to early lithification of

the stromatolite-forming mats, is a result of prokaryotic activity (Visscher et al., 2000; Reid et al., 2003a).

Dominance of prokaryotic communities on the surfaces of modern marine stromatolites, is favored by the selective environmental pressure of periodic burial by sand (Andres and Reid, 2006; Kromkamp et al., 2007). In contrast to eukaryotes, which are typically killed when buried for periods of weeks to months, prokaryotes such as cyanobacteria are able to survive and recover from long burial events (Kromkamp et al., 2007). This attribute may contribute to the success of stromatolites in modern and past environments. Eukaryotic colonization (macroalgae: e.g., *Batophora*), during week- to month-long periods of exposure disrupts the lamination resulting from the cycling of prokaryotic mats, producing intervals of crude to no lamination within the stromatolite head.

It should be noted that trapping and binding alone does not form a stromatolite. The key factor in allowing development of a lithified laminated buildup is a hiatus in sediment accretion and corresponding development and lithification of a microbial biofilm. During long hiatus periods (i.e., several weeks), surfaces infested with the coccoid bacterium *Solentia* forms thicker, well-cemented lamina of fused ooid grains. Precipitation of aragonitic needles associated with the activity of sulfate-reducing bacteria is a key feature in carbonate precipitation within Bahamian stromatolites (Visscher et al., 2000). Precipitation resulting from anaerobic heterotrophic activity is supported by isotopic data (Andres et al., 2005).



Microbialites, Modern, Figure 4 (Continued)

Factors leading to the cycling of surface communities responsible for lamination in Bahamian stromatolites are currently under investigation (Andres and Reid, 2006). Both biological and environmental factors may be important (Figure 6; Seong-Joo et al., 2000; Reid et al., 2003a). Biological factors include microbial ecological interactions, and production and consumption of organic and inorganic compounds. Extrinsic factors include temperature, light, nutrients, and hydrodynamics, which could affect the stickiness of EPS and/or sediment supply (Figure 6).

Hypersaline and saline stromatolites

In contrast to open marine environments, where modern stromatolites are rare, living stromatolites are more common in hypersaline environments. This is because microbial communities are able to cope with high salinity, whereas most eukaryotes, such as macroalgae and burrowing/grazing invertebrates, which compete with or ingest cyanobacteria and disrupt lamination, are largely excluded (Fischer, 1965; Awramik, 1971, 1982, 1992; Walter and Heys, 1985; Riding, 2006).

Stromatolites in Shark Bay, Western Australia are the most famous stromatolites thriving in hypersaline water. They are found in the Hamelin Pool Marine Nature Reserve of the Shark Bay UNESCO World Heritage Site. Ranging from 55–70 ppt throughout the year (Playford, 1990), Hamelin Pool has approximately double normal marine salinity and hosts abundant and diverse stromatolites. Shark Bay stromatolites were discovered in 1954 by Johnstone, Playford, and Chase of the West Australian Petroleum Pty. Ltd (Playford and Cockbain, 1976). They were the first modern stromatolites discovered with sizes and shapes comparable to fossil counterparts (Logan, 1961; Logan et al., 1974; Playford and Cockbain, 1976; Playford, 1979; Bauld et al., 1979; Bauld, 1981; Burns et al., 2004). Like Bahamian stromatolites, Shark Bay structures are formed by both (1) microbial trapping and binding and (2) microbial precipitation. Both processes are important in the intertidal zone, forming coarse grained sandy stromatolites. Microbial precipitation is the primary accretionary mechanism in the subtidal zone of Shark Bay, forming muddy, micritic stromatolites (Reid et al., 2003b).

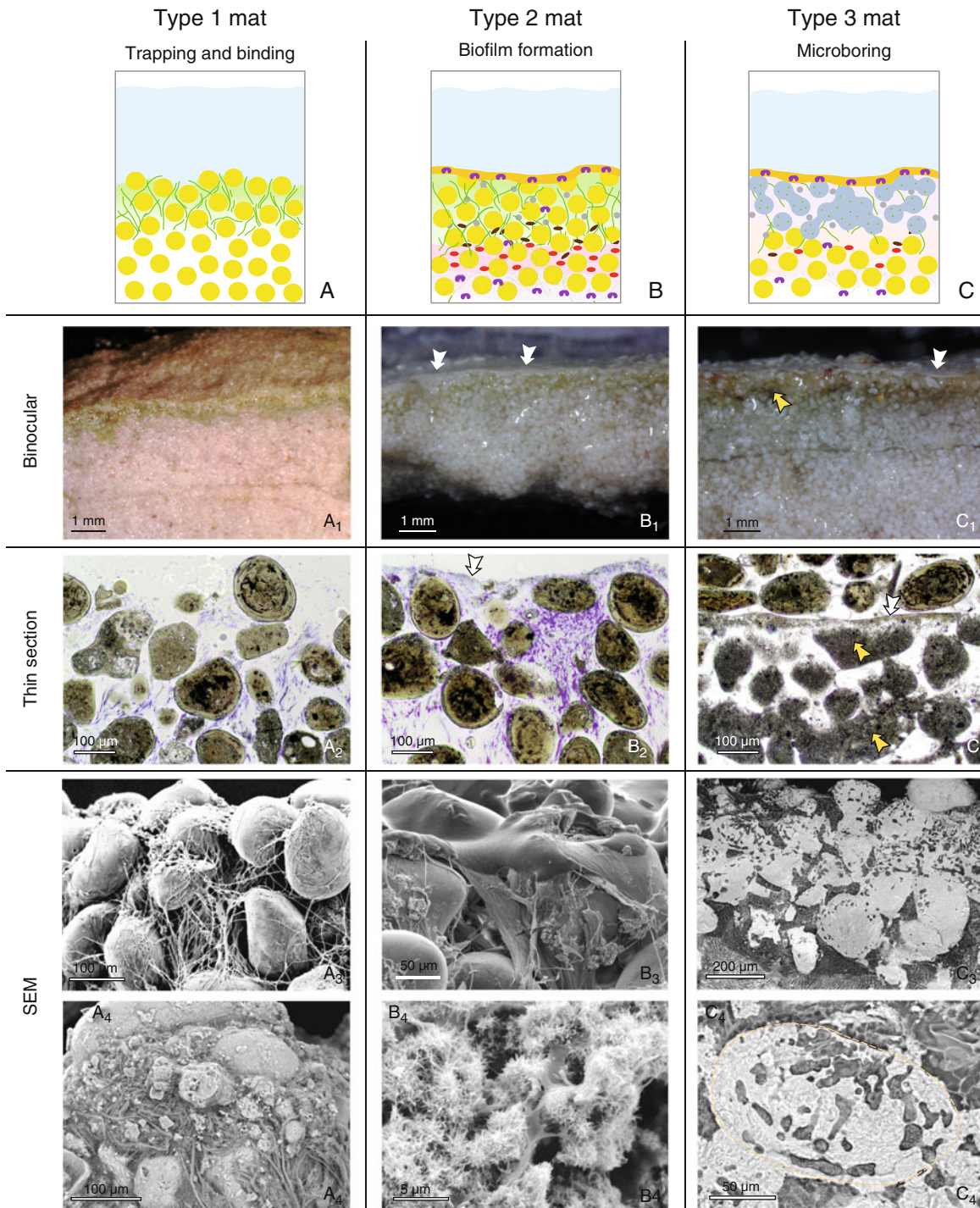
As a result of the dominant sandy textures of Shark Bay stromatolites and open marine stromatolites in the Bahamas, some authors have proposed that these stromatolites are not appropriate analogs for fossil stromatolites, which are generally display fine-grained, micritic microstructures (Awramik and Riding, 1988). Despite their overall coarse-grained texture, these modern stromatolites have micritic laminae formed as a result of microbially-induced precipitation (Reid et al., 2000; Visscher et al., 2000). Moreover, the ecological model developed for Bahamian stromatolites, in which lamination results from the cycling of prokaryotic communities on the stromatolite surface, is likely applicable to the growth of Shark Bay stromatolites. Indeed, a model of iterative accretion of laminae, which record both microbial and environmental fingerprints and progressively shape the emergent morphology, may serve as a conceptual model for the growth of fossil stromatolites.

In addition to Shark Bay, living stromatolites are also present in many other saline and hypersaline environments. Several saline lakes in Australia harbor stromatolites, including Lake Thetis, near Cervantes, lakes on Rottne Island (Reitner et al., 1996), and Lake Clifton near Mandurah (e.g., Grey et al., 1990). Cyanobacterial stromatolitic domes have been described in the intertidal zone of Bermuda (e.g., Sharp, 1969; Golubic and Focke, 1978) and small crudely laminated knobs have been found in a hypersaline lake on Bonaire Island in the Netherlands Antilles (Golubic and Focke, 1978). Various islands in the Bahamas also have hypersaline lakes with well-developed microbial mats and stromatolites, including Storr's Lake on San Salvador. Storr's lake harbors alternating stromatolitic and thrombolitic buildups (Mann and Hoffman, 1984; McNeese, 1988; Neumann et al., 1988; Mann and Nelson, 1989; Pentecost, 1989; Zabielski, 1991).

Fresh water and continental stromatolites

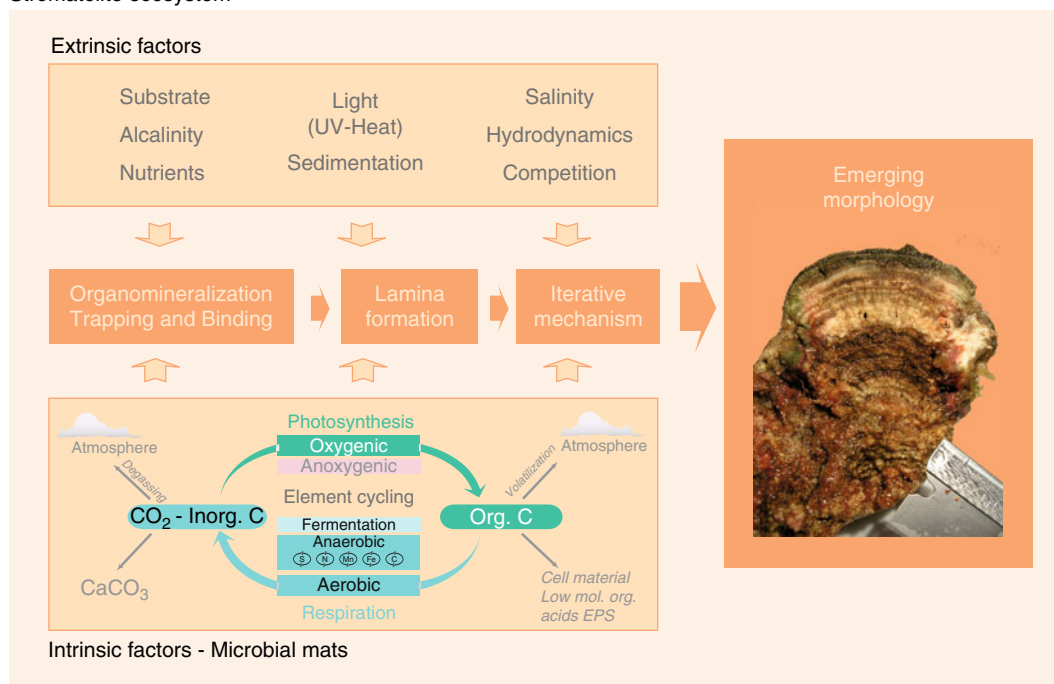
Numerous examples of freshwater laminated microbialite have been published. One of the most widespread examples of these microbialites is travertines. Tufa stromatolites (meteoene travertine following Pentecost (2005)) are freshwater fluviatile tufas, which locally exhibit thick laminated crusts formed by calcified cyanobacteria (Pentecost, 1978; Riding, 1991b, 2000). Most of the precipitation of carbonate in these travertines results from physicochemical

Microbialites, Modern, Figure 4 Model of coarse-grained open-marine stromatolite formation. (a) Cyanobacteria colonize and stabilize mobile ooid sand (trapping and binding). (b) A complex microbial mat develops in the upper few millimeters of the sediment (Cya cyanobacteria; SRB sulfate reducing bacteria; SOB sulfide oxidizing bacteria (aerobic and anaerobic); Het aerobic heterotrophs; see also Figure 8). (c–d) As a result of sediment accretion, the microbial mat migrates upward, continuing trapping and binding. Cyanobacteria (and heterotrophs) can survive massive burial. No significant in situ precipitation is observed at this stage, which corresponds to the Type 1 mat in Figure 5. (e) During a hiatus in sediment accretion, an EPS-rich biofilm develops, draping over the ooids (Type 2 mat, Figure 5). This biofilm rapidly lithifies due to the activity of anaerobic heterotrophs (i.e., SRB), forming a thin lamina of micritic aragonite (see Figure 5b4). (f) A prolonged hiatus in trapping and binding results in colonization of ooid grains under the biofilm by the endolithic cocoid cyanobacteria *Solentia* sp. (Type 3 mat, Figure 5) Precipitation of aragonite in the bore holes made by these endoliths destroys the internal microstructure of ooids, changing their color from golden brown to gray. Precipitation in bore holes crossing between grains fuses them together (Figure 5c2–4). Resumption of trapping and trapping corresponds to formation of a new Type 1 mat (e2, f2). The iterative succession of these three different mat types is responsible for the lamination observed in open-marine stromatolite (Figure 3).



Microbialites, Modern, Figure 5 Micrographs illustrating the main features of the surface mats responsible for the formation of modern marine stromatolites. Stromatolite lamination results from the iterative growth at the surface of the build up of three mat types: (1) Type 1 mats are dominated by filamentous cyanobacteria, which trap carbonate sand grains through EPS stickiness (A₁₋₄); (2) Type 2 mats consist of a continuous biofilm, draping the stromatolite surface (white arrows in B₁₋₃); the biofilm is composed of extracellular polymeric substances (EPS, B₃) containing numerous heterotrophic bacteria. This biofilm rapidly lithifies as a result of precipitation of aragonite needles within the biofilm (B₄); (3) Type 3 mats are characterized by endolith-infested ooid grains (yellow arrows in C₁₋₂), which appear gray and are fused together, below a surface biofilm. (A₁-B₁-C₁) binocular micrographs, (A₂-B₂-C₂) petrographic micrographs, (A₃-B₃-C₃) high vacuum scanning electron microscope images (SEM; chemical drying), and (A₄-C₃-C₄) cryo-SEM (frozen samples) images.

Stromatolite ecosystem



Microbialites, Modern, Figure 6 Interactive processes within the stromatolite ecosystem. Intrinsic (microbial mat-related) and extrinsic (environment-related) factors work together to determine the emergent stromatolite morphology and fabric, which can be described at microscale (biomineralization, trapping, and binding), mesoscale (laminae formation), and macroscale (iterative mechanism leading to morphology) levels.

degassing of CO₂ through resurgence, cascades, or waterfalls. Photosynthetic uptake of CO₂ by cyanobacteria can, however, be responsible for the precipitation of calcium carbonate in slow running and low DIC streams (Verrecchia et al., 1995; Merz-Preiss and Riding, 1999; Arp et al., 2001). Relatively low DIC and high Ca²⁺ concentration is required for photosynthesis to influence carbonate alkalinity and enable precipitation (Arp et al., 2001, 2003).

Fresh water stromatolites are also present in Antarctic lakes, which are permanently ice-covered (Parker et al., 1981). These lakes are seasonally fed by glaciers, which modify salinity from fresh-to-saline while oxygen levels vary from anoxic to supersaturated. The stromatolites are formed through sediment trapping and binding as well as carbonate precipitation in cyanobacterial-dominated (e.g., *Phormidium*, *Oscillatoria*) microbial mats. Other non-marine environments with stromatolites include alkaline lakes, such as the Caldera lakes of Niuafo'ou Island (Tonga), with spectacular laminated buildups (Kazmierczak and Kempe, 2006).

Laminated deposits, referred to as terrestrial stromatolites (Verrecchia et al., 1995; Riding, 2000), are observed in "non-water-saturated" environments. These deposits have also been called lichen stromatolites (Klappa, 1979) and subaerial stromatolites (Riding, 1991b). They consist of laminar calcretes formed by microbial activity or in close association with microbial communities.

Determining between biogenic and non-biogenic calcretes is often difficult as carbonate precipitation may be caused by physicochemical evaporation (e.g., Read, 1976), and/or cyanobacterial activity (e.g., Verrecchia et al., 1995).

Thrombolite

The term thrombolite was introduced by Aitken (1967) for microbial "structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric". Since this initial definition, thrombolites and stromatolites have been recognized as two distinct microbialite structures (Kennard and James, 1986). The key microstructure of thrombolites is mesoclots, which produce the clotted fabric (Shapiro, 2000). The mesoclots consist of polymorphic (simple spheroids to polylobate), millimeter to centimeter-sized, aggregates which display a variety of forms, from simple spheroids to polylobate masses (e.g., Shapiro, 2000). Clotted mesostructures have been described for many fossil thrombolites, which are mostly composed of fine-grained carbonate. Therefore, many authors define mesoclots as mostly composed of micrite or micropeloids (Kennard, and James, 1986; Dupraz and Strasser, 1999; Shapiro, 2000). However, modern thrombolites, as well examples from the Miocene, show agglutinated, clotted fabrics (Riding, 2000). The mesoclots of modern thrombolites thus consist of micrite, micropeloids, or agglutinated particles (Figure 2).

The clotted fabric of thrombolites can have a variety of origins. The fabric can be attributed “to the in situ calcification of coccoid or coccoid-dominated microbial communities” (Kennard and James, 1986). Other mechanisms involve bioturbation and bioerosion, and thrombotic mesostructures within large Bahamian stromatolites are often due to disruption of the lamination by eukaryotic colonization (e.g., *Batophora* sp. and other algae).

In some cases, environmental conditions may trigger changes between thrombolite and stromatolitic fabrics. In Storr’s Lake (San Salvador, Bahamas), well-developed thrombolites alternate with stromatolitic layers (McNeese, 1988; Mann and Nelson, 1989). This alternation might be triggered by lake-level fluctuations and associated turbidity variability. This mechanism results in a succession of phototrophic-dominated (stromatolites) to heterotrophic-dominated communities (thrombolites) as surface communities, as modeled by Dupraz et al. (2006).

Many other modern lakes host thrombolite deposits: Lake Clifton, Lake Tethys, Lake Richmond and different lakes on Rottnest Island (Australia), Kelly and Pavillon lakes (British Columbia, Canada), lakes on Bonaire Island (Netherlands Antilles, southern Caribbean; Kobluk and Crawford, 1990), Lago Sarmiento (Patagonia, Chile), Poza Azul lake (Cuatro Ciénegas, Mexico), Yellow Stone National Park (USA), Green Lake (Fayetteville, New York, USA). In addition, travertines showing thrombotic features have been referred to as “Tufa Thrombolite” (Riding, 2000).

Leiolite

The term leiolite comes from the Greek word “leios,” meaning uniform or smooth and “lithos,” (meaning rock) and was originally applied to late Miocene (Messinian) deposits in Spain (Figure 2; Braga et al., 1995). Leiolite is characterized by relatively structureless, aphanitic, mesostructure, lacking lamination or clots, and can be a synonym for “cryptomicrobial” as used by Kennard and James (1986). Most authors tend to classify microbialites as either stromatolites or thrombolites, despite the fact that many of these deposits lack the defining mesostructures and would more appropriately be termed leiolite (see Riding, 2000). No examples of modern leiolite have been published. However, examples could include non-laminated, non-thrombotic portions of Bahamian or Shark Bay stromatolites, carbonate crust formation in hypersaline lakes (Dupraz et al., 2004), and biological stabilization of sand dune by chasmolithic microorganisms (Hillgaertner et al., 2001).

Other types of microbialites

Microbial processes or the presence of microbes are often intimately related to sedimentary processes in modern carbonate systems, although the resulting deposits may not be considered as true microbialite and classified as stromatolite, thrombolite, or leiolite. These microbial carbonate

deposits include meteogene and thermogene travertine (Pentecost, 2005), where microbial influence creates specific microfabrics. Spectacular examples of travertines can be observed in many alkaline lakes, forming carbonate towers (e.g., Pyramid Lake, Nevada (Benson, 1994)), Lake Can, Turkey (Kempe et al., 1991), and Mono Lake, California (e.g., Bischoff et al., 1993; Riding, 2000). Carbonate chimneys are also formed in hydrothermal systems, where microbial activity is important (e.g., “Lost City” Hydrothermal Field; Kelley et al., 2001; Ludwig et al., 2006).

Other deposits that are difficult to classify include microbial calcrete – a terrestrial carbonate the formation of which is strongly influenced by bacteria and fungi (Jones and Wilson, 1986; Wright et al., 1988; Verrecchia and Verrecchia, 1994; Verrecchia et al., 1995). In addition, in continental environments, the formation of needle fiber calcite (NFC) is related to fungi activity (Verrecchia and Verrecchia, 1994; Cailleau et al., 2004, 2005). Mondmilch or Moonmilch – a soft paste-like or powdery cave deposit – includes important microbial populations, which influence precipitation (Thraillkill, 1976; Canaveras et al., 2006). Microbial mats are also involved in the formation of oncoids, which are unattached spherical stromatolites (e.g., Bathurst, 1966), and microbial activity may play a role in the formation of “whittings” (Thompson, and Ferris, 1990; Robbins, and Blackwelder, 1992).

Processes of microbialite formation

Early lithification is a key process in the formation of modern carbonate microbialites and is critical for the preservation of these structures in the fossil record. Lithification processes start at the time of deposition and continue during burial (diagenesis). Lithification consists of in situ precipitation of minerals, which progressively cement trapped grains (if present), and preserve the biosedimentary structures. Two closely coupled components are fundamental in carbonate precipitation within microbial mats: (1) the alkalinity engine and (2) the organic matrix in which this mineral is forming.

The alkalinity engine

The precipitation of carbonate minerals depends on the availability of carbonate ions, specific cations (e.g., Ca^{2+} , Mg^{2+}), and suitable nucleation sites. Carbonate minerals may precipitate when a solution is saturated with respect to that mineral. The degree of saturation is calculated via the saturation index of the specific mineral:

$$\text{SI} = \log(\text{IAP}/K_{\text{ps}}) \quad (1)$$

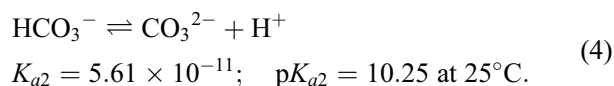
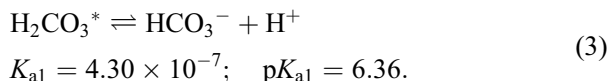
IAP is the “ion activity product” and K_{sp} is the solubility product of the corresponding mineral (solid phase). In the case of CaCO_3 , both the carbonate alkalinity and the activity of the available cations are taken into account

when calculating the saturation index (Stumm and Morgan, 1996):

$$SI_{\text{CaCO}_3} = \text{Log}(\{ \text{Ca}^{2+} \} * \{ \text{CO}_3^{2-} \} / K_{\text{sp}}). \quad (2)$$

The activity of a given chemical compound corresponds to the concentration of that compound multiplied by the activity coefficient. The solubility products for aragonite and calcite are $10^{-6.19}$ and $10^{-6.37}$, respectively, at 25°C, 1 bar atmospheric pressure and 35 PSU salinity (Zeebe and Wolf-Gladrow, 2001). A solution is supersaturated with respect to a given mineral when SI is greater than 0, meaning that K_{sp} is smaller than IAP. Experimental evidence showed that a supersaturation must be greater than 0.8 in order to have spontaneous precipitation of CaCO_3 (Kempe and Kazmierczak, 1994). Arp et al. (2001) used SI greater than 1 (i.e., a 10-fold supersaturation) as a prerequisite for carbonate precipitation in non-marine environment.

Carbonate ion activity, $\{ \text{CO}_3^{2-} \}$, depends on the carbonate equilibrium. CO_2 dissolves in water to form carbonic acid (H_2CO_3). The amount of dissolved CO_2 is proportional to the partial pressure of CO_2 ($p\text{CO}_2$) in the gas phase in contact with the liquid (i.e., Henry's Law). This value varies with pressure and temperature. Low temperature and high pressure allow for more CO_2 dissolution in water. Carbonic acid is weak acid that will only partially dissociate in an aqueous solution to produce H^+ ion and the conjugate base. It will deprotonate as follows (with pK_s value for fresh water):

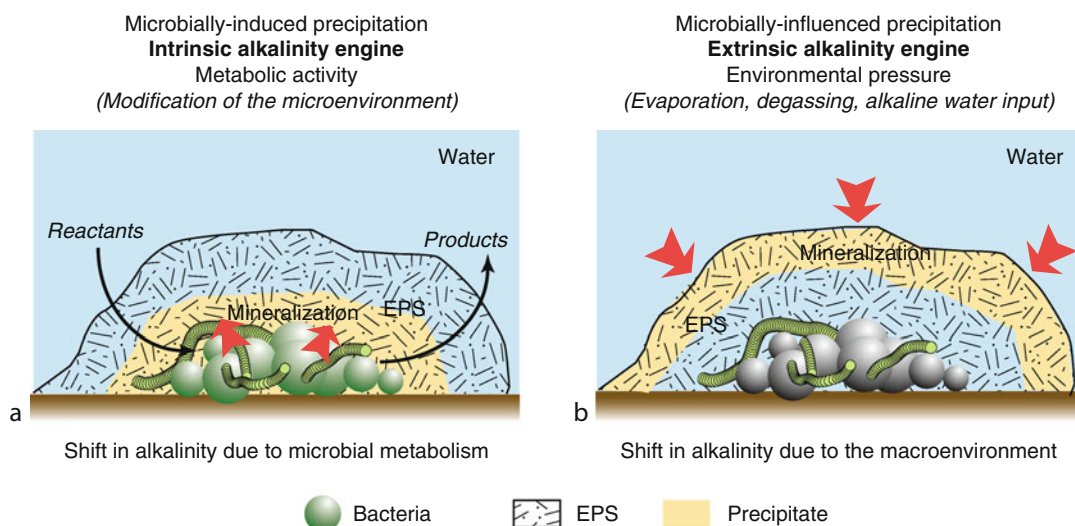


The two pK_s values vary as a function of the salinity. For seawater conditions, pK_{a1} and pK_{a2} are 5.9 and 8.9, respectively (at 25°C, 1 bar atmospheric pressure and 35 PSU salinity; Zeebe and Wolf-Gladrow, 2001).

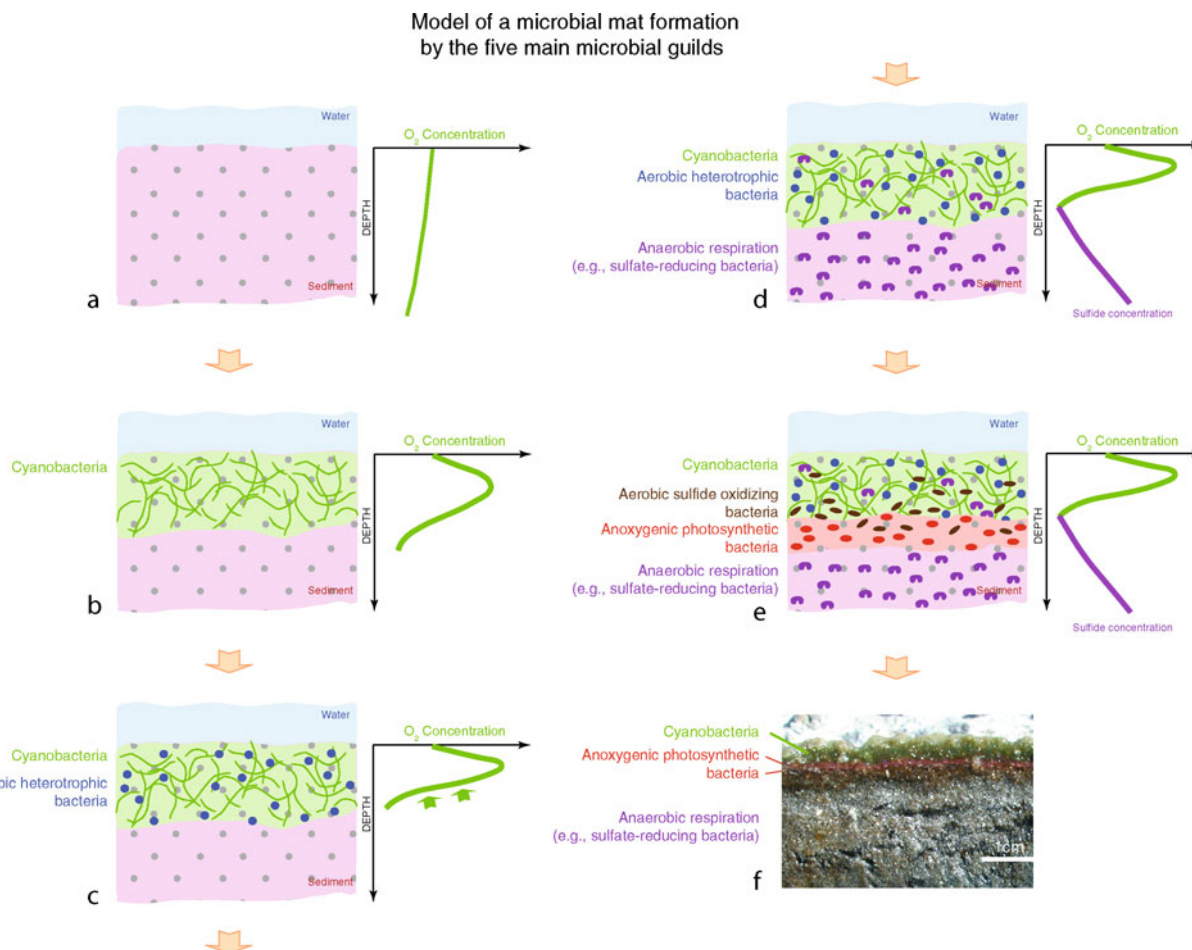
Availability of carbonate ions is a prerequisite for precipitation of carbonate minerals. The amount of bicarbonate and carbonate ions in solution denotes carbonate alkalinity, which is a part of total alkalinity, including borate, hydroxide, phosphate, and silicate. In marine and most of freshwater environments, phosphate and silicate are minor components, and the carbonate equilibrium typically drives global alkalinity. Various processes can have an impact of carbonate alkalinity, indirectly promoting precipitation or dissolution of carbonate mineral. The sum of processes that creates alkalinity represents the “alkalinity engine.” This engine is intrinsically driven when alkalinity is affected by microbial communities, or extrinsically driven when physico-chemical processes of the macro-environment cause alkalinity shifts (Figure 7).

Intrinsic alkalinity engine (metabolism)

The way microbial communities acquire their energy and their source of carbon (i.e., through metabolism) can have a strong impact on local carbonate alkalinity. In a simplified view of the microbial carbon cycle, the production of organic matter through photosynthesis (oxygenic or anoxygenic) is coupled with the oxidation of organic carbon by aerobic (oxygen as oxidant) or



Microbialites, Modern, Figure 7 Model of carbonate precipitation within biofilms. Increases in alkalinity leading to precipitation can be (a) intrinsic, resulting from microbial metabolism or (b) extrinsic, resulting from environmental factors (b). In both the cases, the EPS matrix, which embeds the microbial communities, has a pivotal role in determining mineral morphology, composition, and mineralogy.



Microbialites, Modern, Figure 8 Model of microbial mat formation by the five main guilds (van Gernerden, 1993). (a) Typical diffusion of O₂ through the water-sediment interface in the absence of microbial activity. (b) Cyanobacteria colonize the sediment, fixing (reduce) CO₂ to form biomass using solar energy (photosynthesis) and using water as electron donor. O₂ is a byproduct (visible in the upper millimeter of the depth profile). (c) The autotrophically-produced organic carbon is degraded by aerobic heterotrophs (aerobic respiration) using O₂ as final electron acceptor. The consumption of oxygen results in, or is reflected by, a sharper O₂ gradient in the sediment (arrows) and the formation of an anoxic zone below the active cyanobacterial layer. (d) Anaerobic heterotrophs then develop in the anoxic zone (mostly SRB in this example). (e) SRB produces sulfide compounds, which can then feed anoxygenic photosynthesis and aerobic sulfide oxidation. (f) Examples of a hypersaline mat from the Bahamas showing classical zonation of bacterial guilds.

anaerobic (SO₄²⁻, NO₃⁻, Fe³⁺, etc. as oxidant) respiration (Figure 8). Most microbial metabolites (products of metabolism) are efficiently recycled within the mats, completely closing the cycles of major elements. Many microbial mats function as light-driven engines, fueled only by sunlight.

The biogeochemical niche of microorganisms can be described through three overarching metabolic characteristics related to energy generation and biomass acquisition: the energy source, the electron donor used for energy generation, and the carbon source for biomass production. Each of these three properties have two possibilities: potential energy sources are light (photo-) or chemical redox reactions (chemo-), electron donors can be organic (organo-)

or inorganic (litho-), and biomass can be derived from CO₂ fixation (autotrophy) or from carbon that is already fixed (heterotrophy). For example, a cyanobacterium derives energy from light (photo) uses water as electron donor (litho) and fixes CO₂ for biomass (auto-); it is hence designated as a photolithoautotroph (trophos means “to feed”). A typical aerobic bacterium that uses organic carbon is a chemoorganoheterotroph, etc.

Different types of metabolism impact carbonate precipitation and dissolution by taking up (autotrophy) or releasing (respiration) CO₂ and through production or consumption of organic acids. Changes in organic acid concentrations modify alkalinity and pH of the mats (Ehrlich, 1996; Visscher and Stolz, 2005). Mechanisms

that can promote precipitation of carbonate minerals include the following:

1. *Oxygenic photosynthesis* (producing O_2) by cyanobacteria (photolithoautotrophy). Uptake of CO_2 during photosynthetic activity leads to precipitation of carbonate in the sheath or in the direct vicinity of the cyanobacteria (Pentecost and Riding, 1986; Frey et al. and Verrecchia, 1998, 1999; Merz-Preiss and Riding, 1999; Riding, 2000) (Figure 9). The increase in alkalinity is attributed to an exchange of HCO_3^- and OH^- through the cell membrane (Figure 9). Photosynthesis is an important mechanism for microbialite formation in freshwater environment (Arp et al., 2001), where it may help overcome the kinetic barrier for $CaCO_3$ precipitation, even in highly supersaturated freshwater settings (Shiraishi et al., 2008).
2. *Aerobic respiration* (chemoorganoheterotrophy) generally dissolves calcium carbonate through production of CO_2 . However, precipitation can occur in a well-buffered alkaline environment when CO_2 produces carbonate ions. Carbonate minerals can also be formed when aerobic respiration consumes strong organic acids and produce CO_2 that will be degassed. This process is proposed for carbonate precipitation in tropical soil associated to the oxalate-carbonate cycle (Braissant et al., 2004). In the absence of O_2 , some bacteria can generate energy through fermentation, which process uses the same compound (e.g., organic carbon, inorganic sulfur) both as electron donor and acceptor. Most fermentation of organic carbon will lead to dissolution of carbonate (Visscher and Stolz, 2005).
3. *Anaerobic respiration*: certain bacteria can oxidize organic matter using respiratory chains that do not use O_2 as final electron acceptor (also chemoorganoheterotrophy). These bacteria can use, e.g., sulfate (sulfate-reduction), nitrate (nitrate-reduction), iron (iron-reduction), manganese (manganese reduction), or even HCO_3^- . These types of metabolism can promote precipitation of carbonate through various processes (e.g., Visscher and Stolz, 2005): consumption of organic acid, production of base (e.g., NH_3), removing precipitation inhibition (removing of SO_4^{2-}), etc. Sulfate reduction is the main process in marine environment due to high concentration of SO_4^{2-} in seawater.
4. *Chemolithoautotrophy*: Some bacteria do not oxidize organic matter to generate energy. They will instead use the chemical energy (chemo) produced by the oxidation of inorganic (litho) electron donors such as H_2 , CO , Fe^{2+} , NH_4^+ and HS^- in order to fix CO_2 (autotrophy). Sulfide (in sediments) and ammonium (in the water column) oxidation are the most abundant chemolithoautotrophic electron donor in marine environment. Aerobic sulfide oxidation and ammonium oxidation is likely to induce dissolution of carbonate minerals, whereas anaerobic sulfide oxidation (e.g., using nitrate as electron acceptor) may induce

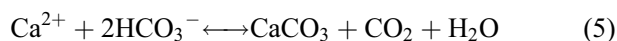
precipitation (Visscher and Stolz, 2005). However, most of these types of metabolism are all autotrophic, i.e., fixing CO_2 . The uptake of CO_2 can increase carbonate alkalinity and induce precipitation of carbonates in similar way that physicochemical degassing of CO_2 does.

Extrinsic alkalinity engine

The determination of alkalinity-driving forces that form microbialites is generally a complex task. In many cases, a combination of intrinsic and extrinsic factors is responsible for precipitation. There are however, a few examples where the macroenvironment acts as a major player in microbial mat lithification. The two important physicochemical processes that can lead to carbonate precipitation in microbial mats are water evaporation and CO_2 degassing. Even in these cases, however, the microbial communities of the mats can serve as substrates for physicochemical carbonate precipitation and affect mineral mineralogy or morphology.

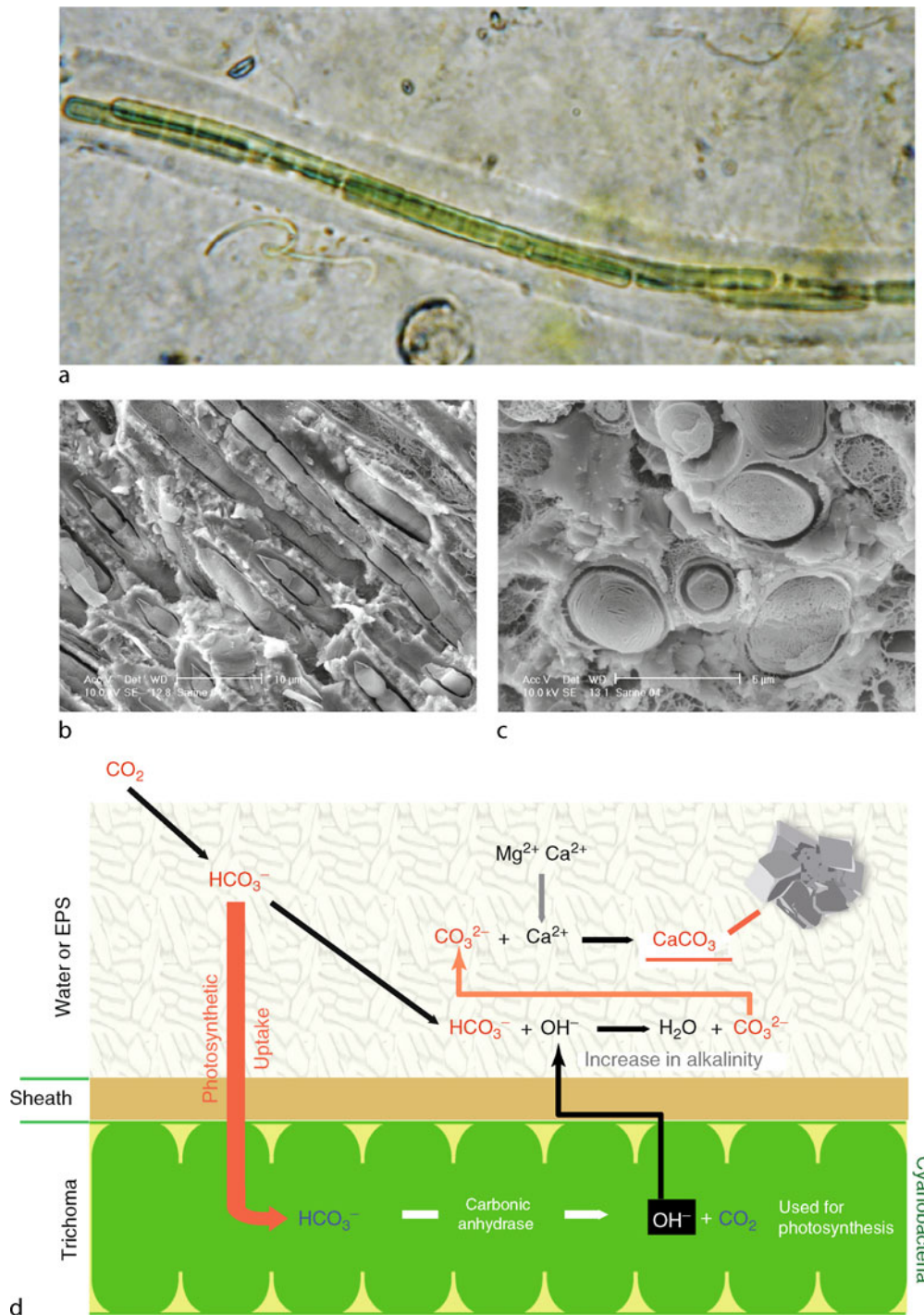
In the marine environment, solar evaporation of water may lead to the formation of evaporitic minerals (e.g., Warren, 2006). Although a variety of carbonate minerals can be produced through this mechanism (e.g., calcite, ikaite, aragonite, Mg-calcite, dolomite, and magnesite), evaporites largely consist of halite and gypsum. Examples of microbial mats associated with gypsum deposits have been described (e.g., Babel, 2004). Moreover, bacteria trapped in gypsum crystals are able to survive for millions of year (Vreeland et al., 2000, 2007). The specific role of microbes in gypsum nucleation is, however, not clear as the evaporation processes generally obscure microbial signatures. For reviews on evaporites, see Yechieli, and Wood (2002) or Warren (2006).

Precipitation of carbonate can also result from degassing of CO_2 and forms travertines. Travertines represent chemically-mediated continental carbonate deposits that form around sinter sources along streams and lakes (Pentecost, 2005). The precipitated minerals, consisting of aragonite or calcite, are mainly related to the release of CO_2 from the system, resulting in supersaturation with respect to calcium carbonate. This can be illustrated by the buffering equation of the carbonate:

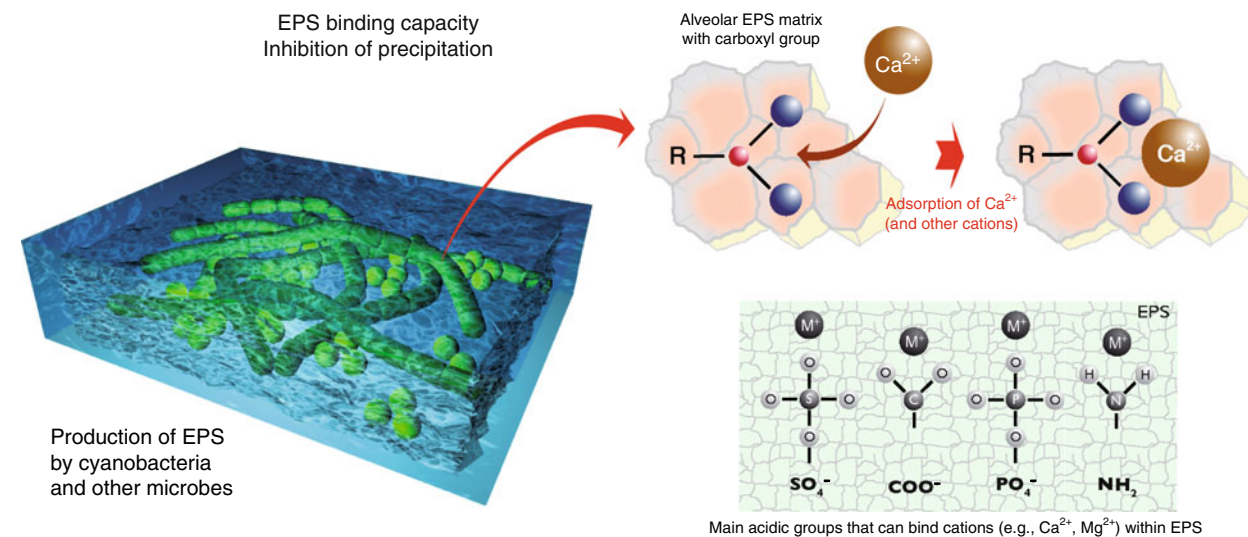


Removal of CO_2 through degassing will drive this equation to the right, promoting precipitation of $CaCO_3$. Similarly, increasing dissolved CO_2 drive the equation to the left, resulting in dissolution of calcium carbonate.

Travertines can be classified based on the source of the CO_2 that is degassed (Pentecost, 2005). "Metogene travertines" are formed by degassing of meteoric carbon dioxide, whereas "thermogene travertines" are formed by the degassing of hydrothermal CO_2 . In metogene travertines, the CO_2 can originate from the atmosphere or from the soil-zone. In thermogene travertines, the bulk of the CO_2

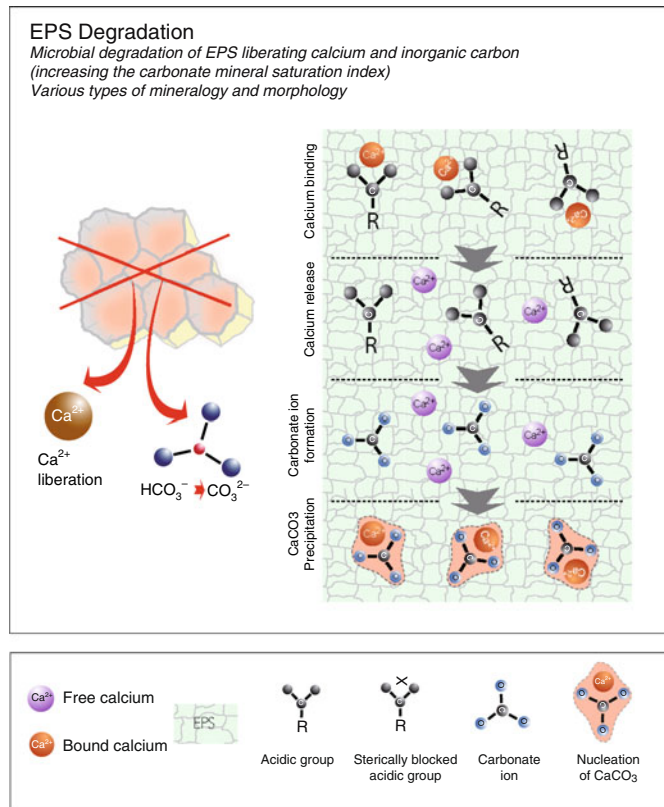


Microbialites, Modern, Figure 9 Role of photosynthesis in the precipitation of carbonate minerals. (a) Photomicrograph of *Microcoleus* spp., a filamentous cyanobacterium showing the trichoma (chains of cells) embedded in a sheath. (b) Longitudinal section through a freshwater travertine (Sarine River, Switzerland) showing precipitation of CaCO_3 in the sheath of the filamentous cyanobacteria *Oscillatoria* sp. and *Phormidium* sp. (c) Perpendicular section through the same travertine. (d) Model of photosynthetically-driven carbonate precipitation. CO_2 dissolve in water, forming bicarbonate ions (the most common form of DIC between pH 5 and 9). The bicarbonate is taken up by cells of the trichoma; the enzyme carbonic anhydrase inside the cells produces CO_2 (used for photosynthesis) and OH^- . The release of hydroxyl ions outside the trichoma increases the alkalinity in the surrounding area of the cyanobacteria and induces precipitation of CaCO_3 (provided Ca^{2+} ions are available).



EPS alteration
leading to precipitation of carbonates

Biologically-induced mineralization



Biologically-influenced mineralization

Extrinsic Supersaturation of EPS

Forcing of mineralization on the EPS matrix.

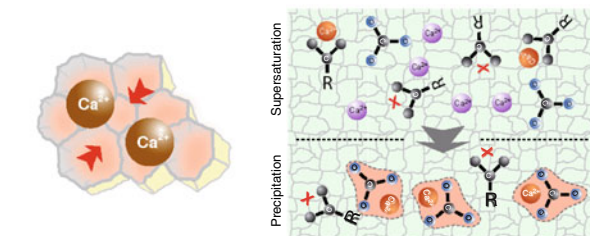
Organic matrix and kinetic of precipitation can control mineral morphology.

Intrinsic Supersaturation of EPS:

Steric Hindrance

Changes in availability of functional groups (steric hindrance).

Cation binding capacity does not reflect abundance of functional groups



Microbialites, Modern, Figure 10 Mechanistic role of the organic EPS matrix in microbial mat lithification. The exopolymeric substances are produced by various groups of bacteria (especially cyanobacteria). EPS can bind large amounts of cations (i.e., Ca²⁺), reducing the calcium carbonate saturation index, inhibiting precipitation. Alteration of the EPS matrix can end this inhibition, allowing carbonate precipitation to commence. EPS alteration is achieved via two main pathways: (1) EPS degradation, and (2b) intrinsic supersaturation of the EPS binding capacity (modified from Dupraz and Visscher, 2005).

originates from thermal processes. These hydrothermal systems are commonly associated with region of recent volcanic or tectonic activities. It is important to note that the term travertine has variable usage in the literature. Many authors use travertine only for hot water deposits, using “tufa” for cold-water deposits (Pedley, 2000). Other authors use tufa for actively-forming deposits and travertine for non-active, fossil forms (see Chapter *Tufa, Freshwater*).

Although carbonate precipitation in travertines and tufas is driven by abiotic processes, precipitation often initiates on organic substrates, e.g., leaves, woods, algae, or microbial mats. In these cases, mineralogy and morphology are strongly influenced by the organic matrix (e.g., Fouke et al., 2000; Farmer, 2000; Pentecost, 2005; Turner and Jones, 2005). Microbial mats at hot springs, for example, can therefore have a profound impact on thermogene travertine by providing templates for mineralization, even though precipitation is due to vigorous CO₂ degassing (Farmer, 2000; Fouke et al., 2000). Travertine formation is thus an example of biologically-influenced mineralization, since the carbonate precipitation is not due to biological activity, but microorganisms indirectly affect the characteristics of the resulting minerals.

The organic matrix (EPS)

Microbial communities generally produce extracellular polymeric substances (EPS; see Chapter *Extracellular Polymeric Substances (EPS)*). This organic matrix is an important part of the microbial mat, preventing desiccation, retaining essential nutrients, protecting against UV radiation, and providing water channels for transport of metabolites and signaling compounds (Decho, 1990, 2000). Cyanobacteria are often considered to be the major producers of EPS. However, many other bacteria can produce EPS matrix, including aerobic and anaerobic (chemoorgano)heterotrophs, anoxygenic phototrophs, and chemolithoautotrophs (De Philippis et al., 1998, 2001; Stal, 2000, 2003; Richert et al., 2005). Anaerobic bacteria, especially sulfate-reducing bacteria, which play a key role in carbon metabolism in marine microbial mats (Thode-Andersen, and Jorgensen, 1989; Canfield and DesMarais, 1991; Visscher et al., 1991), are known to produce large amounts of EPS (Bosak and Newman, 2005; Braissant et al., 2007).

The EPS matrix is generally the location where the carbonate minerals nucleate and grow (Dupraz et al., 2004). EPS contains negatively-charged acidic groups (e.g., carboxyl, sulfhydryl, amine, hydroxyl groups), which can bind a large amount of mono- and divalent cations, notably Ca²⁺ (Braissant et al., 2007). This binding capacity may inhibit the precipitation of carbonate minerals by depleting calcium from the surrounding microenvironment (Figure 10). The saturation index of calcite depends on the carbonate alkalinity and the availability in calcium. Even if carbonate ions are in solution, the lack of free Ca²⁺ may inhibit precipitation (Dupraz and Visscher, 2005). Therefore, the physicochemical properties of the polymer

matrix, such as the acidity or composition of the functional groups associated with the EPS, are key factors in the formation of modern microbialites.

In order to precipitate calcium carbonate in microbial mats, the Ca²⁺-binding capacity of the EPS has to be reduced. Various mechanisms have been proposed (e.g., Dupraz and Visscher, 2005), which can be grouped into two main processes (Figure 10): (1) degradation of the EPS matrix and (2) intrinsic or extrinsic supersaturation of cation-binding sites (e.g., Dupraz and Visscher, 2005). As EPS is a sugar polymer, it serves as metabolic substrate for (chemoorgano) heterotrophic bacteria. Depolymerization (hydrolysis) of EPS, possibly by fungi and heterotrophs, including spirochetes (Harwood and Canale-Parola, 1984; Weaver and Hicks, 1995), must occur before sugar monomers are available. Microbial degradation of EPS liberates the calcium and produces inorganic carbon (under alkaline conditions, the dominant species is carbonate ion). Alternatively, if the amount of Ca exceeds the number of binding sites in the EPS, free calcium will be present and available for precipitation. These conditions can be achieved through extrinsic factors (the environment) through continuous input of Ca. It can also result from the steric hindrance of acidic groups within the EPS. Although EPS may indeed possess abundant functional groups capable of binding Ca²⁺ or Mg²⁺ ions, these groups may be “sterically-inhibited” (i.e., blocked) via molecular-scale interactions into a structurally complex EPS (Figure 10).

Summary

Microbialites are “organosedimentary deposits that accrete as a result of a benthic microbial community, which traps and binds sediment and/or forms the locus of mineral precipitation” (Kennard and James, 1986). Living examples of these structures can be found in many modern environments including marine, hypersaline, freshwater, and even continental settings. Microbialites are classified based on mesostructure as stromatolites (laminated), thrombolites (clotted), and leiolites (structureless). Among the most spectacular examples are modern stromatolites, which were first discovered in a hypersaline lagoon of Shark Bay in 1954. The only known examples of stromatolites presently forming in open marine environments are in the Bahamas. Lamination in these coarse-grained structures results from the iterative growth of three different types of microbial mats on the surfaces of the build-ups. These microbial communities are responsible for successive “trapping and binding” of sediment (type 1) and in situ precipitation of calcium carbonate (types 2 and 3). Both processes are essential for stromatolites formation. Modern thrombolites and leiolites are less well-studied than modern stromatolites.

Early lithification is a key process in the formation of modern microbialites. Lithification consists of in situ precipitation of minerals, which preserves the biosedimentary structures. Two key and closely coupled components

involved in carbonate precipitation within microbial mats are (1) the alkalinity engine and (2) the organic matrix. The alkalinity engine, which effectively changes the saturation index, is *intrinsically driven* when microbial metabolism is the dominant process causing carbonate precipitation. The alkalinity engine is *extrinsically driven* when the macroenvironment is responsible for microbial mat lithification. In both the cases, the organic matrix of extracellular polymeric substances (EPS), which embed the microbial communities, is the physical location where carbonate minerals nucleate and grow. Fresh EPS contains negatively-charged acidic groups, which bind large amount of cations (e.g., Ca^{2+}), inhibiting CaCO_3 precipitation. In order to precipitate carbonate, this inhibition has to be reduced by degradation of the EPS or by oversaturation of the cation-binding capacity. Microbial activity is ultimately responsible for EPS production and degradation leading mineral precipitation.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Altermann, W., Kazmierczak, J., Oren, A., and Wright, D. T., 2006. Cyanobacterial calcification and its rock-building potential during 3.5 billion years of Earth history. *Geobiology*, **4**, 147–166.
- Andres, M. S., and Reid, R. P., 2006. Growth morphologies of modern marine stromatolites: a case study from Highborne Cay, Bahamas. *Sedimentary Geology*, **185**, 319–328.
- Andres, M. S., Sumner, D. Y., Reid, R. P., and Swart, P. K., 2005. Isotopic fingerprints of microbial respiration in aragonite from Bahamas stromatolites. *Geology*, **34**, 973–976.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Awramik, S. M., 1971. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **174**, 825–827.
- Awramik, S. M., 1982. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **216**, 171–173.
- Awramik, S. M., 1992. The history and significance of stromatolites. In Schidlowski, M., Kimberley, M. M., McKirdy, D. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Energy and Mineral Resources*. Berlin: Springer, pp. 435–449.
- Awramik, S. M., and Riding, R., 1988. Role of algal eukaryotes in subtidal columnar stromatolite formation. *Proceeding of National Academy of Science, USA*, **85**, 1327–1329.
- Babel, M., 2004. Models for evaporite, selenite and gypsum microbialite deposition in ancient saline basins. *Acta Geologica Polonica*, **54**, 219–249.
- Barbieri, R., and Cavalazzi, B., 2005. Microbial fabrics from Neogene cold seep carbonates Northern Apennine, Italy. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 143–155.
- Barbieri, R., Ori, G. G., and Cavalazzi, B., 2004. A Silurian cold-seep ecosystem from the Middle Atlas, Morocco. *Palaios*, **19**, 527–542.
- Bathurst, R. G. C., 1966. Boring algae, micrite envelopes and lithification of molluscan biosparite. *Geological Journal*, **5**, 15–32.
- Bauld, J., 1981. Geobiological role of cyanobacterial mats in sedimentary environments: production and preservation of organic matter. *BMR Journal of Australian Geology and Geophysics*, **6**, 307–317.
- Bauld, J., Chambers, L. A., and Skyring, G. W., 1979. Primary productivity, sulfate reduction and sulfur isotope fractionation in algal mats and sediments of Hamelin pool, Shark Bay, W. A. Australia. *Journal of Marine and Freshwater Research*, **30**, 753–764.
- Benson, L., 1994. Carbonate deposition, Pyramid Lake Subbasin, Nevada; 1, Sequence of formation and elevational distribution of carbonate deposits (tufas). *Palaeogeography Palaeoclimatology and Palaeoecology*, **109**, 55–87.
- Benzerara, K., Menguy, N., Lopez-Garca, P., Yoon, T.-H., Kazmierczak, J., Tyliczszak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *PNAS*, **103**, 9440–9445.
- Bhaskar, P. V., and Bhosle, N. B., 2005. Microbial extracellular polymeric substances in marine biogeochemical processes. *Current Science*, **88**, 45–53.
- Bischoff, J. L., Stine, S., Rosenbauer, R. J., Fitzpatrick, J. A., and Stafford, T. W., 1993. Ikaite precipitation by mixing of shoreline springs and lake water, Mono Lake, California, USA. *Geochimica Cosmochimica Acta*, **57**, 3855–3865.
- Bosak, T., and Newman, D. K., 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. *Journal of Sedimentary Research*, **75**, 190–199.
- Braga, J. C., Martin, J. M., and Riding, R., 1995. Controls on microbial dome fabric development along a carbonate-siliciclastic shelf-basin transect, Miocene, SE Spain. *Palaios*, **10**, 347–361.
- Braissant, O., Cailleau, G., Aragno, M., and Verrecchia, E. P., 2004. Biologically induced mineralization in the tree *Milicia excelsa* (Moraceae): its causes and consequences to the environment. *Geobiology*, **2**, 59–66.
- Braissant, O., Decho, A. W., Dupraz, C., Glunk, C., Przekop, K. M., and Visscher, P. T., 2007. Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. *Geobiology*, **5**, 401–411.
- Buick, R., Dunlop, J. S. R., and Groves, D. I., 1981. Stromatolite recognition in ancient rocks: an appraisal of irregularly laminated structures in an Early Archaean chert-barite unit from North Pole, Western Australia. *Alcheringa*, **5**, 161–181.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaios*, **2**, 241–254.
- Burns, B. P., Goh, F., Allen, M., and Nellan, B. A., 2004. Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. *Environmental Microbiology*, **6**, 1096–1101.
- Cailleau, G., Braissant, O., and Verrecchia, E. P., 2004. Biomineralization in plants as a long-term carbon sink. *Naturwissenschaften*, **91**, 191–194.
- Cailleau, G., Braissant, O., Dupraz, C., and Verrecchia, E. P., 2005. Biological control on CaCO_3 accumulations in ferrallitic soils of Biga, Ivory Coast. *Catena*, **59**, 1–17.
- Canaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzales, J. M., and Saiz-Jimenez, C., 2006. *Naturwissenschaften*, **93**, 27–32.
- Canfield, D. E., and DesMarais, D. J., 1991. Aerobic sulfate reduction in microbial mats. *Science*, **251**, 1471–1473.
- Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review*, **28**, 73–154.
- Decho, A. W., 2000. Exopolymer microdomains as a structuring agent for heterogeneity within microbial biofilms. In Riding,

- R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 1–9.
- De Philippis, R., Margheri, M. C., Materassi, R., and Vincenzini, M., 1998. Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. *Applied and Environmental Microbiology*, **64**, 1130–1132.
- De Philippis, R., Sili, C., Papperi, R., and Vincenzini, M., 2001. Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *Journal of Applied Phycology*, **13**, 293–299.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P., 1986. Giant subtidal stromatolites forming in normal salinity water. *Nature*, **324**, 55–58.
- Dravis, J. J., 1983. Hardened subtidal stromatolites, Bahamas. *Science*, **219**, 385–386.
- Dupraz, C., and Strasser, A., 1999. Microbialites and microencrusts in shallow coral bioherms (Middle to Late Oxfordian, Swiss Jura Mountains). *Facies*, **40**, 101–130.
- Dupraz, C., and Strasser, A., 2002. Nutritional modes in coral-microbialite reefs (Jurassic, Oxfordian, Switzerland). *Palaio*, **17**, 449–471.
- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Dupraz, C., Visscher, P. T., Baumgartner, L. K., and Reid, R. P., 2004. Microbe-mineral interactions: early CaCO₃ precipitation in a Recent hypersaline lake (Eleuthera Islands, Bahamas). *Sedimentology*, **51**, 745–765.
- Dupraz, C., Patissina, R., and Verrecchia, E. P., 2006. Simulation of stromatolite morphospace using 'DLA-CA' growth model': translation of energy in morphology. *Sedimentary Geology*, **185**, 185–203.
- Ehrlich, H. L., 1996. *Geomicrobiology*, 3rd edn. (revised and expanded). New York: Marcel Dekker, Inc.
- Farmer, J. D., 2000. Hydrothermal systems: doorways to early biosphere evolution. *GSA Today*, **10**, 1–10.
- Fischer, A. G., 1965. Fossils, early life, and atmospheric history. In *Proceeding National Academy of Science. USA, Washington*, Vol. 53, pp. 1205–1215.
- Fouke, B. W., Farmer, J. D., Des Marais, D. J., Pratt, L., Sturchio, N. C., Burns, P. C., and Discipulo, M. K., 2000. Depositional facies and aqueous-solid geochemistry of travertine-depositing hot springs (Ange Terrace, Mammoth Hot Springs, Yellowstone National Park, USA). *Journal of Sedimentary Research*, **70**, 565–585.
- Freytet, P., and Verrecchia, E. P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*, **45**, 535–563.
- Freytet, P., and Verrecchia, E. P., 1999. Calcitic radial palisadic fabric in freshwater stromatolites: diagenetic and recrystallized feature or physicochemical sinter crust. *Sedimentary Geology*, **126**, 97–102.
- Gebelein, C. D., 1976. The effects of the physical, chemical and biological evolution of the earth. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 499–515.
- Golubic, S., and Focke, J. W., 1978. Phormidium hendersonii Howe: identify and significance of a modern stromatolites building microorganism. *Journal of Sedimentary Petrology*, **48**, 751–764.
- Golubic, S., and Hofmann, H. J., 1976. Comparison of Holocene and mid-Precambrian Entophysalidaceae (Cyanophyta) in stromatolitic algal mats: cell division and degradation. *Journal of Paleontology*, **50**, 1074–1082.
- Grey, K., Moore, L. S., Burne, R. V., Pierson, B. K., and Bauld, J., 1990. Lake Thetis, Western Australia: an example of saline lake sedimentation dominated by benthic microbial processes. *Australian Journal of Marine and Freshwater Research*, **41**, 275–300.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth and Planetary Science*, **27**, 313–358.
- Harwood, C. S., and Canale-Parola, E., 1984. Ecology of spirochetes. *Annual Review of Microbiology*, **38**, 161–192.
- Hillgaertner, H., Dupraz, C., and Hug, W. A., 2001. Microbially induced stabilization of carbonate sands in marine phreatic environments or are micritic meniscus cements good indicators for vadose diagenesis? *Sedimentology*, **48**, 117–131.
- Jones, D., and Wilson, M. J., 1986. Biomineralization in crustose lichens. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals*. The Systematics Association, Special Volume 30. New York: Oxford University Press, pp. 91–105.
- Kalkowsky, E., 1908. Oolith und Stromatolith im nord-deutschen Buntsandstein. *Zeitschrift der deutschen geologischen Gesellschaft*, **60**, 68–125.
- Kazmierczak, J., and Kempe, S., 2006. Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafu'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kelley, D. S., Karson, J. A., Blackman, D. K., Frueh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Shrenk, M. O., Roe, K. K., Lebon, G. T., and Rivizzigno, P., and the AT3–60 Shipboard Party, 2001. An off-axis hydrothermal vent field near the Mid-Atlantic ridge at 30° N. *Nature*, **412**, 145–149.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. *Bulletin de l'Institut oceanographique, Monaco*, **13**, 61–117.
- Kempe, S., Reimer, A., Lipp, A., Kazmierczak, J., Landmann, G., and Konuk, T., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaio*, **1**, 492–503.
- Klappa, C. F., 1979. Lichen stromatolites; criterion for subaerial exposure and a mechanism for the formation of laminar calcretes (caliche). *Journal of Sedimentary Petrology*, **49**, 387–400.
- Kobluk, D. R., and Crawford, D. R., 1990. A modern hypersaline organic mud- and gypsum dominated basin and associated microbialites. *Palaio*, **5**, 134–148.
- Kromkamp, J. C., Perkins, R., Dijkman, N., Consalvey, M., Andres, M., and Reid, R. P., 2007. Resistance to burial of cyanobacteria in stromatolites. *Aquatic Microbial Ecology*, **48**, 123–130.
- Kumar, S., and Pandey, S. K., 2008. Discovery of organic-walled microbiota from the black-bedded chert, Balman Limestone, the Bhandar Group, Lakheri area, Rajasthan. *Current Science*, **94**, 797–800.
- Lepot, K., Benzerara, K., Brown, G. E. Jr., and Philippot, P., 2008. Microbially influenced formation of 2.724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Lindsay, J. F., Brasier, M. D., McLoughlin, N., Green, O. R., Fogel, M., McNamara, K., Steele, A., and Mertzman, S. A., 2003. Abiotic Earth – establishing a baseline for earliest life, data from the Archaean of Western Australia. *Lunar and Planetary Institute, Annual Meeting. Lunar, Planetary Institute Contribution*, **1156**, 1137.
- Logan, B. W., 1961. Cryptozoon and associate stromatolites from the Recent, Shark Bay, Western Australia. *The Journal of Geology*, **69**, 517–533.
- Logan, B. W., Hoffman, P., and Gebelein, C. D., 1974. Algal mats, cryptalgal fabrics and structures, Hamelin Pool, Western Australia. *AAPG Memoir*, **22**, 140–194.

- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 387–390.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press.
- Ludwig, K. A., Kelley, D. S., Butterfield, D. A., Nelson, B., and Früh-Green, G., 2006. Formation and evolution of carbonate chimneys at the Lost City Hydrothermal Field. *Geochemica Cosmochimica Acta*, **70**, 3625–3645.
- Macintyre, I. G., Prufert-Bebout, L., and Reid, R. P., 2000. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology*, **47**, 915–921.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. New York: Oxford University Press.
- Mann, C. J., and Hoffman, L. R., 1984. Algal mounds in Storr's Lake, San Salvador, Bahamas. In *Proceeding of the 2nd Symposium on Geology of the Bahamas*, CCFL Bahamian Field Station, pp. 41–51.
- Mann, C. J., and Nelson, W. M., 1989. Microbialitic structures in Storr's Lake, San Salvador Island, Bahamas Islands. *Palaos*, **4**, 287–293.
- McNeese, L. R., 1988. *The stromatolites of Storr's Lake, San Salvador, Bahamas*. MSc thesis, University of North Carolina at Chapel Hill, Department of Geology.
- Merz-Preiss, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams; ambient environment, chemical thresholds biological processes. *Sedimentary Geology*, **126**, 103–124.
- Neumann, C. A., Bebout, B. M., McNeese, L. R., Paul, C. K., and Paerl, H. W., 1988. Modern stromatolites and associated mats: San Salvador, Bahamas. In *Proceedings of the 4th Symposium on the geology of the Bahamas*. Bahamas Field Station, San Salvador, pp. 235–251.
- Noffke, N., Gerdes, G., and Klenke, T., 2003. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty, and evaporitic carbonatic). *Earth-Science Reviews*, **62**, 163–176.
- Parker, B. C., Simmons, G. M., Love, F. G. Jr., Wharton, R. A., and Seaburg, K. G. Jr., 1981. Modern stromatolites in Antarctic Dry Valley Lakes. *BioScience*, **31**, 656–661.
- Pedley, M., 2000. Ambient temperature freshwater microbial tufas. In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 179–186.
- Pentecost, A., 1978. Blue-green algae and freshwater carbonate deposits. *Proceedings of the Royal Society of London*, **200**, 43–61.
- Pentecost, A., 1995. The quaternary travertine deposits of Europe and Asia Minor. *Quaternary Science Reviews*, **14**, 1005–1028.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer.
- Pentecost, A., and Riding, R., 1986. Calcification in cyanobacteria. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals*. The Systematic Association, Special Volume 30, pp. 73–90.
- Perry, R. S., McLoughlin, N., Lynne, B. Y., Sephton, M. A., Oliver, J. D., Perry, C. C., Campbell, K., Engel, M. H., Farmer, J. D., Brasier, M. D., and Staley, J. T., 2007. Defining biominerals and organominerals: Direct and indirect indicators of life. *Sedimentary Geology*, **201**, 157–179.
- Playford, P. E., 1979. Stromatolite research in Western Australia. *Journal of the Royal Society of Western Australia*, **62**, 13–20.
- Playford, P. E., 1990. Geology of the Shark Bay area, Western Australia. In Berry, P. F., Bradshaw, S. D., and Wilson, B. R. (eds.), *Research In Shark Bay*. West Australian Museum, pp. 13–31.
- Playford, P. E., and Cockbain, A. E., 1976. Modern algal stromatolites at Hamelin Pool, a hypersaline barred basin in Shark Bay, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 389–411.
- Read, J. F., 1976. Calcretes and their distinction from stromatolites. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 55–71.
- Reid, R. P., and Macintyre, I. G., 2000. Microboring versus recrystallization: further insight into the micritization process. *Journal of Sedimentary Research*, **70**, 24–28.
- Reid, R. P., Macintyre, I. G., Browne, K. M., Steneck, R. S., and Miller, T., 1995. Modern marine stromatolites in the Exuma Cays, Bahamas: uncommonly common. *Facies*, **33**, 1–18.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. K., Bebout, B. M., Dupraz, C., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and Des Marais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reid, R. P., Dupraz, C., Visscher, P. T., Decho, A. W., and Sumner, D. Y., 2003a. Microbial processes forming modern marine stromatolites: microbe-mineral interactions with a three-billion-year rock record. In Krumbein, W. E., Paterson, D. M., and Zavarzin, G. A. (eds.), *Fossil and Recent Biofilms - A Natural History of Life on Earth*. Dordrecht: Kluwer, pp. 103–118.
- Reid, R. P., James, N. P., Macintyre, I. G., Dupraz, C. P., and Burne, R. V., 2003b. Shark Bay Stromatolites: microfibrils and reinterpretations of origins. *Facies*, **49**, 243–270.
- Reitner, J., Paul, J., Arp, G., and Hause-Reitner, D., 1996. Lake Thetis Domal Microbialites – a complex framework of calcified biofilms and organomicrites (Cervantes, Western Australia). In Reitner, J., Neuweiler, F., and Gunkel, F. (eds.), *Global and Regional Controls on Biogenetic Sedimentation. I. Reef Evolution*. Research Reports. Göttingen: Göttinger Arb. Geol. Paläont., Sonderband, Vol. 2, pp. 85–89.
- Richert, L., Golubic, S., Le Guedes, R., Ratiskol, J., Payri, C., and Guesennec, J., 2005. Characterization of exopolysaccharides produced by cyanobacteria isolated from Polynesian microbial mats. *Current Microbiology*, **51**, 379–384.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 21–51.
- Riding, R., 1999. The term stromatolite: towards an essential definition. *Lethaia*, **32**, 321–330.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2006. Microbial carbonate abundance compared with fluctuations in metazoan diversity over geological time. *Sedimentary Geology*, **185**, 229–238.
- Robbins, L. L., and Blackwelder, P. L., 1992. Biochemical and ultrastructural evidence for the origin of whittings; a biologically induced calcium carbonate precipitation mechanism. *Geology*, **20**, 464–468.
- Schmid, D. U., 1996. Marine microbolithe und mikroinkrustierer aus dem Oberjura. *Profil*, **9**, 101–251.
- Seong-Joo, L., Browne, K. M., and Golubic, S., 2000. On stromatolites lamination. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 16–24.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaos*, **15**, 166–169.
- Sharp, J. H., 1969. Blue-green algae and carbonates – Schizothrix calcicola and algal stromatolites from Bermuda. *Limnology and Oceanography*, **14**, 568–578.
- Shiraishi, F., Bissett, A., de Beer, D., Reimer, A., and Arp, G., 2008. Photosynthesis, respiration and exopolymer calcium-binding in biofilm calcification (Westerhoefer and Deinschwanger Creek, Germany). *Geomicrobiology Journal*, **25**, 83–94.

- Simkiss, K., and Wilbur, K., 1989. *Biomining. Cell Biology and Mineral Deposition*. San Diego: Academic.
- Stal, L. J., 2000. Microbial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Dordrecht: Kluwer.
- Stal, L. J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal*, **20**, 463–478.
- Stolz, J. F., 2000. Structure of microbial mats and biofilms. In Riding, R. E., Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 1–9.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry*. New York: Wiley, 1022 pp.
- Thode-Andersen, S., and Jorgensen, B. B., 1989. Sulfate reduction and the formation of ^{35}S -labeled FeS, FeS₂, and S(0) (elemental sulfur) in coastal marine sediments. *Limnology and Oceanography*, **34**, 793–806.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Thraillkill, J., 1976. Speleothems. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 73–86.
- Turner, E. C., and Jones, B., 2005. Microscopic calcite dendrites in cold-water tufa: implications for nucleation of micrite and cement. *Sedimentology*, **52**, 1043–1066.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine geology*, **113**, 3–25.
- Verrecchia, E. P., and Verrecchia, K. E., 1994. Needle-fiber calcite: a critical review and a proposed classification. *Journal of Sedimentary Research*, **64**, 650–664.
- Verrecchia, E. P., Freytet, P., Verrecchia, K. E., and Dumont, J. L., 1995. Spherulites in calcareous laminar crusts: biogenic CaCO₃ precipitation as a major contributor to crust formation. *Journal of Sedimentary Research*, **A65**, 690–700.
- Visscher, P. T., and Stolz, J. F., 2005. Microbial mats as bioreactors: populations, processes and products. *Paleogeography Paleoclimatology, Paleoecology*, **219**, 87–100.
- Visscher, P. T., Beukema, J., and van Gemerden, H., 1991. *In situ* characterization of sediments: measurements of oxygen and sulfide profiles. *Limnology and Oceanography*, **36**, 1476–1480.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Vreeland, R. H., Rosenzweig, W. D., and Powers, D. W., 2000. Isolation of a 250 million year old halotolerant bacterium from a primary salt crystal. *Nature*, **407**, 897–900.
- Vreeland, R. H., Lowenstein, T., Timofeeff, M., Satterfield, C., DiFerdinando, J., Jones, J., Monson, A., Rosenzweig, W. D., Cho, B. C., Park, J. S., Wallace, A., and Grant, W. D., 2007. The isolation of live cretaceous (121–112 million years old) halophilic *Archaea* from primary salt crystals. *Geomicrobiology Journal*, **24**, 275–282.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Warren, J. K., 2006. *Evaporites: Sediments, Resources and Hydrocarbons*. New York: Springer.
- Weaver, D. T., and Hicks, R. E., 1995. Biodegradation of *Azotobacter vinelandii* exopolymer by Lake Superior microbes. *Limnology and Oceanography*, **40**, 1035–1041.
- Weiner, S., and Addadi, L., 2002. At the cutting edge. Perspectives. *Science*, **298**, 375–376.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization and the problem of the vital effect. In Dove, P. M., Weiner, S., and De Yoreo, J. J. (eds.), *Biomining. Cell Biology and Mineral Deposition*. Washington: Mineralogical Society of America, Vol. 54, pp. 1–31.
- Wright, V. P., Platt, N. H., and Wimbeldon, W. A., 1988. Biogenic laminar calcareous: evidence of calcified root-mat horizons in paleosols. *Sedimentology*, **35**, 603–620.
- Yechieli, Y., and Wood, W. W., 2002. Hydrogeologic processes in saline systems: playas, sabkhas, and saline lakes. *Earth-Science Reviews*, **58**, 343–365.
- Zabielski, V. P., 1991. The depositional history of Storr's Lake San Salvador, Bahamas. Unpublished PhD thesis, University of North Carolina.
- Zeebe, R. E., and Wolf-Gladrow, D. (eds.), 2001. *CO₂ in Seawater: Equilibrium, Kinetics and Isotopes*. Amsterdam: Elsevier, 346 pp.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomining](#)
[Microbial Mats](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Organomineralization](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Tidal Flats](#)
[Tufa, Freshwater](#)

MICROBIALITES, STROMATOLITES, AND THROMBOLITES

Robert Riding
 University of Tennessee, Knoxville, TN, USA

Microbialite Definition

Microbialites are “organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987, pp. 241–242).

Introduction

Microbial carbonates are produced by the interaction of microbial growth and metabolism, cell surface properties, and extracellular polymeric substances (EPS) with mineral precipitation and grain trapping. The early lithification that is essential for the accretion and preservation of benthic microbial carbonates is both biologically mediated and environmentally dependent. Consequently, microbialite history reflects not only microbial mat evolution, but also long-term changes in seawater and atmospheric chemistry that have influenced microbial metabolism and seawater carbonate saturation state.

Microbialites are in place benthic sediments produced by microbial processes. The term “microbialite” has been most widely used to describe carbonate stromatolites, thrombolites, and similar structures that occur as domes and columns in the shallow waters of lakes and seas, but it can also apply to many additional authigenic accumulations in which microbes are locally conspicuous, such as

some tufa, travertine, speleothem and spring, seep, and vent deposits. A series of terms and definitions were proposed between 1967 and 1987 in attempts to distinguish benthic sediments formed by microbial sediment trapping and/or precipitation. The first term, *cryptalgal*, was proposed by Aitken (1967, p. 1163) for rocks or rock structures “believed to originate through the sediment-binding and/or carbonate precipitating activities of non-skeletal algae.” The second was redefinition of *stromatolite* by Awramik and Margulis’ (1974) as “organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” Both Aitken (1967, p. 1163) and Awramik and Margulis (1974) used blue-green algae to indicate cyanobacteria, and therefore Kennard and James (1986, p. 496) replaced *cryptalgal* by *cryptomicrobial*. The third term was *microbialite* (Burne and Moore, 1987), which effectively repeated Awramik and Margulis’ (1974) definition of *stromatolite*, thereby broadening its scope. The primary focus of all these definitions was stromatolites and thrombolites.

Research during the 1900s revealed many details concerning the nature and history of stromatolites, but it also led to nomenclatural uncertainty as it became necessary to accommodate newly recognized deposits, such as thrombolites. Aitken (1967, p. 1164) sought to clarify use of “algal” in carbonate rock descriptions by distinguishing “rocks composed of the remains of skeletal calcareous algae . . . from those formed by noncalcareous blue-green . . . and green . . . algae.” He termed the latter *cryptalgal*, defined as rocks or rock structures “believed to originate through the sediment-binding and/or carbonate-precipitating activities of nonskeletal algae” (Aitken, 1967, p. 1163). Aitken (1967, Fig. 1) regarded both thrombolites and stromatolites as *cryptalgal* deposits, distinguishing by their respective clotted and laminated macrofabrics. However, Awramik and Margulis’ (1974) proposed a broader definition of *stromatolite* as “megascopic organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” This therefore subsumed thrombolite as a category within *stromatolite*.

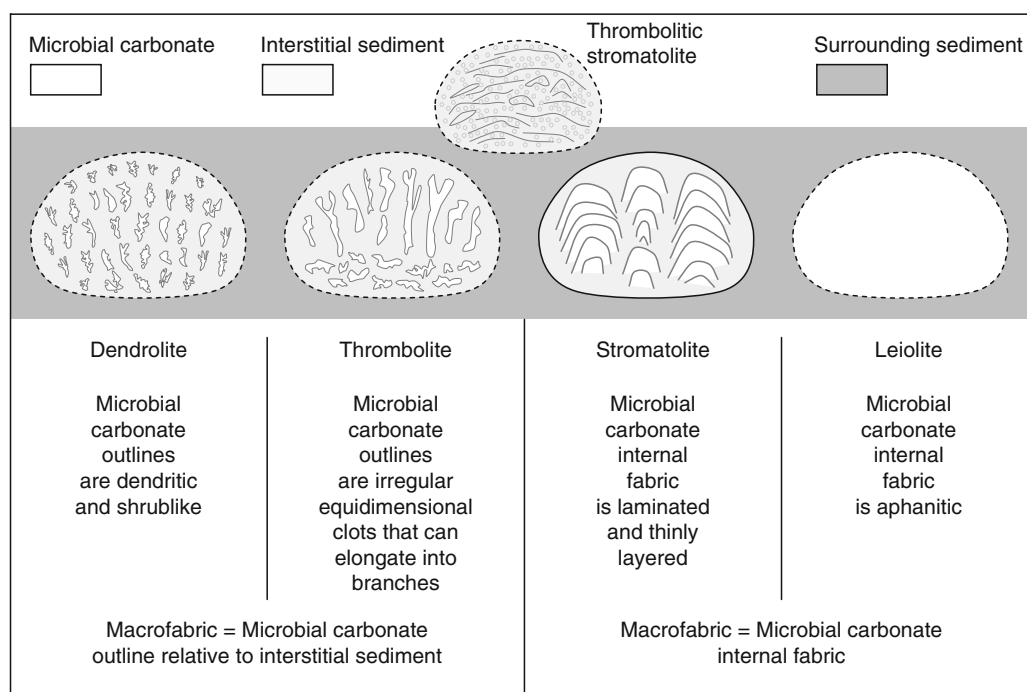
As a result of these and other developments, by the 1980s stromatolites could variously be regarded as (1) microbial and laminated (e.g., Kalkowsky, 1908; Hofmann, 1969; Krumbein, 1983), (2) microbial but not necessarily laminated (Awramik and Margulis, 1974), and (3) laminated but not necessarily organic (Semikhatov et al., 1979) (see Riding, 1999, p. 324). Since the wording of Burne and Moore’s (1987, pp. 241–242) definition of *microbialite* closely followed that of Awramik and Margulis’ (1974) definition of *stromatolite*, it therefore required return to a narrower definition of *stromatolite*, as layered or laminated structures. This relatively narrow definition was intended by Kalkowsky (1908) and is implied by his term *stromatolith*; *stromat*, Greek for “to spread out,” suggests a layer. Burne and Moore’s

(1987) new term, *microbialite*, encompassed more diverse benthic microbial deposits. The outcome of these developments was that *stromatolite* and *thrombolite* could be considered as equal categories within *microbialite*, mirroring Aitken’s (1967, Fig. 1) classification of *stromatolites* and *thrombolites* within *cryptalgal* carbonates. Subsequently, these and other varieties of *microbialite* have commonly been distinguished by their dominant internal macrofabric: *stromatolite* (laminated), *thrombolite* (clotted), *dendrolite* (dendritic), and *leiolite* (aphanitic) (Riding, 2000, pp. 189–195) (Figure 1).

Given the importance of stromatolites in the development of these concepts, this orderly arrangement of terms under the *microbialite* umbrella relied heavily on stromatolites being microbial structures. However, as soon as Kalkowsky’s (1908) article was published, researchers began to point out similarities between stromatolites and abiogenic precipitates (Reis, 1908; Bucher, 1918), and this chorus has continued ever since. Logan et al. (1964, p. 69) suggested recognition of “inorganic stromatolites”; Hofmann (1973, Fig. 5) showed the difficulty of distinguishing stromatolites from other laminites; and Semikhatov et al. (1979, p. 994) proposed a descriptive rather than purely genetic definition of *stromatolite*. Reports of essentially abiogenic stromatolites have persisted, especially in the Proterozoic (Grotzinger and Read, 1983; Grotzinger, 1989a; Pope et al., 2000). Attempts to separate abiogenic and biogenic stromatolites are obstructed by *hybrid stromatolites* (see below, *Stromatolites*) consisting of millimetric alternations of abiogenic crust and lithified microbial mat (Riding, 2008). Consequently, although microbial mat models seem to apply well to all or most thrombolites, dendrolites and leiolites, they do not account for all stromatolite-like deposits, particularly in the Precambrian. The implications of this complication for stromatolite interpretation as well terminology have still to be worked out.

Microbialites in space and time

Microbes occupy a very broad range of environments, including waters of widely differing chemistry and composition, and their involvement in sedimentation is equally varied. Most microbialites are carbonate (e.g., aragonite, calcite, dolomite) in composition, but siliceous, phosphatic, iron, manganese, and sulfate examples also occur. The microbes in microbialites are dominantly bacteria, including cyanobacteria, together with small algae. From the perspective of biocalcification, *microbialite* carbonates are bioinduced. Bacteria in general exert relatively weak control over the organic site and mineralogy of calcification, and present-day microbial carbonates appear to be most widespread in environments where precipitation is inorganically favored. This environmental dependence means that where rapid cooling and degassing strongly favor mineral precipitation irrespective of organic intervention, as in hot water springs and vents, the overall sedimentary contribution of microbes may be



Microbialites, Stromatolites, and Thrombolites, Figure 1 Microbial carbonates defined by macrofabric: leiolite (aphanitic), stromatolite (laminated), thrombolite (clotted), dendrolite (dendritic). Examples show domes and associated sediment. Not to scale. All categories are integrational. In addition to domes/mounds, overall shape can include columns, layers, and irregular masses. A complication is that leiolite and stromatolite macrofabrics are defined by features within (i.e., internal to) the microbial carbonate, such as lamination. In contrast, thrombolite and dendrolite macrofabrics are defined by the external shape of individual masses of microbial carbonate, such as clots or small shrub-like masses. Thrombolitic stromatolite (Aitken, 1967), typified by some Shark Bay columns, is internally weakly clotted and crudely laminated. It is essentially agglutinated, in contrast to Neoproterozoic and early Palaeozoic thrombolites with calcified microbial microfabrics. In the latter, the clots may be prostrate and irregular, and also vertically extended into amalgamated elongate branches (e.g., *Favosamacteria*).

relatively minor. In such cases, they influence the fabric more than the process of deposition. In contrast, where waters are less saturated for minerals, microbial activity can play a major role in sediment precipitation. These differing roles are reflected in the form and fabrics of the resulting deposits.

Sensitivity to environmental influence is also reflected in the secular distribution of microbialites. Without ruling out the influence of other factors – such as competition with other organisms – marine microbial carbonate abundance through time significantly reflects fluctuation in carbonate saturation state (Fischer, 1965; Grotzinger, 1990; Riding and Liang, 2005). Microbial carbonates can therefore be relatively sensitive proxies for seawater carbonate chemistry, and for atmospheric composition, since cyanobacterial sheath calcification is promoted by increased bicarbonate uptake (Merz, 1992) when CO_2 levels decline (Riding, 2006).

Scope

The term microbialite, with its emphasis on benthic microbial deposits, encompasses a wide range of sediments; what does it not cover? “Benthic” would seem to exclude both allochthonous and interstitial microbial sediments.

If so, then biogenic “whiting” deposits produced by photo-synthetically induced nucleation of small CaCO_3 crystals in the water column are not microbialites, and neither are diagenetic bacterially induced precipitates such as cements and concretions. However, these somewhat artificial distinctions could be blurred, as in some soil crust and beachrock deposits. On the other hand, “microbial” must exclude abiogenic stromatolites, identified in both Precambrian and Phanerozoic sediments (Grotzinger and Read, 1983; Pope et al., 2000). This is a complication, especially since microbialite (Burne and Moore, 1987) is based on a preexisting definition of stromatolite proposed by Awramik and Margulis (1974), and arose from a need for a general term to encompass stromatolite, thrombolite, and related deposits. Such difficulties of definition should be resolved by increased understanding of both present-day and ancient examples of these important and geologically widespread deposits.

Stromatolites

Definition

Stromatolites are laminated benthic microbial deposits (Riding, 1991).

Introduction

Kalkowsky (1908) proposed the term stromatolite (*Stromatolith*) for columns and domes in early Triassic playa lake oolites, but similar structures were already long known from examples such as those in the late Cambrian of New York State that Steele (1825, pp. 17–18, pl. 2) described as “calcareous concretions” and Hall (1883) named *Cryptozoon* (Figure 2). The search for present-day analogs of these ancient stromatolites led from freshwater tufa (Walcott, 1914; Roddy, 1915) and marsh deposits (Black, 1933) to marginal marine domes (Ginsburg et al., 1954) and columns (Logan, 1961). These discoveries strongly supported Kalkowsky’s (1908) inference that stromatolites are essentially microbial deposits. But optimism that they could provide appropriate analogs for all ancient marine stromatolites was tempered by studies of Precambrian examples that include significant abiogenic precipitated components (e.g., Serebryakov, 1976, p. 633; Grotzinger and Knoll, 1999, p. 314). Some definitions of stromatolite have therefore encompassed both microbial and abiogenic layered/laminated authigenic crusts that characteristically form domical and columnar morphologies.

Definitions of stromatolite range from that of Kalkowsky (1908) to that of Semikhatov et al. (1979, p. 993): “a stromatolite is . . . an attached, laminated, lithified, sedimentary growth structure, accretionary away from a point or limited surface of initiation. Although characteristically of microbial origin and calcareous composition, it may be of any origin, composition, shape, size, or age.” Understanding of Kalkowsky’s view has been hindered by a definition incorrectly attributed to him by Krumbein (1983, p. 499): “stromatolites are organogenic, laminated, calcareous rock structures, the origin of which is clearly related to microscopic life, which in itself must not be fossilised.” Kalkowsky (1908) did not write this (see Riding, 1999, p. 323), but it has been repeated as if it were a literal translation from his paper (e.g., Ginsburg, 1991, p. 25; Feldmann and McKenzie, 1998, p. 201; Grotzinger and Knoll, 1999, p. 316; McLoughlin et al.,

2008, p. 96). Compounding this mistake, the somewhat awkward wording (use of “must not” rather than “need not”) has been cited as “paradoxical,” and “confusion” to be avoided, and also as an example of the deficiencies of genetic definitions (Grotzinger and Knoll, 1999, p. 316; McLoughlin et al., 2008, p. 96). In his 1908 paper, Ernst Kalkowsky did not provide a specific definition of stromatolite, but he did repeatedly emphasize that it is a laminated organic structure. He thought that the life forms involved were “*niedrig organisierte pflanzliche Organismen*” (simple plantlike organisms, Kalkowsky, 1908, p. 125). It is therefore reasonable to conclude that Kalkowsky essentially regarded stromatolites as laminated microbial deposits (Riding, 1999).

However, at the same time that support was growing for Kalkowsky’s (1908) biogenic interpretation of stromatolites, the contrary view was also being expressed. Reis (1908) interpreted stromatolites as inorganic precipitates, Bucher (1918) compared them with hot spring sinter. Semikhatov et al. (1979, p. 994) were also concerned by the similarities between microbial stromatolites and “morphologically similar structures of nonmicrobial origin” such as those in caves and hot springs, and therefore suggested their descriptive definition. Subsequent research has shown that some stromatolite-like deposits are essentially abiogenic (e.g., Grotzinger and Read, 1983; Grotzinger and James, 2000, p. 9). Pope et al. (2000, p. 1139) contrasted microbial and abiogenic stromatolites, and considered “isopachous stromatolites to have been dominated by chemogenic precipitation in the absence of microbial mats, and the growth of peloidal stromatolites to have been controlled by sedimentation in the presence of microbial mats.”

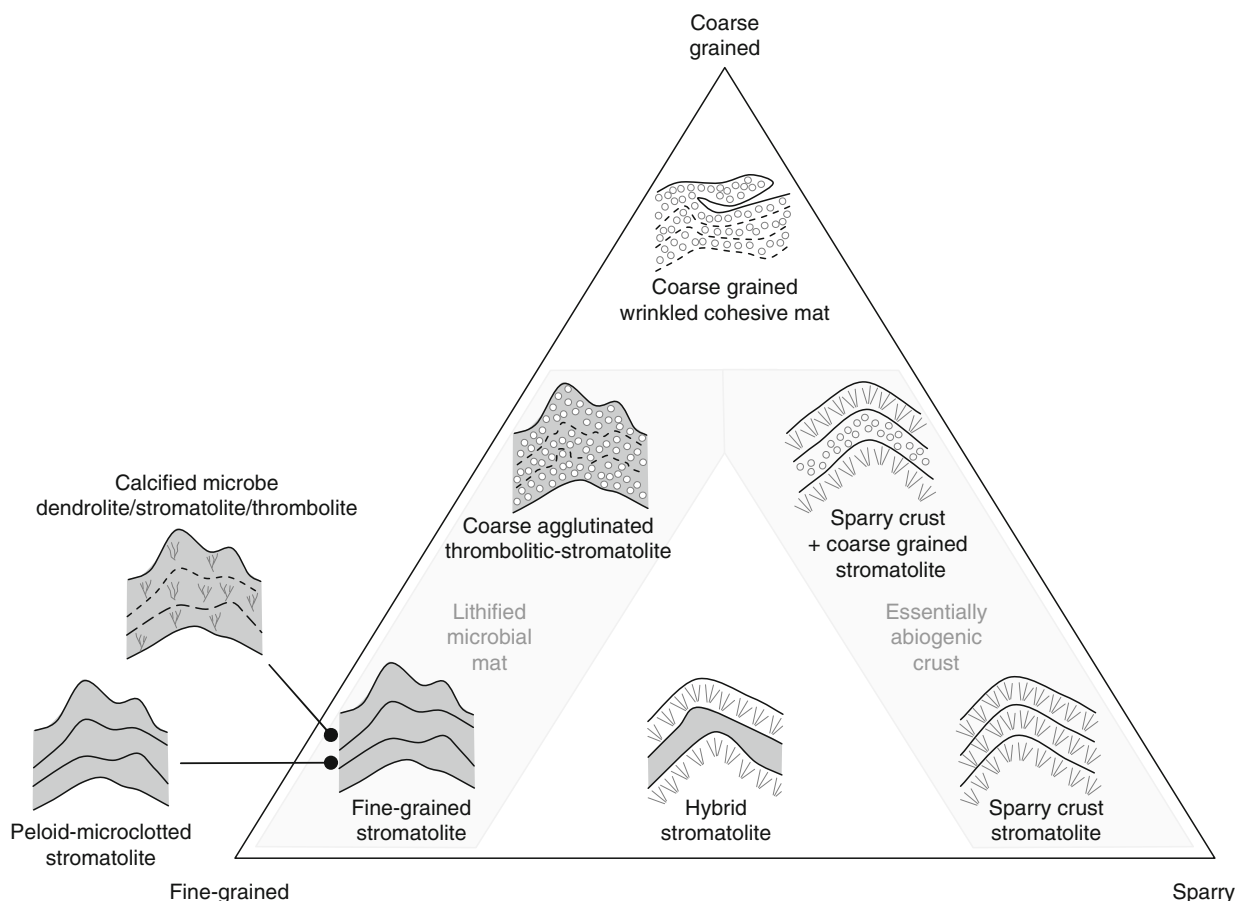
Fabric criteria can be used to distinguish abiogenic evenly layered sparry crust from fine-grained lithified microbial mats with uneven layering. In well-preserved examples, it should therefore be possible to tell abiogenic and biogenic stromatolites apart. However, as noted under microbialite, the millimetric interlayering of these two fabrics that can occur in hybrid stromatolites (Riding, 2008) makes such straightforward separation impossible. Consequently, stromatolites are regarded here, essentially, as laminated benthic microbial deposits, but they can contain abiogenic precipitates and be intimately inter-layered with them. In the marine realm, there is a strong secular perspective to this. Many Phanerozoic and Neoproterozoic stromatolites are probably essentially lithified microbial mats, whereas many older examples probably contain at least some precipitated abiogenic crust.



Microbialites, Stromatolites, and Thrombolites, Figure 2 Late Cambrian (late Franconian–early Trempealeauan) stromatolite (*Cryptozoon*), Hoyt Limestone, Petrified Gardens, Saratoga Springs, New York, USA. Coin diameter ~3 cm.

Stromatolite types

Stromatolite micro- and macrofabrics commonly intergrade with those of dendrolites and thrombolites (Figure 3). Their formation can involve up to three main processes: microbial precipitation, inorganic precipitation, and grain trapping (Figure 4). The main types represent



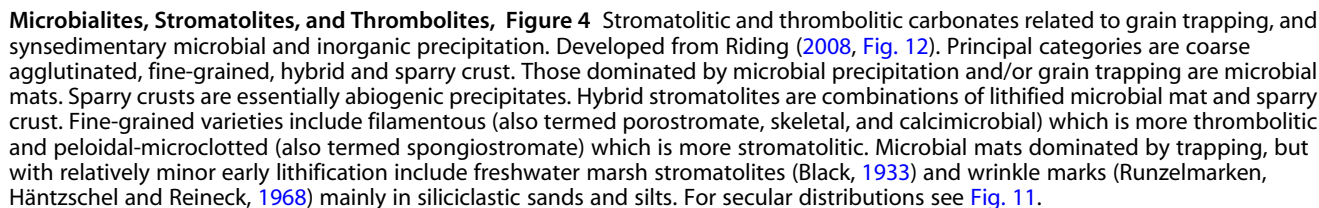
Microbialites, Stromatolites, and Thrombolites, Figure 3 Stromatolites and thrombolites based on micro/macrob fabric. All except wrinkled cohesive mats are syngedimentarily lithified by microbial and/or abiogenic processes. Fine-grained varieties include two distinctive types: (i) stromatolites with peloidal-microclotted microfabrics and (ii) dendrolites and thrombolites with abundant filamentous and other calcified microbial microfossils.

lithified microbial mat, sparry crust, and combinations of these (“hybrid stromatolite”).

Lithified microbial mat stromatolites exhibit a variety of intergradational fabrics. Two main types are fine-grained and coarse agglutinated. They form diverse stratiform, domical and columnar structures and, overall, tend to show relatively uneven to discontinuous layers with relatively poor inheritance and can include abundant fenestrae. Fine-grained stromatolites appear mainly to be products of syngedimentary microbial precipitation and are dominated by fine-grained (micrite, microspar) and filamentous fabrics. Fine-grained microfabrics are typically clotted and peloidal and are probably largely produced by heterotrophic bacterial calcification (e.g., dissimilatory sulfate reduction) of EPS and other cell products. Filamentous microfabrics are dominated by tubiform microfossils such as *Girvanella* that reflect photosynthetic cyanobacterial sheath calcification, but they too have fine-grained matrices. On their own, peloid-microclotted fabrics tend to be stromatolitic, and have been termed spongiostrome (Gürich, 1906; Pia, 1927, p. 36).

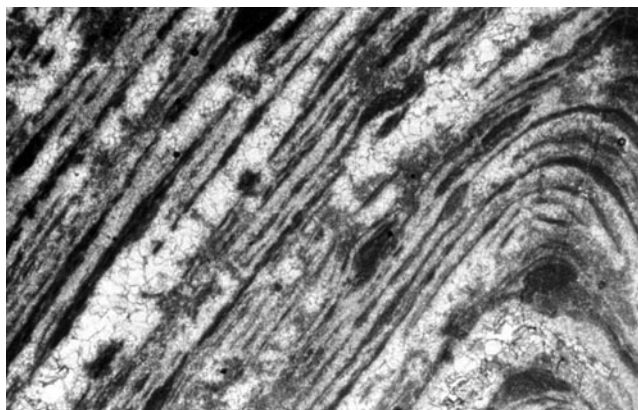
Filamentous fabrics tend to be more crudely layered and grade to thrombolitic. They have been termed porostromate (Pia, 1927, pp. 36–40), skeletal (Riding, 1977), and calcimicrobial (James and Gravestock, 1990). Coarse agglutinated stromatolites/thrombolites are produced by trapping sandy sediment by uncalcified EPS and erect filaments that can include microalgae. Present-day examples include some Shark Bay and Lee Stocking Island columns. They often have crudely layered macrofabrics (Logan, 1961) and have been termed thrombolitic stromatolites (Aitken, 1967).

Sparry crust (Riding, 2008) can form stromatolite-like abiogenic precipitates (Grotzinger and Rothman, 1996) characterized by even, often isopachous, laterally persistent layers with good inheritance (Pope et al., 2000). They have been most widely recognized in the Palaeoproterozoic and Mesoproterozoic (Grotzinger and Knoll, 1999, Fig. 6a, b), with microdigitate forms occupying peritidal environments (Grotzinger and Read, 1983) and isopachous laminite (Jackson, 1989) relatively deeper water facies.



it could resemble some *Conophyton* and *Baicalia* fabrics, as Bertrand-Sarfati (1976) suggested.

At the present day, intertidal and supratidal sediments are commonly colonized by cyanobacteria dominated mats in siliciclastic (e.g., Cameron et al., 1985; Stal et al., 1985), evaporitic (e.g., Gerdes et al., 2000), and carbonate (Black, 1933; Logan et al., 1974) environments (Figure 6). Where these mats are cohesive, but insufficiently early lithified, they do not show significant accretion but nonetheless stabilize layers of sediment. These microbialites that were unlithified during their formation can be preserved in place, and also imprinted and disrupted, e.g., by desiccation and water movement. They and their incorporated sediment may be cracked, curled, and folded, and this syndimentary deformation can be preserved after burial. Such patterned surfaces in



Microbialites, Stromatolites, and Thrombolites,
Figure 5 *Conophyton garganicum*. The fabric is interpreted as Hybrid stromatolite composed of alternating submillimetric layers of fine-grained lithified microbial mat and light-colored essentially abiogenic sparry layers. Middle Riphean, Russia, stratigraphic unit and locality not known. Specimen donated to Geological Survey of Canada by M.A. Semikhatov; photograph courtesy of Hans Hofmann. Width of view 8 mm.



Microbialites, Stromatolites, and Thrombolites,
Figure 6 Wrinkled microbial mat with carbonate sediment; supratidal flat, Crane Key, Florida Bay, USA. Red pen cap ~5 cm long.

Mesoproterozoic limestones were named *Kinneyia* by Walcott (1914, p. 107, pl. 11, Fig. 3) and subsequently linked to “Runzelmarken” (wrinkle marks, Häntzschel and Reineck, 1968) and other mat-related structures (Hagadorn and Bottjer, 1997). They have also been termed microbially induced sedimentary structures (MISS) (Noffke et al., 1996). The exact origins of these bedding plane structures are probably complex and varied (Porada et al., 2008) but they have been widely linked to deformation of microbial mats and have been used to infer the presence of mats in the Proterozoic (Horodyski, 1982) and Archaean (Noffke et al., 2006).

Siliciclastic stromatolites

In contrast to wrinkle structures, siliciclastic stromatolites can possess large domical morphologies and considerable synoptic relief. They are much scarcer than carbonate stromatolites, and are only known from mixed siliciclastic-carbonate environments that provide both siliciclastic grains and the early lithification required to maintain their support (Martín et al., 1993). Most examples are Palaeozoic, e.g., Ordovician (Davis, 1968), Devonian (Draganits and Noffke, 2004), Carboniferous (Bertrand-Sarfati, 1994), and Permian (Harwood, 1990), but diverse examples also occur in the late Miocene of South-east Spain (Martín et al., 1993; Braga and Martín, 2000). Based on the proportion of siliciclastic grains they contained, Martín et al. (1993) recognized three compositional types: “carbonate,” <10%; “siliciclastic-carbonate,” 10–50%; and “sandstone,” >50%. They are typically metric, locally decametric, domes with both stromatolitic and thrombolitic macrofabrics, associated with marginal marine beach (Braga and Martín, 2000), fan-delta and conglomeratic debris-flow deposits (Martín et al., 1993), and also with oolitic stromatolites–thrombolites (Braga et al., 1995). Sediment within the more siliciclastic domes includes abundant quartz, mica, and lithic fragment sand, and occasional quartz and metamorphic rock granules and pebbles.

Thrombolites

Definition

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Introduction

Aitken’s (1967) seemingly straightforward definition of thrombolite contained the seeds of more confusion than might have been anticipated. Since stromatolites are generally regarded as internally laminated; it could be expected that thrombolites are internally clotted. However, stromatolitic laminae are internal features of microbial carbonate, whereas “clot” could be used to describe both the external shape and internal structure of microbial carbonate (Figure 1). Aitken’s (1967) descriptions of Cambro-Ordovician examples, and his emphasis that thrombolite clotted fabric is macroscopic, have directed most researchers to regard “clots” as centimetric patches of microbial carbonate within interstitial sediment. In this view, a thrombolite dome consists of numerous such clots within lighter colored interstitial detrital matrix. Consequently, the dome has an overall macroclotted fabric, but the individual clots themselves are not necessarily internally composed of smaller clots, although they can be. This contrasts with stromatolitic domes in which each individual stromatolite is internally laminated and its shape is described separately, e.g., as domical, columnar, digitate, etc.

Aitken's (1967) concept of clots as patches of microbial carbonate within matrix is appropriate where they are irregularly rounded, but in digitate thrombolites the patches elongate into decimetric branches (e.g., Armella, 1994, Fig. 9) and use of "clot" to describe these is awkward. If some of Aitken's (1967) thrombolite domes contain dendritic fabrics, as seems likely, then why did he stress their clotted macrofabric? A likely explanation is that, in domes composed of radial branches, any section other than a vertical one through the dome center (e.g., Armella, 1994, Fig. 9) tends to show clot-like rounded outlines of oblique sections of branches. If this is correct, then to be consistent the branches should also be regarded as clots. This approach was followed by Armella (1994) and Kennard (1994) (although they used *thromboid* as an equivalent term to clot) to describe rounded lobate patches and elongate columns alike. There seems little doubt that this is what Aitken (1967) intended, and Shapiro (2000, p. 166) recognized that he used clot to describe the thrombolite columns themselves. Nonetheless, in describing similar branched Cambro-Ordovician thrombolites (*Favosamaceria*), Shapiro (2000) and Shapiro and Awramik (2006) broadened "clot" to refer to both millimetric clots within the branches and centimetric clots embedded in interstitial sediment. And there is a further complication. Aitken (1967) suggested that poorly laminated columns at Shark Bay are "thrombolitic stromatolites." As a result, in addition to describing Cambro-Ordovician domes with well-defined fine-grained clots, thrombolite has been applied to agglutinated present-day microbial columns in which the clots are much less distinct patchy fabrics.

These complications of usage still require clarification. At present, it is safe to say that "clot" (and the equivalent terms mesoclot and thromboid) has not been used consistently in thrombolite studies. It has been applied to millimetric patches within microbial carbonate, to centimetric lobate patches and also extended columns of microbial carbonate surrounded by detrital carbonate sediment, to transverse sections of these columns (here termed pseudoclots), and to diffuse patches of trapped sand, as well as to secondarily enhanced clots. Given these complications, it is understandable that earlier workers often referred to thrombolites as "unlaminated stromatolites" (e.g., Aitken, 1967, p. 1166; Pratt, 1982a; Schopf and Klein, 1992, p. 1202). Nonetheless, thrombolites can generally be regarded as benthic microbial carbonates with macroclotted fabric.

Thrombolite types

Two main types of thrombolite are calcified microbe and coarse agglutinated (Riding, 2000, pp. 192–193). Both intergrade with microbial mat stromatolites and primarily reflect the presence of major components that form irregular aggregates rather than thin layers. Nonetheless, the two types have quite distinct origins:

- (i) *Calcified microbe thrombolites* include the classic Cambro-Ordovician examples described by Aitken (1967) and also by Pratt and James (1982) and Kennard and James (1986). Aitken (1967) noted that burrows and trilobite fragments are common in these thrombolites, and examples of similar age often show mottled interiors and stromatolitic outer coatings (Figure 7). They consist of clots composed of framework whose most recognizable components are calcified microfossils, such as *Girvanella* filaments and *Renalcis* botryoids. The clots may be irregular centimetric amoeboid forms (Figure 8) or extend vertically into elongate (Armella, 1994, Fig. 9) meandriform and laterally amalgamated (Pratt and



Microbialites, Stromatolites, and Thrombolites, Figure 7 Late Cambrian (Trempealeauan) thrombolite column with mottled interior (within dotted line) and dark stromatolitic coating (between white arrows). Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA. Pen ~15 cm long.

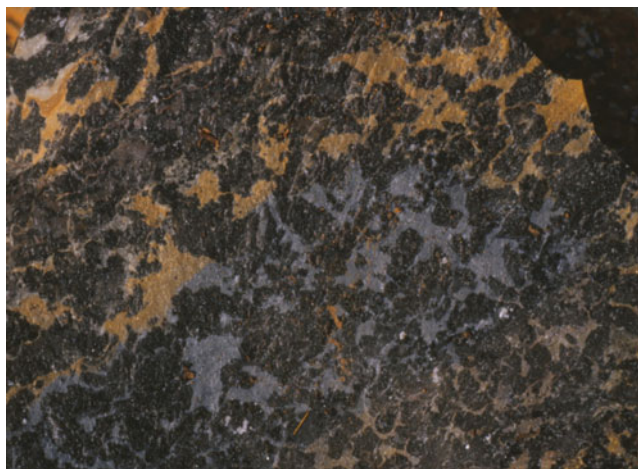


Microbialites, Stromatolites, and Thrombolites, Figure 8 Late Cambrian (Trempealeauan) thrombolite, Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA.

James, 1982, Figs. 9, 12) branches named *Favosamaceria* by Shapiro and Awramik (2006), (Figure 1). The clots are surrounded by detrital sediment infill. Progression from irregular clots to elongate branches has confused descriptive terminology. Shapiro (2000) suggested that in branched forms the clots typical of thrombolites are to be found within the branches, although Aitken (1967) seems to have regarded the columns as clots. James and Gravestock (1990) used “calcimicrobe” (calcified microbial microfossil) to refer to filamentous and botryoidal fossils that are common in Cambrian reefs. These typically form dendrolite (Figure 9) and thrombolite (Figure 10) fabrics and include calcified cyanobacteria such as *Angusticellularia*, *Botomaella*, and *Girvanella*, and also *Epiphyton* and *Renalcis*, whose affinities are less certain. Calcified microbe thrombolites are widespread in shallow marine



Microbialites, Stromatolites, and Thrombolites,
Figure 9 Dendrolite, Middle Cambrian, Zhangxia Formation,
near Jinan, Shandong, China



Microbialites, Stromatolites, and Thrombolites,
Figure 10 Thrombolite, early Cambrian, near Tiout, Anti-Atlas
Mountains, Morocco. Width of view 8 cm.

environments during the Neoproterozoic and early Palaeozoic.

- (ii) *Coarse agglutinated thrombolites* are largely composed of fenestral microbially trapped sandy sediment within finer-grained microbially lithified matrix. They are closely associated with coarse agglutinated stromatolites (Figures 3 and 4) that Aitken (1967, p. 1171) described as “thrombolitic-stromatolites.” They are only known in the late Neogene and their development appears to be linked to the rise of algal-cyanobacterial mats able to trap coarse sediment.

Coarse-grained thrombolites in the late Miocene of South-east Spain are closely associated with stromatolite fabrics in composite domes and columns up to 1.5 m high and 4 m across (Martín et al., 1993; Braga et al., 1995; Feldmann and McKenzie, 1997). They include oolitic examples in which clotted fabric is produced by irregular fenestrae up to 15 mm in size (Riding et al., 1991a, p. 123). Braga et al. (1995, Fig. 8, pp. 358–359) attributed the formation of these thrombolite fabrics to “a complex of irregular agglutination, microbial calcification, skeletal encrustation, and erosional processes.”

Logan (1961) regarded Shark Bay columns as stromatolites but recognized that the lamination is often poor, describing some as “crudely laminated with laminae of 1–10 mm. in thickness” (p. 526, pl. 1, Fig. 4). Playford and Cockbain (1976, p. 403) observed that “Hamelin Pool stromatolites range from unlaminated (thrombolites) to finely laminated; most show only crudely developed lamination.” Walter (1972, p. 64) attributed “the crude lamination of many Shark Bay stromatolites” to the thick irregular mats and coarse sediment. Logan et al. (1974, p. 154) related carbonate fabric to mat type, with thick irregular pustular mat dominated by *Entophysalis* in particular producing “unlaminated to poorly laminated” fenestral fabric, and Gebelein (1974) suggested that clotted fabric could reflect degradation of organic material. Similar Bahamian subtidal columns (Dravis, 1983) are coarse-grained and crudely laminated (Dill et al., 1986) and it has been suggested that abundant algae, including diatoms in these mats contribute to the coarse texture and poor lamination typical of both Shark Bay (Awramik and Riding, 1988) and Bahamian (Riding et al., 1991b) columns.

Mesozoic-Cenozoic thrombolites: Thrombolites are also common in the mid-late Jurassic (e.g., Leinfelder et al., 1993; Parcell, 2002; Kopaska-Merkel, 2003; Mancini et al., 2004), often in association with sponges and stromatolites in deeper water (e.g., Jansa et al., 1988; Leinfelder et al., 1994, p. 37; Dromart et al., 1994) and with corals in shallow-water (e.g., Bertling and Insalaco, 1998; Dupraz and Strasser, 1999; Olivier et al., 2003; Olivier et al., 2006; Helm and Schülke, 1998, 2006). They exhibit a variety of forms, including

arborescent and pendant that commonly occur as thick crusts on frame-building invertebrates, and are also closely associated with a variety of problematic encrusting organisms such as *Bacinella*, *Lithocodium*, and *Shamovella/Tubiphytes*, as well as annelids and foraminifers (Leinfelder et al., 1993, Fig. 6; Olivier et al., 2003, Fig. 4). Similar – but more often stromatolitic – crusts occur in late Neogene, including present-day, scleractinian reefs (Riding et al., 1991c; Montaggioni and Camoin, 1993; Leinfelder et al., 1993, pp. 222–224), and provide important clues to the formation of microbial carbonate microfabrics (Reitner, 1993; Reitner et al., 2000). In addition, the macrofabrics of these Mesozoic-Cenozoic examples broadly resemble those of early Palaeozoic calcified microbe thrombolites, but with significantly different skeletal components. Their microfabrics are fine-grained, with peloids and microclotted micrite (Olivier et al., 2006, Fig. 9), similar to those of many lithified microbial mat stromatolites.

Tufa thrombolite and stromatolite (Riding, 2000, pp. 191, 194) forms in calcifying lakes and streams as a result of cyanobacterial calcification that includes sheath impregnation and encrustation, together with calcification of associated microbes. It was comparisons with freshwater tufa that first led to recognition of links between stromatolites and cyanobacteria (Walcott, 1914; Roddy, 1915). These complex fabrics show considerable variety due to local differences in organisms and the extent of biocalcification and external encrustation. There are similarities between tufa stromatolites and hybrid crust stromatolites (Riding, 2008, p. 88) (Figure 4).

Kennard and James (1986, p. 498) noted that, although “poorly laminated and partially clotted microbial structures” occur on some present-day hypersaline marine shorelines (Laguna Mormona, Shark Bay) and in some lakes of various salinities (Great Salt Lake, Lake Clifton, Green Lake), in these examples “individual microbial clots are generally poorly defined” and “form an irregular, botryoidal-like, mesoscopic fabric that is unlike any of the fabrics observed by us in Lower Paleozoic thrombolites.” However, Moore and Burne (1994, pp. 23) considered that thrombolites in brackish to normal marine Lake Clifton, Western Australia, do closely resemble some early Palaeozoic examples in mesoclot shape and “the interframework of infilled fenestrae” and they pointed out that in Lake Clifton filamentous cyanobacteria (*Scytonema*) are important in thrombolite formation. Moore and Burne (1994, p. 21) noted that fine laminae, initially present in the Lake Clifton structures, are syndimentarily destroyed during mesoclot formation as carbonate precipitation continues, and also that the interframework fenestrae are intrinsic features “primarily related to the topography of the surface of the developing microbialite rather than to excavation of the structure by metazoan activity.” Great Salt Lake “algal mounds,” that consist of precipitated aragonitic framework and internal sediment (Halley, 1976, p. 439, Fig. 3), have a sub-centimetric thrombolitic fabric. Ferris et al. (1997)

suggested that thrombolitic fabrics in Kelly Lake, British Columbia, reflect a greater degree of calcification than in stromatolites.

Post-depositional thrombolites Aitken (1967, p. 1171) noted that thrombolite macroclots are prone to accentuation by recrystallization. This emphasizes a fundamental difference from stromatolites, whose laminae are essentially primary and not likely to be significantly enhanced by diagenesis. In contrast, clots could be significantly enhanced, e.g., by selective dolomitization, and in some cases secondarily produced, e.g., by bioturbation.

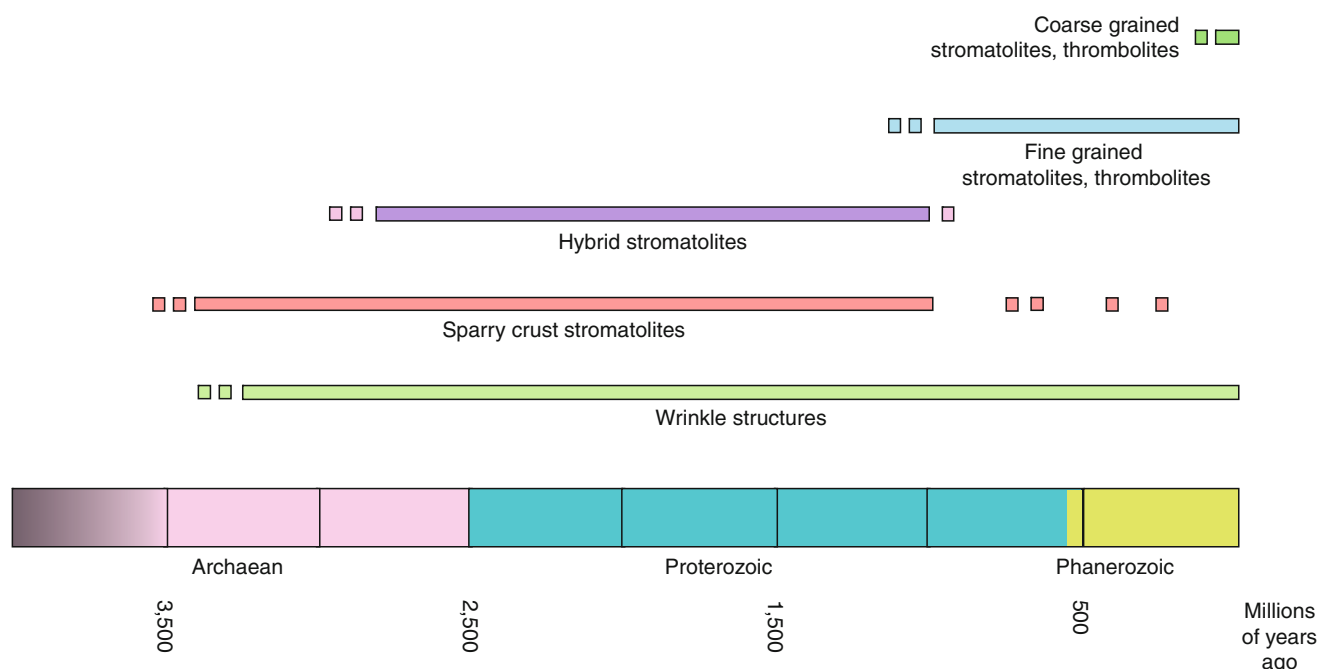
Stromatolites and thrombolites through time

Microbialite types are generally long ranging. Nonetheless, they show some distinctive differences in time distribution (Figure 11). Marine sparry crusts and hybrid stromatolites are typically Precambrian, and fine-grained stromatolites and thrombolites mainly range Neoproterozoic to the present day. Wrinkle structures show the longest range, whereas lithified coarse-grained carbonate thrombolites and stromatolites are only known from the past 10 Ma or less.

Archaean and Proterozoic

Stromatolites are relatively scarce in Archaean rocks until nearly the end of the eon. Their history begins with ~3.45 Ga coniform examples in the Pilbara region of Western Australia. These show fine continuous laminae (Lowe, 1980, 1983) and sparry microfabrics (Hofmann et al., 1999, Fig. 3). Their origins, and those of other Pilbara stromatolites, have been debated (e.g., Lowe, 1994, 1995; Buick et al., 1995), with recent studies supporting a biogenic origin (Hofmann et al., 1999, p. 1260–1261; Allwood et al., 2006, p. 717). Wrinkle and associated structures also suggest the presence of microbial mats in ~2,900 Ma siliclastic sediments of South Africa (Noffke et al., 2008), but these too are generally scarce. However, stromatolites are abundant in the ~2.55 Ga Campbellrand-Malmani carbonate platform of South Africa, locally forming elongate domes 10 m across and 40 m or more in length (Beukes, 1987, p. 9). In addition, these late Archaean carbonates contain distinctive “fenestrate microbialites” (Sumner and Grotzinger, 2004) consisting of millimetric to centimetric areas of light colored cement outlined by thin net-like layers (Figure 12). These have been interpreted as wispy convoluted microbial mats that were encrusted by calcite as they formed (Sumner, 1997a, b).

Large, often decametric, stromatolites are also conspicuous components of Proterozoic carbonate platforms (e.g., Grotzinger, 1986, p. 833; Petrov and Semikhatov, 2001, Fig. 6, p. 269). Where they are well preserved, they often show interlamination of sparry and micritic layers (e.g., Sami and James, 1996, p. 217). These dark-light layers that appear to represent alternations of lithified mat and abiogenic crust, are typical of “hybrid stromatolites” and are well seen in some *Conophyton* and *Baicalia* (Riding,



Microbialites, Stromatolites, and Thrombolites, Figure 11 Approximate time distributions of major categories of marine microbialites. Coarse agglutinated mats are mainly Neogene. Fine-grained mats and thrombolites are mainly Neoproterozoic and Phanerozoic. Marine “sparry” and “hybrid” crusts are mainly Archaean and Proterozoic. Wrinkle structures range Archaean to present-day. For some possible controls, see [Figure 14](#).

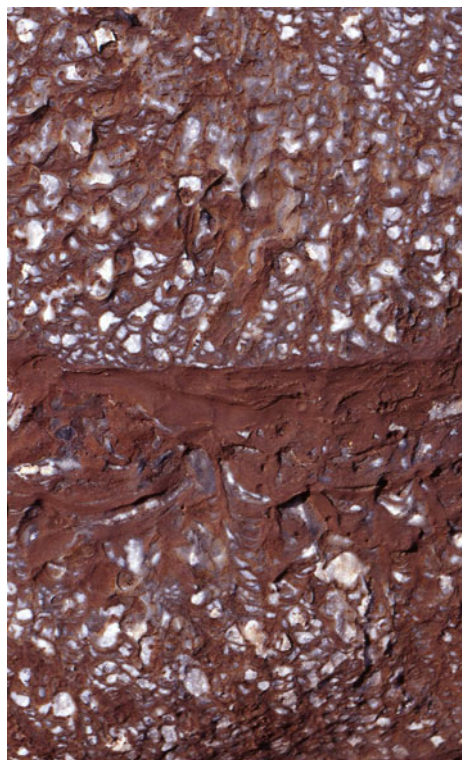
2008). Such incorporation of thin abiogenic sparry crusts appears to have contributed significantly to the size and relief of stromatolites throughout the Palaeo- and Mesoproterozoic. During the same period, extensive sheets of very small “microdigitate” stromatolites, typically <5 mm wide and <20 mm high (Hoffman, 1975, p. 262) were common in shallow peritidal environments ([Figure 13](#)). These “tufas” have been interpreted as essentially abiogenic (Grotzinger and Read, 1983).

An important control on Archaean-Proterozoic stromatolite formation was gradual decline in seawater carbonate saturation state (Grotzinger, 1989b; Grotzinger and Kasting, 1993). This progressively reduced stromatolite abundance (Grotzinger, 1990) and mediated a long-term trend from sparry crust to micritic carbonate sediments (Grotzinger and Kasting, 1993; Kah and Knoll, 1996). Transition to carbonate mud-dominated platforms ~1,400–1,300 Ma ago (Sherman et al., 2000) preceded the appearance of sheath-calcified cyanobacteria ~1,200 Ma ago (Kah and Riding, 2007). This significant transition, which led to Neoproterozoic development of calcimicrobial thrombolites (Aitken and Narbonne, 1989; Turner et al., 1993, 2000a, b), could reflect induction of CO₂-concentrating mechanisms (CCM) in cyanobacteria in response to fall in CO₂ levels below a threshold near ten times present-day levels (Riding, 2006) ([Figure 14](#)). CCMs are responses to reduced availability of inorganic carbon for photosynthesis, and in cyanobacteria include active bicarbonate uptake that

locally increases sheath pH, promoting calcification (Merz, 1992). Calcified filaments, such as *Girvanella*, altered microbial carbonate fabrics, disrupting stromatolite layering and promoting thrombolitic macro-clotted fabric. In subtidal environments, Neoproterozoic stromatolites are commonly interlayered with thrombolites with filamentous, clotted, and spongy “cellular” fabrics (Aitken and Narbonne, 1989) comparable with those of Cambro-Ordovician thrombolitic bioherms (Turner et al., 1993; 1997, p. 441, 449; 2000a, [Figs. 6e, 8h](#) and i).

Stromatolite decline

Stromatolites show long-term decline in abundance that may have commenced as early as the Palaeoproterozoic (Grotzinger, 1990) and is still observed in the Phanerozoic. Fischer (1965) suggested that decline since the Ordovician could reflect both reduction in carbonate saturation and competition by eukaryotes. Competition was subsequently emphasized when it appeared that marked late Proterozoic fall in stromatolite morphotypic diversity coincided with metazoan evolution (Awramik, 1971), but inception of decline prior to the appearance of metazoans implicates reduction in saturation state as the major influence (Grotzinger, 1990). It was also suggested that Cambrian thrombolites reflected disruption of stromatolites by burrowing organisms (Walter and Heys, 1985, pp. 150–151). However, Cambrian-Ordovician thrombolite fabrics are dominated by calcimicrobes that resist disruption (Kennard and James, 1986, p. 494) and



Microbialites, Stromatolites, and Thrombolites,
Figure 12 Fenestrate microbialite. Meshwork of large fenestrae (white) outlined by thin curved dark layers. Late Archaean Campbellrand-Malmani platform, South Africa. Width of field ~ 16 cm.



Microbialites, Stromatolites, and Thrombolites,
Figure 13 Microdigitate stromatolite, silicified after carbonate. Wumishan Formation, Mesoproterozoic, ~ 25 km north of Beijing, China. Width of view ~ 25 cm.

it has since been recognized that thrombolites appeared in the Neoproterozoic (Aitken and Narbonne, 1989), and possibly ~ 1.9 Ga in the Palaeoproterozoic (Kah and Grotzinger, 1992).

This leaves the question of the significance of trends in stromatolite diversity. Stromatolite shape reflects original

synoptic relief, determined by accretion rate relative to adjacent sediment (Figure 15). Low relative accretion rate results in low relief that makes stromatolites more prone to lateral incursion by sediment, thereby fostering complex shapes such as digitate forms (Figure 16). In contrast, high relative accretion rate results in high relief and simple shapes, such as domes and cones. Consequently, although mid-Proterozoic increase in morphotypic diversity, e.g., in branched stromatolites, has been regarded as a proxy for abundance, it more likely reflects low synoptic relief due to reduced relative accretion rate. Paradoxically, therefore, increased diversity could be sign that stromatolite growth was in decline due to reduced microbial growth and/or reduction in syndimentary lithification.

Snowball Earth

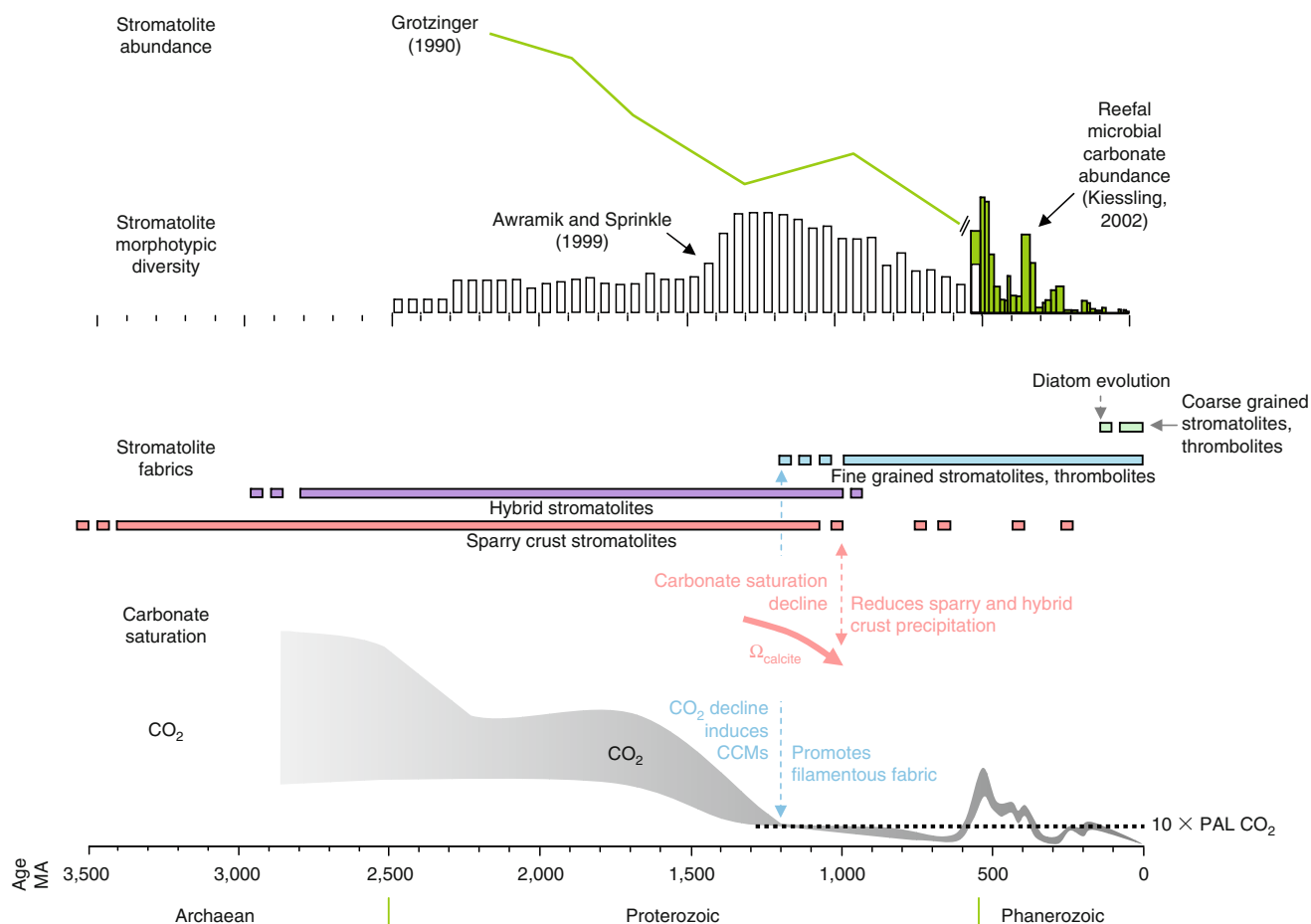
The prolonged warm Mesoproterozoic interval (Jenkins, 2003) was followed by Neoproterozoic (Sturtian, ~ 0.7 Ga Ma, Walter et al., 2000; Marinoan, ~ 0.635 Ga, Bodiselitsch et al., 2005) glaciations. Lower temperature and $p\text{CO}_2$ levels would have decreased seawater saturation state, hindering microbial calcification generally. Cooling would also have favored diffusive entry of CO_2 into cells and therefore may have slowed CCM development, further reducing cyanobacterial calcification. Nonetheless, microbialites are locally conspicuous in Cap Carbonates that immediately follow glacial deposits (e.g., Hoffman and Schrag, 2002; Corsetti and Grotzinger, 2005). Cap Carbonates have been suggested to reflect precipitation from seawater highly saturated for carbonate minerals as a result of alkaline upwelling and enhanced terrestrial weathering (Grotzinger and Knoll, 1995; Hoffman and Schrag, 2002). In the ~ 600 – 700 Ma Noonday Dolomite of California, narrow laterally amalgamated stromatolites define tubes of intervening detrital sediment fill (Figure 17). The overall organization of some Cambro-Ordovician *Favosamaceria* thrombolites (Shapiro and Awramik, 2006) resembles these “tubestones” (Corsetti and Grotzinger, 2005, p. 360).

Following Neoproterozoic “snowball” glaciations, global warming and O_2 rise could have reactivated CCM development, and rising temperature, calcium (Brennan et al., 2004), and $p\text{CO}_2$ (Bernier and Kothavala, 2001) levels would have increased seawater saturation state, stimulating microbial calcification (Riding, 2006). This favored microbialite resurgence, and dendrolites, thrombolites, and stromatolites all became widespread in the early Cambrian (Rowland and Shapiro, 2002) (Figure 18).

Phanerozoic

Secular distribution

Both reefal microbial carbonates (Kiessling, 2002, Fig. 16) and calcified cyanobacteria (Arp et al., 2001) decline in abundance during the Phanerozoic, but this trend shows marked fluctuations. They are common in the late Cambrian-early Ordovician and late Devonian-early Mississippian, and scarce during the Cenozoic.

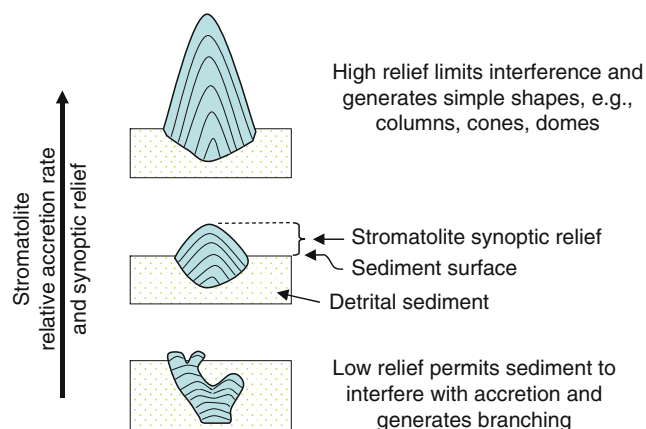


Microbialites, Stromatolites, and Thrombolites, Figure 14 Some secular trends and possible controls on microbial carbonate developments. Proterozoic stromatolite abundance (Grotzinger, 1990) and morphotypic diversity (Awramik and Sprinkle, 1999), together with Phanerozoic reefal microbial carbonate abundance (Kiessling, 2002). These suggest overall long-term decline for at least the past 1,000 Ma. Reduction in both Sparry Crust and Hybrid stromatolites ~1,000 Ma ago may reflect decline in seawater carbonate saturation (Grotzinger, 1990). Stromatolite-like sparry crust occurs sporadically in the Phanerozoic, particularly in evaporite basins (Pope et al., 2000). Increase in thrombolites ~1,200–1,000 Ma ago could reflect development of cyanobacterial sheath calcification, reflecting inception of CO₂-concentrating mechanisms (CCMs) as CO₂ levels declined to ~10 times present atmospheric level (PAL) (Riding, 2006). Inferred Proterozoic CO₂ trend based on Sheldon (2006), Kah and Riding (2007), Hyde et al. (2000) and Ridgwell et al. (2003). Phanerozoic CO₂ trend from Berner and Kothavala (2001, Fig. 13). Threshold for CCMs (10 times PAL CO₂) based on Badger et al. (2002). Late Neogene inception of Coarse agglutinated stromatolites and thrombolite could in part reflect incorporation of diatoms into microbial mats and also generally low values of seawater carbonate saturation.

Fischer (1965) suggested that eukaryote competition and reduction in carbonate saturation state contributed to stromatolite decline from the Ordovician onward.

Carbonate saturation: Comparison with seawater saturation state for CaCO₃ minerals calculated from modeled seawater and CO₂ values shows broad positive correspondence with peaks of microbial/cyanobacterial carbonate abundance during much of the Palaeozoic and Mesozoic (Riding and Liang, 2005), supporting Fischer (1965). Lack of correspondence during the interval ~120–80 Ma ago (when calculated saturation ratio is high but microbial carbonate abundance was low) could reflect removal of carbonate deposition by pelagic plankton that significantly reduced actual saturation state.

Competition: The role of metazoan competition in late Proterozoic and early Palaeozoic stromatolite history is uncertain, and it is debatable whether metazoan grazing significantly affected stromatolite development (Pratt, 1982b) so long as carbonate saturation was high enough to ensure extensive early lithification of microbial mats. Nonetheless, it seems likely that from at least the mid-Ordovician onwards, overgrowth by skeletonized algae and invertebrates inhibited the formation of domical stromatolites and thrombolites. Subsumed within complex reef structures, microbial carbonates would instead have formed patchy and irregular crusts on and around skeletal organisms. Nonetheless, they were often important reef components (Kiessling, 2002, Fig. 16).



Microbialites, Stromatolites, and Thrombolites,

Figure 15 Hypothetical interpretation of the significance of stromatolite shape. Shape is determined by synoptic relief that in turn reflects accretion rate of the stromatolite surface relative to adjacent sediment. In this view, enhanced stromatolite accretion generates high relief and simple shapes, such as columns, cones, and domes. In contrast, relatively low stromatolite accretion results in low relief. As a result, the stromatolite surface is prone to overlap by adjacent sediment, generating branching and irregular margins.



Microbialites, Stromatolites, and Thrombolites,

Figure 16 *Gymnosolen*, digitate stromatolite, Dalien, China, ~1.0 Ga.

Disaster biotas: The concept of stromatolite decline resulting from algal-metazoan diversification (Fischer, 1965; Garrett, 1970; Awramik, 1971) has also given rise to that of stromatolite resurgence in the aftermaths of mass extinctions (Schubert and Bottjer, 1992, p. 885). In this view, if metazoans can competitively exclude microbial carbonates then temporary reduction in metazoan abundance and diversity in the immediate aftermaths of mass



Microbialites, Stromatolites, and Thrombolites,

Figure 17 Laterally amalgamated columnar stromatolites (light color) separated by slightly darker tubular fills of detrital sediment. Noonday Dolomite, late Neoproterozoic, Mesquite Mountains, California, USA.

extinctions should permit temporary increase in microbial carbonates. Schubert and Bottjer (1992, 1995) interpreted early Triassic stromatolites as “post-mass extinction disaster forms.” However, whereas microbial carbonate reefal abundance also increased noticeably in the aftermath of late Devonian extinction, it did not increase following end-Ordovician, end-Triassic, and end-Cretaceous mass extinctions (Riding, 2006). Nonetheless, it is likely that in these situations, unconstrained by algal and invertebrate reef organisms, microbial mats were able to develop distinctive morphologies, and large domes, columns, and digitate structures have been reported, e.g., at the Permian-Triassic boundary in Sichuan, China (Kershaw et al., 1999).

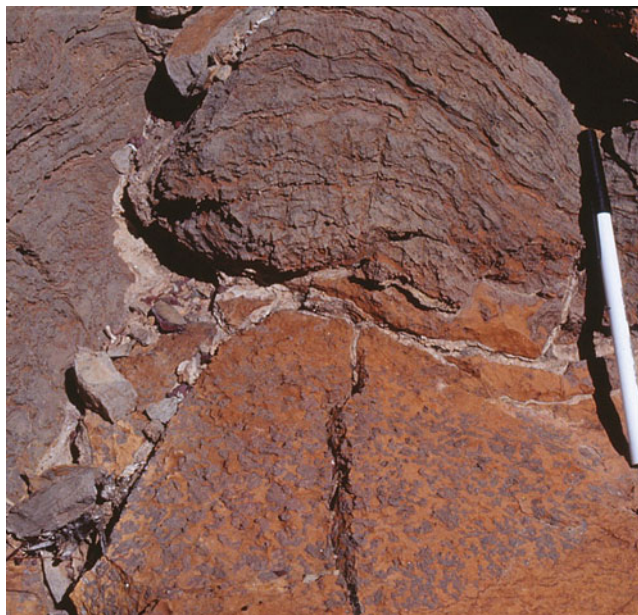
Thrombolites: Flügel (2004, p. 378) suggested that thrombolite abundance also declined after the Cambrian, although they were still locally conspicuous, e.g., in the Silurian (Kahle, 2001), Devonian (Shapiro, 2000, p. 166), Mississippian (Webb, 1987, 2005), and near the Permian-Triassic transition (Kershaw et al., 1999; Ezaki et al., 2008). Thrombolites have been widely reported in the mid-late Jurassic (see Mesozoic-Cenozoic thrombolites, above), broadly coincident with the last major peak of abundance of calcified marine cyanobacteria (Arp et al., 2001, Fig. 3d).

Evaporite stromatolites: In addition to metazoan extinction, localized increase in carbonate saturation in evaporite basins will favor microbialite development. Pope et al. (2000, p. 1139) noted that isopachously laminated stromatolites, which they considered to be dominantly abiogenic, are well developed in association with major evaporite successions and cited examples in the Proterozoic and Phanerozoic, including the Silurian Michigan Basin of North America and the late Permian Zechstein Basin of northern Europe (Pope et al., 2000,

Figs. 7, 9). These conditions therefore marked temporary returns to conditions that promoted formation of the sparry and hybrid crusts typical of the Archaean and early Proterozoic.

Neogene coarse-grained thrombolitic stromatolites

Well-known present-day examples of columns and domes occur in wave- and current-swept environments at Shark Bay (Logan, 1961) and the Bahamas (Dravis, 1983; Dill



Microbialites, Stromatolites, and Thrombolites, Figure 18 Late Cambrian (Trempealeauan) thrombolite overlain by stromatolite, Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA. Pen ~15 cm long.

et al., 1986; Riding et al., 1991b; Reid et al., 2000). They accrete bioclastic and ooid sand, and have crudely layered (Logan, 1961) and thrombolitic (Aitken, 1967, p. 1171) fabrics. Several factors conspire in their formation: water movement, mat community, and seawater chemistry. High-energy grainy conditions facilitate trapping by lifting sand to the accreting mat surface, and simultaneously deter overgrowth by reefal encrusters (Dill et al., 1989, p. 10). At Shark Bay, seasonal hypersalinity also limits competitors. Together with rapid accretion this allows decimetric, and locally metric, columns to develop. Accretion is high because the mats are thick and soft, with abundant EPS (Decho et al., 2005). In addition to cyanobacteria, they contain diatoms and filamentous green algae that enhance trapping ability (Awramik and Riding, 1988; Riding et al., 1991b). The upper mat remains soft and sticky because it is largely uncalcified, and the early lithification necessary to support these large columns mainly occurs in, or below, the lower part of the mat. Microbial lithification by sulfate reduction (Visscher et al., 2000) is limited to very thin micritic crusts (Reid et al., 2000), cyanobacterial sheaths are uncalcified (Reid et al., 2000, p. 992), and calcification of algal filaments mainly occurs in cavities (Dravis, 1983; Whittle et al., 1993, p. 224). Formation of these coarse-grained thrombolitic stromatolites is therefore largely due to their thick, soft, EPS-rich mats. These in turn reflect a combination of Cenozoic circumstances: (i) seawater saturation state that is too low for cyanobacterial sheath impregnation, but permits early lithification and (ii) the presence of fast-growing microalgae, such as diatoms, which – from a geological standpoint – are relative newcomers to mat communities (Figure 14). Coarse-grained thrombolitic stromatolite domes and columns are well developed in the late Miocene of South-east Spain (Riding et al., 1991a; Braga et al., 1995; Feldmann and McKenzie, 1997) (Figure 19) but are not known in older rocks.



Microbialites, Stromatolites, and Thrombolites, Figure 19 Coarse-grained, oolitic, composite leiolite-stromatolite-thrombolite domes, late Miocene (Messinian), Joyazo, Almería, South-east Spain. Red pen, lower left, ~15 cm long.

Summary

During the Archaean and much of the Proterozoic, both microbial mat growth and abiogenic precipitation were involved in stromatolite development that, locally, was particularly abundant from the latest Archaean to early Neoproterozoic (Grotzinger and Knoll, 1999). The combination of these biogenic and abiogenic factors was responsible for the rapid growth that enabled stromatolites, ranging from extensive small microdigitate sheets (Grotzinger and Read, 1983) to decametric domes (e.g., Hofmann, 1998, pl. 8a), to dominate carbonate platforms for 1,500 million years. Well-preserved stromatolites with dark-light lamination (Sami and James, 1996; Petrov and Semikhov, 2001) suggest that lithified mat growth alternated with abiogenic sparry crust precipitation on a millimetric scale to form inter-layered hybrid crust stromatolites (Riding, 2008).

Decline in sparry crusts and hybrid stromatolites ~1,000 Ma ago probably largely reflects reduction in seawater saturation (Grotzinger, 1990). This change also broadly coincided with the inception of cyanobacterial sheath-calcification in the Mesoproterozoic. This important development may reflect induction of CO₂-concentrating mechanisms (CCM) to assist photosynthetic carbon uptake as CO₂ levels declined (Riding, 2006). It transformed microbialite fabrics, and calcified microbe thrombolites and stromatolites with filamentous fabrics were conspicuous in shallow subtidal environments until the early Ordovician. Subsequently, stromatolites and thrombolites were widely subsumed within algal-invertebrate reefs. In these closely packed habitats, they lacked space to develop classic dome and column morphologies, and instead mainly formed reefal crusts and irregular masses. Only where competitors were environmentally excluded, or absent (as in mass extinction aftermaths), did microbial domes briefly develop extensively.

Both competition and declining carbonate saturation limited the abundance of marine microbialites from the Ordovician onwards (Fischer, 1965), and they are much less widespread and abundant in the Cenozoic than in the Palaeozoic. But microbialite communities have the ability to reinvent themselves. Diatoms together with other microalgae have significantly enhanced mat trapping ability. In environments where reefal overgrowth is limited, and coarse grains abundant, these soft mats can create large coarse-grained columns, as at Shark Bay and Lee Stocking Island. Internally these have distinctive crudely layered thrombolitic stromatolite fabrics (Aitken, 1967); in external shape, they closely resemble some Palaeoproterozoic stromatolites.

Microbialites have changed significantly during their extraordinarily long history in shape, size, fabrics, and abundance. They have responded to microbial evolution, to environmental changes that have affected both carbonate sedimentation and microbial metabolism, and to the evolution of other organisms. To add to this complexity, whereas stromatolites are essentially lithified microbial mats, they may be intimately associated with abiogenic crusts.

Microbialites archive important geobiological changes in atmospheric composition, seawater chemistry, mat evolution, and biotic interaction. Their study continues to offer many challenges and opportunities.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Aitken, J. D., and Narbonne, G. M., 1989. Two occurrences of Precambrian thrombolites from the Mackenzie Mountains, northwestern Canada. *Palaios*, **4**, 384–388.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–718.
- Armella, C., 1994. Thrombolitic-stromatolitic cycles of the Cambro-Ordovician boundary sequence, Precordillera Oriental Basin, western Argentina. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 421–441.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Awramik, S. M., 1971. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **174**, 825–827.
- Awramik, S. M., and Margulis, L., 1974. *Stromatolite Newsletter*, **2**, 5.
- Awramik, S. M., and Riding, R., 1988. Role of algal eukaryotes in subtidal columnar stromatolite formation. *Proceedings National Academy of Science USA*, **85**, 1327–1329.
- Awramik, S. M., and Sprinkle, J., 1999. Proterozoic stromatolites: the first marine evolutionary biota. *Historical Biology*, **13**, 241–253.
- Badger, M. R., Hanson, D., and Price, G. D., 2002. Evolution and diversity of CO₂ concentrating mechanisms in cyanobacteria. *Functional Plant Biology*, **29**, 161–173.
- Berner, R. A., and Kothavala, Z., 2001. GEOCARB III. A revised model of atmospheric CO₂ over Phanerozoic time. *American Journal of Science*, **301**, 182–204.
- Bertling, M., and Insalaco, E., 1998. Late Jurassic coral/microbial reefs from the northern Paris Basin - facies, palaeoecology and palaeobiogeography. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **139**, 139–175.
- Bertrand-Sarfati, J., 1976. An attempt to classify Late Precambrian stromatolite microstructure. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 251–259.
- Bertrand-Sarfati, J., 1994. *Siliciclastic-carbonate stromatolite domes in the Early Carboniferous of the Ajers Basin (eastern Sahara, Algeria)*. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic stromatolites II*. Dordrecht: Kluwer, pp. 395–419.
- Beukes, N. J., 1987. Facies relations, depositional environments and diagenesis in a major early Proterozoic stromatolitic carbonate platform to basinal sequence, Campbellrand Subgroup, Transvaal Supergroup, Southern Africa. *Sedimentary Geology*, **54**, 1–46.
- Black, M., 1933. The algal sedimentation of Andros Island Bahamas. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, **222**, 165–192.
- Bodiseltisch, B., Koeberl, C., Master, S., and Reimold, W. U., 2005. Estimating duration of Neoproterozoic snowball glaciations from Ir anomalies. *Science*, **308**, 239–242.
- Braga, J., and Martín, J. M., 2000. Subaqueous siliciclastic stromatolites a case history from Late Miocene beach deposits in the Sorbas Basin, SE Spain. In Riding, R., and Awramik, S. M. (eds), *Microbial sediments*, Berlin: Springer, pp. 226–232.

- Braga, J. C., Martín, J. M., and Riding, R., 1995. Controls on microbial dome fabric development along a carbonate-siliciclastic shelf-basin transect, Miocene, S.E. Spain. *Palaaios*, **10**, 347–361.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Bucher, W., 1918. On oölites and spherulites. *Journal of Geology*, **26**, 593–609.
- Buick, R., Groves, D. I., and Dunlop, J. S. R., 1995. Abiological origin of described stromatolites older than 3.2 Ga: comment and reply. *Geology*, **23**, 191.
- Burne, R. V., and Moore, L., 1987. Microbialites; organosedimentary deposits of benthic microbial communities. *Palaaios*, **2**, 241–254.
- Cameron, B., Cameron, D., and Jones, J. R., 1985. Modern algal mats in intertidal and supratidal quartz sands, northeastern Massachusetts, USA. In Curren, H. A. (ed.), *Biogenic Structures: Their Use in Interpreting Depositional Environments*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 35, pp. 211–235.
- Corsetti, F. A., and Grotzinger, J. P., 2005. Origin and significance of tube structures in Neoproterozoic post-glacial cap carbonates: Example from Noonday Dolomite, Death Valley, United States. *Palaaios*, **20**, 348–362.
- Davis, R. A., 1968. Algal stromatolites composed of quartz sandstone. *Journal of Sedimentary Petrology*, **38**, 953–955.
- Decho, A. W., Visscher, P. T., and Reid, R. P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 71–86.
- Dill, R. F., Kendall, C. G. St. C., and Shinn, E. A., 1989. Giant subtidal stromatolites and related sedimentary features. Field Trip Guidebook T373, 28th International Geological Congress, Washington, DC: American Geophysical Union, 33 pp.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P., 1986. Giant subtidal stromatolites forming in normal salinity waters. *Nature*, **324**, 55–58.
- Draganits, E., and Noffke, N., 2004. Siliciclastic stromatolites and other microbially induced sedimentary structures in an Early Devonian barrier-island environment (Muth Formation, NW Himalayas). *Journal of Sedimentary Research*, **74**, 191–202.
- Dravis, J. L., 1983. Hardened subtidal stromatolites, Bahamas. *Science*, **219**, 385–386.
- Dromart, G., Gaillard, C., and Jansa, L. F., 1994. Deep-marine microbial structures in the Upper Jurassic of western Tethys. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 295–318.
- Dupraz, C., and Strasser, A., 1999. Microbialites and microencrusts in shallow coral bioherms (Middle to Late Oxfordian, Swiss Jura mountains). *Facies*, **4**, 101–129.
- Ezaki, Y., Liu, J., Nagano, T., and Adachi, N., 2008. Geobiological aspects of the earliest Triassic microbialites along the southern periphery of the tropical Yangtze Platform: initiation and cessation of a microbial regime. *Palaaios*, **23**, 356–369.
- Feldmann, M., and McKenzie, J. A., 1997. Messinian stromatolite-thrombolite associations, Santa Pola, SE Spain: an analogue for the Palaeozoic? *Sedimentology*, **44**, 893–914.
- Feldmann, M., and McKenzie, J. A., 1998. Stromatolite-thrombolite associations in a modern environment, Lee Stocking Island, Bahamas. *Palaaios*, **13**, 201–212.
- Ferris, F. G., Thompson, J. B., and Beveridge, T. J., 1997. Modern freshwater microbialites from Kelly Lake, British Columbia, Canada. *Palaaios*, **12**, 213–219.
- Fischer, A. G., 1965. Fossils, early life, and atmospheric history. *Proceedings of the National Academy of Sciences*, **53**, 1205–1215.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks. Analysis, Interpretation and Application*. Berlin: Springer, xx + 976 pp.
- Garrett, P., 1970. Phanerozoic stromatolites: noncompetitive ecological restriction by grazing and burrowing animals. *Science*, **169**, 171–173.
- Gebelein, C. D., 1974. Biological control of stromatolite microstructure: implications for Precambrian time stratigraphy. *American Journal of Science*, **274**, 575–598.
- Gerdes, G., Krumbein, W. E., and Noffke, N., 2000. Evaporite microbial sediments. In R. Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 196–208.
- Ginsburg, R. N., 1991. Controversies about stromatolites: vices and virtues. In Muller, D. W., McKenzie, J. A., and Weissert, H. (eds.), *Controversies in Modern Geology; Evolution of Geological Theories in Sedimentology, Earth History and Tectonics*, London: Academic Press, pp. 25–36.
- Ginsburg, R. N., Isham, L. B., Bein, S. J., and Kuperberg, J., 1954. Laminated algal sediments of South Florida and their recognition in the fossil record. *Marine Laboratory, University of Miami, Coral Gables, Florida*, Unpublished Report, 54–20, 33 pp.
- Grotzinger, J. P., 1986. Cyclicity and paleoenvironmental dynamics, Rocknest platform, northwest Canada. *Geological Society of America Bulletin*, **97**, 1208–1231.
- Grotzinger, J. P., 1989a. Introduction to Precambrian reefs. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Areas*. Canadian Society of Petroleum Geologists Memoir 13, pp. 9–12.
- Grotzinger, J. P., 1989b. Facies and evolution of Precambrian carbonate depositional systems: emergence of the modern platform archetype. In Crevello, P. D., Wilson, J. L., Sarg, J. F., and Read, J. F. (eds.), *Controls on Carbonate Platform and Basin Development*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication 44, pp. 79–106.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., and James, N. P., 2000. Precambrian carbonates: evolution of understanding. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 3–20.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Grotzinger, J. P., and Knoll, A. H., 1995. Anomalous carbonate precipitates: is the Precambrian the key to the Permian? *Palaaios*, **10**, 578–596.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Reviews of Earth and Planetary Sciences*, **27**, 313–358.
- Grotzinger, J. P., and Read, J. F., 1983. Evidence for primary aragonite precipitation, lower Proterozoic (1.9-Ga) Rocknest Dolomite, Wopmay Orogen, Northwest Canada. *Geology*, **11**, 710–713.
- Grotzinger, J. P., and Rothman, D. R., 1996. An abiotic model for stromatolite morphogenesis. *Nature*, **383**, 423–425.
- Gürich, G., 1906. Les spongiostromides du Viséen de la Province de Namur. Musée Royal d'Histoire Naturelle de Belgique, *Mémoires*, **3**(4), 1–55, 13 pls.
- Hagadorn, J. W., and Bottjer, D. J., 1997. Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic-Phanerozoic transition. *Geology*, **25**, 1047–1050.
- Hall, J., 1883. *Cryptozoön*, n.g.; *Cryptozoön proliferum*, nsp. New York State Museum of Natural History, 36th Annual Report of the Trustees, plate 6.

- Halley, R. B., 1976. Textural variation within Great Salt Lake algal mounds. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology*, 20, Amsterdam: Elsevier, pp. 435–445.
- Häntzschel, W., and Reineck, H.-E., 1968. Fazies-Untersuchungen im Hettangium von Helmstedt (Niedersachsen). *Mitteilungen aus dem Geologischen Staatsinstitut in Hamburg*, **37**, 5–39.
- Harwood, G., 1990. 'Sandstone stromatolites' – an example of algal-trapping of sand grains from the Permian Yates Formation, New Mexico, U.S.A. Nottingham, England: 13th International Sedimentological Congress, Abstracts-Posters, p. 97.
- Helm, C., and Schülke, I., 1998. A coral-microbialite patch reef from the late Jurassic (*florigemma*-Bank, Oxfordian) of NW Germany (Süntel Mountains). *Facies*, **39**, 75–104.
- Helm, C., and Schülke, I., 2006. Patch reef development in the *florigemma*-Bank Member (Oxfordian) from the Deister Mts (NW Germany): a type example for Late Jurassic coral thrombolite thickets. *Facies*, **52**, 441–467.
- Hoffman, P. F., 1975. Shoaling-upward shale-to-dolomite cycles in the Rocknest Formation (lower Proterozoic), Northwest Territories, Canada. In Ginsburg, R. N. (ed.), *Tidal Deposits*. New York: Springer, pp. 257–265.
- Hoffman, P. F., and Schrag, D. P., 2002. The snowball Earth hypothesis: testing the limits of global change. *Terra Nova*, **14**, 129–155.
- Hofmann, H. J., 1969. Attributes of stromatolites. *Geological Survey of Canada Paper* 69–39, 58 pp.
- Hofmann, H. J., 1973. Stromatolites: characteristics and utility. *Earth Science Reviews*, **9**, 339–373.
- Hofmann, H. A., 1998. Synopsis of Precambrian fossil occurrences in North America. In Lucas, S. B., and St-Onge, M. R. (co-ords), *Geology of Canada*, no. 7, pp. 271–376.
- Hofmann, H. J., Grey, K., Hickman, A. H., and Thorpe, R. I., 1999. Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. *Geological Society of America Bulletin*, **111**, 1256–1262.
- Horodyski, R. J., 1982. Impressions of algal mats from the Middle Proterozoic Belt Supergroup, northwestern Montana, USA. *Sedimentology*, **29**, 285–289.
- Hyde, W. T., Crowley, T. J., Baum, S. K., and Peltier, W. R., 2000. Neoproterozoic 'snowball Earth' simulations with a coupled climate/ice-sheet model. *Nature*, **405**, 425–429.
- Jackson, M. J., 1989. Lower Proterozoic Cowles Lake foredeep reef, N.W.T., Canada. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Area*. Calgary: Canadian Society of Petroleum Geologists, Memoir 13, 64–71.
- James, N. P., and Gravestock, D. I., 1990. Lower Cambrian shelf and shelf margin build-ups, Flinders Ranges, South Australia. *Sedimentology*, **37**, 455–480.
- Jansa L. F., Pratt, B. R., and Dromart, G., 1988. Deep water thrombolite mounds from the Upper Jurassic of offshore Nova Scotia. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and adjacent areas*. Calgary: Canadian Society of Petroleum Geologists Memoir 13, 725–735.
- Jenkins, G. S., 2003. GCM greenhouse and high-obliquity solutions for early Proterozoic glaciation and middle Proterozoic warmth. *Journal of Geophysical Research*, **108**, D3, 4118, doi:10.1029/2001JD001582, 2003.
- Kah, L. C., and Grotzinger, J. P., 1992. Early Proterozoic (1.9 Ga) thrombolites of the Rocknest Formation, Northwest Territories, Canada. *Palaios*, **7**, 305–315.
- Kah, L. C., and Knoll, A. H., 1996. Microbenthic distribution of Proterozoic tidal flats: environmental and taphonomic considerations. *Geology*, **24**, 79–82.
- Kah, L. C., and Riding, R., 2007. Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. *Geology*, **35**, 799–802.
- Kahle, C. F., 2001. Biosedimentology of a Silurian thrombolite reef with meter-scale growth framework cavities. *Journal of Sedimentary Research*, **71**, 410–422.
- Kalkowsky, E., 1908. Oolith und Stromatolith im norddeutschen Buntsandstein. *Zeitschrift Deutschen geol. Gesellschaft*, **60**, 68–125, pls 4–11.
- Kennard, J. M., 1994. Thrombolites and stromatolites within shale-carbonate cycles, Middle–Late Cambrian Shannon Formation, Amadeus Basin, central Australia. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 443–471.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites; two distinct types of microbial structures. *Palaios*, **1**, 492–503.
- Kershaw, S., Zhang, T., and Lan, G., 1999. A microbialite crust at the Permian-Triassic boundary in south China, and its palaeoenvironmental significance. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **146**, 1–18.
- Kiessling, W., 2002. Secular variations in the Phanerozoic reef ecosystem. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 72, pp. 625–690.
- Kopaska-Merkel, D. C., 2003. "Reefs" as exploration targets in the Smackover Formation. *Gulf Coast Association of Geological Sciences Transactions*, **53**, 411–421.
- Krumbein, W. E., 1983. Stromatolites - the challenge of a term in space and time. *Precambrian Research*, **20**, 493–531.
- Leinfelder, R. R., Nose, M., Schmid, D. U., and Werner, W., 1993. Microbial crusts of the Late Jurassic: composition, palaeoecological significance and importance in reef construction. *Facies*, **29**, 195–229.
- Leinfelder, R. R., Krautter, M., Laternser, R., Nose, M., Schmid, D. U., Schweigert, G., Werner, W., Keupp, H., Brugger, H., Herrmann, R., Rehfeld-Kiefer, U., Schroeder, J. H., Reinhold, C., Koch, R., Zeiss, A., Schweizer, V., Christmann, H., Menges, G., and Luterbacher, H., 1994. The origin of Jurassic reefs: current research developments and results. *Facies*, **31**, 1–56.
- Logan, B. W., 1961. *Cryptozoon* and associated stromatolites from the Recent, Shark Bay, Western Australia. *Journal of Geology*, **69**, 517–533.
- Logan, B. W., Rezak, R., and Ginsburg, R. N., 1964. Classification and environmental significance of algal stromatolites. *Journal of Geology*, **72**, 68–83.
- Logan, B. W., Hoffman, P., and Gebelein, C. D., 1974. Algal mats, cryptalgal fabrics, and structures, Hamelin Pool, Western Australia. *American Association of Petroleum Geologists, Memoir*, **22**, 140–194.
- Lowe, D. R., 1980. Stromatolites 3,400–3,500 Myr old from the Archean of Western Australia. *Nature*, **284**, 441–443.
- Lowe, D. R., 1983. Restricted shallow-water sedimentation of early Archean stromatolitic and avaporitic strata of the Strelley Pool Chert, Pilbara Block, Western Australia. *Precambrian Research*, **19**, 239–283.
- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 387–390.
- Lowe, D. R., 1995. Abiological origin of described stromatolites older than 3.2 Ga: comment and reply. *Geology*, **23**, 191–192.
- Mancini, E. A., Llinas, J. C., Parcell, W. C., Aurell, M., Badenas, B., Leinfelder, R. R., and Benson, D. J., 2004. Upper Jurassic thrombolite reservoir play, northern Gulf of Mexico. *AAPG Bulletin*, **88**, 1573–1602.
- Martin, J. M., Braga, J. C., and Riding, R., 1993. Siliciclastic stromatolites and thrombolites, late Miocene, S.E. Spain. *Journal of Sedimentary Petrology*, **63**, 131–139.
- McLoughlin, N., Wilson, L. A., and Brasier, M. D., 2008. Growth of synthetic stromatolites and wrinkle structures in the absence of

- microbes – implications for the early fossil record. *Geobiology*, **6**, 95–105.
- Merz, M. U. E., 1992. The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**, 81–102.
- Montaggioni, L. F., and Camoin, G. F., 1993. Stromatolites associated with coralgal communities in Holocene high-energy reefs. *Geology*, **21**, 149–152.
- Monty, C. L. V., 1976. The origin and development of cryptalgal fabrics. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology* 20, Amsterdam: Elsevier, pp. 193–249.
- Moore, L. S., and Burne, R. V., 1994. The modern thrombolites of Lake Clifton, western Australia. In Bertrand Sarfati, J., and Monty, C. L. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer Academic Publishers, pp. 3–29.
- Noffke, N., Beukes, N., and Hazen, R., 2006. Microbially induced sedimentary structures in the 2.9 Ga old Brixton Formation, Witwatersrand Supergroup, South Africa. *Precambrian Research*, **146**, 35–44.
- Noffke, N., Beukes, N., Bower, D., Hazen, R. M., and Swift, D. J. P., 2008. An actualistic perspective into Archean worlds - (cyano-) bacterially induced sedimentary structures in the siliciclastic Nhlazatse Section, 2.9 Ga Pongola Supergroup, South Africa. *Geobiology*, **6**, 5–20.
- Noffke, N., Gerdes, G., Klenke, T., and Krumbein, W. E., 1996. Microbially induced sedimentary structures – examples from modern sediments of siliciclastic tidal flats. *Zentralblatt für Geologie und Paläontologie, Teil 1, 1995, Heft 1/2*, 307–316.
- Olivier, N., Hantzpergue, P., Gaillard, C., Pittet, B., Leinfelder, R. R., Schmid, D. U., and Werner, W., 2003. Microbialite morphology, structure and growth: a model of the Upper Jurassic reefs of the Chay Peninsula (Western France). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **193**, 383–404.
- Olivier, N., Lathuilière, B., and Thiry-Bastien, P., 2006. Growth models of Bajocian coral-microbialite reefs of Chargey-lès-Port (eastern France): palaeoenvironmental considerations. *Facies*, **52**, 113–127.
- Parcell, W. C., 2002. Sequence stratigraphic controls on the development of microbial fabrics and growth forms - implication for reservoir quality distribution in the Upper Jurassic (Oxfordian) Smackover Formation, Eastern Gulf Coast, USA. *Carbonates and Evaporites*, **17**, 166–181.
- Petrov, P. Yu., and Semikhatov, M. A., 2001. Sequence organization and growth patterns of late Mesoproterozoic stromatolite reefs: an example from the Burovaya Formation, Turukhansk Uplift, Siberia. *Precambrian Research*, **111**, 257–281.
- Pia, J., 1927. Thallophyta. In Hirmer, M. (ed.), *Handbuch der Paläobotanik* 1, Munich: Oldenbourg, pp. 31–136.
- Playford, P. E., and Cockbain, A. E., 1976. Modern algal stromatolites at Hamelin Pool, a hypersaline barred basin in Shark Bay, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 389–411.
- Pope, M. C., Grotzinger, J. P., and Schreiber, B. C., 2000. Evaporitic subtidal stromatolites produced by in situ precipitation: textures, facies associations, and temporal significance. *Journal of Sedimentary Research*, **70**, 1139–1151.
- Porada, H., Ghergut, J., and Bouougri, E. H., 2008. Kinneyia-type wrinkle structures – critical review and model of formation. *Palaos*, **23**, 65–77.
- Pratt, B. R., 1982a. Stromatolitic framework of carbonate mud-mounds. *Journal of Sedimentary Research*, **52**, 1203–1227.
- Pratt, B. R., 1982b. Stromatolite decline – a reconsideration. *Geology*, **10**, 512–515.
- Pratt, B. R., and James, N. P., 1982. Cryptalgal-metazoan bioherms of early Ordovician age in the St. George Group, western Newfoundland. *Sedimentology*, **29**, 543–569.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reis, O. M., 1908. Kalkowsky: Ueber Oolith und Stromatolith im norddeutschen Buntsandstein. *Neues Jahrbuch für Mineralogie, Geologie und Paläontologie*, **2**, 114–138.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia); formation and concepts. *Facies*, **29**, 3–39.
- Reitner, J., Thiel, V., Zankl, H., Michaelis, W., Wörheide, G., and Gautret, P., 2000. Organic and biogeochemical patterns in cryptic microbialites. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*, Berlin: Springer, pp. 149–160.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Riding, R., 1977. Skeletal stromatolites. In Flügel, E. (ed.), *Fossil Algae, Recent Results and Developments*, Berlin: Springer-Verlag, pp. 57–60.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R., (ed.), *Calcareous algae and stromatolites*. Berlin: Springer-Verlag, pp. 21–51.
- Riding, R., 1999. The term stromatolite: towards an essential definition. *Lethaia*, **32**, 321–330.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47** (Suppl. 1), 179–214.
- Riding, R., 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology*, **4**, 299–316.
- Riding, R., 2008. Abiogenic, microbial and hybrid authigenic carbonate crusts: components of Precambrian stromatolites. *Geologia Croatica*, **61**(2–3), 73–103.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Riding, R., Braga, J. C., and Martín, J. M., 1991a. Oolite stromatolites and thrombolites, Miocene, Spain: analogues of Recent giant Bahamian examples. *Sedimentary Geology*, **71**, 121–127.
- Riding, R., Awramik, S. M., Winsborough, B. M., Griffin, K. M., and Dill, R. F., 1991b. Bahamian giant stromatolites: microbial composition of surface mats. *Geological Magazine*, **128**, 227–234.
- Riding, R., Martín, J. M., and Braga, J. C., 1991c. Coral stromatolite reef framework, Upper Miocene, Almería, Spain. *Sedimentology*, **38**, 799–818.
- Roddy, H. J., 1915. Concretions in streams formed by the agency of blue-green algae and related plants. *Proceedings American Philosophical Society*, **54**, 246–258.
- Rowland, S. M., and Shapiro, R. S., 2002. Reef patterns and environmental influences in the Cambrian and earliest Ordovician. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, **72**, pp. 95–128.
- Sami, T. T., and James, N. P., 1996. Synsedimentary cements as Paleoproterozoic platform building blocks, Pethei Group, northwestern Canada. *Journal of Sedimentary Research*, **66**, 209–222.
- Schopf, J. W., and Klein, C., 1992. Glossary of technical terms. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge, UK: Cambridge University Press, pp. 1189–1204.
- Schubert, J. K., and Bottjer, D. J., 1992. Early Triassic stromatolites as post-mass extinction disaster forms. *Geology*, **20**, 883–886.

- Semikhatov, M. A., Gebelein, C. D., Cloud, P., Awramik, S. M., and Benmore, W. C., 1979. Stromatolite morphogenesis - progress and problems. *Canadian Journal of Earth Science*, **16**, 992–1015.
- Serebryakov, S. N., (1976) Biotic and abiotic factors controlling the morphology of Riphean stromatolites. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology* 20, Amsterdam: Elsevier, pp. 321–336.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaaios*, **15**, 166–169.
- Shapiro, R. S., and Awramik, S. M., 2006. *Favosamaceria cooperi* new group and form: a widely dispersed, time-restricted thrombolite. *Journal of Paleontology*, **80**, 411–422.
- Sheldon, N. D., 2006. Precambrian paleosols and atmospheric CO₂ levels. *Precambrian Research*, **147**, 148–155.
- Sherman, A. G., James, N. P., and Narbonne, G. M., 2000. Sedimentology of a late Mesoproterozoic muddy carbonate ramp, northern Baffin Island, Arctic Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 275–294.
- Stal, L. J., van Gernerden, H., and Krumbein, W. E., 1985. Structure and development of a benthic marine microbial mat. *FEMS Microbiology Ecology*, **31**, 111–125.
- Steele, J. H., 1825. A description of the Oolitic Formation lately discovered in the county of Saratoga, and state of New-York. *American Journal of Science*, **9**, 16–19, part of pl. 2.
- Sumner, D. Y., 1997a. Carbonate precipitation and oxygen stratification in late Archean seawater as deduced from facies and stratigraphy of the Gamohaan and Frisco formations, Transvaal Supergroup, South Africa. *American Journal of Science*, **297**, 455–487.
- Sumner, D. Y., 1997b. Late Archean calcite-microbe interactions: two morphologically distinct microbial communities that affected calcite nucleation differently. *Palaaios*, **12**, 302–318.
- Sumner, D. Y., and Grotzinger, J. P., 2004. Implications for Neoproterozoic ocean chemistry from primary carbonate mineralogy of the Campbellrand-Malmani platform, South Africa. *Sedimentology*, **51**, 1–27.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., James, N. P., and Narbonne, G. M., 1997. Growth dynamics of Neoproterozoic calcimicrobial reefs, Mackenzie mountains, northwest Canada. *Journal of Sedimentary Research*, **67**, 437–450.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N.W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaaios*, **15**, 87–111.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Walcott, C. D., 1914. Cambrian geology and paleontology III: Precambrian Algonkian algal flora. *Smithsonian Miscellaneous Collection*, **64**, 77–156.
- Walter, M. R., 1972. Stromatolites and the biostratigraphy of the Australian Precambrian and Cambrian. *Special Papers in Palaeontology*, **11**, 190 pp, 33 pls.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of the Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research*, **100**, 371–433.
- Webb, G. E., 1987. Late Mississippian thrombolite bioherms from the Pitkin Formation of northern Arkansas. *Geological Society of America, Bulletin*, **99**, 686–698.
- Webb, G. E., 2005. Quantitative analysis and paleoecology of earliest Mississippian microbial reefs, lowermost Gudman formation, Queensland, Australia: not just post-disaster phenomena. *Journal of Sedimentary Research*, **75**, 875–894.
- Whittle, G. L., Kendall, C. G. St. C., Dill, R. F., and Rouch, L., 1993. Carbonate cement fabrics displayed: a traverse across the margin of the Bahama Platform near Lee Stocking Island in the Exuma Cays. *Marine Geology*, **110**, 213–243.

Cross-references

[Biofilms](#)
[Calcified Cyanobacteria](#)
[Cap Carbonates](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Organomineralization](#)
[Snowball Earth](#)

MICROBIAL-METAL BINDING

Kurt O. Konhauser, David A. Fowle
 University of Alberta, Edmonton, Alberta, Canada

Definition

The accumulation of metal cations to microbes, largely through adsorptive processes to the outer cellular surfaces.

Overview

All bacteria have low isoelectric points (below pH 2 in most cases), and consequently, they interact with soluble metal cations and have them intimately associated with their surfaces (Harden and Harris, 1953). Considering their ubiquity in the near-surface environment and their characteristically large surface area-to-volume ratios, bacteria can have a significant influence on metal mobility and speciation in these settings.

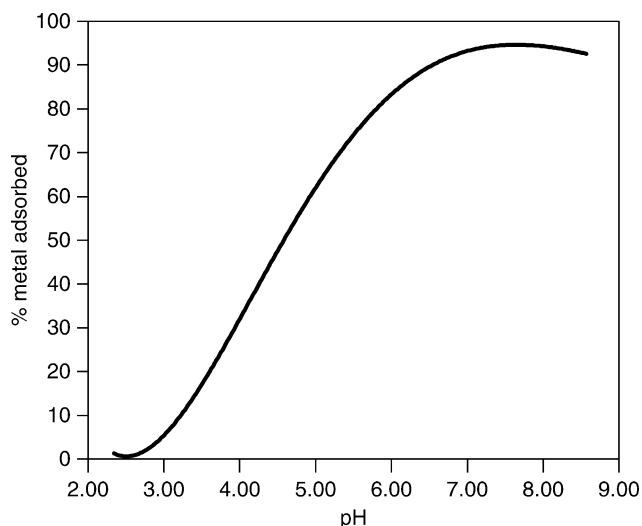
Some bound metals (e.g., Ca and Mg) serve the purpose of stabilizing the negative charges of the anionic functional groups, and thus are relatively “fixed” into place, while other metals are much more exchangeable and provide a temporary positive charge to counter the negative charge induced by the deprotonation of the cell’s surface functional groups (Carstensen and Marquis, 1968). The strength of the metal–microbial bond is quantified by the surface complexation/binding constant (K_M), where M refers to the specific metal of interest. The greater its surface complex formation constant, the less likely

a metal cation will be desorbed into solution. Metal cation sorption is also directly affected by pH, which dictates metal partitioning (or speciation) between solid and soluble phases, and hence, controls its mobility, reactivity, and toxicity in aquatic environments. Of particular importance here is the hydrolysis constant, which measures the tendency of proton release from the hydration sphere of the cation. Figure 1 depicts a generalized adsorption edge curve for a metal binding to a bacterial surface. As pH increases, the cell wall deprotonates and adsorption of the positively charged metal cation increases, but in some instances, adsorption may actually decrease at high pH values as metal hydrolysis is enhanced.

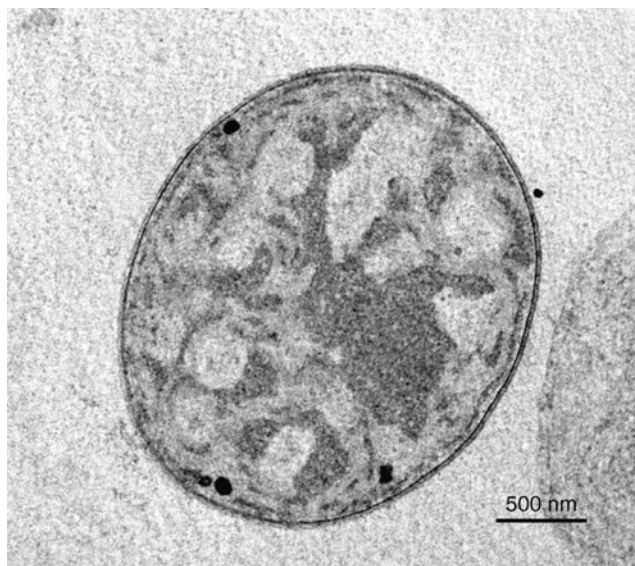
Much of our understanding about how bacteria bind metals stems from the pioneering work of Terry Beveridge on metal accumulation by the Gram-positive bacterium, *Bacillus subtilis* (Beveridge and Murray, 1976). His research demonstrated that it was the carboxyl groups within the peptidoglycan that were the most electronegative sites, and that the bulk of the binding capacity was associated with the cell wall. In similar studies with Gram-negative cells, such as *Escherichia coli*, it was demonstrated that they did not bind as much metal from solution as did their Gram-positive counterparts, a pattern stemming simply from the smaller amounts of peptidoglycan associated with the *E. coli* cell walls (Beveridge and Koval, 1981). Many Gram-positive and Gram-negative bacteria also produce extracellular polymers (EPS), and due to their hydrated nature, dissolved metals can freely diffuse throughout the structure, binding to the anionic carboxyl groups of uronic acids and the neutrally charged hydroxy groups of sugars (Geesey and Jang, 1989). By possessing a large and reactive surface area, it is thus not

unexpected that a number of studies have also documented that encapsulated bacteria bind more metals than nonencapsulated varieties (Rudd et al., 1983). Indeed, species producing EPS can tolerate higher metal concentrations than those that do not, and it has been shown that the proportion of encapsulated bacteria increases in metal-polluted sediment, whereas mutants that cannot produce capsules die off (Aislabie and Loutit, 1986).

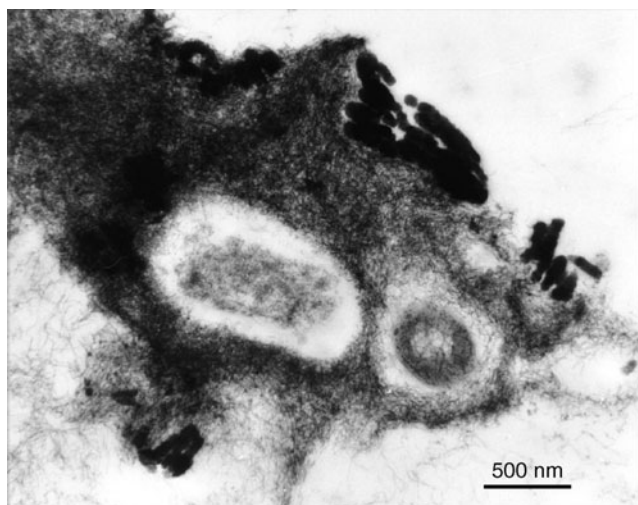
Irrespective of the bacteria studied, what was repeatedly observed was that considerable variations in metal immobilization could be displayed by a single species. In some instances, cell walls were diffusely stained (Figure 2), while at other times so much metal was fixed to the cell surface that it formed a distinct mineral phase (Figure 3). This led Beveridge and Murray (1976) to originally propose a two-step mechanism for the metal adsorption process; the first step in time is an electrostatic interaction between the metal cations and the anionic sites in the cell wall/EPS. This interaction then acts as a nucleation site for the deposition of more metal cations (and anions) from solution, potentially leading to biomineralization. The size of the deposit depends on a number of variables, including the concentration of ions and the amount of time through which the reactions proceed. If sufficient time exists, then the mineral product grows in size within the intermolecular spaces until it is physically constrained by the organic polymers. The end result is a bacterial wall/EPS that contains copious amounts of metal, often approaching the mass of the bacterial cell itself (Beveridge, 1984).



Microbial-Metal Binding, Figure 1 Generic adsorption curve for a metal cation binding to a bacterial surface. Metal adsorption increases as pH increases and cell wall functional groups deprotonate.



Microbial-Metal Binding, Figure 2 Transmission electron micrograph (TEM) of the lab-cultured cyanobacterium *Anabena* sp. that was stained with uranium acetate and lead citrate to reveal cell ultrastructure. Note the dark staining of the cell wall and some of the cytoplasmic constituents.



Microbial-Metal Binding, Figure 3 TEM image of two unidentified bacteria surrounded by iron-stained extracellular polymers. The dark grains at the top right are ferric hydroxide, $\text{Fe}(\text{OH})_3$. These cells grew naturally in Fe-rich hydrothermal fluids at the Lysuholl hot spring in Iceland. From Konhauser (2007).

Although binding of cations to a microbial surface is largely an electrostatic phenomenon, the structural and compositional complexity of the wall or EPS, as well as the unique physicochemical properties of each element, adds a level of complexity to the overall process. In other words, protons and each different cation should be capable of interacting in a distinctive way with surface ligands, such that a cell's surface will display varying affinities for binding different cations. Moreover, in natural systems, a multitude of cations (and anions) exist that should compete with one another for complex formation. This realization has led to a number of metal binding studies that have compared the relative affinities of protons and various cations for the anionic ligands on the cell surfaces of different microorganisms, by techniques that involve displacing one by another. Those studies have highlighted two very important points, the first being that metals and protons compete for the same surface sites, and as solution pH decreases, the functional groups become protonated, displacing loosely bound metal cations (Fowle and Fein, 2000). The second finding is that microbial-metal interactions are largely abiotic, nonspecific, and reliant upon thermodynamics (Warren and Haack, 2001). Some of the most important factors that influence metal binding to cells are (1) the ionic potential of the solution, (2) cell wall/EPS ligand spacing and their stereochemistry, (3) the ligand composition, and (4) the balance between the initial electrostatic attractions between a soluble metal cation and the organic ligands, and the subsequent covalent forces that arise from electron sharing across a metal cation-ligand molecular orbital (Williams, 1981). These properties are largely understood, and given

sufficient information about the environment in which a microorganism is growing, it is possible to extrapolate and predict metal binding patterns on a cell surface.

In the past decade, metal binding experiments have begun to emphasize the stability constants for metal-organic ligand interactions and elucidate how metal binding correlates with cell surface reactivity during changes in solution chemistry. Moreover, unlike many of the early biosorption studies that were carried out in supersaturated conditions with respect to the metal of interest, more recent studies have focused on describing metal-microbe interactions at more realistic undersaturated conditions. The goal for much of this research is to develop geochemical speciation models that describe how microorganisms interact with metals and mineral surfaces under varying geochemical conditions, thus enabling their use in reactive transport models (Fein, 2000 for review). The reactions can be quantified using two different approaches: (1) bulk partitioning relationships or (2) surface complexation models (SCM). In the first instance, partitioning models, such as K_d , Freundlich and Langmuir isotherms, can easily be applied to complex systems because they do not require a detailed understanding of the nature of the surfaces or the adsorption/desorption mechanisms involved. However, they are system-specific, meaning that the results from a set of experiments can be inapplicable to different systems. In contrast, the SCM takes into account the effects of changing pH, solution composition and ionic strength, the acid-base properties of surface functional groups, competitive sorption with other solutes, and solid-phase reactivity and mineralogy. It then draws upon that information to extrapolate to conditions beyond those tested in the laboratory. However, in order to utilize these models in more complex settings, more detailed understanding is required of not only surface and aqueous speciation but also the adsorption and desorption mechanisms.

New experimental techniques are helping to elucidate both the mechanisms and stoichiometry of metal-microbe interactions. Spectroscopic data provide more direct evidence of the metal coordination environment than traditional SCM and partitioning models. For example, metal binding onto *B. subtilis* has been examined by X-ray absorption spectroscopy (XAS) and provided estimates of metal:functional group stoichiometry (Boyanov et al., 2003; Kelly et al., 2002). Mechanisms of metal binding have been directly probed via time-resolved laser-induced fluorescence spectroscopy (TRLFS) and have shown that some metals preferentially bond to one functionality in the cell wall (Panak et al., 2000), while others are less specific (Texier et al., 2000; Markai et al., 2003). A notable recent advance in constraining the thermodynamics of metal-microbe adsorption reactions was the combination of calorimetric data with SCM to generate site-specific enthalpies and entropies of metal adsorption onto the cell wall of *B. subtilis*. This work indicated that heavy metals bind to the cell wall via inner sphere complexation (e.g., no interlayer water molecules) with multiple anionic

oxygen ligands. Stoichiometry and temperature dependence of the metal–bacteria adsorption reactions can also be extracted using this approach (Gorman-Lewis et al., 2006).

Summary

Microbial–metal adsorption includes the electrostatic and chemical association of metals with the organic functional groups of microbial cell surfaces. This can be considered a passive process driven by the electronegativity of the microbial cell wall/EPS and the Gibbs free energy of the adsorption reactions. These adsorption reactions seem to be dominated by binding of cations to the oxygen containing moieties on the cell surface. Future advances in elucidating the mechanisms of metal adsorption to microbes will come from combining novel analytical techniques, such as colorimetry and spectroscopy, with bulk partitioning studies and site-specific adsorption models.

Bibliography

- Aislabie, J., and Loutit, M. W., 1986. Accumulation of Cr(III) by bacteria isolated from polluted sediment. *Marine Environmental Research*, **20**, 221–232.
- Beveridge, T. J., 1984. Mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In Reddy, C. A., and Klug, M. J. (eds.), *Current Perspectives in Microbial Ecology*. Washington, DC: American Society for Microbiology, pp. 601–607.
- Beveridge, T. J., and Koval, S. F., 1981. Binding of metals to cell envelopes of *Escherichia coli* K-12. *Applied and Environmental Microbiology*, **42**, 325–335.
- Beveridge, T. J., and Murray, R. G. E., 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *Journal of Bacteriology*, **127**, 1502–1518.
- Boyanov, M. L., Kelly, S. D., Kemner, K. M., Bunker, B. A., Fein, J. B., and Fowle, D. A., 2003. Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: a pH dependent X-ray absorption fine structure spectroscopy study. *Geochimica et Cosmochimica Acta*, **67**, 3299–3311.
- Carstensen, E. L., and Marquis, R. E., 1968. Passive electrical properties of microorganisms. III. Conductivity of isolated bacterial cell walls. *Biophysics Journal*, **8**, 536–548.
- Fein, J. B., 2000. Quantifying the effects of bacteria on adsorption reactions in water-rock systems. *Chemical Geology*, **169**, 265–280.
- Fowle, D. A., and Fein, J. B., 2000. Experimental measurements of the reversibility of metal-bacteria adsorption reactions. *Chemical Geology*, **168**, 27–36.
- Geesey, G. G., and Jang, L., 1989. Interactions between metal ions and capsular polymers. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: John Wiley, pp. 325–357.
- Gorman-Lewis, D., Fein, J. B., and Jensen, M. P., 2006. Enthalpies and entropies of proton and cadmium adsorption onto *Bacillus subtilis* bacterial cells from calorimetric measurements. *Geochimica et Cosmochimica Acta*, **70**, 4862–4873.
- Harden, V. P., and Harris, J. O., 1953. The isoelectric point of bacterial cells. *Journal of Bacteriology*, **65**, 198–202.
- Kelly, S. D., Boyanov, M. I., Fein, J. B., Fowle, D. A., Yee, N., and Kemner, K. M., 2002. X-ray absorption fine structure determination of pH-dependent U-bacterial cell wall interactions. *Geochimica et Cosmochimica Acta*, **66**, 3855–3871.
- Markai, S., Andrés, Y., Montavon, G., and Grambow, B., 2003. Study of the interaction between europium (III) and *Bacillus subtilis*: fixation sites, biosorption modelling and reversibility. *Journal of Interface and Colloid Science*, **243**, 73–80.
- Panak, P., Raff, J., Selenska-Pobell, S., Geipel, G., Bernhard, G., and Nitsche, H., 2000. *Radiochimica Acta*, **88**, 71–76.
- Rudd, T., Sterritt, R. M., and Lester, J. N., 1983. Mass balance of heavy metal uptake by encapsulated cultures of *Klebsiella aerogenes*. *Microbial Ecology*, **9**, 261–272.
- Texier, A. C., Andrés, Y., Illemassene, M., and Le Cloirec, P., 2000. Characterization of lanthanide ions binding sites in the cell wall of *Pseudomonas aeruginosa*. *Environmental Science and Technology*, **34**, 610–615.
- Warren, L. A., and Haack, E. A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. *Earth-Science Reviews*, **54**, 261–320.
- Williams, R. J. P., 1981. Physico-chemical aspects of inorganic element transfer through membranes. *Philosophical Transactions of the Royal Society of London*, **B294**, 57–74.

Cross-references

Carbonates
Clay Authigenesis, Bacterial
Dolomite, Microbial
Metalloenzymes
Microbial Biomineralization
Microbial Surface Reactivity
Nanocrystals, Microbially Induced
Organomineralization

MICROBIOCORROSION

Aline Tribollet¹, Stjepko Golubic², Gudrun Radtke³,
Joachim Reitner⁴
¹Institut de Recherche pour le Développement, Nouméa,
Nouvelle-Calédonie, France
²Boston University, Boston, MA, USA
³Hessisches Landesamt für Umwelt und Geologie,
Wiesbaden, Germany
⁴University of Göttingen, Göttingen, Germany

Definition

Destruction of rocks and minerals by biological activities has been termed bioerosion (Neumann, 1966). It includes mechanical as well as chemical effects, that is, bioabrasion and biocorrosion (Schneider, 1976; Golubic and Schneider, 1979). However, both the processes often co-occur; they are functionally interconnected and mutually supportive. Biocorrosion can result from the activity of macro- or microorganisms and, thus, is called macrobiocorrosion and microbiocorrosion. Microbiocorrosion can also be closely associated with microbial rock formation and consolidation in stromatolitic structures (Reid et al., 2000; Macintyre et al., 2000; Garcia-Pichel et al., 2004; Dupraz and Visscher, 2005). In fact, the oldest known fossils of microboring organisms were located in lithified horizons of silicified stromatolites (Zhang and Golubic, 1987).

Bibliography

- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–338.
- Garcia-Pichel, F., Al-Horani, F., Ludwig, R., Farmer, J., and Wade, B., 2004. Balance between calcification and bioerosion in modern stromatolites. *Geobiology*, **2**, 49–57.
- Golubic, S., and Schneider, J., 1979. Carbonate dissolution. In Trudinger, P. A., and Swaine, D. J. (eds.), *Biogeochemical Cycling of Mineral-Forming Elements*. Amsterdam: Elsevier, pp. 107–129.
- Macintyre, I. G., Prufert-Bebout, L., and Reid, R. P., 2000. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology*, **47**, 915–921.
- Neumann, A. C., 1966. Observations on coastal erosion in Bermuda and measurements of the boring rate of the sponge *Cliona lampa*. *Limnology and Oceanography*, **11**, 92–108.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C. P., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Schneider, J., 1976. Biological and inorganic factors in the destruction of limestone coasts. *Contributions to Sedimentology*, **6**, 1–112.
- Zhang, Y., and Golubic, S., 1987. Endolithic microfossils (cyanophyta) from early Proterozoic stromatolites, Hebei, China. *Acta Micropaleont Sinica*, **4**, 1–12.

MICROSENSORS FOR SEDIMENTS, MICROBIAL MATS, AND BIOFILMS

Dirk de Beer
Max Planck Institute for Marine Microbiology, Bremen,
Germany

Synonyms

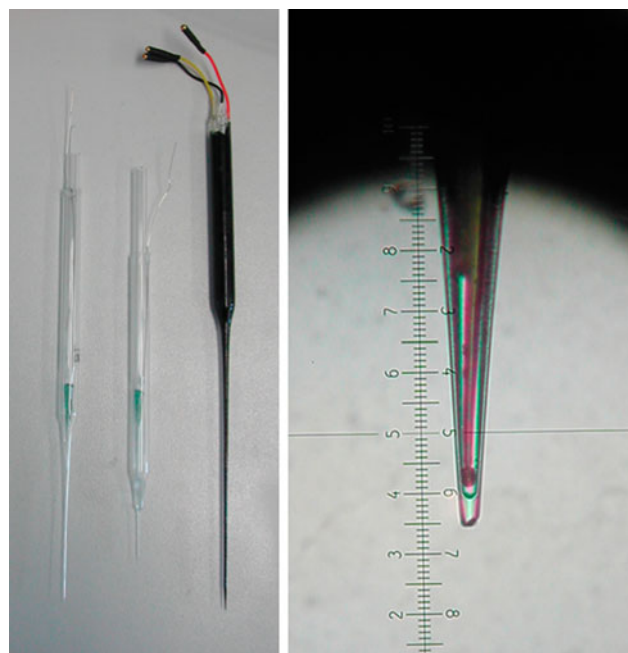
Amperometry; Diagenesis; Diffusion-reaction; Micro-electrode; Microenvironment; Potentiometry

Definition

Microsensors are needle-shaped sensors ([Figure 1](#)) that can be inserted in biologically active matrices, such as sediments, to measure directly concentrations of certain compounds.

Introduction

In sediments, microbial mats, and biofilms steep gradients of substrates and products develop, due to high metabolic activities and mass transfer limitations (see Chapters [Biofilms](#); [Microbial Mats](#); [Sediment Diagenesis – Biologically Controlled](#)). Stratifications of microenvironments develop that determine the existence and activity of microbial consortia. For example, in active biofilms oxygen can penetrate less than 50 μm (de Beer et al., 1994c; de Beer et al., 1993), in sediments oxygen penetrates typically less than 2 mm (de Beer, 2001; Jørgensen and Revsbech, 1985; Meyers et al., 1987; Revsbech,



Microsensors for Sediments, Microbial Mats, and Biofilms, Figure 1 Left panel, microsensors for oxygen (left sensor), for pH (middle), and for H_2S (right sensor). The right panel zooms further in on the tip of an oxygen sensor, showing the gold-plated electrode behind a silicon membrane, the ticks on the ruler are 1 μm .

1983; Sorensen et al., 1981; Sweerts and de Beer, 1989). Below the oxic zone anaerobic microbial processes can occur, such as denitrification and sulfate reduction (see Chapter [Sulfur Cycle](#)), that determine the element cycling (the coupled degradative and chemolithotrophic processes) in the system as a whole. Stratifications on such a small scale cannot be studied by more classical techniques such as pore-water extraction and chemical analyses. Extraction is impossible on such small scales, as it is highly destructive for the system and concentrations will have changed during the extraction procedure. To study such stratified systems we need microsensors that are minimally invasive and directly measure the concentrations without disturbing the system in which we measure. Moreover, as the system is not disturbed by microsensor analyses, we can perform sequences of measurements and, for example, study how the system responds to environmental changes. Microsensor measurements have enormously enhanced the insight in the vertical distribution of microbial processes in sediments. Microsensors were originally developed for physiological studies in animal and plant tissues, and even for intracellular measurements (Ammann, 1986; Hinke, 1969; Thomas, 1978), but soon applied in environmental studies (Bungay et al., 1969). They became an established technique for ecological studies by the developing work of Revsbech, who made them more robust and invented a large diversity of sensors (Kühl and Revsbech, 2000; Revsbech and Jørgensen, 1986).

Principle

The microsensors most commonly used for environmental studies are of the electrochemical principle, i.e., the compound to be measured induces a current (amperometric) or a potential (potentiometric) proportional to the concentration of the solute. The sensors are commonly made of glass, as this is easy to pull to fine tips, is inert and has excellent isolation properties. It involves the pulling of micropipettes in flames and electrical coils to exactly the desired geometry, thickness, and tip opening, followed by inserting the actual electrodes into these capillaries (Figure 1). The electrodes are mostly platinum wires, etched to micron thickness, and coated with a thin insulating glass layer. The making of microsensors is a fine art. For detailed descriptions of sensor preparation and electrochemical principles the reader is referred to various manuals and reviews (Gieseke and de Beer, 2004; Kühl and Revsbech, 2000; Revsbech and Jørgensen, 1986; Thomas, 1978).

The amperometric sensors consume the compound they measure, but since microsensors are so small this effect can be ignored. The tip diameter of some types can be less than 1 μm , but for most studies ranges between 3 and 15 μm are common, so the sensors are of similar size as the microbes studied. The size of the sensor must be carefully chosen. Small sensors respond faster and disturb the local microenvironment less, but are more fragile than larger sensors. The size of the sensor is a compromise between robustness and spatial resolution desired. For soft and extremely active biofilms microsensors of 1–3 μm are ideal; for measurements in coarse sand, tip diameters of 300 μm are used. Also for such big sensors, the actual

sensing surface is ca 1 μm , the size is determined by the thick glass wall (Table 1).

Essential equipment

Microsensors can be used in the laboratory and in the field. They can function also under high pressure, and can provide direct data from deep sea environments. Typical applications are measurements of concentration profiles and concentration changes upon a perturbation at a defined location. Microsensors are always positioned by micromanipulators mounted on a stable, heavy stand. The micromanipulator is preferably motorized, and controlled by a computer that is also used for data-acquisition. One can determine the exact position of the sensor to the surface of the studied object, with help of a dissection microscope. For in situ use all electronic components must be packed in water-sealed pressure housings. The most reliable in situ profiler consists of a single cylinder with amplifiers and computer for data storage and motor control. The sensors are mounted on special plugs directly on the cylinder, which can be moved vertically along a rail in steps of 12.5 μm (or multiples of this) by an external motor, according to a preprogrammed protocol. It is powered by a battery and can operate up to 72 h, at a depth of up to 6,300 m.

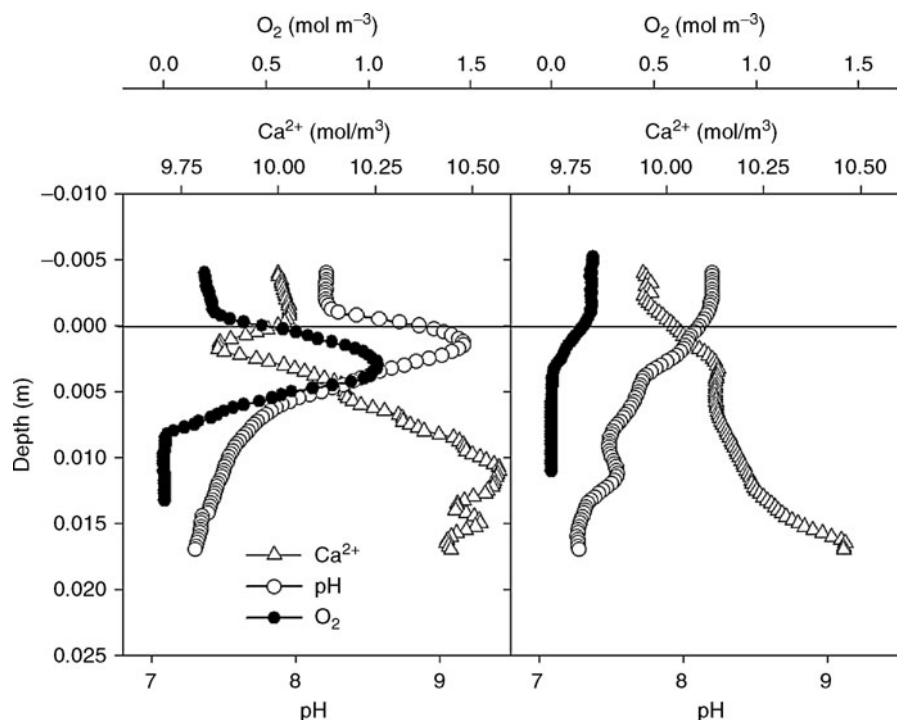
Applications

Microsensors have been intensively used in studies on sediments, biofilms, and microbial mats (Kühl and Revsbech, 2000). The microscale distribution of substrates is a function of both mass transfer and local conversion rates, so if we have two of the phenomena quantified

Microsensors for Sediments, Microbial Mats, and Biofilms, Table 1 Overview of microsensors suitable for environmental studies

Compound measured	Main interferences	Tip size (μm)	Reference
<i>Amperometric</i>			
O ₂	H ₂ S	3	Revsbech and Ward (1983)
N ₂ O	H ₂ S, methanethiol	3	Andersen et al. (2001)
H ₂ S		8	Jeroschewski et al. (1996)
Glucose	H ₂ O ₂	15	Cronenberg et al. (1991)
NO ₃ [−]	N ₂ O, NO ₂ [−] , H ₂ S	20	Larsen et al. (1996, 1997)
NO ₂ [−]	N ₂ O, H ₂ S	20	Nielsen et al. (2002)
H ₂ O ₂		5	Cronenberg et al. (1991)
HClO [−]		10	de Beer et al. (1994a)
H ₂		3	Ebert and Brune (1997)
CH ₄		10	Damgaard and Revsbech (1997)
NO	H ₂ S, methanethiol	30	Schreiber et al. (2008)
<i>Potentiometric</i>			
pH	—	1	de Beer et al. (1997b); Thomas, (1978)
CO ₃ ^{2−} #	Cl [−]	15	Meyerhoff et al. (1987)
NO ₃ [−] #	Cl [−]	1	de Beer and Sweerts (1989)
NO ₂ [−] #	Cl [−]	15	de Beer et al. (1997b)
Ca ²⁺	H ₂ S, Mg ²⁺	1	de Beer et al. (2000); Tsien and Rink (1981)
CO ₂	H ₂ S	3	de Beer et al. (1997a)
NH ₄ ⁺ #	K ⁺ , Na ⁺	1	de Beer and Van den Heuvel (1988)
S ^{2−}	—	6	Revsbech et al. (1983)

The sensors marked with # are only suitable for freshwater studies, the others can be used in all salinities



Microsensors for Sediments, Microbial Mats, and Biofilms, Figure 2 Microprofiles of O_2 , pH and Ca^{2+} in calcareous sediments from a coral reef (Heron Island Australia). The effect of photosynthesis is demonstrated.

we can calculate the third: from the microprofiles and mass transfer we can determine the distribution of microbial activity (Berg et al., 1998; Gieseke and de Beer, 2004; Revsbech et al., 1986), and from the microprofiles and the local conversion rates we can determine the mass transfer rates (de Beer et al., 2006; de Beer et al., 2004). The latter possibility is rather rarely considered, but the most direct way to determine advection and areal conversion rates in advection-dominated sediments.

Calcification is for geology a highly relevant (micro) biological process (see Chapters *Microbial Biomineralization*; *Stromatolites*). The large background calcium concentrations of ca 8–10 mM make it difficult to determine calcification rates from a mass balance. With microsensors it is possible to measure micromolar concentration changes in seawater, in time or over distance, and thus to study the relation between (de)calcification and metabolic activities (*photosynthesis* and respiration). In Figure 2 the effect of light on coral reef sediments (Heron Island, Great Barrier Reef) is demonstrated: by photosynthesis the oxygen concentration peaks in the photic zone, due to CO_2 fixation the pH shifts up and calcium carbonate precipitates (concentration minimum). In the dark (right panel) calcium diffuses out of the sediment. The photic layer in which these processes occur is less than 5 mm thick.

Microsensors are extensively used to study photosynthesis in microbial mats (Hoehler et al., 2002; Polerecky

et al., 2007; Revsbech et al., 1983; Wieland and Kuehl, 2000; Wieland et al., 2005) (see Chapter *Hypersaline Environments*), (de Beer and Schramm, 1999; de Beer et al., 1994b; Santegoeds et al., 1998; Schramm et al., 1996, 1999), in sediments (Revsbech, 1983; Sweerts, 1990; Sweerts and de Beer, 1989; Sweerts et al., 1990). Using diver-operated microprofilers well-controlled in situ measurements are possible (Weber et al., 2007; Ziebis et al., 1998). However, the most exciting applications are autonomous deployments on sites too remote or too harsh for human guidance: (1) direct in situ measurements during tidal cycles give insight in transport processes in permeable intertidal flats (de Beer et al., 2004; Røy et al., 2007; Werner et al., 2006a,b), (2) the in situ measurements in the deep sea supply physiological data communities associated with gas hydrates and high pressure volcanism (de Beer et al., 2006; Glud et al., 1993, 1994; Wenzhöfer et al., 2001a,b; Wenzhöfer and Glud, 2002). It is thinkable that some sort of microsensors will be used in future exploration of other planets.

Summary

Microsensors can measure the microenvironment of dense and highly active microbial communities on the scale of microbes. Microsensors are used in field studies and in controlled laboratory experiments. As the sensors are very small they do not disturb the structure or the

pore-water chemistry, allowing precise, rapid, and online measurements of steady-state microprofiles, or concentration dynamics upon a perturbation. Both types of measurements are useful for high spatial resolution studies on the distribution of microbial activities, and on studies on mass transfer phenomena.

Bibliography

- Ammann, D., 1986. *Ion-Selective Microelectrodes: Principles, Design and Applications*. Berlin: Springer.
- Andersen, K., Kjær, T., and Revsbech, N. P., 2001. An oxygen insensitive microsensor for nitrous oxide. *Sensors and Actuators*, **81**, 42–48.
- Berg, P., Risgaard-Pedersen, N., and Rysgaard, S., 1998. Interpretation of measured concentration profiles in sediment pore water. *Limnology and Oceanography*, **43**, 1500–1510.
- Bungay, H. R., Whalen, W. J., and Sanders, W. M., 1969. Microprobe techniques for determining diffusivities and respiration rates in microbial slimes. *Biotechnology and Bioengineering*, **11**, 765–772.
- Cronenberg, C. C. H., Van Groen, H., de Beer, D., and Van den Heuvel, J. C., 1991. Oxygen-independent glucose microsensor based on glucose oxidase. *Analytica Chimica Acta*, **242**, 275–278.
- Damgaard, L. R., and Revsbech, N. P., 1997. A microscale biosensor for methane containing methanotrophic bacteria and an internal oxygen reservoir. *Analytical Chemistry*, **69**, 2262–2267.
- de Beer, D., 2001. Microsensor studies of oxygen, carbon and nitrogen cycles in lake sediments and microbial mats. In Taillefert, M., and Rozan, T. F. (eds.), *Environmental Electrochemistry: Analyses of Trace Element Biogeochemistry*. New York: Oxford University Press, pp. 227–246.
- de Beer, D., and Schramm, A., 1999. Micro-environments and mass transfer phenomena in biofilms studied with microsensors. *Water Science and Technology*, **39**, 173–178.
- de Beer, D., and Sweerts, J. P. R. A., 1989. Measurements of nitrate gradients with an ion-selective microelectrode. *Analytica Chimica Acta*, **219**, 351–356.
- de Beer, D., and Van den Heuvel, J. C., 1988. Response of ammonium-selective microelectrodes based on the neutral carrier nonactin. *Talanta*, **35**, 728–730.
- de Beer, D., van den Heuvel, J. C., and Ottengraf, S. P. P., 1993. Microelectrode measurements of the activity distribution in nitrifying bacterial aggregates. *Applied and Environmental Microbiology*, **59**, 573–579.
- de Beer, D., Srinivasan, R., and Stewart, P. S., 1994a. Direct measurement of chlorine penetration into biofilms during disinfection. *Applied and Environmental Microbiology*, **60**, 4339–4344.
- de Beer, D., Stoodley, P., and Lewandowski, Z., 1994b. Liquid flow in heterogeneous biofilms. *Biotechnology and Bioengineering*, **44**, 636–641.
- de Beer, D., Stoodley, P., Roe, F., and Lewandowski, Z., 1994c. Effect of biofilm structures on oxygen distribution and mass transfer. *Biotechnology and Bioengineering*, **43**, 1131–1138.
- de Beer, D., Glud, A., Epping, E., and Kühl, M., 1997a. A fast responding CO₂ micro-electrode for profiling sediments, microbial mats and biofilms. *Limnology and Oceanography*, **42**, 1590–1600.
- de Beer, D., Schramm, A., Santegoeds, C. M., and Kühl, M., 1997b. A nitrite microsensor for profiling environmental biofilms. *Applied and Environmental Microbiology*, **63**, 973–977.
- de Beer, D., Kühl, M., Stambler, N., and Vaki, L., 2000. A microsensor study of light enhanced Ca²⁺ uptake and photosynthesis in the reef-building hermatypic coral *Favia* sp. *Marine Ecology. Progress Series*, **194**, 75–85.
- de Beer, D., Wenzhöfer, F., Ferdelman, T. G., Boehme, S., Huettel, M., van Beusekom, J., Boettcher, M., Musat, N., and Dubilier, N., 2004. Transport and mineralization rates in North Sea sandy intertidal sediments (Sylt-Rømø Basin, Waddensea). *Limnology and Oceanography*, **50**, 113–127.
- de Beer, D., Sauter, E., Niemann, H., Kaul, N., Foucher, J. P., Witte, U., Schlüter, M., and Boettius, A., 2006. In situ fluxes and zonation of microbial activity in surface sediments of the Håkon Mosby Mud Volcano. *Limnology and Oceanography*, **51**, 1315–1331.
- Ebert, A., and Brune, A., 1997. Hydrogen concentration profiles at the oxicanoxic interface: A microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). *Applied and Environmental Microbiology*, **63**, 4039–4046.
- Gieseke, A., and de Beer, D., 2004. Use of microelectrodes to measure in situ microbial activities in biofilms, sediments, and microbial mats. In Akkermans, A. D. L., and van Elsas, D. (eds.), *Molecular Microbial Ecology Manual*. Dordrecht: Kluwer, pp. 1581–1612.
- Glud, R. N., Gundersen, J. K., Revsbech, N. P., and Jørgensen, B. B., 1993. In situ measurements of total and diffusive oxygen uptake of sediments. *Trends in Microbial Ecology*, 427–430.
- Glud, R. N., Gundersen, J. K., Jørgensen, B. B., Revsbech, N. P., and Schulz, H. D., 1994. Diffusive and total oxygen uptake of deep-sea sediments in the eastern South Atlantic Ocean: in situ and laboratory measurements. *Deep-Sea Research*, **41**, 1767–1788.
- Hinke, J., 1969. Glass microelectrodes for the study of binding and compartmentalisation of intracellular ions. In Lavalley, M., Schanne, O. F., and Herbert, N. C. (eds.), *Glass Microelectrodes*. New York: Wiley, pp. 349–375.
- Hoehler, T. M., Albert, D. B., Alperin, M. J., Bebout, B., Martens, C. S., and Des Marais, D., 2002. Comparative ecology of H₂ cycling in sedimentary and phototrophic ecosystems. *Antonie van Leeuwenhoek*, **81**, 575–585.
- Jeroschewski, P., Steukart, C., and Kühl, M., 1996. An amperometric microsensor for the determination of H₂S in aquatic environments. *Analytical Chemistry*, **68**, 4351–4357.
- Jørgensen, B. B., and Revsbech, N. P., 1985. Diffusive boundary layers and the oxygen uptake of sediments and detritus. *Limnology and Oceanography*, **30**, 111–122.
- Kühl, M., and Revsbech, N. P., 2000. Biogeochemical microsensors for boundary layer studies. In Boudreau, B., and Jørgensen, B. B. (eds.), *The Benthic Boundary Layer*. New York: Oxford University Press, pp. 180–210.
- Larsen, L. H., Revsbech, N., and Binnerup, S. J., 1996. A microsensor for nitrate based on immobilized denitrifying bacteria. *Applied and Environmental Microbiology*, **62**, 148–1251.
- Larsen, L. H., Kjaer, T., and Revsbech, N. P., 1997. A microscale NO₃[−] biosensor for environmental applications. *Analytical Chemistry*, **69**, 3527–3531.
- Meyerhoff, M. E., Pretch, E., Welti, D. H., and Simon, W., 1987. Role of trifluoroacetophenone solvents and quaternary ammonium salts in carbonate-selective liquid membrane electrodes. *Analytical Chemistry*, **59**, 144–150.
- Meyers, M. B., Fossing, H., and Powell, E. N., 1987. Microdistribution of interstitial meiofauna, oxygen and sulfide gradients, and the tubes of macro-fauna. *Marine Ecology. Progress Series*, **35**, 223–241.
- Nielsen, M., Revsbech, N. P., Larsen, L. H., and Lynnggaard-Jensen, A., 2002. On-line determination of nitrite in wastewater treatment by use of a biosensor. *Water Science and Technology*, **45**, 69–75.
- Polerecky, L., Bachar, A., Schoon, R., Grinstein, M., Jørgensen, B. B., de Beer, D., and Jonkers, H., 2007. Contribution of Chloroflexus respiration to oxygen cycling in a hypersaline

- microbial mat from Lake Chiprana, Spain. *Environmental Microbiology*, **9**, 2007–2024.
- Revsbech, N. P., 1983. In situ measurement of oxygen profiles of sediments by use of oxygen microelectrodes. In Gnaiger, E., and Forstner, H. (eds.), *Polarographic Oxygen Sensors: Aquatic and Physiological Applications*. Berlin: Springer, pp. 265–273.
- Revsbech, N. P., and Jørgensen, B. B., 1986. Microelectrodes: their use in microbial ecology. *Advances in Microbial Ecology*, **9**, 293–352.
- Revsbech, N. P., and Ward, D. M., 1983. Oxygen microelectrode that is insensitive to medium chemical composition: use in an acid microbial mat dominated by *Cyanidium caldarium*. *Applied and Environmental Microbiology*, **45**, 755–759.
- Revsbech, N. P., Jørgensen, B. B., Blackburn, T. H., and Cohen, Y., 1983. Microelectrode studies of the photosynthesis and O₂, H₂S and pH profiles of a microbial mat. *Limnology and Oceanography*, **28**, 1062–1074.
- Revsbech, N. P., Madsen, B., and Jørgensen, B. B., 1986. Oxygen production and consumption in sediments determined at high spatial resolution by computer simulation of oxygen microelectrode data. *Limnology and Oceanography*, **31**, 293–304.
- Røy, H., Lee, J. S., Jansen, S., and de Beer, D., 2007. Tide-driven deep pore-water flow in intertidal sand flats. *Limnology and Oceanography*, **53**, 1521–1530.
- Santegoeds, C. M., Ferdelman, T. G., Muyzer, G., and de Beer, D., 1998. Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. *Applied and Environmental Microbiology*, **64**, 3731–3739.
- Schramm, A., Larsen, L. H., Revsbech, N. P., Ramsing, N. B., Amann, R., and Schleifer, K. H., 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology*, **62**, 4641–4647.
- Schramm, A., de Beer, D., van den Heuvel, J. C., Ottengraf, S. P. P., and Amann, R., 1999. Microscale distribution of populations and activities of *Nitrosospora* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridisation and the use of microsenors. *Applied and Environmental Microbiology*, **65**, 3690–3696.
- Schreiber, F., Polerecky, L., and de Beer, D., 2008. Nitric oxide microsensor for high spatial resolution measurements in biofilms and sediments. *Analytical Chemistry*, **80**, 1152–1158.
- Sorensen, J., Jørgensen, B. B., and Revsbech, N. P., 1981. A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. *Microbial Ecology*, **5**, 105–115.
- Sweerts, J.-P. R. A., 1990. Oxygen consumption processes, mineralization and nitrogen cycling at the sediment-water interface of North temperate lakes, University of Amsterdam, 136 pp.
- Sweerts, J.-P. R. A., and de Beer, D., 1989. Microelectrode measurements of nitrate gradients in the littoral and profundal sediments of a meso-eutrophic lake (lake Vechten, The Netherlands). *Applied and Environmental Microbiology*, **55**, 754–757.
- Sweerts, J.-P. R. A., de Beer, D., Nielsen, L. P., Verdouw, H., van den Heuvel, J. C., Cohen, Y., and Cappenberg, T. E., 1990. Denitrification by sulphur oxidizing *Beggiatoa* spp. mats on freshwater sediments. *Nature*, **344**, 762–763.
- Thomas, R. C., 1978. *Ion-Sensitive Intracellular Microelectrodes, How to Make and Use Them*. New York: Academic.
- Tsien, R. Y., and Rink, T. J., 1981. Ca²⁺-selective electrodes: a novel PVC-gelled neutral carrier mixture compared with other currently available sensors. *Journal of Neuroscience Methods*, **4**, 73–86.
- Weber, M., Faerber, P., Meyer, V., Lott, C., Eickert, G., Fabricius, K. E., and de Beer, D., 2007. In situ applications of a new diver-operated motorized microsensor profiler. *Environmental Science and Technology*, **41**, 6210–6215.
- Wenzhöfer, F., and Glud, R. N., 2002. Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. *Deep-sea Research I*, **49**, 1255–1279.
- Wenzhöfer, F., Adler, M., Kohls, O., Hensen, C., Strotmann, B., Boehme, S., and Schulz, H. D., 2001a. Calcite dissolution driven by benthic mineralization in the deep-sea: in situ measurements of Ca²⁺, pH, pCO₂ and O₂. *Geochimica et Cosmochimica Acta*, **65**, 2677–2690.
- Wenzhöfer, F., Holby, O., and Kohls, O., 2001b. Deep penetrating oxygen profiles measured in situ by oxygen optodes. *Deep-sea Research I*, **48**, 1741–1755.
- Werner, U., Billerbeck, M., Polerecky, L., Franke, U., Huettel, M., van Beusekom, J. E. E., and de Beer, D., 2006a. Spatial and temporal patterns of mineralization rates and oxygen distribution in a permeable intertidal sand flat (Sylt, Germany). *Limnology and Oceanography*, **51**, 2549–2563.
- Werner, U., Bird, P., Wild, C., Ferdelman, T. G., Polerecky, L., Eickert, G., Jonstone, R., Hoegh-Guldberg, O., and de Beer, D., 2006b. Spatial patterns of aerobic and anaerobic mineralization rates and oxygen penetration dynamics in coral reef sediments (Heron Island, Australia). *Marine Ecology. Progress Series*, **309**, 93–105.
- Wieland, A., and Kuehl, M., 2000. Irradiance and temperature regulation of oxygenic photosynthesis and O₂ consumption in a hypersaline cyanobacterial mat (Solar Lake, Egypt). *Marine Biology*, **137**, 71–85.
- Wieland, A., Zopfi, J., Benthien, M., and Kühl, M., 2005. Biogeochemistry of an iron-rich hypersaline microbial mat (Camargue, France). *Microbial Ecology*, **49**, 34–49.
- Ziebis, W., Pillen, T., and Unger, B., 1998. A diver observatory for in situ studies in sublittoral sediments. *Journal of the Society for Underwater Technology*, **23**, 63–69.

Cross-references

[Biofilms](#)
[Biofilms and Fossilization](#)
[Cyanobacteria](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Hypersaline Environments](#)
[Microbial Biomineralization](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Photosynthesis](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Sulfur Cycle](#)

MOLAR-TOOTH STRUCTURE

Brian R. Pratt
 University of Saskatchewan, Saskatoon, SK, Canada

Definition

Molar-tooth structure is a synsedimentary, combined deformation and early diagenetic feature occurring in calcareous strata of mainly Precambrian age. It consists of arrays of closely spaced, sharply defined, upright veins, and subordinate horizontal sheets and spheroids composed of calcite microspar. Veins are vertically to obliquely oriented, discontinuous, typically strongly squashed or crumpled, and often brecciated.

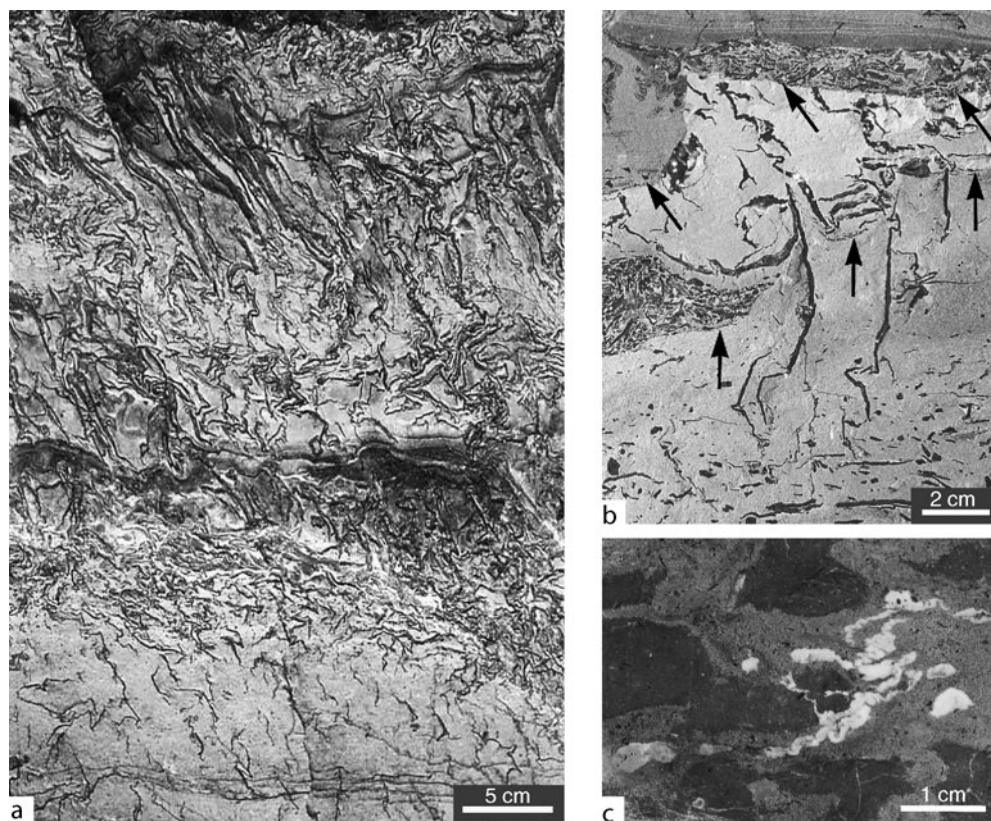
Geological age

Molar-tooth structure (MTS) was first named in 1885 from its appearance on limestone bedding planes in the Mesoproterozoic Purcell Supergroup (= Belt Supergroup in USA.) along the border between southwestern Canada and adjacent northwestern Montana. Almost all occurrences are in Mesoproterozoic to late Neoproterozoic (Ediacaran) strata, representing a 1 billion-year time span from about 1,600 to 600 Ma, but there are several examples noted from the Paleoproterozoic and one from the Neoarchean (James et al., 1998; Pratt, 1998b; Shields, 2002; Meng and Ge, 2002). Importantly, a few Phanerozoic cases have also been reported (Figure 1c; Pratt, 1982; Rossetti, 2000).

Geobiological implications

Organisms and organic matter have been alleged to be implicated in MTS formation in various ways. Its rarity

after the Neoproterozoic has been ascribed generally to substrate modification by animals. However, the drastic reduction of MTS occurrences took place before the known appearance of infaunal invertebrates and, instead, Shields (2002) saw the decline as due to changes in the chemistry of seawater, especially a decrease in CaCO_3 saturation caused by a drop in atmospheric pCO_2 . There may have been an evolutionary control on the shape and mineralogy of lime mud, leading to the precipitation of granular lime mud which was thus prone to fluidization (Pratt, 1998b, 2001). Pratt (1998b) postulated that the rheology of carbonate sediment altered with the changing nature of organic matter and the microbiota brought about by the “Cambrian explosion.” Biomarkers in late Neoproterozoic examples represent cyanobacterial, anaerobic bacterial, and eukaryotic algal precursors in the sediment (Kuang et al., 2004). According to some interpretations, MTS was originally void space created by CO_2 generated by the biodegradation of organic matter



Molar-tooth Structure, Figure 1 (a) Lime mudstone riddled with molar-tooth structure (MTS) veins (dark): initially sparse and upright, passing upward to dense and strongly oblique, thence, in the upper two thirds, to upright but markedly crumpled and tilted. George Formation, Muskwa Group (~1.6 Ga), northern British Columbia. (b) Mudstone with three generations of MTS and erosion surfaces. First-generation veins in lower part are fragmented and reworked while second-generation veins are upright. Lowest erosion surface (vertical arrows) is cusate around upright veins, with vein fragments collected in gutter at left. Overlying mudstone contains third-generation veins that are variably oriented. Second erosion surface (oblique arrows) truncates veins; vein fragments are collected in gutter at left and on horizontal surface at right. Overlying cross-laminated grainstone contains a fourth generation of rare upright veins. George Formation. (c) Wackestone (dolomitic areas light gray) with crumpled veins (whitish). Coronach Member, Herald Formation Red River Group (late Ordovician), 14-26-6-11W2 (2,565.5 m), southern Saskatchewan (see El Taki and Pratt, 2009).

(Furniss et al., 1998; Pollock et al., 2006) or by CO₂-clathrate destabilization (Marshall and Anglin, 2004). James et al. (1998) suggested that a surface microbial mat sealed the sediment. If not physicochemical, precipitation of CaCO₃ crystals in the veins (Pollock et al., 2006) or as sediment grains before being fluidized (Pratt, 1998b, 2001) may have been triggered by dissolved organic molecules, nucleated on organic particles, or induced by photosynthesis.

Shape

MTS occurs mostly in both clean and argillaceous subtidal lime mudstones (Figures 1a–c, 2a and b), but a few are in shales (Figure 2c). Individual vein arrays crosscut up to ~0.5 m of sedimentary bedding; locally, they can comprise more than 50% of the host rock. Veins are discontinuous, range from fairly uniform to variable in width up to ~5 mm along their length, and typically exhibit tapered terminations. Although more or less straight to gently curved in some beds, mostly veins are strongly squashed or crumpled. The whole array may be tilted in one direction. On bedding planes, veins describe variably continuous subparallel, crisscrossing, or irregularly reticulate lines. The upper and lower surfaces of horizontal veins match, indicating that they are dilatational sheet cracks. Crudely spheroidal blobs are up to ~1 cm across. Two crosscutting episodes of vein formation are common, and they typically show differing degrees of deformation. In some argillaceous and silty units, as many as four consecutive phases of vein generation can be delineated, with each containing progressively less calcite and more

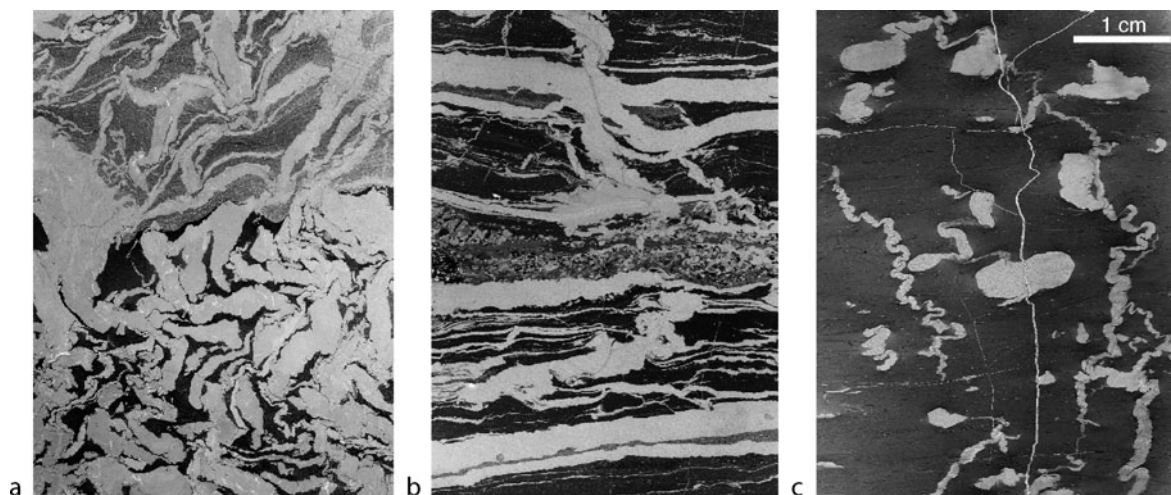
terrigenous particles, and consequently appearing less and less distinct from the matrix.

The matrix usually shows soft-sediment deformation especially in the form of small-scale folding. Typically, the matrix along with the enclosed MTS was deformed plastically together. Veins may be dislocated and the segments shingled in both vertical and horizontal directions, but they are also often stretched, boudinaged, or smeared. In many cases, a subsequent event caused the still plastic matrix to be deformed around veins that had become stiff due to calcite cementation, which also resulted in brecciation of the veins. After consolidation of the matrix, a further phase of deformation caused small mode I cracks in the veins but little displacement. These features are evidence for variably directed compressional, tensile and shear stresses that were imposed often repeatedly during the separate rheological evolution of both components.

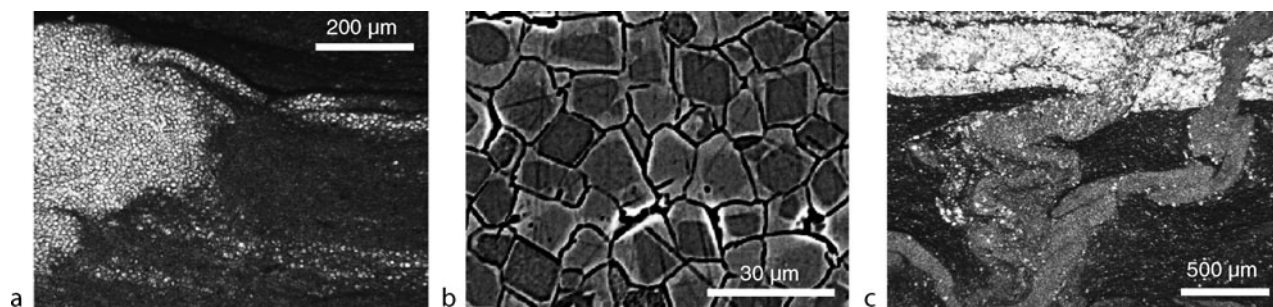
Seafloor erosion affected many beds with MTS. The more resistant veins commonly protruded above the substrate, and vein segments and fragments were winnowed out and concentrated into interbedded intraclastic grainstones (Figures 1b and 2b). In a so far unique case, cross-lamination in vein fills indicates that they were exhumed and reworked by waves before cementation (Bishop and Sumner, 2006).

Composition

Locally MTS consists of micrite. However, in most cases it is composed of interlocking, equant, inclusion-free microspar crystals 7–15 µm across (Figure 3a). Back-scattered electron microscopy and cathode-luminescence reveal that in some examples these crystals consist of



Molar-tooth Structure, Figure 2 Thin section photomicrographs of molar-tooth structure (MTS) (light gray); scale bar same for all. (a) C_{org}-rich mudstone with densely packed squashed and broken veins, overlain by distorted, laminated mudstone with tilted and crumpled upright and subhorizontal veins. George Formation. (b) Dolomitic mudstone with two crosscutting generations of horizontal and squashed upright veins. In middle, grainstone lamina (composed of micrite peloids and grains of MTS) is crossed by veins and part of its interparticle porosity is filled with microspar. I-5 unit, Atar Group (~1.1 Ga), Mauritania. (c) Slightly calcareous shale with spheroidal and crumpled upright veins. (Vertical crack in center is an artifact.) Chamberlain Formation, Belt Supergroup (1.45 Ga), central Montana.



Molar-tooth Structure, Figure 3 (a) Thin section photomicrograph of calcite microspar in pod-shaped molar-tooth structure (MTS) and smeared, variably distinct, horizontal veins, and stringers; isolated microspar grains are also dispersed in shale matrix. Chamberlain Formation. (b) Back-scattered electron photomicrograph showing microspar mosaic with euhedral mud-sized cores (dark gray). Cores are rhombs and less commonly multifaceted crystals. Monteville Formation, Transvaal Supergroup (~2.6 Ga), South Africa (see Bishop and Sumner, 2006, fig. 9C; photograph courtesy of J. W. Bishop). (c) Thin section photomicrograph of squashed and shingled veins crossing argillaceous lime mudstone overlain by sandstone. Veins contain quartz silt and very fine-grained sand (whitish grains near the sandstone and sourced from it; those elsewhere are authigenic quartz crystals). Helena Formation, Belt Supergroup, northwestern Montana.

well-sorted rhombs and multifaceted euhedra with calcite cement overgrowths (Figure 3b; Pollock et al., 2006; Bishop and Sumner, 2006). These cores may be the original mud particles or a product of syndimentary recrystallization. Similar grains occur in the matrix mudstone or shale. Microspar in MTS is typically uniform in size but locally there is variation that describes crude wall-parallel lamination; indistinct shear veins or cracks can be detected in some examples. In some units the MTS is recrystallized to pseudospar.

Where veins penetrate interbedded siltstone or sandstone layers the microspar may contain silt or fine sand plus minor amounts of clay (Figure 3c; Pratt, 1998b, 1999; Bishop and Sumner, 2006). MTS may grade into dikelets and thin sills of injected silt and sand of the kind that have been termed “syneresis” cracks (Pratt, 1998a; cf. Tanner, 1998; Shi et al., 2009).

The calcite in MTS exhibits $\delta^{13}\text{C}_{\text{PDB}}$ values in the range of about -1.5‰ to 3.5‰ , which are similar to those in co-occurring sedimentary carbonate particles (Frank and Lyons, 1998; Pratt, 1998b; Marshall and Anglin, 2004; Bishop et al., 2006; Kuang et al., 2007).

Formation

For more than a century, the enigmatic nature of MTS was tolerated but largely ignored. Because fragments of MTS comprise sedimentary particles, it was eventually recognized as having formed intrastratally just below the sediment–water interface. Some geologists presumed MTS to be a fossil algal or microbial object. Later it was accepted as a deformation feature involving substantial shrinkage of the host sediment – although not due to desiccation because there is no evidence for associated subaerial exposure.

Pore-lining cement fabrics are absent in MTS even though they are present in co-occurring grainstones. Nevertheless, it is still debated whether or not MTS was initially a system of cavities, in which case CaCO_3 precipitated later as tiny crystals inside them (Fairchild

et al., 1997; Furniss et al., 1998; Pollock et al., 2006; Bishop and Sumner, 2006; Bishop et al., 2006). The presence in MTS of sand, silt, and clay impurities as suspended particles, clusters of grains, or seams is difficult to reconcile with this interpretation.

The possibility that MTS began as voids forced open by bubbles of CO_2 passively generated by the oxidation of organic matter in the sediments is unlikely because of the extreme amount of organic matter required, the shallow-water, oxidizing depositional setting, the linear to reticulate shape of vein arrays, the evidence for concomitant sand and silt injection, and the complex nature of the stresses demonstrated by accompanying physical deformation (Pratt, 1999). Because of the tropical sedimentary environment of host limestones, the involvement of CO_2 -clathrates is implausible. In most units, the absence of closely associated high-energy features is further evidence against the possibility of void opening by wave-induced loading (Bishop et al., 2006).

Pratt (1998b) explained MTS as a seismically induced deformation feature of calcareous sediment possessing unique geotechnical properties for which modern analogues are lacking. By this model, cyclic loading during earthquakes caused elevated fluid pressure and loss of shear strength in the muddy sediment. During abrupt dewatering, veins, sheet cracks, and spheroidal pockets were jacked open by the injection of granular lime mud that was segregated from the sediment. Examples where more than 50% of limestones consist of MTS indicate that a substantial proportion of the lime mud was mobilized. However, shaly host rocks still contain poorly defined seams and residual disseminated calcite grains that did not find their way into veins. Incorporated sand and silt show that dewatering and injection were both downwardly and upwardly directed. Spheroids formed where dewatering was localized and fluids did not escape to the surface. All this caused a striking shrinkage of the matrix and compaction of upright veins. As shaking proceeded,

they were squeezed, sheared, and stretched in different directions. After cementation of the mud infill, subsequent events deformed the matrix again, generated more veins, and brecciated the first set of veins, until the sediment was too stiff to deform further. Indeed, in almost every case, distortion of MTS and the matrix testifies to differential rheologies and progressive evolution from liquefaction through plastic deformation to brittle failure, as would be expected if affected by repeated earthquakes during dewatering and early diagenesis. A similar seismic model has been invoked for sand and silt dykelets (syneresis cracks), except that synsedimentary cementation was not involved (Pratt, 1998a).

Bibliography

- Bishop, J. W., and Sumner, D. Y., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. I: constraints on microcrystalline CaCO_3 precipitation. *Sedimentology*, **53**, 1049–1058.
- Bishop, J. W., Sumner, D. Y., and Huerta, N. J., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. II: a wave-induced fluid flow model. *Sedimentology*, **53**, 1069–1082.
- El Taki, H., and Pratt, B. R., 2009. Synsedimentary deformation in laminated dolomites and evaporites of the Herald Formation (Red River Group): signature of Late Ordovician tectonic activity in southern Saskatchewan. In Summary of investigations 2009, Vol. 1. Saskatchewan Geological Survey, Saskatchewan Ministry of Energy and Resources, Miscellaneous Report 2009-4.1, Paper A-3, p. 10.
- Fairchild, I. J., Einsele, G., and Song, T., 1997. Possible seismic origin of molar tooth structures in Neoproterozoic carbonate ramp deposits, North China. *Sedimentology*, **44**, 611–636.
- Frank, T. D., and Lyons, T. W., 1998. “Molar-tooth” structures: a geochemical perspective on a Proterozoic enigma. *Geology*, **26**, 683–686.
- Furniss, G., Rittel, J. F., and Winston, D., 1998. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, Western Montana. *Journal of Sedimentary Research*, **68**, 104–114.
- James, N. P., Narbonne, G. M., and Sherman, A. G., 1998. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic. *Journal of Sedimentary Research*, **68**, 716–722.
- Kuang, H., Li, Y., Zeng, Y., Meng, X., and Ge, M., 2004. Biomarkers in the molar tooth (MT)-bearing limestones in the Jilin–Liaoning area of China. *Chinese Journal of Geochemistry*, **23**, 334–341.
- Kuang, H., Liu, Y., Meng, X., and Ge, M., 2007. A study on the environmental conditions of the microsparite (molar-tooth) carbonates. *Chinese Journal of Geochemistry*, **26**, 28–34.
- Marshall, D., and Anglin, C. D., 2004. CO_2 -clathrate destabilization: a new model of formation for molar tooth structures. *Precambrian Research*, **129**, 325–341.
- Meng, X., and Ge, M., 2002. The sedimentary features of Proterozoic microspar (molar-tooth) carbonates in China and their significance. *Episodes*, **25**, 185–195.
- Pollock, M. D., Kah, L. C., and Bartley, J. K., 2006. Morphology of molar-tooth structures in Precambrian carbonates: influence of substrate rheology and implications for diagenesis. *Journal of Sedimentary Research*, **76**, 310–323.
- Pratt, B. R., 1982. Limestone response to stress: pressure solution and dolomitization—discussion and examples of compaction in carbonate sediments. *Journal of Sedimentary Petrology*, **52**, 323–327.
- Pratt, B. R., 1998a. Syneresis cracks: subaqueous shrinkage in argillaceous sediments caused by earthquake-induced dewatering. *Sedimentary Geology*, **117**, 1–10.
- Pratt, B. R., 1998b. Molar-tooth structure in Proterozoic carbonate rocks: origin from synsedimentary earthquakes, and implications for the nature and evolution of basins and marine sediment. *Geological Society of America Bulletin*, **110**, 1028–1045.
- Pratt, B. R., 1999. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, western Montana—Discussion. *Journal of Sedimentary Research*, **68**, 1136–1140.
- Pratt, B. R., 2001. Oceanography, bathymetry and syndepositional tectonics of a Precambrian intracratonic basin: integrating sediments, storms, earthquakes and tsunamies in the Belt Supergroup (Helena Formation, ca. 145 Ga), Western North America. *Sedimentary Geology*, **141–142**, 371–394.
- Rossetti, D. F., 2000. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic—Discussion. *Journal of Sedimentary Research*, **70**, 1246–1248.
- Shi, X. Y., Jiang, G. Q., Zhang, C. H., Gao, L. Z., and Liu, J., 2009. Sand veins and MISS from the Mesoproterozoic black shale (ca. 1.7 Ga) in North China: implications for methane degassing from microbial mats. *Science in China Series D: Earth Sciences*, **51**, 1525–1536.
- Shields, G. A., 2002. ‘Molar-tooth microspar’: a chemical explanation for its disappearance ~750 Ma. *Terra Nova*, **14**, 108–113.
- Tanner, P. W. G., 1998. Interstratal dewatering origin for polygonal patterns of sand-filled cracks: a case study of late Proterozoic metasediments of Islay, Scotland. *Sedimentology*, **45**, 71–89.

Cross-references

Calcite Precipitation, Microbially Induced
Carbonate Environments
Carbonates
Deep Fluids
Microbialites, Modern

MOONMILK

Joachim Reitner
University of Göttingen, Göttingen, Germany

Synonyms

Bergmilch; Lac lunae; Lac montanum; Mannmilch; Mondmilch; Montmilch; Mundmilch

Definition

Moonmilk is a carbonate deposit that occurs within various subterranean systems. Moonmilk has a white to gray color and, in contrast to rigid cave deposits (*speleothems*) such as stalactites and stalagmites, exhibits a soft, muddy texture of microcrystalline aggregates. These aggregates are mainly composed of calcite, and to a lesser extent of aragonite, monohydrocalcite, hydromagnesite, sulfates, and nitrates (Martínez-Arkarazo et al., 2007; Richter et al., 2008; Cañaveras et al., 2006; Borsato et al., 2000). The calcite shows an aragonite-like needle form (“lublinite”), which is normally associated

with soil bacteria. The crystal needles have a diameter of about 0.1 μm and a length of ca. 8–10 μm . The name “moonmilk – Mondmilch” is derived from the name of a cave, “Mondmilchloch,” located at the Pilatus mountain (Emmental Alps, Switzerland) (Fischer, 1988).

Geobiological implications

Moonmilk differs in many aspects from the traditional cave carbonates such as stalagmites and stalactites (Moore and Sullivan, 1997; Bögli, 1978). Its formation is strongly influenced by microbial activity, as suggested by the presence of microbial aggregates, biofilms, and/or fungal mycelia (Cañaveras et al., 1999, 2001, 2006; Borsato et al., 2000; Gadd, 2004; Burford et al., 2003). Moonmilk is a result of (1) microbially mediated weathering and *microbiocorrosion* of carbonate host rock under highly humid conditions and (2) microbially mediated reprecipitation of various carbonate minerals. After dissolution of the mineral components, the residue exhibits high amounts of organic remains. Besides many fungal and actinomycetes, the bacterium *Macromonas bipunctata* plays a major role in the formation of moonmilk. *M. bipunctata* is a lophotrichous, heterotrophic and strictly aerobic betaproteobacterium that has been classified as a colorless sulfur bacterium capable of oxidizing sulfide (Dubinia and Grabovich, 1984). Within *extracellular polymeric substances (EPS)* of *M. bipunctata* and further microbes, calcite seed nuclei are formed to precipitate “lubinite” moonmilk crystals. *M. bipunctata* was originally described as *Pseudomonas bipunctata* by Gicklhorn (1920) and renamed by Utermöhl and Koppe (1924).

Bibliography

- Bögli, A., 1978. *Karsthydrographie und physische Speläologie*. Springer, Berlin/Heidelberg/New York.
- Borsato, A., Frisia, S., Jones, B., and Van den Borg, K., 2000. Calcite moonmilk: crystal morphology and environment of formation in caves in Italian alps. *Journal of Sedimentary Research*, **70**, 1179–1190.
- Burford, E. P., Fomina, M., and Gadd, G., 2003. Fungal involvement in bioweathering and biotransformations of rocks and minerals. *Mineralogical Magazine*, **67**(6), 1172–1155.
- Cañaveras, J. C., Hoyos, M., Sanchez-Moral, S., Sanz-Rubio, E., Bedoya, J., Soler, V., Groth, I., Schumann, P., Laiz, L., Gonzalez, I., and Saiz-Jimenez, C., 1999. Microbial communities associated with hydromagnesite and needle-fiber aragonite deposits in a Karstic cave (Altamira, Northern Spain). *Geomicrobiology Journal*, **16**, 9–25.
- Cañaveras, J. C., Sanchez-Moral, S., Sloer, V., and Saiz-Jimenez, C., 2001. Microorganisms and microbially induced fabrics in cave walls. *Geomicrobiology Journal*, **18**, 223–240.
- Cañaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J. M., and Saiz-Jimenez, C., 2006. On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften*, **93**, 27–32.
- Dubinia, G. A., and Grabovich, M. Y., 1984. Isolation, cultivation and characteristics of *Macromonas bipunctata*. *Mikrobiologiya*, **53**, 748–755.
- Fischer, H., 1988. Etymologie von Mondmilch bzw. Mondmilchloch. *Zeitschrift der deutschen Geologischen Gesellschaft*, **139**, 155–159.

- Gadd, G. M., 2004. Mycotransformation of organic and inorganic substrates. *Mycologist*, **18**, 60–70.
- Gicklhorn, J., 1920. Über neue farblose Schwefelbakterien.-Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt.2, **50**, 415–427.
- Martínez-Arkarazo, I., Angulo, M., Zuloaga, O., Usobiaga, A., and Madariaga, J. M., 2007. Spectroscopic characterisation of moonmilk deposits in Pozalagua tourist cave (Karrantza, Basque Country, North of Spain). *Spectrochimica Acta Part A*, **68**, 1058–1064.
- Moore, G. W., and Sullivan, N., 1997. *Speleology: Caves and the Cave Environment*. Huntsville: National Speleological Society, p. 176.
- Richter, D., Immenhauser, A., and Neuser, R. D., 2008. Electron backscatter diffraction documents randomly orientated c-axes in moonmilk calcite fibers: evidence for biologically induced precipitation. *Sedimentology*, **55**, 487–497.
- Utermöhl, H., and Koppe, F., 1924. Genus *Macromonas*. In Koppe, F. (ed.), *Die Schlammflora der ostholsteinischen Seen und des Bodensees; Archiv für Hydrobiologie*, **14**, 619–672.

Cross-references

[Biofilms](#)
[Carbonate Environments](#)
[Carbonates](#)
[Karst Ecosystems](#)
[Microbial Mineralisation](#)
[Microbial Mats](#)

MUD MOUNDS

Marta Rodríguez-Martínez
 Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Synonyms

Biodebital mound; Carbonate mounds; Carbonate mud mound; Lime mud mound; Microbial mound; Mudbank; Reef mound; Stromatactis mounds

Definition

Mud mounds are biosedimentary buildups, part of the reef system and are dominated by fine-grained carbonates (up and more than 50% of rock volume), which form heterogeneous polygenetic matrix-supported fabrics (stromatolitic, thrombolitic, leiolitic, fenestral, laminar, reticulate, stromatactoid, etc.). These fabrics are composed of both allomicrites and automicrites (abiogenetic, biogenetically induced, and biogenetically controlled). Production and accretion mechanisms in mud mounds are varied and not always clear, but are associated with the activity of microbial benthic communities (in different degrees of participation). Metazoans, being important colonizers, can or cannot occur but never produce a primary skeletal framework. Mud mounds colonized the oceans from the Proterozoic times, and they grow up from deep aphotic basinal settings to shallow water platforms.

Evolution of the term mud mound

Probably, the core of the Silurian Wabash buildup (Indiana, EEUU) was the first carbonate deposit referred to in the literature as a “carbonate mud mound” and was described as a mound of calcisiltite containing crinoid, bryozoan, ostracode bioclasts, and stromatactis, but essentially no stromatoporoids or corals (Textoris, 1966). The Silurian mud mound facies were compared by Textoris with similar buildups reported from other parts of the world (Devonian of Canada, Carboniferous of Europe and EEUU).

When reefs turned mud mounds

In the 1960s, the lack of a recognizable skeletal framework in these buildups pointed out their differences with the “classical” reef idea. During some years, most works on mud mounds were focused on the study of their scarce fauna and few were mentions to the origin of the carbonate mud, paradoxically the main component. Wilson (1975) extended in his book the definition and denomination of “lime mud mound” to many other Phanerozoic buildups, which were seen as dominated by detrital, bioclastic micrites with minor organic boundstone and *perceived to accumulate both through hydrodynamic processes and in situ organic production*. Thus, during the 1970s, the origin of the matrix was explained by the baffling theory: the carbonate mud, externally produced, was baffled by metazoans (crinoids, bryozoans, sponges, etc.). The role of early submarine cementation was also taken into consideration to explain the stabilization of mud mound depositional slopes. Baffled micrites and subsequent cementation was the proposed origin; thus, mud mounds were compared with very different types of Holocene buildups: shallow-water seagrass mudbanks and deep-water coral lithoherms.

When some mud mounds turned into microbial reefs

From the 1970s to the 1980s, some fabrics and textures present in the mud mounds were related with “cryptalgal” and other biosedimentary structures as stromatolites and thrombolites (Monty, 1976; Pratt, 1982). The mud mound matrix could be now bound, trapped, and precipitated by the activity of microorganisms (“blue-green algae” – cyanophytes and cyanobacteria).

From then, the source or origin of the carbonate mud, the matrix problem, will be the leitmotiv in the study of mud mounds. Bosence et al. (1985), from the recent carbonate mounds from Florida Keys, proposed three mud mound models based on the relation between the external and the internal sediment (carbonate mud) supplies in the mound: export, import, and self-sufficient models, respectively. From the 1970s and clearly in the 1990s, mud mounds began to be seen as “self-sufficient” mounds, and the role of microbial communities in their construction gained more importance (Tsien, 1985; Lees and Miller, 1985; Camoin and Maurin, 1988).

James and Bourque (1992) proposed a conceptual classification of reefs and “mounds,” where mounds are *those structures which were built by smaller, commonly delicate and/or solitary elements in tranquil settings*. They differentiated three types of mounds: “microbial” mounds, “skeletal” mounds, and “mud” mounds. Microbial and skeletal mounds are grouped into “biogenic mounds” because they are organically controlled, whereas the term “mud mound” was restricted by the authors to those *formed by inorganic accumulation of mud with variable amounts of fossils*. The authors pointed out that *the division between biogenic mounds and mud mounds depends on the nature of the accumulation/construction controls, not on the percentage of fossils*.

One of the main outstanding questions concerning the mud mounds is related to the modes of formation of the carbonate mud matrix and their recognition. The increase in the number of studies on microbial carbonates and their formation and fabric classifications (Kennard and James, 1986; Burne and Moore, 1987; Riding, 1991) led to new horizons in the matrix problem. Fabrics and geochemical signatures from automicrites of recent cryptic microbialites and controls in their formation (Reitner, 1993) were found to be similar to those recorded in Cretaceous and Jurassic mud mounds (Neuweiler, 1993; Keupp et al., 1993; Reitner et al., 1995; Leinfelder and Keupp, 1995). Thus, modern automicrites began to be used as modern counterparts of some mud mound end products.

Some authors began to use the term mud mound in a descriptive nongenetic way as *carbonate buildup consisting of more than 50% of mud and/or peloidal mud* (Reitner and Neuweiler, 1995), having depositional relief, and forming part of a polygenetic and continuum spectrum ranging from “biodetritral” to “microbial” mud mounds (Bosence and Bridges, 1995). Pratt (1995) considered mud mounds as ecologic reefs because they possess “rigid frameworks” produced by both microbial activity and syndimentary cementation.

Last formal definition of mud mound has been given by Riding (2002), as *carbonate mud-dominated (micrite and fine-silt) deposits with topographic relief and with few or no stromatolites, thrombolites, or in-place skeletons*. Riding added that *the deposits can be organic and/or inorganic in origin and it can be difficult to distinguish their origins*.

General features

Mud mounds have varied external forms; the term mud mound is used here not in a morphological way due to the fact that not all of them are mounds or dome-shaped; they can be tabular-shaped and can occur as isolated massive bodies or as stacked or amalgamated bodies, spreading over kilometers. The biggest mud mound complex is the Waulsortian mudbank from Ireland.

They could develop as a high syndimentary relief and show depositional dips from 10° up to 50°, can be or cannot be flanked by bioclastic limestones, and grow up from

basinal to platform settings. Mud mounds are composed mainly of fine-grained carbonate-matrix-supported fabrics, which are called, depending on the classification schemes followed, mudstones, wackestones, biomicrites, etc. These carbonate muds are texturally and genetically varied. The presence of cavities can be a characteristic feature in some mud mounds. Their macrobiota are diverse and can be important volumetrically in some mounds, although they never constitute a skeletal framework.

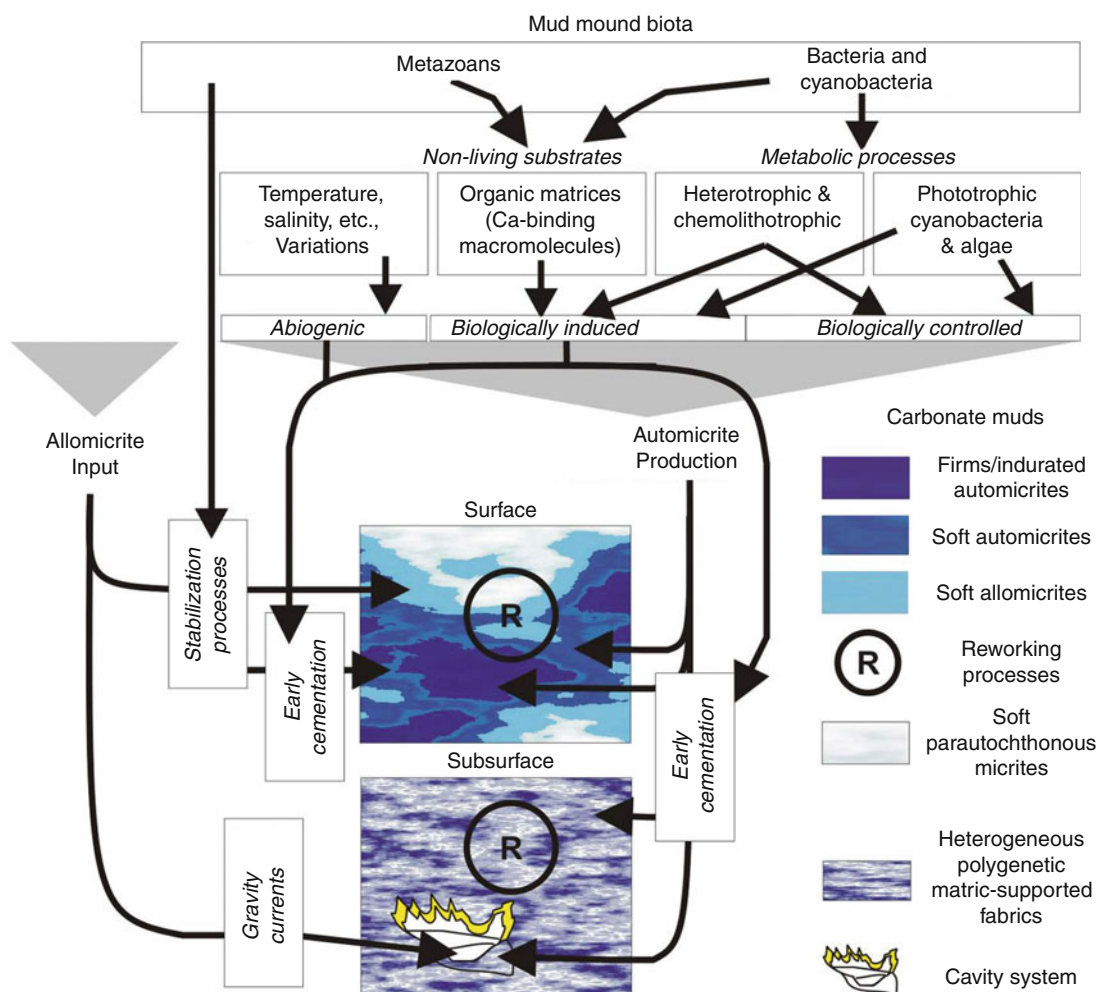
Types of carbonate muds

Carbonate muds are formed by pure aphanitic micrites and/or mixture of silt-sized calcite particles (up to 62 μm) as peloids. Then, they can be texturally grouped into aphanitic and peloidal micrites. Both types occur separately, or there is grading between them; they also occur as neomorphosed microspars. Microstructurally, they appear as dense and/or clotted/grumular/spongiostromic microfabrics, which constitute at mesoscale, stromatolitic,

thrombolitic, fenestral, and/or cryptalgal/cryptomicrobial/leiolitic fabrics (summarized in Riding, 2000).

The carbonate muds can be classified in relation to the *locus* of their production. The muds externally produced and later imported into the mound are named allomicrites (Figure 1). They are stabilized at mound surface by mound biota (eukaryotes and prokaryotes) through baffling, trapping, and/or binding mechanisms and early cementation.

The in situ produced carbonate muds are named automicrites. They are formed through abiogenic, biologically induced, and biologically controlled mechanisms (summarized in Flügel, 2004). The biologically related mechanisms are known as nonenzymatically and enzymatically controlled carbonates. Mud mounds seem to be specially dominated by the nonenzymatically carbonate production of automicrites via nonliving substrates (organomineralization of the organic matter derived from decaying organisms) and/or via metabolic processes (by heterotrophic, chemolithotrophic, and phototrophic bacteria and cyanobacteria). In the first case, the



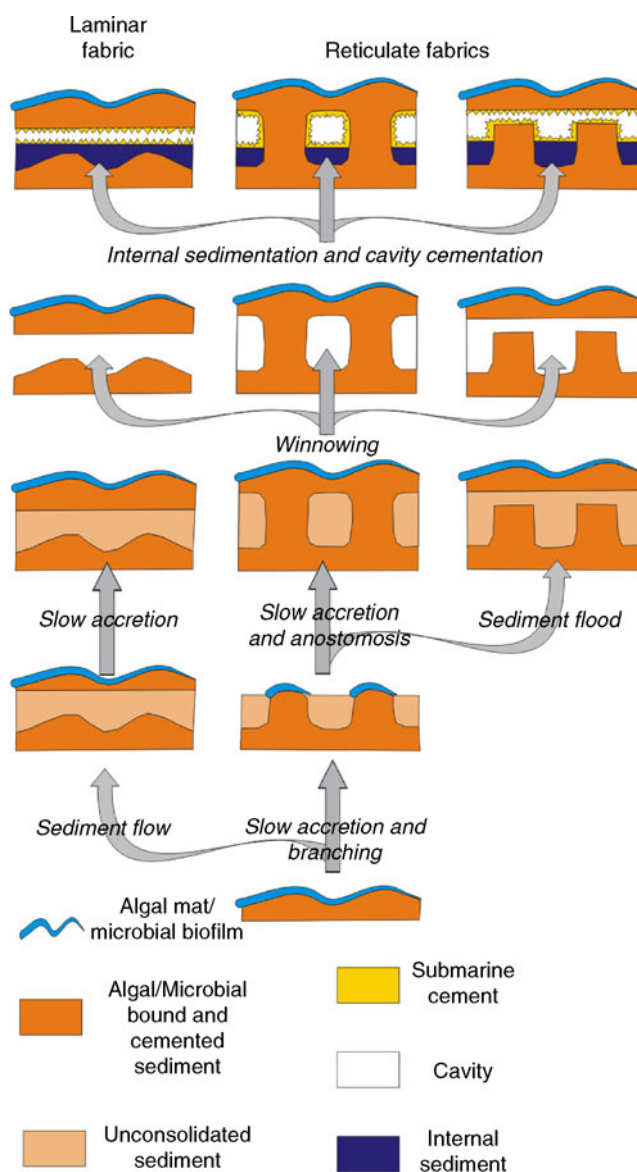
Mud Mounds, Figure 1 Relationships between the mud mound biota and carbonate mud stabilization and production processes.

resulting automicrites are known as organomicrites. The automicrite production occurs at mound surface as well as at subsurface.

The mud mound system comprises both soft (allomicrites and automicrites) as well as firm/early indurated carbonate muds (automicrites), which can suffer from reworking processes (chemical, physical, and/or biological ones) resulting in parautochthonous muds. These secondary muds or the next successive generations of in situ muds, therefore, resemble a mixture of previous ones. This system should be analyzed as a continuous series of processes of automicrite production, allomicrite input, and internal reworking.

The filling of mound cavity system records many of the last mud mound synsedimentary episodes: (1) the entry of allomicrite by gravity or currents, (2) the colonization by cryptic biota, (3) production of automicrites, (4) reworking processes, and (5) the early marine cementation phases as well as burial-related phases.

So mud mounds have polygenetic carbonate mud-dominated fabrics resulting from automicrites production, allomicrites input, and recycling processes (Figure 1). But some microfabrics have been related at the same time with different types of biogenic as well as abiogenic processes, and discriminating between some processes and their products is not always possible.



Mud Mounds, Figure 2 Microbial mat-based model for accretion of mud mounds and development of cavities. (Modified from Pratt, 1982.)

Biota

Dominant invertebrate macrofossils on mud mounds were suspension feeders as pelmatozoans, bryozoans, and sponges – siliceous and calcareous types, although others such as brachiopods, molluscs, and arthropods could be sporadically important. Solitary and colonial corals occasionally occurred on mud mound records as well as calcareous algae, but they were much less common (not as colonizers of the shallow-water mud mound phases). Others such as benthic foraminifera and polychaete worms also occurred as part of the mud mound biota from the Carboniferous times. In general, the biota of mud mounds was mainly dominated by heterozoan assemblages.

In mud mounds, excluding the evident microbial fabrics (stromatolitic, thrombolitic and others), the main signal or product of microbial activity is hidden in the carbonate mud (biogenic automicrites) and in some authigenic mineralizations (iron pigments, Fe/Mn surfaces, framboidal pyrites, etc.). There is also direct evidence of calcified microbes (*Girvanella*, *Renalcis*, *Epiphyton*, *Bacinella*, *Rivularia*-like forms, etc.), and most of them have been interpreted as cyanobacterial fossils. Other evidence of microbial activity is the presence of unidentified filament-rich microfibrils, filaments and spherulites as moulds or casts, and peloids.

Sponge–bacterial assemblages have also played an important role in the initiation and first stages of many mud mound developments (early cementation of sponge tissues is related with the activity of symbiotic bacteria as well as the organomicrite production through the decaying of their tissues).

Cavities

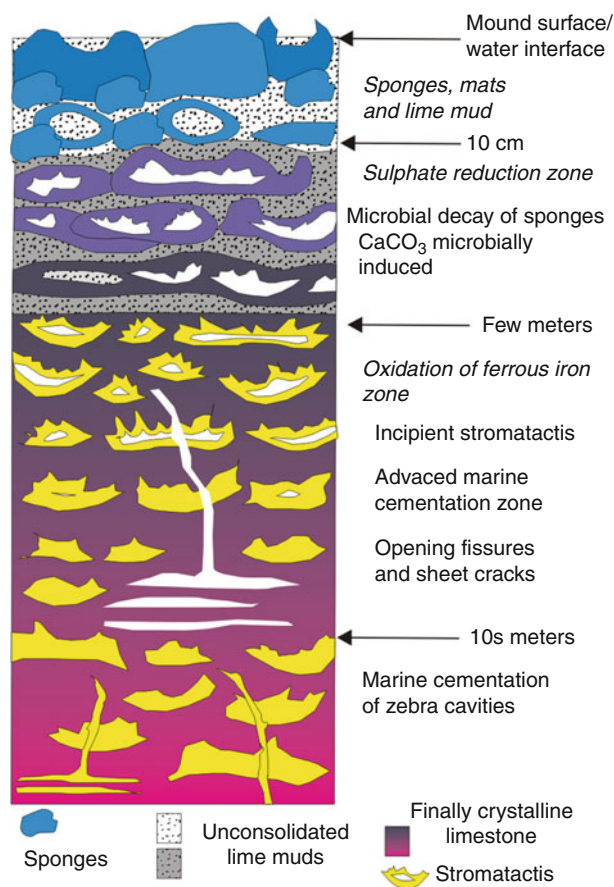
There is a wider spectrum of cavity types in mud mounds (stromatactis, zebra, irregular, and sheet-like shapes). Probably, the most famous are the stromatactis, with smooth, flat base and irregularly digitate roofs, matrix-supported in general, although some can be partially or totally sheltered by remains of fossils. Filled with centripetal marine fibrous cement crusts and varied internal sediments (allomicrites and automicrites), stromatactis were abundant in Paleozoic mud mounds, especially from the Ordovician–Mississippian interval.

The origin of stromatactis has been largely debated, and today it remains controversial. Several hypothesis has been used (summarized in Flügel, 2004, 194 p.), which can be grouped in “organic” (recrystallized fossils, related with decaying organism, burrowing, etc.) and “inorganic” theories (dissolution, collapse, internal erosion between submarine crusts or bioclasts, dewatering and compaction of the muds, recrystallization, enlargement of preexisting cavities, presence of gas hydrates, etc.). In the organic cases, there are two models: the cryptalgal/microbial mat model and the sponge model.

The microbial mat model was initially proposed by Pratt (1982) to explain the mud mound accretion and its

final observed fabrics (Figure 2). The fabrics and the resulting cavities were the result of the combination of three variables: (1) the distribution of the microbial mat, (2) the rate of sediment loading onto the mat, and (3) the degree of winnowing. The distribution of the microbial mat (laterally continuous or in patches) produces the resulting fabrics (laminar or reticulate, respectively). The winnowing of the unconsolidated sediment between the microbial bounds and cemented sediment produces the cavities.

In the sponge model (Bourque and Boulvain, 1993), the cavities are formed by the partial collapse of the sponge body as result of early diagenetic processes through four stages (Figure 3): (1) zone of the living community, (2) zone of sulphate reduction, (3) zone of oxidation of ferrous iron, and (4) zone of marine cementation. In the living surface, the mud is trapped by sponges and/or produced by microbial activity. Below the surface, the microbial decay of sponges occur; part of the sponge body and the mud are cemented, and other sponge parts are in collapsed state; the later internal erosion of the unconsolidated material produces the open cavities. A few meters



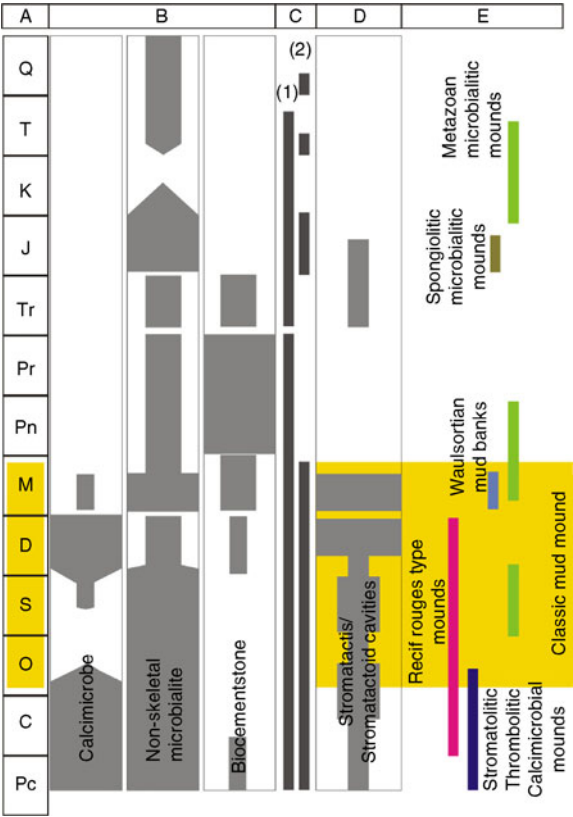
Mud Mounds, Figure 3 Sponge model for the Paleozoic red stromatolitic mud mounds. (Modified from Bourque and Boulvain, 1993.)

below, in the ferrous iron oxidation zone began the incipient marine cementation of these cavities forming the stromatactis.

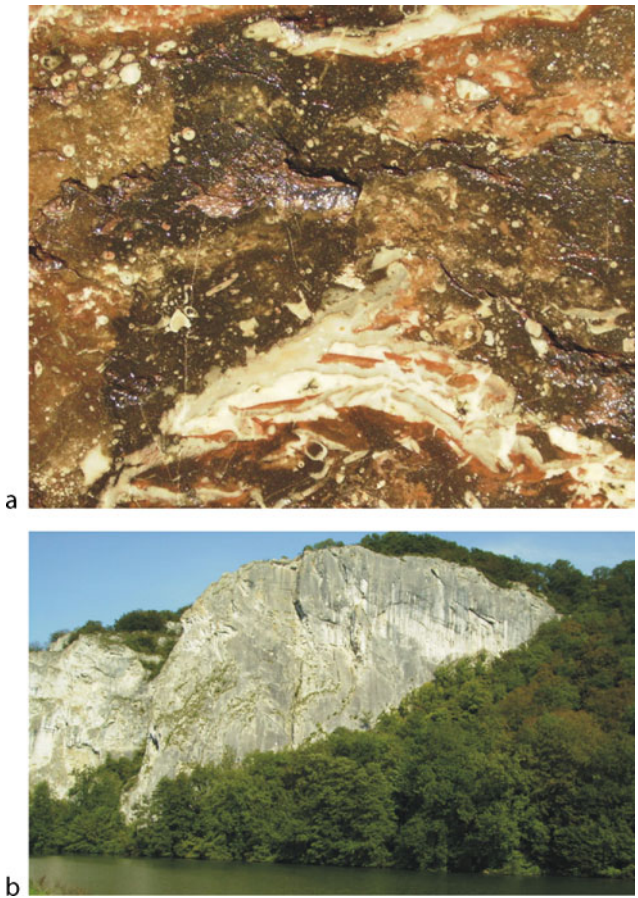
Types of mud mounds through the Phanerozoic record

The classification and distribution of mud mounds through the record depend on the “mud mound versus reef concept” of almost each researcher and thus remain controversial in several aspects. Mud mounds have existed from Proterozoic times and through the Phanerozoic record (see reviews by Monty, 1995; Pratt, 1995), and important growth episodes occurred in early Cambrian, late Devonian, and Mississippian times. Cambrian to Ordovician mud mounds (Figure 4) were characterized by different fabrics and assemblages (stromatolitic, thrombolitic, and massive fabrics with calcimicrobes, and cambrian archaeocyathid/sponge-calcimicrobial

assemblages). From the Ordovician to the Mississippian, some of the carbonate mud-rich buildups, which typify this interval, have been regarded as the classical massive mud mounds with abundant stromatactis (Figure 4) as the ‘red stromatactis mounds’ (Recif Rouges-type mound) and the Waulsortian mudbanks (Figure 5a and b). Clear microbilite-related fabrics (stromatolites and thrombolites) are extremely rare in classical mud mounds, although they show other nonskeletal reef frameworks (calcimicrobial and biocemenstones *sensu*, Webb, 1996), as well as filaments and some characteristic features (clotted, peloidal micrites, and fenestral microfabrics). The parallel diversification of skeletal mound macro- and micro-biota produced more varied metazoan–microbialitic assemblages. One of the most persistent through the mud mound record is the sponge–microbialitic association, very common in Jurassic mud mounds as well as in the deeper initial stages of many Paleozoic mounds. Early Cretaceous is considered the last significant moment of organomineralic mud mound development (Neuweiler et al., 1999), although



Mud Mounds, Figure 4 Distribution of mud mounds through the geologic record. (A) Precambrian to Quaternary time divisions. (B) Distribution and relative importance of nonenzymatic reef frameworks (from Webb, 1996), characteristic also in mud mounds. (C) Distribution of (1) microbial and (2) biotrital mud mounds. (Modified from Bosence and Bridges, 1995.) (D) Distribution of stromatactis and stromatactoid cavities, the size of the bar represents their relative importance in mud mounds. (E) Interval distribution of the main typologies of mud mounds.



Mud Mounds, Figure 5 Classical mud mounds. (a) Close view of a Devonian Recifs Rouges-type mound, Beauchateau Quarry, Belgium. (b) Carboniferous Waulsortian mudbank, Waulsort, Belgium.

other authors mentioned that the latest mud mounds are Miocene (Pratt, 2000).

Ecological zonation models

The Récifs Rouges-type mounds and the Waulsortian mudbanks are probably the most famous and are well known as “classical mud mounds” (Figures 4 and 5) Devonian (Frasnian) mounds from Belgium show a shallowing upward succession from deep basal greenish-grey shales through their mud mound facies: red lime mudstones to wackestones with stromatactis, pink wackestones with millimeter stromatactis to crudely bedded grey wackestones to packstones, and bindstones, and their associated biota. Similar facies (red stromatactis mounds) and assemblages have been found in other mud mounds through the Paleozoic record (Bourque, 1997). The carboniferous Waulsortian mudbanks of Belgium show a four grain-type assemblages related with the growth of these mounds from deep subtidal, aphotic to photic conditions. A depth-related phase model was recognized by Lees and coworkers (summarized in Lees and Miller, 1995) and applied to Waulsortian mudbanks of Europe and North America. Bourque (1997) compared Récifs Rouges-type mounds from Silurian of Canada and Devonian of France and Belgium with carboniferous mounds from Algeria and the Waulsortian mudbanks model and established an ecological zonation model formed by four benthic communities zones (Figure 6): (1) the sponge zone, (2) the fenestellid–sponge zone, (3) the delicate skeletons–sponge zone, and (4) the delicate skeletons–microbial encrusters zone.

Controlling factors

Today, many parameters about the initiation, accretion, and spread of many mud mounds seem to be enigmatic. However, some others begin to be more clear.

Locations of some mud mounds have been related with submarine cold seeps, vents, faults, halokinetic and argilokinetic settings, and local antecedent topographic irregularities, have been associated to nutrient-enriched areas (by endo-, down- and/or up-welling currents), and could be also related with stratified basin waters and fluctuating oxygen minimum zone. Many of them grew up into aphotic to disphotic depths (more than 200 m); however, they could reach and colonize the euphotic zone. Some grew at lower sedimentation rates and/or during sedimentary starvation during TST episodes, although this is not a general trend for all of them. Most of these factors are oceanographic and/or tectonically basin-related parameters and could co-occur together in many cases.

Those factors (biological and chemical parameters) associated with the carbonate mud precipitation related with eukaryotes and prokaryotes should be taken into consideration:

- Production of acidic organic macromolecules (AOM) and an increase in carbonate alkalinity via (1) bacterial heterotrophic pathways (ammonification and sulphate reduction), (2) carbonate, and (3) silicate weathering. AOM and high alkalinity favor the organomicrite production in microbialites and mud mounds (Reitner and Neuweiler, 1995).
- Extracellular polymeric substances (EPS)-biomineralization of microbial mats and biofilms.
- Calcification of cyanobacteria.

Cambrian-devonian Récifs rouges-type mounds			Carboniferous mud mounds			
Silurian (Canada)	Devonian (France)	Devonian (Belgium)	Viséan (Algerian)	Waulsortian mud banks phases	Depth-related communities	
<i>Fenestellids</i> <i>Stromatoporoids</i> <i>Calcareous algae</i> <i>Microbial encrusters</i>		<i>Corals</i> <i>Stromatoporoids</i> <i>Microbial encrusters</i>	<i>Rugose corals</i> <i>Microbial encrust.</i> <i>Calcareous algae</i>	Phase D: C + grain coating calcareous algae	<i>Delicate skeletons and microbial encrusters</i>	Photic
	<i>Fenestellid</i> <i>Sponge (spicules)</i> <i>Crinoids</i> + mud	<i>Corals</i> <i>Crinoids</i> <i>Bryozoans</i> + mud	<i>Crinoids</i> <i>Fenestellids</i> <i>Sponge (spicules)</i> <i>Red algae</i> + mud	Phase C: B + Plurilocular foraminifera	<i>Delicate skeletons and sponges + mud</i>	Aphotic
			<i>Sponges</i> <i>Fenestellids</i>	Phase B: A + Sponger spicules Phase A: fenestellids, crinoids, ostracods	<i>Fenestellids and sponges</i>	
<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i>		<i>Sponges</i>	

Mud Mounds, Figure 6 Ecological zonation assemblages in some Paleozoic mud mounds. (Modified from Bourque, 1997.)

- (d) CO₂ depletion of the medium through autotrophic pathways by methanogenic archaea, sulfurous and nonsulfurous purple and green photosynthetic bacteria, and cyanobacteria (Castanier et al., 2000).

Conclusions

Mud mounds are considered mainly as nonenzymatic carbonate deposits that formed a polygenetic and very continuous record of buildups through the Phanerozoic and reached a maximum development during the Paleozoic times. They are of considerable importance not from an economic point of view (hydrocarbon reservoirs, major mineral host-rock and base deposits, ornamental rocks) alone. In fact, they represent an excellent record for the study and establishment of (1) the interactions between the marine microbial and nonmicrobial benthic communities (paleoecologic and paleoenvironmental parameters), (2) marine carbonate production models (mud mound factory), and (3) proxies in palaeoceanographic reconstructions (nonenzymatic carbonates are formed in equilibrium with ambient sea water).

Mud mounds are characterized by different modes of carbonate mud production (abiogenic, biologically induced, and biologically controlled automicrites) and sedimentation input (allomicrites). The key of mud mounds understanding lies behind here: to recognize and differentiate between the automicrite and allomicrite relationship and their contribution to the mud mound accretion.

Bibliography

- Bosence, D. W. J., and Bridges, P. H., 1995. A review of the origin and evolution of carbonate mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt B. R. (eds.), *Carbonate Mud-mounds: Their Origin and Evolution*. Oxford: Blackwell Science for the International Association of Sedimentologists, Special Publication, **23**, pp. 3–9.
- Bosence, D. W. J., Rowlands, R. J., and Quine, M. L., 1985. Sedimentology and budget of a recent carbonate mound, Florida keys. *Sedimentology*, **32**, 317–343.
- Bourque, P. A., 1997. Paleozoic finely crystalline carbonate mounds: cryptic communities, petrogenesis and ecological zonation. *Facies*, **36**, 250–253.
- Bourque, P.-A., and Boulvain, F., 1993. A model for the origin and petrogenesis of the red stromatolite limestone of Paleozoic carbonate mounds. *Journal of Sedimentary Petrology*, **63**, 607–619.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaaios*, **2**(3), 241–254.
- Camoin, G., and Maurin, A.-F., 1988. Roles of micro-organisms (bacteria, cyanobacteria) in the formation of mud mounds: examples from the Turonian strata of Jebels Bireno and Mrhila (Tunisia). *Compte Rendu des Académie des Sciences de Paris, Série II*, **307**, 401–407.
- Castanier, S., Le Métyer-Levrel, G., and Perthuisot, J. P., 2000. Bacterial roles in the precipitation of carbonate minerals. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 32–39.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks: Analysis, Interpretation and Application*. Berlin: Springer, 976 p.
- James, N. P., and Bourque, P.-A., 1992. Reefs and mounds. In Walker, R. G., and James, N. P. (eds.), *Facies Models: Response to Sea Level Change*. St. John's, Newfoundland: Geological Association of Canada, pp. 323–347.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaaios*, **1**(5), 492–503.
- Keupp, H., Jenisch, A., Herrmann, R., Neuweiler, F., and Reitner, J., 1993. Microbial carbonate crusts – a key to the environmental analysis of fossil spongiolites? *Facies*, **29**, 41–54.
- Lees, A., and Miller, J., 1985. Facies variation in Waulsortian buildups. Part 2. Mid-Dinantian buildups from Europe and North America. *Geological Journal*, **20**, 159–180.
- Leinfelder, R. R., and Keupp, H., 1995. Part III Upper Jurassic mud mounds: allochthonous sedimentation versus autochthonous carbonate production. *Facies*, **32**, 17–26.
- Monty, C. L. V., 1976. The origin and development of cryptalgal fabrics. In Walter, M. R. (ed.), *Stromatolites: Developments in Sedimentology*. Amsterdam: Elsevier, Vol. 20, pp. 193–249.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, Their Origin and Evolution*. International Association of Sedimentologists. Special publication, **23**, pp. 11–48.
- Neuweiler, F., 1993. Development of Albian microbialites and microbialites reefs at marginal platform areas of the Vasco-Cantabrian Basin (Soba Reef, Cantabria, N. Spain). *Facies*, **29**, 231–250.
- Neuweiler, F., Gautret, P., Thiel, V., Langes, R., Michaelis, W., and Reitner, J., 1999. Petrology of lower cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Pratt, B. R., 1982. Stromatolitic framework of carbonate mud-mounds. *Journal of Sedimentary Petrology*, **52**(4), 1203–1227.
- Pratt, B. R., 1995. The origin, biota and evolution of deep water mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, Their Origin and Evolution*. Oxford: Blackwell Science for the International Association of Sedimentologists, Special Publication, **23**, pp. 49–123.
- Pratt, B. R., 2000. Microbial contribution to reefal mud-mounds in ancient deep-water settings: evidence from Cambrian. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 282–287.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan rich-facies from Lizard Island (Great Barrier Reef, Australia) – formation and concepts. *Facies*, **29**, 3–40.
- Reitner, J., and Neuweiler, F., 1995. Part I, mud mounds: recognizing a polygenetic spectrum of fine-grained carbonate buildups. *Facies*, **32**, 2–4.
- Reitner, J., Neuweiler, F., and Gautret, P., 1995. Part II modern and fossil automicrites: implications for mud mounds genesis. *Facies*, **32**, 4–17.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 21–51.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2002. Structure and composition of organic reef and carbonate mud mounds: concepts and categories. *Earth-Science Reviews*, **58**, 163–231.
- Textoris, D. A., 1966. Algal cap for a Niagaran (Silurian) carbonated mud mound of Indiana. *Journal of Sedimentary Petrology*, **36**(2), 455–461.

- Tsien, H. H., 1985. Algal-bacterial origin of micrites in mud mounds. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 290–296.
- Webb, G. E., 1996. Was Phanerozoic reef history controlled by the distribution of non-enzymatically secreted reef carbonates (microbial carbonate and biologically induced cement)? *Sedimentology*, **43**, 947–971.
- Wilson, J. L., 1975. *Carbonate Facies in Geologic History*. Berlin: Springer, 471 p.

Cross-references

[Bacteria](#)
[Biofilms](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Reefs](#)
[Waulsortian Mud Mounds](#)

MUTUALISM

Mutualism is a form of a symbiosis, in which both organisms involved benefit. See entry “[Symbiosis](#)” for further reading.

MYCORRHIZAE

A mycorrhiza (pl mycorrhizae, from the Greek “*mycos*” = fungus and “*rhiza*” = root) is a plant-fungal association that comprises the most widespread type of terrestrial symbiosis. See entry “[Symbiosis](#)” for further reading.

- Bouougri, E. H., and Porada, H., 2007. Siliciclastic biolaminites indicative of widespread microbial mats in the Neoproterozoic Nama Group of Namibia. *Journal of African Earth Sciences*, **48**, 38–48.
- Bouougri, E., Gerdes, G., and Porada, H., 2007. Inherent problems of terminology: definition of terms frequently used in connection with microbial mats. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp.145–151.
- Eriksson, P. G., Schieber, J., Bouougri, E., Gerdes, G., Porada, H., Banerjee, S., Bose, P. K., and Sarkar, S., 2007. Classification of structures left by microbial mats in their host sediments. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp. 39–52.
- Gehling, J. G., 1999. Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. *Palaaios*, **14**, 40–57.
- Gerdes, G., Klenke, Th., and Noffke, N., 2000. Microbial signatures in peritidal siliciclastic sediments: a catalogue. *Sedimentology*, **47**, 279–308.
- Hagadorn, J. W., and Bottjer, D. J., 1997. Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic-Phanerozoic transition. *Geology*, **25**, 1047–1050.
- Hagadorn, J. W., and Bottjer, D. J., 1999. Restriction of a late neoproterozoic biotope: suspect-microbial structures and trace fossils at the Vendian-Cambrian transition. *Palaaios*, **14**, 73–85.
- Krumbein, W. E., Paterson, D. M., and Stal, L. J. (eds.), 1994. *Biostabilization of Sediments*, Oldenburg: BIS Verlag, 526 p.
- McKenzie, D. B., 1972. Tidal sand flat deposits in Lower Cretaceous Dakota Group near Denver, Colorado. *The Mountain Geologist*, **9**, 269–277.
- Noffke, N., 1998. Multidirected ripple marks rising from biological and sedimentological processes in modern lower supratidal deposits (Mellum Island, southern North Sea). *Geology*, **26**, 879–882.
- Noffke, N., 1999. Erosional remnants and pockets evolving from biotic-physical interactions in a recent lower supratidal environment. *Sedimentary Geology*, **123**, 175–181.
- Noffke, N., Gerdes, G., Klenke, T., and Krumbein, W. E., 2001. Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. *Journal of Sedimentary Research*, **71**, 649–656.
- Noffke, N., Gerdes, G., and Klenke, T., 2003a. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty, and evaporitic carbonatic). *Earth-science Reviews*, **62**, 163–176.
- Noffke, N., Hazen, R., and Nhlenko, N., 2003b. Earth's earliest microbial mats in a siliciclastic marine environment (2.9 Ga Mozaan Group, South Africa). *Geology*, **31**, 673–676.
- Pflüger, F., 1999. Matground structures and redox facies. *Palaaios*, **14**, 25–39.
- Porada, H., and Bouougri, E. H., 2007. Wrinkle structures – a critical review. *Earth-science Reviews*, **81**, 199–215.
- Porada, H., and Löffler, T., 2000. Microbial shrinkage cracks in siliciclastic rocks of the Neoproterozoic Nosib Group (Damara Supergroup) of central Namibia. *Communications of the Geological Survey of Namibia*, **12**, 63–72.
- Porada, H., Ghergut, J., and Bouougri, E. H., 2008. Kinneyia-type wrinkle structures –critical review and model of formation. *Palaaios*, **23**, 63–77.
- Schieber, J., 2004. Microbial mats in the siliciclastic rock record: a summary of diagnostic features. In Eriksson, P. G., Altermann, W., Nelson, D., Mueller, W. U., Catuneanu, O., and Strand, K. (eds.), *The Precambrian Earth: Tempos and Events. Developments in Precambrian Geology*. Amsterdam: Elsevier, pp. 663–672.
- Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O., 2007. *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, 311 p.
- Stal, L. J., 2000. Cyanobacterial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Amsterdam: Kluwer, pp. 61–120.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine Geology*, **113**, 3–25.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Iron Sulfide Formation](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Sulfate-Reducing Bacteria](#)
[Tidal Flats](#)

METAGENOMICS

Wolfgang Liebl
 Technische Universität München,
 Freising-Weihenstephan, Germany

Definition

Metagenomics is a research discipline that uses molecular biology methods to acquire, analyze, and interpret sequence data and/or functional data from the metagenomes (the collectivity of genomes) present in the environmental samples, without the need to cultivate the organisms in the samples. Related methodologies are so-called metatranscriptomics and metaproteomics that focus on the collective transcriptomes and proteomes, respectively, rather than on the genomes.

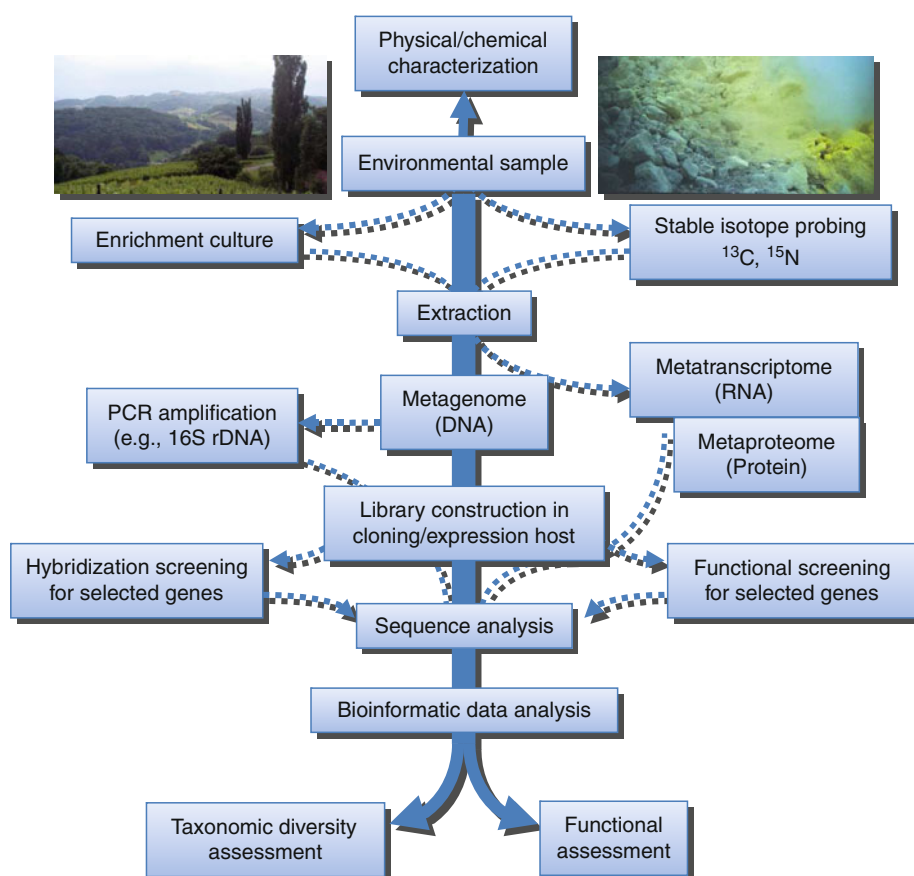
Introduction

Microorganisms constitute major life forms on earth with respect to cell numbers and total biomass, and fulfill essential functions in global element cycles. It has been estimated that the earth's biosphere harbors at least two to three orders of magnitude more prokaryotic cells ($4\text{--}6 \times 10^{30}$) than the number of plant and animal cells combined. The total carbon fixed in prokaryotic cells amounts to $3.5\text{--}5.5 \times 10^{17}$ g of C which is 60–100% of the estimated total carbon in plants (Whitman et al., 1998).

For many decades since the beginnings of microbiology, research in this field has depended on the isolation and cultivation of individual microorganisms (best illustrated for the area of medical microbiology with the work of Robert Koch on the isolation and characterization of pathogenic bacteria in monocultures, leading to the Koch's postulates) or well-defined syntrophic consortia, and the subsequent in-depth analysis of pure cultures. Until today, microbiological research with traditional cultivation and

subsequent chemical, genetic, and physiological characterization has led to the description of roughly 10^4 bacterial and archaeal species deposited as pure cultures in microbial culture collections. However, in the course of the past 2 decades, it has become more and more evident that the microbial diversity in most aquatic habitats, soil, as well as oceanic and terrestrial subsurfaces is enormous (Amann et al., 1995; Hugenholtz et al., 1998; Quince et al., 2008; Sleator et al., 2008) and that at least at present the majority (>99%) of microorganisms in many complex habitats are not cultivated by using standard techniques (Amann et al., 1995). Thus, it must be acknowledged that the number of described species contrasts by orders of magnitude with estimates about the number of prokaryotic taxa on earth which may amount to 10^6 – 10^8 distinct genospecies (Simon and Daniel, 2010). Metagenomics, a relatively young research discipline, can aid in the detection and acquisition of data about this vast majority of uncultured microorganisms. For this methodology, which importantly is independent of cultivation, the collectivity of genomes (the metagenome) present in a given environmental sample is extracted and subjected to completely random or gene-targeted sequence analysis (Figure 1).

Although bacteria and archaea normally are unicellular, they usually occur as highly diverse but often poorly characterized consortia in their natural habitats. Individual environmental samples can contain a breathtaking prokaryotic diversity. For example, the numbers of different molecular species or operational taxonomic units (OTUs) in soil samples could be over 10^6 (Curtis et al., 2002). Statistics-based predictions for a specific Canadian soil sample range from 2×10^4 to 1.4×10^5 taxa (Quince et al., 2008). Highly interesting, based on taxonomy assessment results from deep-sea hydrothermal vent samples and statistical follow-up considerations, even samples from extreme environments – which often are believed to contain only limited biodiversity due to the extreme conditions – have revealed enormous microbial biodiversity, for example, 3×10^5 bacterial taxa at one specific sample site (Quince et al., 2008). These numbers illustrate the problems one faces to reach near-complete metagenomic datasets for complex natural habitats with today's analysis techniques. Thus, even with high-throughput methods, it is difficult if not impossible to generate a complete microbial inventory of a habitat including also most of the rare taxa. However, the methods available do allow the reliable



Metagenomics, Figure 1 Flowchart summarizing steps of environmental meta-analysis.

identification of the abundant taxa that presumably account for the major biogeochemical conversions.

Uses and applications of metagenome analysis

One major advantage of metagenomic analysis is that one can obtain a wealth of genetic information about organisms that live together in more- or less-complex communities, most of which cannot be cultivated with today's standard techniques. It can be used for extremely diverse purposes, such as the analysis of fundamental aspects of microbial community structure, function, and adaptation in environmental microbiology, but also to explore and exploit the microbial genetic, metabolic, and enzymatic diversity space for the development or improvement of biotechnological processes. Examples for the application of metagenomic techniques in geobiology are the analysis of microbial communities in rock material, sediments, aquifers, as well as in biofilms, on rock surfaces or in subsurface caves and mines, etc. It must be stressed that it is of utmost importance for most metagenomic studies to comprehensively record the sample-associated metadata (the data about the data), like date and position of sampling, physical and chemical characteristics, etc., as has been pointed out by Wooley et al. (2010).

Principally, metagenome analysis can be applied to any kind of habitat, irrespective of the complexity of the microbial communities present. For low-diversity habitats, high-throughput metagenome sequencing can yield a comprehensive overview of genetic information present and can even allow the near-complete or complete assembly of whole prokaryotic genomes, which was nicely shown for the organisms present in acid mine drain fluids (Tyson et al., 2004) or community genome sequencing of an anammox bacterium (Strous et al., 2006). However, the more diverse the community, the higher the sequencing and/or screening effort has to be to arrive at results that cover as comprehensively as possible the target genes of interest present in the sample. In some cases, it is possible to reduce the amount of work and costs by choosing a more specific than a completely random metagenome analysis. For example, if the main interest of a study lies in the analysis of genes involved in a certain metabolic trait (like breakdown and utilization of a specific substrate), one can try to enrich for the corresponding genes by carrying out enrichment cultures wherein the growth of organisms which display this metabolic trait is favored (e.g., by supplying the said specific substrate as the sole carbon source). Enrichment cultivation also has the advantages that (i) more cell material becomes available and that (ii) less organic and inorganic contaminating compounds from the original habitat sample that may interfere with DNA isolation yield and quality are present. An alternative is to enrich for genomic DNA or RNA from microorganisms with a certain substrate utilization capability by feeding a natural microbial community with ^{13}C - or ^{15}N -labeled substrate and then to isolate and process only the heavy nucleic acid that stems from

organisms that utilized the added ^{13}C - or ^{15}N -substrate and incorporated the label into their nucleic acids (stable isotope probing) (Friedrich, 2006; Neufeld et al., 2007). Using enrichment cultures to preselect for microorganisms with certain physiological traits has been successfully done in several cases (e.g., Entcheva et al., 2001) and may be of advantage especially for very complex microbial communities, but it has to be kept in mind that any kind of cultivation step before DNA isolation can result in dramatic changes in community composition and diversity.

Assessment of phylogenetic and functional diversity

Sequence analysis of conserved marker genes, usually 16S rRNA genes, is widely used for community diversity assessment. To this end, 16S rRNA gene sequences can be amplified with PCR directly from metagenomic DNA samples, followed by cloning into appropriate vectors and sequence analysis. Also, the sequences of conserved marker genes (rRNA genes, but also protein-encoding marker genes) can be retrieved from large sequence datasets compiled by large-scale random sequencing of metagenome DNA and then used for phylogenetic community composition assessment (von Mering et al., 2007).

Recently, massively parallel sequencing specifically of rRNA gene fragments amplified from metagenomic DNA has become possible by the use of the 454 Life Sciences (Roche) high-throughput tag pyrosequencing technology (Huse et al., 2008), but due to the fact that read-length limitations presently only allow the sequencing of incomplete rRNA gene fragments, this technology is suited for the trustworthy phylogenetic assignment at the phylum level but is not as reliable at the genus or species level. Nonetheless, this method allows interesting insights into the affiliation of microorganisms from complex communities to the large phylogenetic lineages, and technological improvements pertaining to the read lengths will surely allow more precise analyses at the genus or species level in the near future.

Bioinformatic tools are available for the comprehensive phylogenetic tree calculation from alignments of conserved marker sequences, for example, the ARB software package (Ludwig et al., 2004). Also, other sequence information than merely phylogenetically conserved genes available from large metagenomic random DNA sequence datasets can be used for diversity assessment. In the past years, various software tools have been developed that are useful for the so-called taxonomic binning of such sequence information (see Simon and Daniel, 2010). While some algorithms work on the basis of oligonucleotide sequence frequencies (composition-based binning, e.g., PhyloPhytia, TETRA; Teeling et al., 2004; McHardy et al., 2007), which are known to vary between different genomes, others use comparative orthologous ORF analysis for this purpose (CARMA, MEGAN, Sort-ITEMS; Huson et al., 2007; Krause et al., 2008; Monzoorul et al., 2009). Importantly, however, since most of these

bioinformatic tools depend on genome sequences and annotations from the available reference genome databases, which presently cover only a minority of the (readily) culturable species (not to speak of the enormous uncultured species diversity present in nature [see above]), our present possibilities for the assignment of short metagenomic DNA sequences to microbial taxa are quite limited.

Current large-scale efforts to sequence the genomes of the prokaryotic species deposited in major microbiological culture collections (GEBA, a Genomic Encyclopedia of Bacteria and Archaea; <http://www.jgi.doe.gov/programs/GEBA/>; Wu et al., 2009) will surely dramatically improve the reference genome sequence database necessary for a more accurate phylogenetic assignment of metagenomic sequence fragments. Nevertheless, considering that there are still in some cases not a single, in other cases only very few cultured representatives for some deep-rooting phyla, severe limitations in our ability to reliably assign short metagenomic DNA sequences to phylogenetic taxa will remain at least in the near future.

Screening for genes in metagenomes

Metagenomic studies often include the screening of metagenomic libraries or metagenomic sequence datasets for genes of interest, for example, for genes encoding key enzymes characteristic for habitat-specific physiological traits, or for genes for biotechnologically relevant enzymes. Two fundamentally different approaches are used for this: the sequence-based approach and the function-based approach. Both methods have individual advantages and disadvantages and are explained briefly in the following.

The *sequence-based approach* uses in silico sequence mining, PCR amplification or hybridization methods to detect and isolate genes of interest. Metagenomic sequence datasets can be scored for fragments encoding orthologs of known genes deposited in the available reference databases. Various bioinformatic tools such as BLAST, Pfam, SEED, TIGRFAM, MEX, RAMMCAP, or MG-RAST are available which are helpful for the functional annotation of metagenome sequence data (see Simon and Daniel, 2010; Wooley et al., 2010). Another sequence-driven approach is to design degenerate PCR primers directed against conserved sequence motifs in a gene family of interest and to amplify partial ORFs from metagenomic DNA. The fragments can then be used to isolate the complete ORFs, for example, by using them as hybridization probes to identify clones or metagenomic DNA fragments containing the adjacent, missing ORF sequences, or by metagenome walking (see Simon and Daniel, 2010). Alternatively, DNA microarrays carrying oligonucleotides derived from consensus sequences of known genes have been used for the sequence-directed screening of a metagenomic library for a light, oxygen and voltage (LOV) domain blue light photoreceptor gene (Pathak et al., 2009). The major disadvantage of sequence-based gene detection is that this method depends on prior sequence information from known gene families.

Therefore, truly novel genes unrelated to the already known genes encoding for a given function cannot be identified with this approach.

In the *function-based screening approach*, clones from metagenomic libraries are subjected to activity-based assays that indicate the function of interest. The major advantage of this approach is that it allows the recovery of complete genes or gene clusters and of novel types of enzymes without any prior knowledge of the sequence. However, in order to work efficiently, the genes of interest, which often originated from uncultured microorganisms with unknown genetics and physiology, must be compatible with the expression machinery provided by the heterologous screening host with respect to transcription, translation, protein folding, and eventually secretion (Gabor et al., 2004). Therefore, the fraction of the existing enzymatic diversity that can be accessed via functional screening approaches is strongly influenced by the choice of the host organism used. The phylogenetic distance between the gene donor and the cloning/expression host may be crucial for the success of gene expression. Mostly, *Escherichia coli* is used to construct metagenomic libraries for functional screening, but it is clear that the expression of metagenomic genes can be severely biased in this host. Therefore, it is important to establish and improve an alternative host/vector systems in order to enhance the probability of detecting genes from metagenomic samples (Angelov et al., 2009; Steele et al., 2009; Uchiyama and Miyazaki, 2009).

Some restrictions and problems

Despite the power of metagenomic studies to unravel genetic information from microbes in communities that are not amenable to cultivation and in-depth characterization, a number of restrictions and problems remain, only some of which are listed hereafter.

Huge amounts of data are generated, especially in metagenome random sequencing projects. These data need to be generated, processed, and stored in a complex succession of steps. Special care must be taken with respect to library construction and sequencing quality. Exemplarily, the procedure of a metagenomic sequencing project is described by Kunin et al. (2008). A useful compilation of software tools for composition-based and similarity-based taxonomic binning, functional annotation of metagenomic data, and comparative metagenomics can be found in Wooley et al. (2010).

Interpretation of the sequence data and their connection with biochemical functions of biocatalysts remains a challenging task. The tools for ORF extraction from metagenomic sequence data and bioinformatics-driven function prediction are being improved constantly, but it is of course not feasible to check all gene function predictions experimentally, for example, by heterologous expression and biochemical analysis. Also, if only metagenomic DNA sequence data are available, it is unclear if the genes found are expressed at all. Thus, it has to be kept in mind that in many cases sequence data

obtained from metagenomes only indirectly point to (predicted) enzymes/pathways of the organisms in the sample. In future, performing metatranscriptomics and/or metaproteomics experiments in parallel with metagenomics can help to overcome this problem.

Also, linking phylogenetic and functional metagenomic data with the physiology of living microorganisms in the environmental sample is a crucial but not a trivial step for many studies attempting to correlate environmental data with a microbial community. Due to the species complexity in most microbial communities, it is only rarely possible to obtain assembled genomes. Even if conserved marker sequences point to close relatives of certain well-known microorganisms this does not necessarily prove that the organisms from which the metagenomic sequence information was derived have identical physiologies as the reference organisms. Also, some traits believed to be characteristic for certain phylogenetic groups of microorganisms can get lost by simple mutations. Still, it is possible to extract environment-specific gene fingerprints from unassembled random environmental DNA sequencing data that reflect known characteristics of the sampled environments, thus linking metagenomic sequence information with metabolic functions of the organisms present (Tringe et al., 2005).

Finally, since most conclusions reached from metagenome data analysis rely on comparative sequence analysis and thus are indirect, it will be important to develop and improve new microbiological cultivation and characterization techniques that also allow the cultivation and in vivo analysis of physiologically and quantitatively dominant microbial representatives within the studied communities.

Summary

Considering the enormous microbial diversity in most environmental samples on one hand and the current shortcoming of the microbiologists' ability to cultivate the vast majority of these organisms on the other hand, it makes sense that metagenomics has become one of the key methods to study the composition and functions of complex microbial communities. The rapid advance of massively parallel sequencing and high-throughput screening technologies will enable even faster and cheaper access to environmental sequence datasets in the future, but connecting the metagenomic data with the physiology of the microorganisms and the environmental data remains challenging, and it will be crucial that the development of experimental setups and bioinformatic tools keep pace in order to be able to derive meaningful conclusions from metagenomic sequence data.

Bibliography

- Amann, R. I., Ludwig, W., and Schleifer, K. H., 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**, 143–169.
- Angelov, A., Mientus, M., Liebl, S., and Liebl, W., 2009. A two-host fosmid system for functional screening of (meta)genomic

- libraries from extreme thermophiles. *Systematic and Applied Microbiology*, **32**, 177–185.
- Curtis, T. P., Sloan, W. T., and Scannell J. W., 2002. Estimating prokaryotic diversity and its limits. *Proceedings of the National Academy of Science USA*, **99**, 10494–10499.
- Entcheva, P., Liebl, W., Johann, A., Hartsch, T., and Streit, W., 2001. Direct cloning from enrichment cultures, a reliable strategy for isolation of complete operons and genes from microbial consortia. *Applied and Environmental Microbiology*, **67**, 89–99.
- Friedrich, M. W., 2006. Stable-isotope probing of DNA: insights into the function of uncultivated microorganisms from isotopically labeled metagenomes. *Current Opinion in Biotechnology*, **17**, 59–66.
- Gabor, E. M., Alkema, W. B., and Janssen, D. B., 2004. Quantifying the accessibility of the metagenome by random expression cloning techniques. *Environmental Microbiology*, **6**, 879–886.
- Hugenholtz, P., Goebel, B. M., and Pace, N. R., 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology*, **180**, 4765–4774.
- Huse, S. M., Dethlefsen, L., Huber, J. A., Welch, D. M., Relman, D. A., and Sogin, M. L., 2008. Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing. *PLoS Genetics*, **4**, e1000255.
- Huson, D. H., Auch, A. F., Qi, J., and Schuster, S. C., 2007. MEGAN analysis of metagenomic data. *Genome Research*, **17**, 377–386.
- Krause, L., Diaz, N. N., Goesmann, A., Kelley, S., Nattkemper, T. W., Rohwer, F., Edwards, R. A., and Stoye, J., 2008. Phylogenetic classification of short environmental DNA fragments. *Nucleic Acids Research*, **36**, 2230–2239.
- Kunin, V., Copeland, A., Lapidus, A., Mavromatis, K., and Hugenholtz, P., 2008. A bioinformaticians guide to metagenomics. *Microbiology and Molecular Biology Reviews*, **72**, 557–578.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadukumar, Buchner, A., Lai, T., Steppi, S., Jobb, G., Förster, W., Brettiske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., Liss, T., Lüßmann, R., May, M., Nonhoff, B., Reichel, B., Strehlow, R., Stamatakis, A., Stuckmann, N., Vilbig, A., Lenke, M., Ludwig, T., Bode, A., and Schleifer, K.-H., 2004. ARB: a software environment for sequence data. *Nucleic Acids Research*, **32**(4), 1363–1371.
- McHardy, A. C., Martin, H. G., Tsirigos, A., Hugenholtz, P., and Rigoutsos, I., 2007. Accurate phylogenetic classification of variable-length DNA fragments. *Nature Methods*, **4**, 63–72.
- Neufeld, J. D., Vohra, J., Dumont, M. G., Lueders, T., Manefield, M., Friedrich, M. W., and Murrel, J. C., 2007. DNA stable-isotope probing. *Nature Protocols*, **2**, 860–866.
- Pathak, G. P., Ehrenreich, A., Losi, A., Streit, W. R., and Gärtner, W., 2009. Novel blue light-sensitive proteins from a metagenomic approach. *Environmental Microbiology*, **11**, 2388–2399.
- Simon, C., and Daniel, R., 2010. Achievements and new knowledge unraveled by metagenomic approaches. *Applied Microbiology and Biotechnology*, **85**, 265–276.
- Sleator, R. D., Shortall, C., and Hill, C., 2008. Metagenomics. *Letters in Applied Microbiology*, **47**, 361–366.
- Steele, H. L., Jaeger, K.-E., Daniel, R., and Streit, W. R., 2009. Advances in recovery of novel biocatalysts from metagenomes. *Journal of Molecular Microbiology and Biotechnology*, **16**, 25–37.
- Strous, M., Pelletier, E., Mangenot, S., Rattei, T., Lehner, A., Taylor, M. W., Horn, M., Daims, H., Bartol-Mavel, D., Wincker, P., Barbe, V., Fonknechten, N., Vallenet, D., Segurens, B., Schenowitz-Truong, C., Médigue, C., Collingro, A., Snel, B., Dutilh, B. E., Op den Camp, H. J., van der Drift, C., Cirpus, I., van de Pas-Schoonen, K. T., Harhangi, H. R., van Niftrik, L., Schmid, M., Keltjens, J., van de Vossenberg, J., Kartal, B., Meier, H., Frishman, D., Huynen, M. A., Mewes, H. W., Weissenbach, J., Jetten, M. S., Wagner, M., and Le Paslier, D.,

2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature*, **440**, 790–794.
- Teeling, H., Waldmann, J., Lombardot, T., Bauer, M., and Glöckner, F. O., 2004. TETRA: a web-service and a stand-alone program for the analysis and comparison of tetranucleotide usage patterns in DNA sequences. *BMC Bioinformatics*, **5**, 163.
- Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., Detter, J. C., Bork, P., Hugenholtz, P., and Rubin, E. M., 2005. Comparative metagenomics of microbial communities. *Science*, **308**, 554–557.
- Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., and Banfield, J. F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, **428**, 37–43.
- Uchiyama, T., and Miyazaki, K., 2009. Functional metagenomics for enzyme discovery: challenges to efficient screening. *Current Opinion in Biotechnology*, **20**, 616–622.
- von Mering, C., Hugenholtz, P., Raes, J., Tringe, S. G., Doerks, T., Jensen, L. J., Ward, N., and Bork, P., 2007. Quantitative phylogenetic assessment of microbial communities in diverse environments. *Science*, **315**, 1126–1130.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceeding of the National Academy of Science USA*, **95**, 6578–6583.
- Wooley, J. C., Godzik, A., and Friedberg, I., 2010. A primer on metagenomics. *PLoS Computational Biology*, **6**, e1000667.
- Wu, D., Hugenholtz, P., Mavromatis, K., Pukall, R., Dalin, E., Ivanova, N. N., Kunin, V., Goodwin, L., Wu, M., Tindall, B. J., Hooper, S. D., Pati, A., Lykidis, A., Spring, S., Anderson, I. J., D'haeseleer, P., Zemla, A., Singer, M., Lapidus, A., Nolan, M., Copeland, A., Han, C., Chen, F., Cheng, J.-F., Lucas, S., Kerfeld, C., Lang, E., Gronow, S., Chain, P., Bruce, D., Rubin, E. D., Kyrpides, N. C., Klenk, H.-P., and Eisen, J. A., 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. *Nature*, **462**, 1056–1060.

Cross-references

Archaea
Bacteria
Biofilms
Endosymbiosis
Exoenzymes
Symbiosis
Terrestrial Deep Biosphere

METALLOENZYMES

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms

Metalloprotein (broader term)

Definition

Enzyme protein containing one or more metal cofactor(s).

Introduction

Metalloenzymes are enzyme proteins containing metal ions (metal cofactors), which are directly bound to the

protein or to enzyme-bound nonprotein components (prosthetic groups). About one-third of all enzymes known so far are metalloenzymes (see Holm et al., 1996 for a general overview). Besides enzymes, other metalloproteins are involved in non-enzyme electron transfer reactions (cytochromes), may act as storage (e.g., ferritin for iron) or transport proteins (e.g., transferrin for iron). In the latter groups of proteins, the metal storage is reversible and the metal is a temporary component. Also ribozymes, i.e., RNA molecules with enzyme function may contain structurally and/or functionally important metal ions (mostly divalent metal ions such as Mg^{2+}) and may be therefore termed as metalloenzymes in a broader sense (Sigel and Pyle, 2007).

Though primarily focused on enzyme proteins, i.e., proteins acting as biocatalysts in biochemical reactions, also important non-enzyme proteins (without catalytic activity in the strict sense) and their metal centers are discussed in this article.

In enzyme catalysis, transition metals are of special relevance due to their redox activity, and/or a high charge density, which allows the polarization of enzyme substrates. Vanadium, molybdenum, tungsten, manganese, iron, cobalt, nickel, and copper metal centers have been characterized so far; the metal atoms can occur in mono-, di-, and multinuclear centers with nitrogen, oxygen, and sulfur atoms of the polypeptide chain as possible ligands (Holm et al., 1996; Degtyarenko, 2000; Wilson et al., 2004; Table 1). For analysis of metal centers in proteins, spectroscopic tools for the investigation of small molecules are of relevance, namely electron paramagnetic resonance (EPR) or the related electron-nuclear double resonance (ENDOR) spectroscopy, electron spin resonance (ESR), resonance Raman or Mössbauer spectroscopy. Data obtained from X-ray protein crystallography are inevitable to locate the metal center and its ligands inside a protein molecule. Combination of proteomic approaches with technologies for elemental trace analysis (inductively coupled plasma mass spectrometry or ICP-MS) introduced tools for determination of the entire set of metalloproteins (the metalloproteome) and/or all trace metals (metallome) in an organism (Szpunar, 2005; Barton et al., 2007).

Metalloenzymes in early evolution

In early evolution, precursors of metal enzymes may have played a key role in catalytic reactions relevant for the synthesis of prebiotic macromolecules. According to the hypotheses originally postulated by Wächtershäuser, surface-bound iron, cobalt, nickel, and other transition metal centers with sulphido, carbonyl, and other ligands were catalysts involved in the synthesis of macromolecular compounds by carbon fixation, thus promoting growth of organic “superstructures” (Wächtershäuser, 1990). The metabolic type of this prebiotic scenario was chemolithoautotrophic, driven by the reducing potential of the outflow from deep-sea hydrothermal vents during

2006. Deciphering the evolution and metabolism of an anammox bacterium from a community genome. *Nature*, **440**, 790–794.
- Teeling, H., Waldmann, J., Lombardot, T., Bauer, M., and Glöckner, F. O., 2004. TETRA: a web-service and a stand-alone program for the analysis and comparison of tetranucleotide usage patterns in DNA sequences. *BMC Bioinformatics*, **5**, 163.
- Tringe, S. G., von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., Detter, J. C., Bork, P., Hugenholtz, P., and Rubin, E. M., 2005. Comparative metagenomics of microbial communities. *Science*, **308**, 554–557.
- Tyson, G. W., Chapman, J., Hugenholtz, P., Allen, E. E., Ram, R. J., Richardson, P. M., Solovyev, V. V., Rubin, E. M., Rokhsar, D. S., and Banfield, J. F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. *Nature*, **428**, 37–43.
- Uchiyama, T., and Miyazaki, K., 2009. Functional metagenomics for enzyme discovery: challenges to efficient screening. *Current Opinion in Biotechnology*, **20**, 616–622.
- von Mering, C., Hugenholtz, P., Raes, J., Tringe, S. G., Doerks, T., Jensen, L. J., Ward, N., and Bork, P., 2007. Quantitative phylogenetic assessment of microbial communities in diverse environments. *Science*, **315**, 1126–1130.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceeding of the National Academy of Science USA*, **95**, 6578–6583.
- Wooley, J. C., Godzik, A., and Friedberg, I., 2010. A primer on metagenomics. *PLoS Computational Biology*, **6**, e1000667.
- Wu, D., Hugenholtz, P., Mavromatis, K., Pukall, R., Dalin, E., Ivanova, N. N., Kunin, V., Goodwin, L., Wu, M., Tindall, B. J., Hooper, S. D., Pati, A., Lykidis, A., Spring, S., Anderson, I. J., D'haeseleer, P., Zemla, A., Singer, M., Lapidus, A., Nolan, M., Copeland, A., Han, C., Chen, F., Cheng, J.-F., Lucas, S., Kerfeld, C., Lang, E., Gronow, S., Chain, P., Bruce, D., Rubin, E. D., Kyrpides, N. C., Klenk, H.-P., and Eisen, J. A., 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. *Nature*, **462**, 1056–1060.

Cross-references

Archaea
Bacteria
Biofilms
Endosymbiosis
Exoenzymes
Symbiosis
Terrestrial Deep Biosphere

METALLOENZYMES

Michael Hoppert
University of Göttingen, Göttingen, Germany

Synonyms

Metalloprotein (broader term)

Definition

Enzyme protein containing one or more metal cofactor(s).

Introduction

Metalloenzymes are enzyme proteins containing metal ions (metal cofactors), which are directly bound to the

protein or to enzyme-bound nonprotein components (prosthetic groups). About one-third of all enzymes known so far are metalloenzymes (see Holm et al., 1996 for a general overview). Besides enzymes, other metalloproteins are involved in non-enzyme electron transfer reactions (cytochromes), may act as storage (e.g., ferritin for iron) or transport proteins (e.g., transferrin for iron). In the latter groups of proteins, the metal storage is reversible and the metal is a temporary component. Also ribozymes, i.e., RNA molecules with enzyme function may contain structurally and/or functionally important metal ions (mostly divalent metal ions such as Mg^{2+}) and may be therefore termed as metalloenzymes in a broader sense (Sigel and Pyle, 2007).

Though primarily focused on enzyme proteins, i.e., proteins acting as biocatalysts in biochemical reactions, also important non-enzyme proteins (without catalytic activity in the strict sense) and their metal centers are discussed in this article.

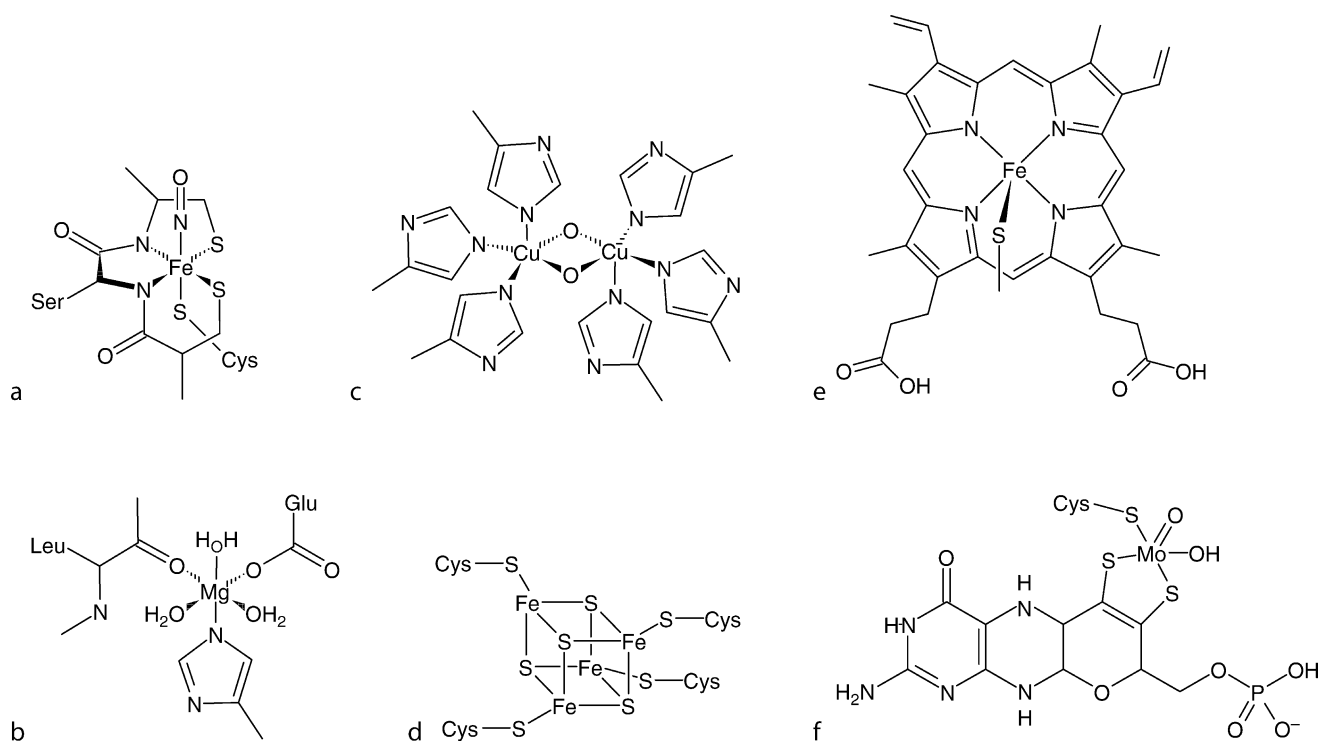
In enzyme catalysis, transition metals are of special relevance due to their redox activity, and/or a high charge density, which allows the polarization of enzyme substrates. Vanadium, molybdenum, tungsten, manganese, iron, cobalt, nickel, and copper metal centers have been characterized so far; the metal atoms can occur in mono-, di-, and multinuclear centers with nitrogen, oxygen, and sulfur atoms of the polypeptide chain as possible ligands (Holm et al., 1996; Degtyarenko, 2000; Wilson et al., 2004; Table 1). For analysis of metal centers in proteins, spectroscopic tools for the investigation of small molecules are of relevance, namely electron paramagnetic resonance (EPR) or the related electron-nuclear double resonance (ENDOR) spectroscopy, electron spin resonance (ESR), resonance Raman or Mössbauer spectroscopy. Data obtained from X-ray protein crystallography are inevitable to locate the metal center and its ligands inside a protein molecule. Combination of proteomic approaches with technologies for elemental trace analysis (inductively coupled plasma mass spectrometry or ICP-MS) introduced tools for determination of the entire set of metalloproteins (the metalloproteome) and/or all trace metals (metallome) in an organism (Szpunar, 2005; Barton et al., 2007).

Metalloenzymes in early evolution

In early evolution, precursors of metal enzymes may have played a key role in catalytic reactions relevant for the synthesis of prebiotic macromolecules. According to the hypotheses originally postulated by Wächtershäuser, surface-bound iron, cobalt, nickel, and other transition metal centers with sulphido, carbonyl, and other ligands were catalysts involved in the synthesis of macromolecular compounds by carbon fixation, thus promoting growth of organic “superstructures” (Wächtershäuser, 1990). The metabolic type of this prebiotic scenario was chemolithoautotrophic, driven by the reducing potential of the outflow from deep-sea hydrothermal vents during

Metalloenzymes, Table 1 Abundance of some metals in bacterial and algal organisms in g/100 g (dry weight) and some important key functions in enzymes (Barton et al., 2007)

Element	Algae	Bacteria	Some specific roles in enzymes
K	1.81	1.7	Protein stabilization
Ca	0.55	0.1	Protein stabilization
Mg	0.35	0.25	Protein stabilization
Fe	0.011	0.015	O ₂ transport, storage and/or activation; electron transport; superoxide breakdown
Mn	0.006	0.005	O ₂ evolution; peroxide and superoxide breakdown
Zn	0.001	0.005	Protein stabilization; hydrolytic cleavage
Cu	0.0007	0.001	O ₂ transport, storage and/or activation; electron transport; superoxide breakdown
Mo	0.0001	0.001	Oxygen transfer; N ₂ activation
Co	0.0003	0.001	Free radical reactions; nucleophil

**Metalloenzymes, Figure 1** Various metal centers (from Degtyarenko, 2000) (a) mononuclear iron center (nitrile hydratase), (b) mononuclear magnesium center (Ni-Fe-hydrogenase from *Desulfovibrio*), (c) dinuclear copper center (oxyhemocyanin), (d) polynuclear iron-sulfur center, (e) heme iron coordination (cytochrome P450), and (f) molybdopterin (sulfite oxidase).

the Hadean period. Namely, the acetyl-CoA biosynthetic pathway may be seen as a model for prebiotic energy metabolism, since in this pathway enzymes and other proteins with FeS and (Fe,Ni)S centers are involved (acetyl CoA-synthetase/carbon monoxide dehydrogenase, hydrogenases, ferredoxin); these metal centers are in principle the cubic Fe₄S₄ as well as (SNiS)(Fe₄S₄)(SFeS) units of greigite (Fe(II)Fe(III)₂S₄). Later on in evolution, the prebiotic metal centers became parts of peptides and, finally, defined proteins (Wächtershäuser, 1990; Russell and Martin, 2004). Among all metalloproteins, iron metalloproteins and, within this class, iron-sulfur proteins

are supposed to be the most important group and ubiquitous in organisms.

Besides FeS-centers, tetrapyrroles as metal-chelating prosthetic groups are interesting from the evolutionary viewpoint. Tetrapyrroles are as cyclic molecules (corrins and porphyrins; e.g., Figure 1e) ideal chelators of cobalt, copper, iron, manganese, and nickel. At the same time, the highly conjugated porphyrin molecules increase the catalytic activities of transition metal ions in electron transfer reactions by several orders of magnitude. Since porphyrins are present in all kingdoms of life, they must have evolved early in evolution, perhaps even in the

prebiotic state. As for FeS centers, the prosthetic groups are found in otherwise completely unrelated metalloproteins, such as methyl-coenzyme M reductase and chlorophylls (see below; Mauzerall, 1998).

Until the Paleozoic era, availability of bioessential transition metals changed by several orders of magnitude. Whereas Cu, Zn, Ni, and Mo concentrations increased in the ocean water, Co, Mn, and particularly iron decreased by several orders of magnitude during the Proterozoic eon (Anbar, 2008). These changes must have had an impact on the biogenic production of greenhouse gases such as nitrous oxide (Cu-dependant) or methane (Ni-dependant) and, hence, respective impact on global climate. The molybdenum cofactor (see below), in particular, plays a key role in the biological nitrogen cycle. It may even be speculated that molybdenum availability in oceans had a tremendous impact on early evolution. Under the reducing atmosphere, molybdenum was depleted in the proterozoic ocean, generating a “bottleneck” for the nitrogen cycle. The low availability of nitrogen must have had a retarding effect of total biomass production of all organisms (especially non-nitrogen-fixing eukaryotes), until the oxidizing atmosphere also provided high molybdenum availability and stopped nitrogen limitation (Zerkle et al., 2006; Anbar, 2008). Limited molybdenum availability may also have been overcome in part by (less effective) molybdenum-independent nitrogenases. The evolutionary history of the enzymes is, however, difficult to reconstruct. There is no phylogenetic evidence that these “alternative” nitrogenases preceeded the molybdenum-dependant enzymes (Raymond et al., 2004).

In recent oceans, though, most metal ions relevant for enzyme metal centers are just present in trace amounts in seawater, and/or form hardly soluble salts. Thus, most organisms utilize specialized chelating compounds, uptake and transport systems for metal ion intake.

Biomimetic metalloenzymes

Metal centers from enzyme proteins may give essential data for the design of biomimetic enzymes in bioorganic synthesis. Biomimetic metal centers from enzymes such as cytochrome oxidases (with Fe-porphyrin), catechol oxidase (based on Mn(IV)-complexes with *o*-aminophenol ligands), or hydrogenase (based, e.g., on six-coordinate Ni-Ru-centers with hydrogen as one of the bridging ligands for NiFe hydrogenase) reveal the biotechnological potential of catalysts derived from biological metal enzymes (Zbaida and Kariv, 1989; Chaudhuri et al., 2005; Song et al., 2005; Ogo et al., 2007).

Classification of metalloenzymes

Since metalloenzymes are found in all enzymes families, simple classification of metal-containing subgroups based on sequence similarities cannot be applied. Reasonable classification schemes are based upon the bioinorganic motifs (Degtyarenko, 2000; Figure 1), i.e., the metal atom and its ligands (Degtyarenko and Contrino, 2004).

Metal centers may be classified by the number of bound metal atoms as mononuclear (for one metal atom, Figure 1a and b) or polynuclear (more than one metal atom, Figure 1c and d) with endogenous ligands (i.e., the polypeptides of the enzyme molecule form a mostly polydentate coordination shell) or exogenous ligands (non-protein component of a conjugated protein such as heme or molybdopterin; Figure 1e and f).

The description given below is, for the sake of simplicity, based on the central atoms. It has to be kept in mind that different metal atoms can be bound to identical or similar bioorganic motifs (e.g., cobalt, copper, iron, magnesium, and nickel by porphyrins or corrins), and different motifs coordinate the same type of metal atom (e.g., either molybdopterin or Fe₇MoS₉ homocitrate cofactor for molybdenum; see below).

Metal centers in metalloenzymes and other metal proteins

Mononuclear iron proteins are oxidoreductases (e.g., aromatic amino acid hydroxylases, aromatic ring cleavage dioxygenases, Fe-superoxide dismutase, lipooxygenases, nitrile hydratase, and Rieske oxygenases), or involved in electron transfer (desulfuroredoxins, rubredoxins, and photosynthetic reaction centers). The amino acid ligands are mostly histidine and/or cysteine such as in nitrile hydratase with three cysteine residues and two amide nitrogens (Figure 1a). *Diiron carboxylate proteins* bind a coupled binuclear iron center using, e.g., four carboxylate and two histidine residues. Oxidoreductases (alkene hydroxylase, methane monooxygenase, and phenol hydroxylase), iron storage (bacterioferritin, ferritin), and oxygen storage/transport proteins are members of this group (hemerythrin, myohemerythrin).

Hemoproteins are characterized by an iron porphyrin as prosthetic group (Figure 1e). While four of the iron ligands are occupied by the porphyrin ring, the other two are used for binding of the protein. Hemoproteins are oxidoreductases (catalases, peroxidases), electron transfer proteins (cytochromes), or oxygen transport and storage proteins (globins) (Nordlund and Eklund, 1995; Que and Ho, 1996).

Iron-sulfur proteins are characterized by the presence of iron-sulfur clusters containing sulfide-linked di-, tri-, and tetrairon centers in variable oxidation states (Figure 1d). Iron-sulfur clusters are found in ferredoxins, NADH hydrogenases, dehydrogenases, cytochrome c reductases, nitrogenases, and other proteins. Proteins with iron-sulfur clusters have key roles in nearly all respiratory chains, either in bacteria and archaea or in mitochondria of eukaryotic organisms. Other functions include catalysis of stereo-specific isomerization of citrate to isocitrate (aconitase) in the tricarboxylic acid cycle or the redox-dependent gene regulation. The simplest iron-sulfur cluster type consists of two iron ions bridged by two sulfide ions and is coordinated by four cysteinyl ligands or by two cysteines and two histidins. The oxidized proteins

contain two Fe^{3+} ions, whereas the reduced proteins contain one Fe^{3+} and one Fe^{2+} ion. These species exist in two oxidation states, Fe(III)_2 and Fe(III)Fe(II) . Besides $[\text{2Fe-2S}]$, $[\text{3Fe-4S}]$, $[\text{4Fe-4S}]$, or $[\text{8Fe-7S}]$ clusters are known (Bertini et al., 1995).

Also in *copper-containing proteins*, several metal center types occur. Similar to iron proteins, copper proteins are involved in oxygen transport or activation processes and electron transport. *Type I copper centers* have a single copper atom coordinated by two histidine residues and a cysteine residue in a trigonal planar structure and a variable axial amino acid ligand. Type I proteins are, e.g., ascorbate oxidase, ceruloplasmin, laccase, nitrite reductase, auracynin, and azurin. *Type II copper centers* are square planar coordinated by N or N/O ligands (e.g., in galactose oxidase). *Type III centers* consist of two copper atoms (Figure 1c), each coordinated by three histidine residues (catechol oxidase, hemocyanins, tyrosinase). The *copper A center* in cytochrome c-oxidase consists of two copper atoms, coordinated by two histidine imidazole residues, methionine, carbonyl-oxygen, and two cysteine-S bridging the copper atoms. In cytochrome oxidase *copper B centers*, the copper atom is coordinated by three histidine imidazole residues. The *four copper atom center* of nitrous oxide oxidase is coordinated by seven histidine residues. The four centers are bridged by one sulfur atom. Besides cytochrome oxidase, also other copper proteins are frequently oxygenases or dioxygenases (monoamine-oxidase, laccase, caeruloplasmin, superoxide-dismutase, hemocyanine) (Adman, 1991).

Zinc proteins represent the third large group of metalloproteins, with one, two, or three zinc ions. Zinc ions in proteins are classified as catalytic, co-catalytic, and structural, where zinc is either directly involved in the opening or closing a chemical bond, or zinc acts in the catalytic reaction in concert with other metal ions, or zinc ions maintain structural integrity of the enzymes. Zinc ions in catalytic sites are generally coordinated to the side chains of three amino acid residues and a water molecule. In structural zinc sites, the ion is coordinated by four amino acid side chains in a tetrahedral symmetry. With a filled *d* orbital, zinc ions do not participate in redox reactions, but rather function as Lewis acids to accept a pair of electrons, which is favorable for reactions like proteolysis or the hydration of carbon dioxide. Important zinc-containing enzymes are the alcohol dehydrogenase (containing both a catalytic and a structural zinc site), metalloproteases, and carbonic anhydrases with catalytic zinc sites. Cocatalytic zinc sites are found in superoxide dismutases, phosphatases aminopeptidases, and β -lactamases. In alcohol dehydrogenases, the zinc catalytic site is responsible for binding of the hydroxyl group oxygen from the alcohol substrate by replacing the zinc-bound water molecule (Lipscomb and Sträter, 1996; McCall et al., 2000).

Nickel in metalloenzymes occurs as Ni-4Fe-5S (e.g., nickel-containing CO dehydrogenases), coordinated in a *tetrapyrrole complex* (e.g., in methyl-coenzyme

M reductase with glutamine as axial ligand) and as *dinuclear NiNi or NiFe complexes* (e.g., in hydrogenases and urease) or a $[\text{4Fe-4S}]^{2+}$ cluster bridged by a cysteine thiolate to a dinuclear NiNi center (acetyl-CoA synthase). Nickel-containing CO-dehydrogenases occur in anaerobic bacteria and archaea (CO-dehydrogenases from aerobic bacteria are molybdopterin enzymes), whereby CO is bound by the nickel atom and hydroxyl oxygen (from water) is transferred to the bound carbon atom. After CO_2 is released, nickel is reduced ($\text{Ni}^{2+} \rightarrow \text{Ni}^+$) and transfers two electrons to ferredoxin. In the methyl-coenzyme M-reductase reaction, methane and a heterodisulfide are generated from the substrate methyl coenzyme M that reacts with coenzyme B (7-thioheptanoylthreoninephosphate). In nickel-iron hydrogenases, nickel of the Ni-Fe center is coordinated by one apical and three equatorial S-cysteins. The Ni-Fe hydrogenases catalyze both H_2 evolution and uptake in vitro, with cytochromes acting as either electron donors or acceptors. Acetyl-CoA synthase catalyzes the reversible formation of acetyl-CoA from carbon monoxide, a methylated corrinoid protein and coenzyme A, and is complexed with a nickel CO dehydrogenase (Ermler et al., 1998).

Manganese in metalloenzymes may also act as a simple Lewis acid catalyst. Manganese (normally Mn(II)) can also be oxidized to the (III)- or (IV)-state in biological systems, which allows redox-cycling reactions. *Manganese metal centers* may be mono-, bi-, tri-, or tetranuclear. Mn-superoxide dismutases (SODs) exhibit *mononuclear centers* and are antioxidant metalloenzymes catalyzing the redox disproportionation of the superoxide radical, O_2^- . SOD decomposes the highly reactive superoxide anion by a two-step disproportionation reaction which generates oxygen hydrogen peroxide. The active site manganese is five-coordinate, with the metal ligands arranged in distorted trigonal bipyramidal geometry. Two known enzymes with *binuclear centers* are arginase and manganese catalase. Arginase catalyzes the conversion of arginine to urea and ornithine. The manganese ions are five- and six-coordinate, respectively, with square-pyramidal and (distorted) octahedral coordination symmetries. The metal centers are interconnected by a solvent-derived hydroxide and carboxylate residues. This metal-bridging hydroxide acts as nucleophile that attacks the arginine guanidinium carbon. Other binuclear manganese enzymes are aminopeptidase P from *Escherichia coli* and the dinitrogenreductase-activating glycohydrolase from *Rhodospirillum rubrum*.

One of the most important biochemical catalytic reactions in our biosphere, the oxygen-generating photolysis of water, is brought about by the *tetranuclear manganese cluster* in oxygenic photosynthesis of cyanobacteria, eukaryotic algae, and higher plants. In oxygenic photosynthesis, water is oxidized by a cluster of Mn ions. Light is captured by antenna chlorophyll and funneled to the primary reaction center, which contains chlorophyll (P680). The oxidized P680 first oxidizes a redox-active tyrosine. Subsequent oxidation of the Mn cluster occurs via this

generated tyrosine radical. The enzyme can exist in five oxidation levels, corresponding to various oxidation states of the cluster (Wiegardt, 1994; Yocum and Pecoraro, 1999; Carrell et al., 2002).

Molybdenum and *tungsten* enzymes catalyze basic metabolic reactions in the nitrogen, sulfur, and carbon cycles. With the exception of the nitrogenase cofactor, molybdenum is associated with the heterocyclic pterin derivative (molybdopterin) that contains a mononucleate center, coordinated to the thiols of the cofactor (Figure 1f). These *molybdenum-cofactor-containing (MoCo) enzymes* catalyze the transfer of an oxygen atom, derived from or incorporated into water, to or from a substrate in a two-electron redox reaction. Three classes of MoCo enzymes are now distinguished, represented by xanthine oxidase and sulfite oxidase families (with single pterin ligands and either ligand-Mo(VI)OS(OH) or ligand-Mo(VI)O₂(S-cys) cores), and DMSO reductase family (with two pterin ligands). The *nitrogenase molybdenum metal center* is not related to the pterin derivatives. In molybdenum nitrogenases, each Mo forms a part of a polynuclear cluster containing 1 Mo, 7 Fe, and 9 S organized into an elongated structure. Within the protein the terminal Fe of the cluster is coordinated by a cysteine residue, while the Mo is coordinated by a histidine residue and a homocitrate ligand.

Tungsten, which lies in the chromium group of the periodic system immediately below molybdenum exhibits similar chemical properties and is complexed in a similar way to the pterin cofactors as described for molybdenum. Due to the higher sensitivity of tungsten toward high redox potential, tungsten enzymes are found in (thermophilic) anaerobic bacterial and archaeal microorganisms (Hille, 2002; Williams and Fausto da Silva, 2002).

Cobalt is the central metal ion in the tertrapyrrol *corrin ring* in the coenzyme B₁₂. Similar to porphyrin rings, four coordination sites are provided by the corrin ring. The B₁₂-bearing enzymes catalyze either the transfer of methyl groups between two molecules or isomerization reactions, i.e., rearrangements of hydrogen atoms with concomitant exchange of a substituent (e.g., an hydroxyl group) between two adjacent carbon atoms in a molecule. In *non-corrin cobalt enzymes*, cobalt is complexed by aminoacids such as in nitrile hydratase (catalyzing the hydration of nitriles to amides; Kobayashi and Shimizu, 1999).

Vanadium occurs as V(III), V(IV), or V(V) in biological systems. The respective enzymes catalyze the two-electron oxidation of a halide by hydrogen peroxide. Besides the vanadium-containing enzymes, also heme-containing haloperoxidases and metal-free enzymes are known. In tunicates (ascidians) vanabin binds 20 V(IV) ions. However, the role of vanabins in vanadium accumulation by the ascidian has not yet been determined. In vanadium nitrogenases, vanadium, instead of molybdenum is present in a FeVCo-center (Michibata et al., 2003; Crans et al., 2004).

Though frequently not classified as metalloenzymes in the strict sense, numerous (enzyme) *proteins bind Na⁺ and*

K⁺ ions. The structures of Na⁺ and K⁺-complexes with proteins have a variety of coordination schemes and functions. Usual coordination numbers are five and six. Though the ions are not directly involved in catalytic steps, they activate numerous enzymes. Prominent examples are Na⁺-activated β -galactosidase or the K⁺-dependent activity of pyruvate-kinase and glycerol dehydratases (Page and Di Cera, 2006).

Specific *binding of calcium ions* frequently leads to conformational changes and increased protein stability. One large family of calcium-binding proteins is denoted *EF-hand proteins*, comprising a calcium-binding structural domain ("helix-turn-helix" motif). In an EF-hand loop the calcium ion is coordinated in a pentagonal bipyramidal configuration, by atoms of the protein backbone and side chains especially of aspartate and glutamate. Examples for EF-hand proteins are calmodulin and parvalbumin. In *non-EF-hand calcium-binding proteins*, the binding motifs are not conserved, such as α -lactalbumin and thermitase (Yang et al., 2002).

Also *magnesium* has a key function in activation and stabilization of enzymes. Isocitrate lyase and glutamine synthetase are two examples of Mg²⁺-activated metabolic enzymes. Enzymes catalyzing phosphorylation of proteins typically use magnesium chelates of ATP as cosubstrate. The bound Mg²⁺ serves to facilitate nucleophilic attack at the γ -phosphate of the ATP substrate. Phosphate and phosphoryl transfer reactions require Mg²⁺ as an essential cofactor. In chlorophyll molecules of photosynthetic reaction centers, magnesium ions are coordinated in a terrapyrrole ring system and to an axial *N*-histidin, *O*-aspartate, *O*-formyl-methionin, *O*-leucin, or water as ligands (Figure 1b; Cowan, 2002).

Conclusion

Since metal centers are present in all enzyme families, and enzymes with metal centers also catalyze a wide variety of different reactions, metal enzymes cannot be assigned to a special group of enzymes or proteins. Thus it is very likely that metal centers developed early in evolution and evolved in several different lineages. Since free transition metal ions are ideal catalysts for numerous reactions in organic chemistry (and biochemistry) at moderate temperatures, they appear to be the natural "precursors" of functional enzyme proteins. Peptides and proteins are, on the other hand, ideal chelators for metal ions. Thus, protein and metal partners may have easily come together frequently during evolution.

Bibliography

- Adman, E. T., 1991. Copper protein structures. *Advances in Protein Chemistry*, **42**, 145–197.
- Anbar, A. D., 2008. Oceans. Elements and evolution. *Science*, **322**, 1481–1483.
- Barton, L. L., Goulhen, F., Bruschi, M., Woodards, N. A., Plunkett, R. M., and Rietmeijer, F. J. M., 2007. The bacterial metallome: composition and stability with specific reference to the anaerobic bacterium *Desulfovibrio desulfuricans*. *BioMetals*, **20**, 291–302.

- Bertini, I., Ciurli, S., and Luchinat, C., 1995. The electronic structure of FeS centers in proteins and models. A contribution to the understanding of their electron transfer properties. *Structure and Bonding*, **83**, 1–53.
- Carrell, T. G., Tyryshkin, A. M., and Dismukes, G. C., 2002. An evaluation of structural models for the photosynthetic water-oxidizing complex derived from spectroscopic and X-ray diffraction structures. *Journal of Biological Inorganic Chemistry*, **7**, 2–22.
- Chaudhuri, P., Wieghardt, K., Weyhermüller, T., Paine, T. K., Mukherjee, S., and Mukherjee, C., 2005. Biomimetic metal-radical reactivity: aerial oxidation of alcohols, amines, aminophenols and catechols catalyzed by transition metal complexes. *Biological Chemistry*, **386**, 1023–1033.
- Cowan, J. A., 2002. Structural and catalytic chemistry of magnesium-dependent enzymes. *BioMetals*, **15**, 225–235.
- Crans, D. C., Smee, J. J., Galdamauskas, E., and Yang, L., 2004. Chemistry and biology of vanadium and the biological activities exerted by vanadium compounds. *Chemistry Reviews*, **104**, 849–902.
- Degtyarenko, K., 2000. Bioorganic motifs: towards functional classification of metalloproteins. *Bioinformatics Review*, **16**, 851–864.
- Degtyarenko, K., and Contrino, S., 2004. COMe: the ontology of bioinorganic proteins. *BMC Structural Biology*, **4**, 3.
- Ermler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer R. K., 1998. Active sites of transition-metal enzymes with a focus on nickel. *Current Opinon Structural Biology*, **8**, 749–758.
- Hille, R., 2002. Molybdenum and tungsten in biology. *Trends in Biochemical Sciences*, **27**, 360–367.
- Holm, R., Kennepohl, P., and Solomon, E. I., 1996. Structural and functional aspects of metal sites in biology. *Chemistry Reviews*, **96**, 2239–2314.
- Kobayashi, M., and Shimizu, S., 1999. Cobalt proteins. *European Journal of Biochemistry*, **261**, 1–9.
- Lipscomb, W. N., and Sträter, N., 1996. Recent advances in zinc enzymology. *Chemistry Reviews*, **96**, 2375–2433.
- Mauzerall, D. C., 1998. Evolution of porphyrins. *Clinics Dermatology*, **16**, 195–201.
- McCall, K. A., Huang, C.-C., and Fierke, C. A., 2000. Function and mechanism of zinc metalloenzymes. *Journal of Nutrition*, **130**, 1437S–1446S.
- Michibata, H., Yamaguchi, N., Uyama, T., and Ueki, T., 2003. Molecular biological approaches to the accumulation and reduction of vanadium by ascidians. *Coordination Chemistry Reviews*, **237**, 41–51.
- Nordlund, P., and Eklund, H., 1995. Diiron-carboxylate proteins. *Current Opinion in Structural Biology*, **5**, 758–766.
- Ogo, S., Kabe, R., Uehara, K., Kure, B., Nishimura, T., Menon, S. C., Harada, R., Fukuzumi, S., Higuchi, Y., Ohhara, T., Tamada, T., and Kuroki, R., 2007. A dinuclear Ni(μ -H)Ru complex derived from H₂. *Science*, **316**, 585–587.
- Page, M. J., and Di Cera, E., 2006. Role of Na⁺ and K⁺ in enzyme function. *Physiological Reviews*, **86**, 1049–1092.
- Que, L. Jr., and Ho, R. Y. N., 1996. Dioxygen activation by enzymes with mononuclear nonheme iron active sites. *Chemistry Reviews*, **96**, 2607–2624.
- Raymond, J. R., Siefert, J. L., Staples, C. R., and Blankenship, R. E., 2004. The natural history of nitrogen fixation. *Molecular Biology and Evolution*, **21**, 541–554.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Sigel, R. K. O., and Pyle, A. M., 2007. Alternative roles for metal ions in enzyme catalysis and the implications for ribozyme chemistry. *Chemistry Reviews*, **107**, 97–113.
- Song, L.-C., Yang, Z.-Y., Bian, H.-Z., Liu, Y., Wang, H.-T., Liu, X.-F., and Hu, Q.-M., 2005. Diiron oxadithiolate type models for the active site of iron-only hydrogenases and biomimetic hydrogen evolution catalyzed by Fe₂(μ -SCH₂OCH₂S- μ)(CO)₂. *Organometallics*, **24**, 6126–6135.
- Szpunar, J., 2005. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst*, **130**, 442–465.
- Wächtershäuser, G., 1990. Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 200–204.
- Wieghardt, K., 1994. A structural model for the water-oxidizing manganese cluster in photosystem II. *Angewandte Chemie International Edition in English*, **33**, 725–728.
- Williams, R. J. P., and Frausto da Silva, J. J. P., 2002. The involvement of molybdenum in life. *Biochemical and Biophysical Research Communications*, **292**, 293–299.
- Wilson, C. J., Apiyo, D., and Wittung-Stafshede, P., 2004. Role of cofactors in metalloprotein folding. *Quarterly Review of Biophysics*, **37**, 285–314.
- Yang, W., Hsiao-Wei, L., Hellings, H., and Yang, J. D., 2002. Structural analysis, identification, and design of calcium-binding sites in proteins. *Proteins Structure, Function, and Genetics*, **47**, 344–356.
- Yocum, C. F., and Pecoraro, V. L., 1999. Recent advances in the understanding of the biological chemistry of manganese. *Current Opinon Structural Biology*, **3**, 182–187.
- Zbaida S., and Kariv, R., 1989. Biomimetic models for monooxygenases. *Biopharmaceutics and Drug Disposition*, **10**, 431–442.
- Zerkle, A. L., House, C. H., Cox, R. P., and Canfield, D. E., 2006. Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle. *Geobiology*, **4**, 285–297.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Methane, Organ](#)
[Methane Oxidation \(Aerobic\)](#)
[Nickel, Biology](#)
[Siderophores](#)
[Zinc](#)

METALLOGENIUM

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition

Metallogenium s.str. is a star-shaped enigmatic bacterium oxidizing Mn and iron. It preferentially lives in limnic environments.

Metallogenium is a relatively large (2–10 μ m) microorganism with bacterial affinities that concentrates manganese and iron oxides in significant amounts. The genus *Metallogenium* has not yet been assigned to any taxonomic group. The cells lack rigid walls and develop flexible, tapering threads from which new cells probably arise. The organism is therefore, star-shaped and is found in various facies, predominantly in limnic environments (Figures 1 and 2). The threads are often heavily encrusted with oxides of iron and/or manganese. The oldest

SUBSURFACE FILAMENTOUS FABRICS

Beda A. Hofmann

Natural History Museum Bern, Bern, Switzerland

Synonyms

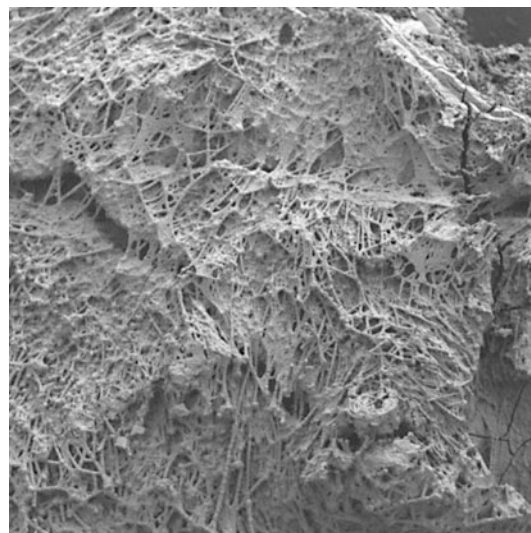
Moss agate p.p.; SFF; Stalactitic minerals p.p.

Definition

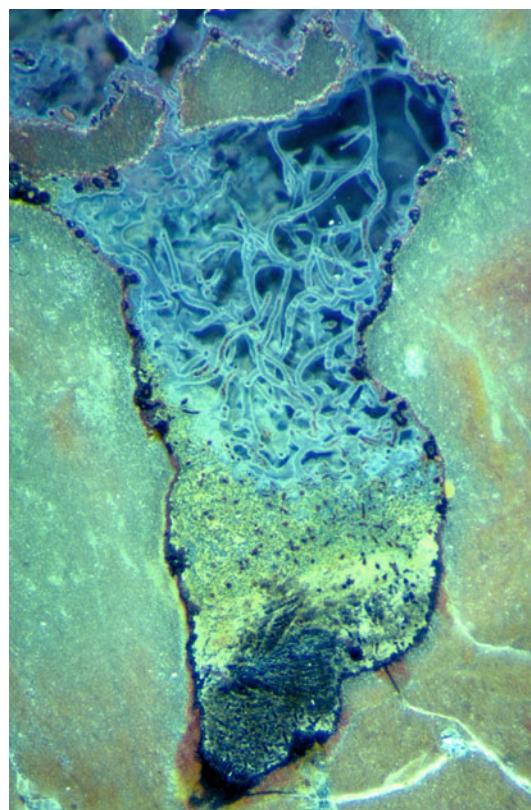
Subsurface filamentous fabrics (SFF, [Figures 1–5](#)) result from the encrustation of microbial filaments with minerals in subsurface geological environments, followed by oxidation of organic matter ([Hofmann, 2007](#); [Hofmann and Farmer, 2000](#); [Hofmann et al., 2008](#)). Encrustations of fibrous minerals may yield similar fabrics, but a biogenic origin is indicated by evidence for high flexibility, and a low variability of the smallest diameter of the filamentous forms, typically 1–3 μm . SFF textures vary from delicate mineral webs to massive rocks containing enclosed filaments, depending on the degree of mineral precipitation after initial filament encrustation in voids. SFF are most commonly found in voids in volcanic rocks and in the oxidation zone of ore deposits rich in reduced elements (S, Fe), but also occur in paleokarst environments ([Baele, 1998](#); [Feldmann et al., 1997](#); [Kretzschmar, 1982](#)), Mississippi Valley-type base metal deposits ([Hofmann et al., 2008](#)), cavities in fossils ([Weitschat, 1986](#)), mineral veins ([Hofmann, 1989](#); [Reitner, 2004](#); [Trewin and Knoll, 1999](#)), and cavities in impact rocks ([Hofmann et al., 2008](#)). Many of these occurrences show low-temperature hydrothermal influences. Historically, SFF have aroused the interest of researchers because of their often conspicuous nature. Examples of long-known forms are particularly the so-called moss agates, for which various interpretations have



Subsurface Filamentous Fabrics, Figure 1 Characteristic example of pseudostalactitic SFF. Encrustation of mat-like, vertically draped fabric by goethite. From the oxidation zone of a sulfide deposit, Kintore open cut, Broken Hill, New South Wales, Australia. Scale in cm.



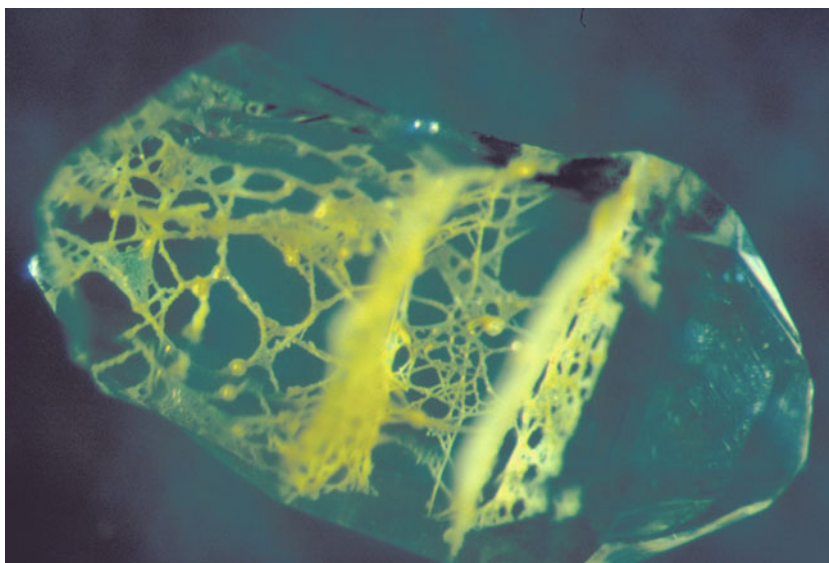
Subsurface Filamentous Fabrics, Figure 2 SEM image of goethite-encrusted microbial filaments from the oxidation zone (in contact with glacial till) of a pyrite-rich small orebody, Lengenbach, Binntal, Switzerland. Image width: 2.9 mm.



Subsurface Filamentous Fabrics, Figure 3 Image of filamentous forms enclosed within chalcedony in a fracture within a lithophysa in Tertiary rhyolite, Priday Ranch, Oregon, USA. Image width: 4 mm.



Subsurface Filamentous Fabrics, Figure 4 Filaments encrusted by iron hydroxides, associated to numerous folded mats, and enclosed by chalcedony. Host rocks are basaltic volcanics of Jurassic/Cretaceous age. This assemblage is known as moss agate. Image width: 18 mm. Arts Bogd Uul, Mongolia.



Subsurface Filamentous Fabrics, Figure 5 Filaments encrusted by iron hydroxides, enclosed in a single quartz crystal. Paleokarst within Devonian limestones (mineralization probably of Tertiary age), Hohenlimburg, Germany. Image width: 2.8 mm.

been brought forward (Bowerbank, 1842; Daubenton, 1782; Razumovsky, 1835). Similarities do exist with some types of dendrites (present in some moss agates) and speleothems. Many examples of “stalactitic” minerals (e.g., goethite, chalcedony, chrysocolla) were formed around filamentous centers with diameters in the order of microns, much too small for true speleothems the size of which is governed by the diameter of a water droplet (Hill

and Forti, 1997). Features discriminating SFF from similar fabrics are (Hofmann et al., 2008) (a) median width of filamentous substrate of $<5\ \mu\text{m}$ with narrow, near-normal distribution; (b) evidence of bending (e.g., U-loops) and aggregation to mat-like (biofilms), stromatolite-like, or rope-like fabrics; (c) filamentous cores show a high degree of irregular bending ($>0.5^\circ/\mu\text{m}$); (d) filamentous cores show many direction changes ($>10/\text{mm}$);

(e) association of SFF with or occurrence within SFF of other morphological bioforms such as fossil *Gallionella* or *Metallogenium*-like structures; (f) presence of vertically oriented elements (may resemble stalactites) resulting from mineral encrustation of filaments, filament strands, or mats while they are still flexible; and (g) inferred environmental conditions compatible with microbial growth, such as an abundant energy source and temperatures between ambient and $<120^{\circ}\text{C}$.

Types of organisms/substrates involved: The micro-morphology of SFF indicates that only filamentous micro-organisms are involved in their formation. Filamentous substrates of microbiological origin may include archaea, bacteria, and fungi. Some fabrics formed by encrustation of mineral fibers (these may be dissolved later) may show similar morphologies, but can usually be discriminated by evidence for characteristics typical of mineral fibers: Radial growth, variable fiber thickness, straight development, brittle behavior, and remnants of mineral fibers.

Conclusions

SFF represent a common, but often overlooked, type of biosignature from subsurface environments. SFF often display macroscopically visible fabrics that are easily recognizable and may be regarded as subsurface microbialites and the most conspicuous expression of the deep biosphere, representing in some ways an analog to stromatolites from surface environments. The very common occurrence in a wide variety of different rock types demonstrates that SFF are a common expression of subsurface life, wherever filamentous forms generate a large amount of biomass in relatively short time, allowing mineralization before organic filaments are destroyed. Organisms involved were chemolithotrophs (based on oxidation of Fe, S), but may also include heterotrophs living on chemolithotrophic primary producers. The formation in subsurface environments implies that no photosynthesizing organisms are involved. SFF-like features represent a target in the search for past life on Mars (Hofmann, 2007), as they might potentially be found in nearly all rock types.

Bibliography

- Baele, J. M., 1998. Reliques silicifiées et minéralisées de paléokarsts post-varisques sur le Dévonien en Belgique méridionale (Entre-Sambre-et-Meuse). *Annales de la Société Géologique du Nord*, **6**(2), 127–133.
- Bowerbank, J. S., 1842. On the spongy origin of moss agates and other siliceous bodies. *AMNH*, **10**, 9–18, 84–91.
- Daubenton, L. J. M., 1782. Sur les causes qui produisent trois sortes d'herborisations dans les pierres. *Mémoires de l'Académie Royale des Sciences*, 667–673.
- Feldmann, M., Neher, J., Jung, W., and Graf, F., 1997. Fungal quartz weathering and iron crystallite formation in an Alpine environment, Piz Alv, Switzerland. *Eclogae Geologicae Helvetiae*, **90**, 541–556.
- Hill, C., and Forti, P., 1997. *Cave Minerals of the World*. Huntsville: National Speleological Society.
- Hofmann, B., 1989. Genese, Alteration und rezentess Fließ-System der Uranlagerstätte Krunkelbach (Menzenschwand, Südschwarzwald). *Nagra Technical Report* 88–30, Nagra, Baden.
- Hofmann, B. A., 2007. Morphological biosignatures from subsurface environments: recognition on planetary missions. *Space Science Reviews*, DOI 10.1007/s11214-007-9147-9.
- Hofmann, B. A., and Farmer, J. D., 2000. Filamentous fabrics in low-temperature mineral assemblages: are they fossil biomarkers? implications for the search for a subsurface fossil record on the early Earth and Mars. *Planetary and Space Science*, **48**, 1077–1086.
- Hofmann, B. A., Farmer, J. D., von Blanckenburg, F., and Fallick, A. E., 2008. Subsurface filamentous fabrics: an evaluation of possible modes of origins based on morphological and geochemical criteria, with implications for exoplaeontology. *Astrobiology*, **8**, 87–117.
- Kretzschmar, M., 1982. Fossile Pilze in Eisen-Stromatolithen von Warstein (Rheinisches Schiefergebirge). *Facies*, **7**, 237–260.
- Razumovsky, G., 1835. Les agates mousseuses. *Bulletin de la société géologique de France*, **6**, 165–168.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie*. Göttingen: Universitätsdruckerei Göttingen.
- Trewin, N. H., and Knoll, A. H., 1999. Preservation of Devonian chemotrophic filamentous bacteria in calcite veins. *Palaos*, **14**, 288–294.
- Weitschat, W., 1986. Phosphatisierte Ammonoideen aus der Mittleren Trias von Central-Spitzbergen. *Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg*, **61**, 249–279.

Cross-references

[Biofilms and Fossilization](#)
[Biosignatures in Rocks](#)
[Chemolithotrophy](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Gallionella](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Karst Ecosystems](#)
[Microbialites, Stromatolites, and Thrombolites](#)

SULFATE-REDUCING BACTERIA

Heribert Cypionka
 University of Oldenburg, Oldenburg, Germany

Synonyms

Dissimilatory sulfate reducers; Sulfate-reducing prokaryotes

Definition

Sulfate reducers are prokaryotic microorganisms (Archaea or Bacteria) that use sulfate as electron acceptor of an anaerobic respiration process.

Cross-references

[Acetogens](#)
[Archaea](#)
[Asteroid and Comet Impacts](#)
[Basalt \(Glass, Endoliths\)](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Geobacter](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Iron Sulfide Formation](#)
[Metalloenzymes](#)
[Methane, Origin](#)
[Nickel, Biology](#)
[Shewanella](#)

ORIGINS OF THE METAZOA

Daniel J. Jackson
 University of Göttingen, Göttingen, Germany

Synonyms

Animals

Definition

Metazoans – heterotrophic, multicellular organisms that possess eukaryotic cells.

Heterotrophs – organisms that must ingest organic carbon to support growth, unlike plants that are autotrophic and can use light to photosynthesize organic compounds.

Evolutionary concepts

A major event in the time line of Earth's history was the evolution of multicellular animal life, known scientifically as the Metazoa (Knoll and Carroll, 1999; Knoll et al., 2006; Budd, 2008; Love et al., 2009; Xiao and Laflamme, 2009). Metazoans possess eukaryotic cells which contain a distinct membrane bound nucleus, nuclear material (DNA) packaged into multiple linear chromosomes, membrane bound organelles, and modes of cellular division distinct from that of Bacteria and Archaea (Cooper and Hausman, 2009). Metazoans are primarily set apart from plants (also multicellular eukaryotes) in being heterotrophic, and in lacking a rigid cell wall (Campbell et al., 2008).

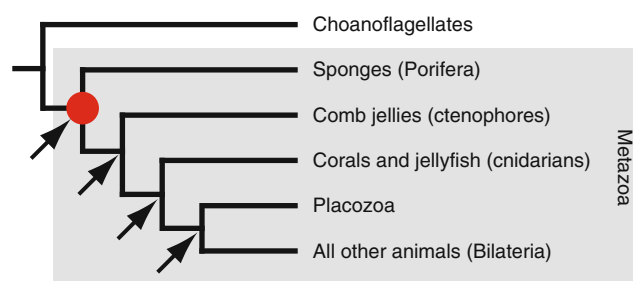
While all other forms of life (Bacteria, Archaea, and plants) display extensive genetic and metabolic diversity (Venter et al., 2004), the metazoans through 600+ million years of evolution (Peterson et al., 2008) display the greatest variety of locomotory habits, behaviors, and morphologies. Indeed, a fundamental feature of the Metazoa is the morphological diversity displayed by the 35+ (Collins and Valentine, 2001) major groups of animals that constitute the Kingdom Animalia (Carroll, 2001).

Metazoan morphological diversity is essentially generated by two means. Firstly through an ability of

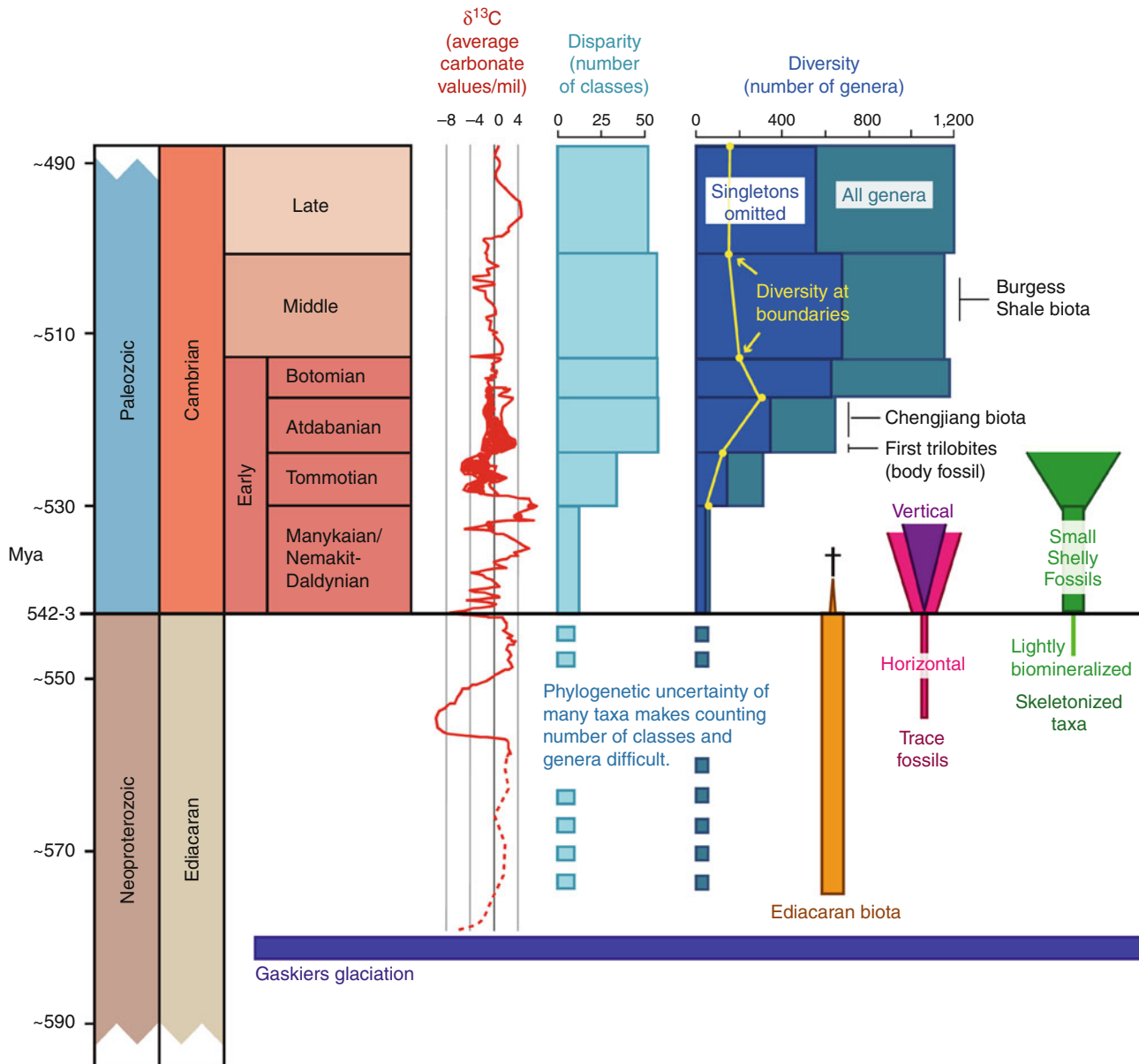
a collection of cells to accurately communicate with one another during the process of development, and secondly for members of that collection of cells to adopt fates with distinct functions. This is most clearly conceptualized when considering the process of metazoan development. Typically, unicellular gametes (sperm and egg cells) fuse to form a zygote (a fertilized egg), which then divides to produce a multicelled embryo and eventually a reproductively mature adult (Gilbert, 2006). During this process, undifferentiated embryonic cells become progressively specified to a particular cell fate (e.g., muscle, neuron, skin, gonad, and eye). Without cell fate specification, tissues (e.g., muscle), organs (e.g., hearts), and organ systems (e.g., digestive system) and the variety of functions that these collections of cells fulfill would not develop (Arendt, 2008).

Evolutionary changes to the metazoan developmental program are ultimately responsible for generating diversity in the adult phenotype. This realization has fuelled the growth of the field of evolutionary-developmental biology (Carroll, 2005). Comparisons of the genes that regulate different metazoan developmental programs now holds the exciting promise of explaining on a molecular level how metazoan morphological diversity is generated (Martindale et al., 2003; Matus et al., 2006). Additionally, recent developments in high-throughput DNA sequencing technologies have seen the completion of a variety of metazoan genomes (King et al., 2008; Putnam et al., 2008; Chapman et al., 2010). These large-scale datasets allow exhaustive comparisons to be made between disparate animal phyla, and deepen our understanding of the genetic innovations that accompanied the diversification of metazoan life.

Related to these efforts are attempts to understand how metazoan life first arose. This requires that we accurately understand how metazoan and pre-metazoan groups are evolutionarily related, and calls on the field of



Origins of the Metazoa, Figure 1 A phylogenetic tree represents a hypothesis regarding the evolutionary relationships of a given set of organisms. Speciation events (indicated by an arrow) generate new lineages of organisms that in turn speciate to generate new "sublineages" thereby increasing diversity. This simplified phylogenetic tree summarizes a recently proposed hypothesis (Pick et al., 2010) that suggests the last common ancestor of the Metazoa (indicated by the red circle) was a sponge-like animal that was descended from a choanoflagellate-like organism.



Origins of the Metazoa, Figure 2 The expansion of metazoan life primarily took place during the early and pre-Cambrian. Trace fossils and the soft-bodied remains of Ediacaran fauna are of uncertain phylogenetic affinities and therefore may, or may not, be ancestral life forms to those that inhabit the world today. However, the abundance of well-preserved specimens from Cambrian strata indicates that both the majority of modern day animal groups had been established during this time, and that the widespread adoption of various biomineralization strategies accompanied this diversification. (Reprinted with permission from Marshall, 2006.)

phylogenetics (Felsenstein, 2004). Although phylogenetics was arguably born over 150 years ago with Darwin's publication of the *Origin of Species* (Darwin, 1859), our understanding of metazoan relationships is far from settled, and remains an exciting and dynamic field of scientific research which now benefits from modern techniques in molecular biology (high-throughput DNA sequencing) and super computing (Stamatakis et al., 2008). Arguably, the current and most widely accepted

theory of the evolutionary origins of the Metazoa (based on a recent meta-analysis of DNA sequence data from a large collection of metazoan groups), maintains that sea sponges (phylum Porifera) are the most ancestral-like metazoan, with choanoflagellates (a primarily unicellular eukaryotic organism) representing the closest non-metazoan cousin (Figure 1; Pick et al., 2010). This theory is attractive because it is conceivable that a choanoflagellate ancestor once formed a colony of

individuals (modern choanoflagellates are known to do this), which then evolved into a primitive sponge, the first metazoan. However, because the Metazoa has origins dating back 600+ million years thus obscuring many of the phylogenetic signals used to infer kinship, and our understanding of the ways in which the mechanisms of molecular evolution operate are still being developed, competing theories should also be considered (Dunn et al., 2008; Schierwater et al., 2008).

Exactly when the first metazoan arose, and what it looked like remain topics of intense research. It is clear that approximately 542 million years ago (MYA), well after the birth of the Metazoa (Wray et al., 1996; Wang et al., 1999; Xiao and Laflamme, 2009), there was a period in the evolution of animal body plans that saw the generation of an unsurpassed diversity of phenotypes (Figure 2; Knoll, 2003a). Because of the rapid generation of this diversity, and the geological period in which it occurred, this evolutionary radiation has come to be known as the “Cambrian Explosion” (Gould, 1989; Marshall, 2006), and is typified by the sudden appearance in the fossil record of a range of biologically mineralized structures (Butterfield, 2003; Knoll, 2003b; Budd, 2008). While the underlying causes of this widespread adoption of biomineralization strategies is also an area of active research, likely candidates are changes to ocean calcium levels (Brennan et al., 2004), increased genetic potential (Peterson et al., 2009), and ecological factors such as increased predation pressure (Bengston and Yue, 1992). Satisfyingly, recent molecular estimates of early metazoan divergence times using molecular clocks (Bromham, 2008) have been shown to agree with paleontological data (Peterson et al., 2008).

Summary

Contemporary metazoans consist of animals representing a wide range of body plans ranging from the relatively simple sponges, cnidarians (jellyfish and corals), and ctenophores (comb jellies) through to more complex organisms such as snails, worms, insects, and vertebrates. Metazoan diversity has universal origins that lie in, or even prior to, the Cryogenian 635–850 MYA, with a watershed in metazoan evolution recognized as being in the early Cambrian (542 MYA) by which time most modern, and now extinct, metazoan body plans had been established. The last common ancestor of the Metazoa is likely to have shared morphological and genetic similarities with modern day sponges. Recent advances across a range of scientific fields are constantly forcing scientists to revise the evolutionary relationships of animals at all levels, but a clearly resolved and robust metazoan tree of life is gradually emerging.

Bibliography

Arendt, D., 2008. The evolution of cell types in animals: emerging principles from molecular studies. *Nature Reviews Genetics*, **9**, 868–882.

- Bengston, S., and Yue, Z., 1992. Predatorial borings in late Precambrian mineralized exoskeletons. *Science*, **257**, 267–369.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Bromham, L., 2008. Molecular evolution: rates. In *Encyclopedia of Life Sciences*. Chichester: Wiley, pp. 1–6.
- Budd, G. E., 2008. The earliest fossil record of the animals and its significance. *Philosophical Transactions of the Royal Society*, **363**, 1425–1434.
- Butterfield, N., 2003. Exceptional fossil preservation and the Cambrian explosion. *Integrative and Comparative Biology*, **43**, 166–177.
- Campbell, N. A., Reece, J. B., Urry, L. A., Cain, M. L., Wasserman, S. A., Minorsky, P. V., and Jackson, R. B., 2008. *Biology*. San Francisco: Benjamin Cummings.
- Carroll, S. B., 2001. Chance and necessity: the evolution of morphological complexity and diversity. *Nature*, **409**, 1102–1109.
- Carroll, S. B., 2005. *Endless Forms Most Beautiful: The New Science of Evo Devo and the Making of the Animal Kingdom*. New York: Norton.
- Chapman, J. A., Kirkness, E. F., Simakov, O., Hampson, S. E., Mitros, T., Weinmaier, T., Rattei, T., Balasubramanian, P. G., Borman, J., Busam, D., Disbennett, K., Pfannkoch, C., Sumin, N., Sutton, G. G., Viswanathan, L. D., Walenz, B., Goodstein, D. M., Hellsten, U., Kawashima, T., Prochnik, S. E., Putnam, N., Shu, S., Blumberg, B., Dana, C. E., Gee, L., Kibler, D. F., Law, L., Lindgens, D., Martinez, D. E., Peng, J., Wigge, P. A., Bertulat, B., Guder, C., Nakamura, Y., Ozbek, S., Watanabe, H., Khalturin, K., Hemmrich, G., Franke, A., Augustin, R., Fraune, S., Hayakawa, E., Hayakawa, S., Hirose, M., Hwang, J. S., Ikeo, K., Nishimiya-Fujisawa, C., Ogura, A., Takahashi, T., Steinmetz, P. R., Zhang, X., Aufschnaiter, R., Eder, M., Gorny, A., Salvenmoser, W., Heimberg, A. M., Wheeler, B. M., Peterson, K. J., Böttger, A., Tischler, P., Wolf, A., Gojobori, T., Remington, K. A., Strausberg, R. L., Venter, J. C., Technau, U., Hobmayer, B., Bosch, T. C., Holstein, T. W., Fujisawa, T., Bode, H. R., David, C. N., Rokhsar, D. S., and Steele, R. E., 2010. The dynamic genome of Hydra. *Nature*, **464**, 592–596.
- Collins, A. G., and Valentine, J. W., 2001. Defining phyla: evolutionary pathways to metazoan body plans. *Evolution and Development*, **3**, 432–442.
- Cooper, G. M., and Hausman, R. E., 2009. *The Cell: A Molecular Approach*. Sunderland: Sinauer.
- Darwin, C., 1859. *On the Origin of Species*. London: John Murray.
- Dunn, C. W. D., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., Seaver, E. C. S., Rouse, G. W., Obst, M., Edgecombe, G. D., Sørensen, M. V., Haddock, S. H., Schmidt-Rhaesa, A., Okusu, A., Kristensen, R. M., Wheeler, W. C., Martindale, M. Q. M., and Giribet, G., 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, **452**, 745–749.
- Felsenstein, J., 2004. *Inferring Phylogenies*. Sunderland: Sinauer.
- Gilbert, S. F., 2006. *Developmental Biology*. Sunderland: Sinauer.
- Gould, S. J., 1989. *Wonderful Life: The Burgess Shale and the Nature of History*. New York: W.W. Norton.
- King, N., Westbrook, M. J., Young, S. L., Kuo, A., Abedin, M., Chapman, J., Fairclough, S., Hellsten, U., Isogai, Y., Letunic, I., Marr, M., Pincus, D., Putnam, N., Rokas, A., Wright, K. J., Zuzow, R., Dirks, W., Good, M., Goodstein, D., Lemons, D., Li, W., Lyons, J. B., Morris, A., Nichols, S., Richter, D. J., Salamov, A., Sequencing, J. G., Bork, P., Lim, W. A., Manning, G., Miller, W. T., McGinnis, W., Shapiro, H., Tjian, R., Grigoriev, I., and Rokhsar, D., 2008. The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. *Nature*, **451**, 783–788.

- Knoll, A. H., 2003a. *Life on a Young Planet: The First Three Billion Years of Evolution on Earth*. Princeton: Princeton University Press.
- Knoll, A. H. K., 2003b. Biomineralization and evolutionary history. *Reviews in Mineralogy and Geochemistry*, **54**, 229–356.
- Knoll, A. H. K., and Carroll, S. B. C., 1999. Early animal evolution: emerging views from comparative biology and geology. *Science*, **284**, 2129–2137.
- Knoll, A. H. K., Javaux, E. J., Hewitt, D., and Cohen, P., 2006. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society*, **361**, 1023–1038.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D., Grotzinger, J., Bradley, A., Kelly, A., Bhatia, M., Meredith, W., Snape, C., Bowring, S., Condon, D., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–721.
- Marshall, C. R., 2006. Explaining the Cambrian “Explosion” of animals. *Annual Review of Earth and Planetary Sciences*, **34**, 355–384.
- Martindale, M. Q., Pang, K., Matus, D. Q., and Finnerty, J. R., 2003. Expression of mesodermal genes in the anthozoan *Nematostella vectensis*. *Integrative and Comparative Biology*, **43**, 942–942.
- Matus, D. Q., Pang, K., Marlow, H., Dunn, C. W. D., Thomsen, G. H., and Martindale, M. Q. M. 2006. Molecular evidence for deep evolutionary roots of bilaterality in animal development. *Proceedings of the National Academic Sciences USA*, **103**, 11195–11200.
- Peterson, K. J., Cotton, J. A., Gehling, J. G., and Pisani, D., 2008. The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Philosophical Transactions of the Biological Society*, **363**, 1435–1443.
- Peterson, K. J., Dietrich, M. R., and Mcpeek, M. A., 2009. MicroRNAs and metazoan macroevolution: insights into canalization, complexity, and the Cambrian explosion. *Bioessays*, **31**, 736–747.
- Pick, K., Hervé, P., Fabian, S., Erpenbeck, D., Jackson, D. J., Wrede, P., Matthias, W., Alie, A., Burkhard, M., Manuel, M., and Wörheide, G. 2010. Improved phylogenomic taxon sampling noticeably affects non-bilaterian relationships. *Molecular Biology and Evolution*, doi:10.1093/molbev/msq089.
- Putnam, N., Butts, T., Ferrier, D. E., Furlong, R., Hellsten, U., Kawashima, T., Robinson-Rechavi, M., Shoguchi, E., Terry, A., Yu, J., Benito-Gutiérrez, E. L., Dubchak, I., Garcia-Fernández, J., Gibson-Brown, J., Grigoriev, I., Horton, A., De Jong, P., Jurka, J., Kapitonov, V., Kohara, Y., Kuroki, Y., Lindquist, E., Lucas, S., Osoegawa, K., Pennacchio, L., Salamov, A., Satou, Y., Sauka-Spengler, T., Schmutz, J., Shin, -I T., Toyoda, A., Bronner-Fraser, M., Fujiyama, A., Holland, L., Holland, P. W., Satoh, N., and Rokhsar, D. S., 2008. The amphioxus genome and the evolution of the chordate karyotype. *Nature*, **453**, 1064–1071.
- Schierwater, B., De Jong, D., and Desalle, R. 2008. Placozoa and the evolution of Metazoa and intrasomatic cell differentiation. *The International Journal of Biochemistry & Cell Biology*, **41**, 370–379.
- Stamatakis, A., Hoover, P., and Rougemont, J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Systematic Biology*, **57**, 758–771.
- Venter, J. C., Remington, K., Heidelberg, J. F., Halpern, A. L., Rusch, D., Eisen, J. A., Wu, D., Paulsen, I., Nelson, K. E., Nelson, W., Fouts, D. E., Levy, S., Knap, A. H., Lomas, M. W., Nealson, K., White, O., Peterson, J., Hoffman, J., Parsons, R., Baden-Tillson, H., Pfannkoch, C., Rogers, Y. H., and Smith, H. O. 2004. Environmental genome shotgun sequencing of the Sargasso Sea. *Science*, **304**, 66–74.
- Wang, D. Y., Kumar, S., and Hedges, S. B. 1999. Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society of London B*, **266**, 163–171.
- Wray, G. A., Levinton, J. S., and Shapiro, L. H. 1996. Molecular evidence for deep Precambrian divergences among metazoan phyla. *Science*, **274**, 568–573.
- Xiao, S., and Laflamme, M., 2009. On the eve of animal radiation: phylogeny, ecology and evolution of the Ediacara biota. *Trends in Ecology and Evolution*, **24**, 31–40.

Cross-references

[Critical Intervals in Earth History](#)
[Early Precambrian Eukaryotes](#)
[Ediacaran Biota](#)
[Silica Biomineralization](#)
[Sponges \(Porifera\) and Sponge Microbes](#)

- mediated by microbial iron(III)-binding ligands. *Nature*, **413**, 409–413.
- Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., and Butler, A., 2003. The photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria, based on characteristic iron(III)-binding groups. *Limnology and Oceanography*, **48**, 1069–1078.
- Bergeron, R. J., Huang, G., Smith, R. E., Bharti, N., McManis, J. S., and Butler, A., 2003. Total synthesis of petrobactin. *Tetrahedron*, **59**, 2007–2014.
- Butler, A., 2007. Siderophores. In Bertini, I., Gray, H. B., and Valentine, J. S. (eds.), *Biological Inorganic Chemistry: Structure and Reactivity (Metal Transport and Storage Proteins section)*. Sausalito: University Science Books, (Chap. VIII.3), pp. 151–156.
- Butler, A., and Martin, J. D., 2005. The marine biogeochemistry of iron. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *“Biogeochemical Cycles,” Metal Ions in Biological Systems*. New York: M. Dekker, Vol. 43, pp. 21–46.
- Crosa, J. H., Mey, A. R., and Payne, S. M., 2004. *Iron Transport in Bacteria*. Washington, DC: ASM, p. 499.
- Haygood, M. J., Holt, P., and Butler, A., 1993. Aerobactin production by a planktonic marine *Vibrio* sp. *Limnology and Oceanography*, **38**, 1091–1097.
- Hickford, S. J. H., Küpper, F. C., Zhang, G., Carrano, C. J., Blunt, J. W., and Butler, A., 2004. Petrobactin sulfonate, a new siderophore produced by the marine bacterium *Marinobacter hydrocarbonoclasticus*. *Journal of Natural Products*, **67**, 1897–1899.
- Ito, Y., and Butler, A., 2005. Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002. *Limnology and Oceanography*, **50**, 1918–1923.
- Kanoh, K., Kamino, K., Leleo, G., Adachi, K., and Shizuri, Y., 2003. Pseudoalterobactin A and B, new siderophores excreted by marine bacterium *Pseudoalteromonas* sp. KP20–4. *The Journal of Antibiotics*, **56**, 871–875.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Biogenic ligands and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry: Molecular Geomicrobiology*, **59**, 53–76.
- Küpper, F. C., Carrano, C. J., Kuhn, J. U., and Butler, A., 2006. Photoreactivity of iron(III)-aerobactin: Photoproduct structure and iron(III) coordination. *Inorganic Chemistry*, **45**, 6026–6033.
- Martin, J. D., Ito, Y., Homann, V. V., Haygood, M. G., and Butler, A., 2006. Structure and membrane affinity of new amphiphilic siderophores produced by *Ochrobactrum* sp. SP18. *Journal of Biological Inorganic Chemistry*, **11**(5), 633–641.
- Martinez, J. S., Zhang, G. P., Holt, P. D., Jung, H. T., Carrano, C. J., Haygood, M. G., and Butler, A., 2000. Self-assembling amphiphilic siderophores from marine bacteria. *Science*, **287**, 1245–1247.
- Martinez, J. S., Carter-Franklin, J. N., Mann, E. L., Martin, J. D., Haygood, M. G., and Butler, A., 2003. Structure and membrane affinity of a new suite of amphiphilic siderophores produced by a marine bacterium. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(7), 3754–3759.
- Reid, R. T., Live, D. H., Faulkner, D. J., and Butler, A., 1993. A siderophore from a marine bacterium with an exceptional ferric ion stability constant. *Nature*, **366**, 455–457.

Cross-references

Bacteria
 Cyanobacteria
 Fe(II)-Oxidizing Prokaryotes
 Metalloenzymes
 Siderophores

METEORITICS

Mark A. Sephton

Imperial College London, London, UK

Definition

Meteorites are fragments of extraterrestrial material that fall on the Earth's surface. Most meteorites are parts of asteroids propelled into Earth-crossing orbits by relatively recent collisions in the asteroid belt, initiated by the gravitational effects of Jupiter's orbit. Relatively small numbers of meteorites originate from larger objects such as the Moon and Mars. The Earth acquires 10^2 to 10^3 t of such material each day, but only 1% or less arrives in pieces large enough for identification and recovery (Dodd, 1981). The surface of the meteorite usually melts and emits a glowing tail and a trail of smoke, but the interior of the meteorite is unaffected by passage through the Earth's atmosphere and remains at the temperature of interplanetary space.

Collection of meteorites

“Falls” are meteorites that have been observed to fall and were subsequently collected; “finds” are meteorites that were not seen to fall. Before the 1970s, the total number of known meteorites amounted to less than 1,000 falls and 1,706 authenticated finds. Since then, it has been recognized that certain parts of the Earth's surface preserve and concentrate meteorites. To date, tens of thousands of meteorites have been collected from the Antarctic ice. Similar numbers of meteorite fragments have also been collected from hot deserts. Most recently, telescope and camera network surveys have produced meteorites with photographed fireballs allowing the calculation of their orbits and assignment to a particular source within the solar system (Bland et al., 2009).

Chondrites

About 85% of the meteorites observed to fall on the Earth are chondrites, conglomerate rocks with an overall elemental composition similar to the Sun, if the loss of the most volatile elements is ignored. When their near solar makeup is considered alongside radiometric ages of 4.56×10^9 years, it becomes clear that chondrites are rocks formed during, or shortly after, the birth of the Sun.

The record in chondrites

Their status as some of the earliest formed solids in the solar system combined with a lack of subsequent processing ensures that chondrites represent a valuable record of extraterrestrial environments. Contributing regions include interstellar space, the solar nebula and the asteroidal meteorite parent bodies.

Carbonaceous chondrites

Of all the chondrites, the carbonaceous chondrites are considered the most primitive and these objects represent an enthralling window back in time providing views of the earliest solar system (Figure 1). The name “carbonaceous chondrite” is highly descriptive as these meteorites contain a carbon-rich matrix composed primarily of clay minerals, oxides, and organic matter, and most contain spherules of silicate glass termed chondrules.

Classification of carbonaceous chondrites

The carbonaceous chondrites are classified according to their primary and secondary characteristics (Figure 2). The primary classification (CI, CM, CR, CO, CV, CK)



Meteoritics, Figure 1 The Murchison carbonaceous chondrite.

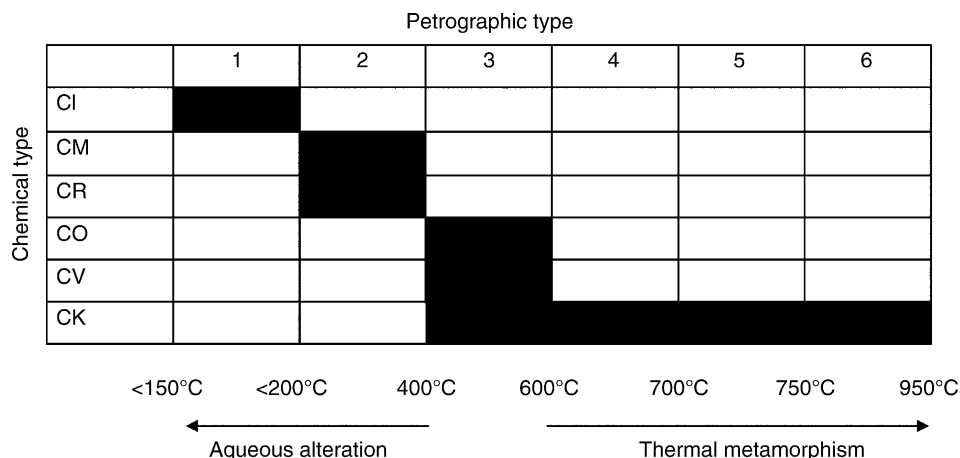
reflects the original properties of the meteorites such as bulk chemistry and mineralogy. The label is derived from the carbonaceous chondrite accepted as the type specimen, for example, CI = Ivuna. The secondary numerical classification reflects petrographic type, which indicates the extent and type of alteration that the meteorite has undergone on its parent body.

Carbon in meteorites

The carbon in carbonaceous chondrites is present in several forms and each has its specific source region(s) (Table 1; Sephton et al., 2003). Materials like silicon carbide and graphite condensed from the atmospheres of stars that existed long before our Sun. Nanometer-sized diamonds also originate as condensation products of stellar outflows as well as from catastrophic supernova explosions. Carbonate minerals were formed in the early solar system as aqueous alteration took place on asteroids. Yet all these components are quantitatively subordinate to the main carbonaceous material in carbonaceous chondrites – organic matter.

Types of organic matter in meteorites

Much of our current understanding of meteoritic organic matter has come from investigations of the Murchison carbonaceous chondrite, approximately 100 kg of which fell in Australia in 1969 (Figure 1). Not all of the organic molecules in meteorites are present in the same form. A minor portion of the organic matter in meteorites is present as small “free” molecules, including various hydrocarbons and the biologically useful amino acids and carboxylic acids (Figure 3), but the majority of organic matter in the carbon-rich meteorites is present as a highly cross-linked organic network or “macromolecular material”.



Meteoritics, Figure 2 The classification system for carbonaceous chondrites.

Free compounds

Amino acids

The amino acids found in meteorites have attracted a great deal of attention owing to their role as building blocks for proteins. The search for amino acids in meteorites accelerated in the 1960s (Degens and Bajor, 1962; Kaplan et al., 1963) following reports of microfossils in certain samples (Claus and Nagy, 1961). Amino acids were discovered, but these initially promising results were later found to be the result of terrestrial contamination. It was only when the organic-rich Murchison meteorite fell and was rapidly analyzed that both protein and, importantly, nonprotein amino acids were found in this fresh sample (Kvenvolden

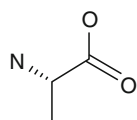
et al., 1970). Today it is known that the amino acids in Murchison are present at around 60 ppm. Compounds containing two through eight carbon atoms exist and they exhibit several features, which suggest that they are not terrestrial contaminants but are extraterrestrial and nonbiological or “abiotic” in origin. The meteoritic amino acids display broad structural diversity, a decline in abundance with increasing molecule size, and the presence of unusual and even unique molecular configurations.

A particular characteristic of amino acids is chirality or handedness. One chiral form rotates the polarized light to the left and is termed levorotatory or “L,” while the other form rotates the light to the right, and is called dextrorotatory or “D.” Chirality is important for theories that link extraterrestrial organic matter with the origin of life, because terrestrial biology preferentially generates L-amino acids whereas abiotic reactions generally have no preference and produce racemic mixtures with equal amounts of L and D forms.

The discussion of whether amino acids in meteorites were racemic (with equal amounts of L and D forms) or nonracemic (with a preference for one or the other) began in the 1950s and continued through the 1960s, (e.g., Mueller, 1953; Nagy et al., 1964) but early results implied that the dominance of L forms was caused by terrestrial contamination and analytical artefacts. Yet following the fall of Murchison in 1969, four protein and seven

Meteoritics, Table 1 The carbon-bearing components in carbonaceous chondrites

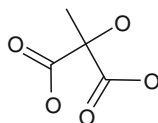
Carbon-bearing component	Abundance (wt. %)	Environment of formation
Organic matter	2.0	Interstellar space, solar nebula, asteroids
Carbonate	0.2	Asteroids
Diamond	0.04	Stars, supernovae
Graphite	0.005	Stars
Silicon carbide	0.009	Stars



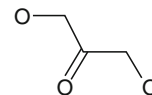
Amino acids
(Alanine)



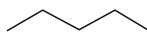
Carboxylic acids
(Ethanoic acid)



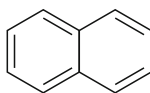
Hydroxyacids
(2-Hydroxy-2-methyl
propanedioic acid)



Polyols
(Dihydroxyacetone)



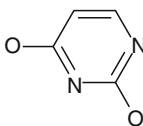
Aliphatic hydrocarbons
(Pentane)



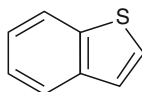
Aromatic hydrocarbons
(Naphthalene)



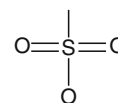
Amines
(Methylamine)



Nitrogen heterocycles
(Uracil)



Sulfur heterocycles
(Benzothiophene)



Sulfonic acids
(Methylsulfonic acid)

Meteoritics, Figure 3 Some free compound classes and examples found in carbonaceous chondrites.

nonprotein amino acids were found to be racemic (Kvenvolden et al., 1970, 1971). More modern analyses have reinvigorated the debate over the racemic nature of meteorite amino acids and a slight L-excess in both protein and nonprotein amino acids has been reported (Engel and Nagy, 1982; Pizzarello and Cronin, 2000).

Proposed mechanisms for producing this L-excess in meteoritic amino acids rely on selective destruction of the right-handed form of the amino acids. Currently, popular hypotheses involve ultraviolet circularly polarized light from a neutron star or reflection nebulae (Bailey, 2001; Bonner and Rubenstein, 1987). It is a necessary consequence of this theory that at least some amino acids were already formed in interstellar space so that preferential destruction of D-amino acids could take place. The recent detection of the simplest amino acid, glycine, in interstellar spectra supports this assertion (Kuan et al., 2003).

Carboxylic acids

Carboxylic acids are another type of biologically useful molecule found in meteorites. In life, they are a convenient and compact store of energy and are common cell membrane molecules. Carboxylic acids in Murchison contain between two and five carbon atoms and display complete structural diversity, a general decrease in abundance with increasing carbon number, and an equal concentration of branched and straight-chain isomers (Lawless and Yuen, 1979; Yuen and Kvenvolden, 1973). The monocarboxylic acids are present in a much higher concentration (332 ppm) than the amino acids and are the most abundant solvent extractable compounds. A structurally diverse suite of dicarboxylic acids containing up to nine carbon atoms are also present in much smaller amounts (26 ppm) (Lawless et al., 1974).

Hydroxyacids

Hydroxycarboxylic acids (or hydroxyacids) in carbonaceous chondrites contain up to nine carbon atoms and are structurally diverse and present in concentrations of 15 ppm (Peltzer and Bada, 1978). Intriguingly, the hydroxyacids correspond in structure to the more abundant amino acids suggesting that some hydroxy and amino acids may have been produced together on the parent asteroid. The relevant chemical reaction is known as the Strecker-cyanohydrin synthesis. Hydrogen cyanide and aldehydes combine in aqueous solution to produce hydroxyacids but if ammonia is also present then the products become amino acids.

Sugar-related compounds

Collectively, sugars and their related compounds are called polyhydroxylated compounds or "polyols." They are compounds containing a number of hydroxyl (OH) groups attached to their carbon skeleton. Sugars are

important biologically as a source of energy for organisms and, when combined to form larger molecules, they can act as food stores and give structural support. Experiments in the 1960s detected polyols in carbonaceous chondrites, but terrestrial contamination was implied (Degens and Bajor, 1962). More recent experiments on Murchison have discovered a number of sugar-related compounds (sugars, sugar alcohols, and sugar acids) in similar abundances to the amino acids (Cooper et al., 2001). The Murchison polyols display many of the features of extraterrestrial compounds. Progressively larger polyols become less abundant, display almost complete structural diversity, and contain some terrestrially rare compounds implying an abiotic source.

Aliphatic and aromatic hydrocarbons

Short aliphatic molecules in Murchison are present in amounts of 1.4 ppm and display many nonbiological features, which imply they are indigenous to the meteorite, such as structural diversity and a decrease in amount with increasing molecule size (Yuen et al., 1984). Aromatic hydrocarbons, in free form are present in carbonaceous chondrites at around 30 ppm (Pering and Ponnamperna, 1971). Compound sizes range from one to seven aromatic rings with the smaller compounds generally being most abundant (Sephton et al., 1998).

Amines and amides

Amines are derivatives of ammonia (NH₃) where hydrogen atoms are replaced by other structures. In Murchison, amine concentration is 8 ppm, amounts of individual amines decrease with increasing carbon number and structural diversity is observed with almost all amine isomers with up to five carbon atoms present (Pizzarello et al., 1994).

Two possible sources of the amines in Murchison have been proposed: they may be directly inherited from the presolar molecular cloud, an assertion supported by the detection of methylamine in interstellar space, or as with the hydroxyl acids, the amines may share a common origin with the amino acids. By simply removing the carboxylic acid group of amino acids, 16 of the 20 amines can be made, a reaction that would take place if the molecules were heated on their parent asteroid.

Amides are structurally analogous to carboxylic acids and, in Murchison, the amide equivalents of monocarboxylic acids, dicarboxylic acids, and hydroxyacids are found in concentrations greater than 70 ppm (Cooper and Cronin, 1995). The amides display complete structural diversity up to and including molecules with eight carbon atoms, a decline in abundance with carbon number, and the presence of many compounds with no terrestrial source. Cyclic amides (lactams) are less abundant (2 ppm) but they are very interesting to

investigators of the origin of life. The chemical structures of cyclic amides permit hydrogen-bonded pair formation and may have acted as parts of a primitive genetic coding apparatus, possible forerunners of the present day nucleic acids.

Nitrogen heterocycles

Another compound class essential for terrestrial life is that of the nitrogen heterocycles. Some of these compounds are building blocks for nucleic acids that store genetic information. The possible presence of nitrogen heterocycles in water extracts of carbonaceous meteorites was first reported in the early 1960s but the initial optimism was dispelled when they turned out to be laboratory contaminants (Oró, 1963). Later work did confirm the presence of nitrogen heterocycles in meteorites such as Murchison and several classes, including purines, pyrimidines, quinolines/isoquinolines, and pyridines were found. All of the purines and pyrimidines found are biologically common and together account for 1.3 ppm in Murchison (Stoks and Schwartz, 1979, 1981). The quinolines, isoquinolines, and pyridines are structurally diverse, contain a large number of isomeric alkyl derivatives, and are present at concentrations of 7 ppm (Pizzarello et al., 2001; Stoks and Schwartz, 1982). Recent work has confirmed the presence and extraterrestrial origin of xanthine and uracil in the Murchison meteorite (Martins et al., 2008).

Sulfur heterocycles

Murchison contains small amounts of sulfur-containing compounds such as thiophenes, benzothiophenes, dibenzothiophenes, and benzonaphthothiophenes. The abundance pattern of thiophenes in Murchison is markedly different from those in terrestrial sediments, indicating an abiotic origin for these molecules (Shimoyama and Katsumata, 2001).

Phosphonic and sulfonic acids

Phosphorus compounds have many biological roles and play a part in cell membranes, energy transactions during metabolism, and the storage and transfer of genetic information. Sulfur compounds are also common in living systems and take part in key biochemical reactions. In Murchison, a series of alkyl phosphonic acids and alkyl sulfonic acids with up to four carbon atoms have been detected (Cooper et al., 1992). As observed for many of the other compound classes in Murchison, the sulfonic and phosphonic acids display an exponential decline in amount with increasing carbon number and exhibit complete structural diversity.

Macromolecular materials

The solvent soluble substances are only trace components, yet they are often the focus of studies on meteorite organic

matter due to their analytical amenability. Essentially, the macromolecular materials can be considered as a cross-linked agglomeration of some of the free compounds but with an overwhelming dominance of aromatic hydrocarbons. The macromolecular materials are generally assumed to be completely indigenous to their meteorite host due to their high molecular weight and immobility.

As the major organic component, the macromolecular material is key to theories of the origin of meteoritic organic matter as a whole. Hence it is interesting to consider that meteoritic organic matter is often compared with that observed in interstellar space. Some scientists believe that the molecular cloud that collapsed to form the solar system bequeathed a significant amount of interstellar organic matter, aliquots of which are preserved in primitive asteroids and the meteorites derived from them. For many years, only one- to four-ring polycyclic aromatic hydrocarbons (PAH) were commonly observed in Murchison macromolecular material breakdown products (Sephton et al., 1998), which contrasted sharply with the greater than 20-ring PAH proposed for the interstellar medium (Pendleton and Allamandola, 2002). Recently, using modern analytical techniques the meteoritic macromolecular material has yielded more information. Up to seven-ring PAH units have been liberated from the macromolecular material in Murchison and it appears that even larger entities are present in the experimental residue (Sephton et al., 2004). These discoveries partly reconcile the apparent disharmony between the meteoritic and interstellar organic inventories and point to a partly presolar origin for this abundant organic component.

Terrestrial contamination

Although some compound classes in meteorites may contain a record that extends back to interstellar environments before the solar system formed others can be much more recent in the form of terrestrial contamination. Unusual and subtle contamination sources exist and an example is provided by data from the Orgueil carbonaceous chondrite, which has a long curation history since its fall in 1864. Terpene-related compounds have been identified in this meteorite (Watson et al., 2003) and these compounds are structurally specific, and are unlikely to be produced by abiotic reactions. Terpenes are common in essential plant oils, used extensively in cleaning products, and may have percolated their way into the porous Orgueil stones.

A more widespread type of terrestrial contaminant is provided by the long-chain aliphatic hydrocarbons (normal alkanes) in meteorites (Sephton et al., 2001). Unlike the short-chain aliphatic hydrocarbons, the normal alkanes are structurally specific with all carbon atoms arranged in a straight chain. Normal alkanes in meteorites were first detected in Orgueil and were attributed to extraterrestrial biology based on similar distributions

for molecules in the meteorite and those found in butter and marine sediments (Nagy et al., 1961). Subsequent analyses on many different meteorites led to the discovery that these compounds were present in several tens of ppm, were concentrated near to the surfaces of samples, and that they were frequently associated with other, obviously terrestrial, compounds (Oró et al., 1966). A subsequent revival in the belief that these compounds were indigenous occurred when normal alkanes were interpreted as products of an abiotic catalytic reaction called the Fischer–Tropsch synthesis (Studier et al., 1968). Modern measurements of individual normal alkanes reveal terrestrial values that are distinct from the majority of meteoritic organic matter (Sephton et al., 2001) and imply that normal alkanes are simply contributions from our terrestrial environment once the meteorite has fallen on the Earth.

The location of organic matter in meteorites

In chondrites, organic matter is present within the fine-grained inorganic meteorite matrix, which contains evidence of alteration by liquid water on the parent asteroid. Hence, a general relationship between extraterrestrial organic matter and inorganic aqueous alteration products in carbonaceous chondrites has been recognized for some time. Recently, however, it has been discovered that the association is highly specific to a particular inorganic phase – clay minerals (Pearson et al., 2002). Clay minerals, therefore, may have trapped and concentrated organic matter in the early solar system and organic–inorganic interactions may have played a role in the assembly of increasingly complex organic entities 4.56×10^9 years ago.

Relevance to the origin of life on Earth

With the recognition that meteorites contain many biologically useful molecules it is enlightening to consider that the amounts of organic matter extraterrestrial objects can deliver to the Earth's surface are considerable. For example, calculations indicate that comet Halley contains organic matter equivalent to approximately 10% of the current biomass of the Earth (Greenberg, 1993). A connection between life and extraterrestrial material was first proposed in the early 1960s by the biochemist Jan Oró (Oró, 1961). It was suggested that extraterrestrial objects may have seeded the early Earth with an extensive list of ingredients for use in the recipe of life. Modern theories imply that the early Earth had a nonreducing atmosphere composed mainly of carbon dioxide and nitrogen in which the in situ generation of organic matter would be rather sluggish and inefficient. Even if only a small fraction of the molecules delivered by extraterrestrial infall proved biologically relevant, they would have had a significant impact on the prebiotic chemistry of the Earth.

Outlook

Until space missions begin to return large quantities of extraterrestrial material back to the Earth for analysis in laboratories, meteorites will remain the most accessible and most frequently studied objects to reveal the origin and evolution of the solar system. In particular, the study of carbonaceous meteorites, which contain bona fide prebiotic organic matter is likely to remain our most important means of determining how chemical evolution led to the origin of life.

Bibliography

- Bailey, J., 2001. Astronomical sources of circularly polarized light and the origin of homochirality. *Origins Life Evolution Biosphere*, **31**, 167–183.
- Bland, P. A., Spurny, P., Towner, M. C., Bevan, A. W., Singleton, A. T., Botke, W. F., Greenwood, R. C., Chesley, S. R., Shrubny, L., Borovicka, J., Ceplecha, Z., McClafferty, T. P., Vaughan, D., Benedix, G. K., Deacon, G., Howard, K. T., Franchi, I. A., and Hough, R. M., 2009. An anomalous basaltic meteorite from the innermost main belt. *Science*, **325**, 1525–1527.
- Bonner, W. A., and Rubenstein, E., 1987. Supernovae, neutron-stars and biomolecular chirality. *Biosystems*, **20**, 99–111.
- Chang, S., Mack, R., and Lennon, K., 1978. Carbon chemistry of separated phases of Murchison and Allende meteorites. *Lunar and Planetary Science*, **9**, 157–159.
- Claus, G., and Nagy, B., 1961. A microbiological examination of some carbonaceous chondrites. *Nature*, **192**, 594–596.
- Cooper, G. W., and Cronin, J. R., 1995. Linear and cyclic aliphatic carboxamides of the Murchison meteorite – hydrolyzable derivatives of amino-acids and other carboxylic-acids. *Geochimica et Cosmochimica Acta*, **59**, 1003–1015.
- Cooper, G., Kimmich, N., Belisle, W., Sarinana, J., Brabham, K., and Garrel, L., 2001. Carbonaceous meteorites as a source of sugar-related organic compounds for the early Earth. *Nature* **414**, 879–883.
- Cooper, G. W., Onwo, W. M., and Cronin, J. R., 1992. Alkyl phosphonic-acids and sulfonic-acids in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **56**, 4109–4115.
- Cooper, G. W., Thieme, M. H., Jackson, T. L., and Chang, S., 1997. Sulfur and hydrogen isotope anomalies in meteorite sulfonic acids. *Science*, **277**, 1072–1074.
- Cronin, J. R., Pizzarello, S., and Cruikshank, D. P., 1988. Organic matter in carbonaceous chondrites, planetary satellites, asteroids and comets. In Kerridge, J. F., and Matthews, M. S. (eds.), *Meteorites and the Early Solar System*. Tucson, AZ: University of Arizona Press, pp. 819–857.
- Degens, E. T., and Bajor, M., 1962. Amino acids and sugars in the Bruderheim and Murray meteorite. *Naturwissenschaften*, **49**, 605–606.
- Dodd, R. T., 1981. *Meteorites: A Chemical and Petrological Synthesis*. London: Cambridge University Press, p. 386.
- Engel, M. H., and Nagy, B., 1982. Distribution and enantiomeric composition of amino-acids in the Murchison meteorite. *Nature*, **296**, 837–840.
- Greenberg, J. M., 1993. Physical and chemical composition of comets – from interstellar space to the Earth. In Greenburg, J. M., and Pirronello, V. (eds.), *Chemistry of Life's Origins*. Dordrecht: Kluwer, pp. 195–207.
- Hayatsu, R., Anders, E., Studier, M. H., and Moore, L. P., 1975. Purines and triazines in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **39**, 471–488.

- Jungclauss, G., Cronin, J. R., Moore, C. B., and Yuen, G. U., 1976a. Aliphatic amines in the Murchison meteorite. *Nature*, **261**, 126–128.
- Jungclauss, G. A., Yuen, G. U., Moore, C. B., and Lawless, J. G., 1976b. Evidence for the presence of low molecular weight alcohols and carbonyl compounds in the Murchison meteorite. *Meteoritics*, **11**, 231–237.
- Kaplan, I. R., Degens, E. T., and Reuter, J. H., 1963. Organic compounds in stony meteorites. *Geochimica et Cosmochimica Acta*, **27**, 805–834.
- Kuan, Y.-J., Charnley, S. B., Huang, H.-C., Tseng, W.-L., and Kisiel, Z., 2003. Interstellar glycine. *The Astrophysical Journal*, **593**, 848–867.
- Kvenvolden, K., Lawless, J., Pering, K., Peterson, E., Flores, J., Ponnamperna, C., Kaplan, I. R., and Moore, C., 1970. Evidence for extra-terrestrial amino acids and hydrocarbons in the Murchison meteorite. *Nature*, **228**, 928–926.
- Kvenvolden, K. A., Lawless, J. G., and Ponnamperna, C., 1971. Nonprotein amino acids in the Murchison meteorite. *Proceedings of the National Academy of Sciences of the United States of America*, **68**, 86–490.
- Lawless, J. G., and Yuen, G. U., 1979. Quantification of monocarboxylic acids in the Murchison carbonaceous meteorite. *Nature*, **282**, 396–398.
- Lawless, J. G., Zeitman, B., Pereira, W. E., Summons, R. E., and Duffield, A. M., 1974. Dicarboxylic acids in the Murchison meteorite. *Nature*, **251**, 40–42.
- Martins, Z., Botta, O., Fogel, M. L., Sephton, M. A., Glavin, D. P., Watson, J. S., Dworkin, J. P., Schwartz, A. W., and Ehrenfreund, P., 2008. Extraterrestrial nucleobases in the Murchison meteorite. *Earth and Planetary Science Letters*, **270**, 130–136.
- Meierhenrich, U. J., Munoz Caro, G. M., Bredehoft, J. H., Jessberger, E. K., and Thiemann, W. H. P., 2004. Identification of diamino acids in the Murchison meteorite. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 9182–9186.
- Mueller, G., 1953. The properties and genesis of the carbonaceous complex within the Cold Bokkeveld meteorite. *Geochimica et Cosmochimica Acta*, **4**, 1–10.
- Nagy, B., Meinschein, W. G., and Hennessy, D. J., 1961. Mass spectroscopic analysis of the Orgueil meteorite; evidence for biogenic hydrocarbons. *Annals of the New York Academy of Sciences*, **93**, 25–35.
- Nagy, G., Murphy, T. J., Modzeleski, V. E., Rouser, G. E., Claus, G., Hennessy, D. J., Colombo, U., and Gazzarini, F., 1964. Optical activity in saponified organic matter isolated from the interior of the Orgueil meteorite. *Nature*, **202**, 228–223.
- Oró, J., 1961. Comets and the formation of biochemical compounds on the primitive Earth. *Nature*, **190**, 389–390.
- Oró, J., 1963. Ultraviolet absorbing compounds reported present in the Murray meteorite. *Nature*, **197**, 756–758.
- Oró, J., Nooner, D. W., Zlatkis, A., and Wisktrom, S. A., 1966. Paraffinic hydrocarbons in the Orgueil, Murray, Mokoia and other meteorites. *Life Sciences and Space Research*, **4**, 63–100.
- Pearson, V. K., Sephton, M. A., Kearsley, A. T., Bland, P. A., Franchi, I. A., and Gilmour, I., 2002. Clay mineral-organic matter relationships in the early Solar System. *Meteoritics and Planetary Science*, **37**, 1829–1833.
- Peltzer, E. T., and Bada, J., 1978. α -Hydroxycarboxylic acids in the Murchison meteorite. *Nature*, **272**, 443–444.
- Peltzer, E. T., Bada, J. L., Schlesinger, G., and Miller, S. L., 1984. The chemical conditions on the parent body of the Murchison Meteorite; some conclusions based on amino, hydroxy and dicarboxylic acids. *Advances in Space Research*, **4**, 69–74.
- Pendleton, Y. J., and Allamandola, L. J., 2002. The organic refractory material in the diffuse interstellar medium: mid-infrared spectroscopic constraints. *Astrophysical Journal Supplement Series*, **138**, 75–98.
- Pering, K. L., and Ponnamperna, C., 1971. Aromatic hydrocarbons in the Murchison meteorite. *Science*, **173**, 237–239.
- Pizzarello, S., and Cronin, J. R., 2000. Non-racemic amino acids in the Murray and Murchison meteorites. *Geochimica et Cosmochimica Acta*, **64**, 329–338.
- Pizzarello, S., Feng, X., Epstein, S., and Cronin, J. R., 1994. Isotopic analyses of nitrogenous compounds from the Murchison meteorite – ammonia, amines, amino-acids, and polar hydrocarbons. *Geochimica et Cosmochimica Acta*, **58**, 5579–5587.
- Pizzarello, S., Huang, Y. S., Becker, L., Poreda, R. J., Nieman, R. A., Cooper, G., and Williams, M., 2001. The organic content of the Tagish Lake meteorite. *Science*, **293**, 2236–2239.
- Sephton, M. A., Love, G. D., Watson, J. S., Verchovsky, A. B., Wright, I. P., Snape, C. E., and Gilmour, I., 2004. Hydropyrolysis of insoluble carbonaceous matter in the Murchison meteorite: new insights into its macromolecular structure. *Geochimica et Cosmochimica Acta*, **68**, 1385–1393.
- Sephton, M. A., Pillinger, C. T., and Gilmour, I., 1998. $\delta^{13}\text{C}$ of free and macromolecular aromatic structures in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **62**, 1821–1828.
- Sephton, M. A., Pillinger, C. T., and Gilmour, I., 2001. Normal alkanes in meteorites: molecular $\delta^{13}\text{C}$ values indicate an origin by terrestrial contamination. *Precambrian Research*, **106**, 47–58.
- Sephton, M. A., Verchovsky, A. B., Bland, P. A., Gilmour, I., Grady, M. M., and Wright, I. P., 2003. Investigating the variations in carbon and nitrogen isotopes in carbonaceous chondrites. *Geochimica et Cosmochimica Acta*, **67**, 2093–2108.
- Shimoyama, A., and Katsumata, H., 2001. Polynuclear aromatic thiophenes in the Murchison carbonaceous chondrite. *Chemistry Letters*, **3**, 202–203.
- Stoks, P. G., and Schwartz, A. W., 1979. Uracil in carbonaceous meteorites. *Nature*, **282**, 709–710.
- Stoks, P. G., and Schwartz, A. W., 1981. Nitrogen-heterocyclic compounds in meteorites – significance and mechanisms of formation. *Geochimica et Cosmochimica Acta*, **45**, 563–569.
- Stoks, P. G., and Schwartz, A. W., 1982. Basic nitrogen-heterocyclic compounds in the Murchison meteorite. *Geochimica et Cosmochimica Acta*, **46**, 309–315.
- Studier, M. H., Hayatsu, R., and Anders, E., 1968. Origin of organic matter in early solar system; I, Hydrocarbons. *Geochimica et Cosmochimica Acta*, **32**, 151–173.
- Watson, J. S., Pearson, V. K., Gilmour, I., and Sephton, M. A., 2003. Sesquiterpenoid derivatives in the Orgueil carbonaceous chondrite. *Organic Geochemistry*, **34**, 37–47.
- Yuen, G., Blair, N., DesMarais, D. J., and Chang, S., 1984. Carbon isotope composition of low molecular weight hydrocarbons and monocarboxylic acids from the Murchison meteorite. *Nature*, **307**, 252–254.
- Yuen, G. U., and Kvenvolden, K. A., 1973. Monocarboxylic acids in Murray and Murchison carbonaceous meteorites. *Nature*, **251**, 40–42.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Cosmic Molecular Clouds](#)
[Origin of Life](#)

METHANE OXIDATION (AEROBIC)

Helmut Bürgmann

Eawag, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

Synonyms

Methanotrophy

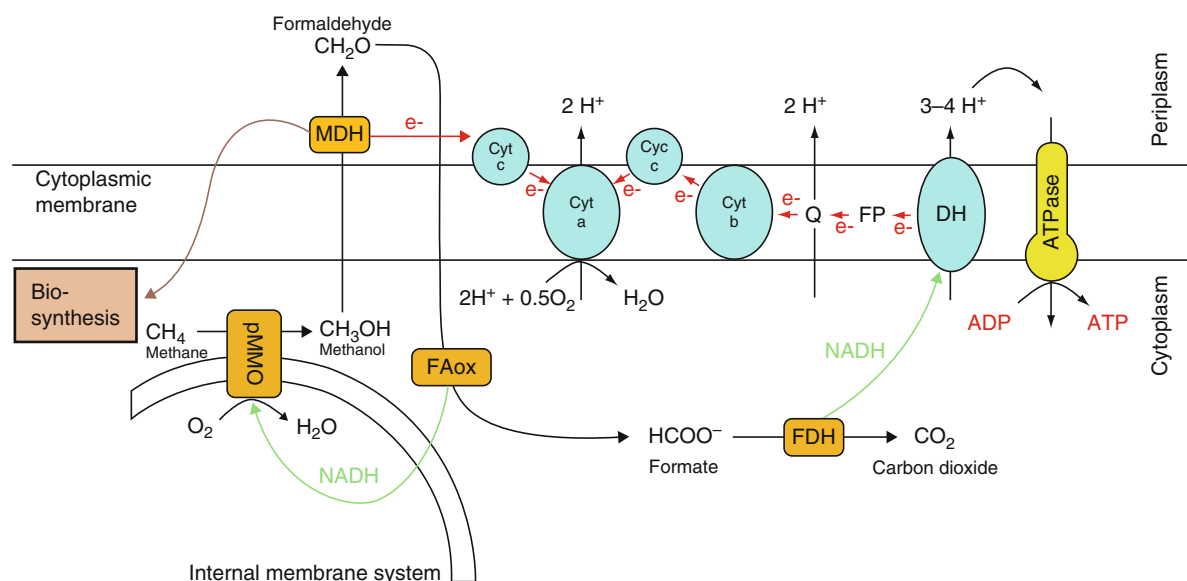
Definition

Methane oxidation is a microbial metabolic process for energy generation and carbon assimilation from methane that is carried out by specific groups of bacteria, the methanotrophs. Methane (CH_4) is oxidized with molecular oxygen (O_2) to carbon dioxide (CO_2).

Biochemical basis

Methanotrophy is the microbially mediated process of the oxidation of CH_4 with O_2 to methanol, formaldehyde, formate, and finally CO_2 (Figure 1) (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). The process is performed by a specialized group of bacteria (qv), the methanotrophs (CH_4 oxidizing bacteria). They are a subgroup of the methylotrophs, bacteria capable of utilizing single-carbon compounds (Bowman, 2006).

Methane is both the energy source (electron donor) and the sole or partial carbon source for methanotrophs. Aerobic oxidation of methane requires specific enzymes, most importantly methane monooxygenase (MMO), which catalyzes the first step in the reaction: the oxidation of methane with molecular oxygen to methanol and water. Two forms of this enzyme are known: particulate methane monooxygenase (pMMO), which is ubiquitous in methanotrophs and contains copper, and the iron-containing soluble methane monooxygenase (sMMO) (Hanson and Hanson, 1996). The reaction mediated by MMO requires additional electrons, which are supplied by cellular redox carriers such as cytochrome C (for pMMO) or NADH (sMMO). Energy is conserved in the subsequent stepwise oxidations of methanol, formaldehyde, and formate. In these reactions, electrons are donated back to a membrane-bound electron transport chain (see Chapter *Aerobic Metabolism*) via a pyrroloquinoline quinone cofactor to cytochrome C (methanol dehydrogenase) or NAD (in formaldehyde oxidation systems and formate dehydrogenase). Electron flow through the membrane ultimately produces a proton motive force that is converted to the cellular energy carrier ATP by the ATPase enzyme complex. O_2 is the terminal electron acceptor (see Chapter *Aerobic Metabolism*) (Figure 1) (Madigan et al., 2003). Thus, aerobic methane oxidation can be considered a special case of aerobic



Methane Oxidation (Aerobic), Figure 1 Pathway of aerobic methane oxidation for energy conservation and carbon assimilation in Type I and II methanotrophs. pMMO, particulate methane monooxygenase; MDH, methanol dehydrogenase; FAox, formaldehyde oxidation system (tetrahydrofolate- or methanopterin-dependent) FDH, formate dehydrogenase. Methane is oxidized with oxygen by pMMO, a step which requires reducing equivalents (e.g., from NADH) supplied by later oxidations in the pathway. Energy is generated by shuttling electrons generated in the subsequent oxidations of methanol (CH_3OH), formaldehyde (CH_2O), and formate (HCOO^-) through a membrane-bound electron transport chain that creates a proton gradient across the cell membrane (proton motive force) that can be converted into ATP by ATPase (oxidative phosphorylation). Formaldehyde (CH_2O) is the starting point for carbon assimilation via the ribulose monophosphate or serine pathways.

respiration. Differences between methanotrophs are thought to exist regarding the enzymes used, especially with regard to formaldehyde oxidation, where different systems are known (Lidstrom, 2006).

Methanotrophs

Most known methanotrophs fall into two well-defined phylogenetic groups belonging to the gamma- (Type I methanotrophs) and alpha-proteobacteria (Type II) that are also distinguished by morphological traits and differences in carbon assimilation (Hanson and Hanson, 1996; Madigan et al., 2003; Bowman, 2006). Both types share the characteristic extensive intracytoplasmic membrane system (ICM), which is the site of methane oxidation. However, in Type I methanotrophs, the ICM occurs as disk-shaped membrane stacks within the cell, while in Type II methanotrophs, the ICM is located around the periphery of the cell. Both groups are strict aerobes and obligate methylotrophs. The presence of soluble methane monooxygenase (sMMO) seems to be mostly limited to Type II methanotrophs (Hanson and Hanson, 1996; Bowman, 2006). Type I and II methanotrophs employ different carbon assimilation strategies, although both incorporate methane carbon at the level of formaldehyde (Figure 1). Type I methanotrophs use the ribulose monophosphate pathway and obtain all their carbon from methane. Type II methanotrophs use the serine pathway, which in addition to formaldehyde incorporation further involves fixation of one molecule of CO₂ per molecule of formaldehyde assimilated. The former pathway is energetically more favorable, requiring only 1/3 ATP per molecule formaldehyde fixed, instead of 1 ATP and reducing power for the serine pathway. This is in accordance with the observed higher growth rates in Type I methanotrophs (Madigan et al., 2003). Most methanotrophs can also grow using other single-carbon compounds, e.g., methanol, which is the first intermediate of methane oxidation.

Within the Type I methanotrophs, the genera *Methylosphaera*, *Methylobacter*, *Methyломicrobium*, *Methylomonas*, *Methylococcus*, and *Methylocaldum* have been described. The latter two genera are often distinguished as “type X methanotrophs,” as they are phylogenetically and morphologically distinct from the other Type I genera. The cultivated type II methanotrophs belong to the genera *Methylosinus* and *Methylocystis* (Bowman, 2006). Environmental clone libraries suggest the existence of additional, yet uncultured, genera. Recently, acidophilic methanotrophs belonging to the *Verrucomicrobia* phylum have been isolated, showing for the first time that not all methanotrophs belong to the proteobacteria and that methanotrophs are phylogenetically more diverse than previously thought (Dunfield et al., 2007; Pol et al., 2007). The genome sequence of one isolate revealed the presence of pMMO genes (pmoA), but lacked homologs to known methanol

monooxygenases and formaldehyde oxidation genes, indicating a methane oxidation system that deviates from those known from Type I and II methanotrophs (Dunfield et al., 2007).

Habitats

Although current isolates require high methane partial pressures for growth, some methanotrophs can apparently utilize methane with high affinity at low partial pressures, down to atmospheric levels (Hanson and Hanson, 1996). Therefore, they are ubiquitous in soil and water. Increased populations and activity occur wherever increased methane concentrations, whether from biogenic or other sources, meet oxic conditions. Typical habitats are thus closely tied to sites with methanogenesis (see Chapters *Methane, Origin*; *Methanogens*), e.g., rice paddies, swamps, aquatic and terrestrial sediments, land-fill coverings, and the oxycline of stratified water bodies (Hanson and Hanson, 1996). In stratified systems, (e.g., in stratified water bodies or sediments) aerobic methane oxidation will often be located within a relatively narrow band within the oxycline, where methanotrophs can become highly enriched. Methanotrophs also occur as endosymbionts, e.g., of marine mussels found at cold seeps (qv) that use methane released to supply their hosts with carbon and energy (Hanson and Hanson, 1996; Bowman, 2006).

Methods to study aerobic methane oxidation and methanotrophs

Methane oxidation rates in environmental samples are frequently determined by incubation experiments. Samples are placed in a gas-tight container and the consumption of methane over time is determined in the headspace. Alternatively, ¹⁴C labeled methane can be used to quantify the conversion to CO₂ and incorporation into the bacterial biomass. Field methods include the use of push–pull tests (Urmann et al., 2005), or modeling of rates based on methane gradients.

Methanotrophs can be cultured from almost any environment using a nitrate mineral salts medium and a CH₄/air atmosphere (Madigan et al., 2003; Bowman, 2006). Since they are relatively easily cultured, viable count methods can be used but will severely underestimate the actual population, due to the prevalence of viable but nonculturable cells. Since they are phylogenetically well defined, methanotrophs can be detected and quantified using ribosomal RNA as a genetic marker, e.g., using specific oligonucleotide probes (e.g., for fluorescent in-situ hybridization) or primers for polymerase chain reaction based detection (PCR) (McDonald et al., 2008). In addition, the gene sequences of functional genes, most frequently the gene encoding pMMO (pmoA), but also genes for sMMO (mmoX) and methanol dehydrogenase (mxaF), are frequently used as molecular markers for

PCR-based detection (Hanson and Hanson, 1996; Bowman, 2006; McDonald et al., 2008). A hybridization microarray was developed for rapid phylogenetic typing of PCR amplified *pmoA* (Bodrossy et al., 2003). Type I and II methanotrophs also contain biomarkers (see Chapter *Biomarkers (Organic, Compound-Specific Isotopes)*), e.g., specific lipids and phospholipid fatty acids that are useful in tracing community changes (Hanson and Hanson, 1996; Bowman, 2006; Schubert et al., 2006; Blumenberg et al., 2007).

Geochemical impact

Locally, methanotrophs can be an important basis of the food web, if sufficiently large fluxes of methane are converted to biomass and passed on down the food chain. Since biogenic methane (see Chapters *Methane, Origin; Methanogens*) has a strongly ^{13}C depleted carbon isotope signature, carbon flow originating from oxidized methane can be studied using isotope measurements (see Chapter *Isotopes (Methods)*).

The primary importance of aerobic methane oxidation for the global *carbon cycle* (qv) is that it represents (together with *anaerobic methane oxidation* (qv) and photochemical methane oxidation in the atmosphere) a major sink for this radiatively active and therefore, important greenhouse gas. Globally, photochemical methane oxidation is by far the dominant process (94%), but microbial oxidation is an important factor to take into account when projecting global warming effects. Obtaining global estimates of microbial aerobic methane oxidation is not trivial due to high spatial and temporal variability and the difficulty in performing rate measurements in environments where methanogenesis and methanotrophy occur simultaneously. The total sink function of soils has been estimated at 29 Tg year^{-1} , with an uncertainty range of 7–100 Tg year^{-1} (Smith et al., 2000). In rice paddies, methane oxidation can occur in the rhizosphere and at the soil surface, and is estimated to remove 80% of the methane produced in these systems, but there is uncertainty that CH_4 bypass would possibly occur through vascular plants' parenchyma. Methane oxidation rates in soil depend on various factors, e.g., soil bulk density, water content, temperature, and pH (Hanson and Hanson, 1996). The process is also often limited by the rate of methane diffusion into the oxic zone. Further, ammonium is a competing substrate for MMO that is not coupled to energy generation and therefore acts as a poison to methanotrophs at higher concentrations (Madigan et al., 2003). Thus, the methanotroph activity in soil is subject to considerable variability and sensitive to land use changes, and is one factor to be considered for mitigation strategies to reduce atmospheric methane concentrations (Hanson and Hanson, 1996).

Lakes were long considered a minor source of methane, a view that has somewhat changed based on observation of significant methane fluxes from reservoirs (St. Louis

et al., 2000). In permanently or temporally stratified water bodies such as lakes, reservoirs, or the Black Sea, a large proportion of the methane is oxidized by aerobic methanotrophs in the chemocline (Schubert et al., 2006; Blumenberg et al., 2007).

While the open ocean is not considered a major source of methane, and methane oxidation activity is generally low, *cold seeps* (qv) release large amounts of methane from sediments and hydrocarbon deposits (including *gas hydrates* (qv)), creating high local fluxes of methane (see Chapter *Methane, Origin*). This creates a situation where symbiotic as well as free-living methane oxidizers are the basis of large oceanic food webs. However, in anoxic marine sediments *anaerobic methane oxidation* (qv) coupled to sulfate reduction is the most important process.

Summary

Aerobic oxidation of methane is performed by specific bacteria, the methanotrophs, which use a specific metabolic pathway for carbon assimilation and energy generation from methane. Most known methanotrophs belong to the Proteobacteria, but recent discoveries of novel methanotroph *Verrucomicrobia* indicated that the diversity of methanotrophs is not yet fully known. Aerobic methane oxidation is one of the major sinks for the greenhouse gas methane, along with *anaerobic methane oxidation* (qv) and photochemical oxidation in the atmosphere. It is, thus, an important process for the global *carbon cycle* (qv) and an important element in the regulation of the atmospheric concentration of methane.

Bibliography

- Blumenberg, M., Seifert, R., and Michaelis, W., 2007. Aerobic methanotrophy in the oxic-anoxic transition zone of the Black Sea water column. *Organic Geochemistry*, **38**(1), 84–91.
- Bodrossy, L., Stralis-Pavese, N., Murrell, J. C., Radajewski, S., Weilharter, A., and Sessitsch, A., 2003. Development and validation of a diagnostic microbial microarray for methanotrophs. *Environmental Microbiology*, **5**(7), 566–582.
- Bowman, J., 2006. The methanotrophs - the families methylcocccaceae and methylcystaceae. In Dworkin, M. (ed.), *The Prokaryotes*. New York: Springer, Vol. 5, pp. 266–289.
- Dunfield, P. F., Yuryev, A., Senin, P., Smirnova, A. V., Stott, M. B., Hou, S., Ly, B., Saw, J. H., Zhou, Z., Ren, Y., Wang, J., Mountain, B. W., Crowe, M. A., Weatherby, T. M., Bodelier, P. L. E., Liesack, W., Feng, L., Wang, L., and Alam, M., 2007. Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature*, **450**(7171), 879–882.
- Hanson, R. S., and Hanson, T. E., 1996. Methanotrophic bacteria. *Microbiological Reviews*, **60**(2), 439–471.
- Lidstrom, M. E., 2006. Aerobic methylotrophic prokaryotes. In Dworkin, M. (ed.), *The Prokaryotes*. New York: Springer, Vol. 2, pp. 618–634.
- Madigan, M. T., Martinko, J. M., and Parker, J., 2003. *Brock Biology of Microorganisms*. Upper Saddle River, NJ: Pearson Education.

- McDonald, I. R., Bodrossy, L., Chen, Y., and Murrell, C. J., 2008. Molecular ecology techniques for the study of aerobic methanotrophs. *Applied and Environmental Microbiology*, **75**(5), 1305–1315.
- Pol, A., Heijmans, K., Harhangi, H. R., Tedesco, D., Jetten, M. S. M., and Op den Camp, H. J. M., 2007. Methanotrophy below pH1 by a new Verrucomicrobia species. *Nature*, **450**(7171), 874–878.
- Schubert, C. J., Coolen, M. J. L., Neretin, L. N., Schippers, A., Abbas, B., Durisch-Kaiser, E., Wehrli, B., Hopmans, E. C., Damste, J. S. S., Wakeham, S., and Kuypers, M. M. M., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**(10), 1844–1856.
- Smith, K. A., Dobbie, K. E., Ball, B. C., Bakken, L. R., Sitaula, B. K., Hansen, S., Brumme, R., Borken, W., Christensen, S., Prieme, A., Fowler, D., Macdonald, J. A., Skiba, U., Klemmedtsson, L., Kasimir-Klemmedtsson, A., Degorska, A., and Orlanski, P., 2000. Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Global Change Biology*, **6**(7), 791–803.
- St. Louis, V. L., Kelly, C. A., Duchemin, E., Rudd, J. W. M., and Rosenberg, D. M., 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. *BioScience*, **50**(9), 766–775.
- Urmann, K., Gonzalez-Gil, G., Schroth, M. H., Hofer, M., and Zeyer, J., 2005. New field method: gas push-pull test for the in-situ quantification of microbial activities in the vadose zone. *Environmental Science & Technology*, **39**(1), 304–310.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Carbon Cycle](#)
[Cold Seeps](#)
[Isotopes \(Methods\)](#)
[Methane, Origin](#)
[Methanogens](#)

METHANE, ORIGIN

Carsten J. Schubert
 Eawag, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

Definition

Methane is a colorless and odorless gas, with the chemical formula CH₄. Due to its radiative force, it is a strong greenhouse gas and contributes to the warming of the earth. It is formed in the environment by methanogenesis. The main natural sources are wetlands and termites (30% of total emissions), while anthropogenic sources include rice fields, cattle farming, and energy production (70% of total emissions).

General aspects

Methane is a colorless and odorless gas and the major component (97% vol.) of natural gas. Methane has a boiling point of −161°C at a pressure of one atmosphere. As a gas, it is flammable only over a narrow range of concentrations (5–15%) in air. It is mainly produced by microorganisms (methanogens) under anoxic conditions in a process called methanogenesis. These conditions exist under water-covered soils, such as rice paddies, tundra, swamps, and marshes, but also in freshwater and marine sediments. Other anoxic environments include ruminant stomachs and termite guts (see [Table 1](#)).

Methanogenesis is the final step in the anaerobic degradation of organic carbon, with methane being formed as a waste product. Methanogenesis is performed by strict anaerobes called methanogens, organisms belonging to the Archaea (a domain of life alongside Bacteria and Eukaryota). Methanogens are rather cosmopolitan in respect to environmental conditions (i.e., temperature, pH, salinity; as long as the environment is anoxic) and represent the largest and most diverse group in the Archaea domain (Magonigal et al., 2004; c.f. Boone et al., 1993). Methanogens are able to grow at temperatures between 4°C and 100°C, at pH values from 3 to 9, and at salinities ranging from brines to freshwater. Despite this huge taxonomic diversity, the substrates that can be used by methanogens are rather limited – hydrogen, acetate, formate, some alcohols, and methylated compounds (Zinder, 1993). The most important of these are hydrogen and acetate, which give rise to two different kinds of methanogenesis: (1) the fermentation of acetate to CO₂ and CH₄ and (2) the reduction of CO₂ with H₂.

Garcia et al. (2000) showed that over 70% of methanogens use the latter pathway, which is known as hydrogenotrophic methanogenesis or carbon dioxide/hydrogen reduction. Hydrogen in this reaction is used both as an energy source and electron donor, whereas CO₂ is the electron acceptor and the carbon source. This reaction yields the most energy of all methanogenic processes – much more than acetate fermentation, which yields the least (see [Table 2](#)). Since hydrogen occurs only in very low concentrations in natural environments, it is the limiting substrate in CO₂/H₂ reduction.

Only 10% of the methanogenic genera, i.e., *Methanosarcina* and *Methanosaeta*, are able to use the other pathway, called acetate fermentation or acetoclastic

Methane, Origin, Table 1 Methane properties

Methane properties	
Chemical formula	CH ₄
Mass	16.0425 g/mol
Melting point	−182.5°C
Boiling point	−161.6°C
Solubility in water	3.5 mg/100 ml

- Hein, R., Crutzen, P. J., and Heimann, M., 1997. An inverse modeling approach to investigate the global atmospheric methane cycle. *Global Biogeochemical Cycles*, **11**(1), 43–76.
- Holzapfel-Pschorn, A., and Seiler, W., 1986. Methane emission during a cultivation period from an Italian rice paddy. *Journal of Geophysical Research-Atmospheres*, **91**(D11), 1803–1814.
- Hornafius, J. S., Quigley, D., and Luyendyk, B. P., 1999. The world's most spectacular marine hydrocarbon seeps (Coal Oil Point, Santa Barbara Channel, California): quantification of emissions. *Journal of Geophysical Research-Oceans*, **104**(C9), 20703–20711.
- Hovland, M., and Judd, A. G., 1988. *Seabed Pockmarks and Seepages: Impact on Geology, Biology and the Marine Environment*. London: Graham and Trotman.
- IPCC, 2001. Climate change 2001: the scientific basis. In Houghton, J. T., Ding, Y., Griggs, D. J., Noguer, M., van der Linden, P. J., Dai, X., Maskell, K., and Johnson, C.A. (eds.), *Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK: Cambridge University Press, 881 p.
- Judd, A. G., Hovland, M., Dimitrov, L. I., Garcia Gil, S., and Jukes, V., 2002. The geological methane budget at continental margins and its influence on climate change. *Geofluids*, **2**(2), 109–126.
- Kalff, J., 2001. Limnology: Inland water ecosystems. Prentice Hall, 592 p.
- Kelly, C. A. et al., 1997. Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir. *Environmental Science & Technology*, **31**(5), 1334–1344.
- Khalil, M. A. K., 2000. Atmospheric methane: an introduction. In Khalil, M.A.K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 1–8.
- Kvenvolden, K. A., and Lorensen, T. D., 2001. The global occurrence of natural gas hydrate. In Paull, C. K., and Dillon, W. D. (eds), *Natural Gas Hydrates: Occurrence, Distribution and Detection*. Geophysical Monograph. Washington, DC: American Geophysical Union.
- Kvenvolden, K. A., and Rogers, B. W., 2005. Gaia's breath - global methane exhalations. *Marine and Petroleum Geology*, **22**(4), 579–590.
- McGinnis, D. F., Wüest, A., Schubert, C. J., Klauser, L., Lorke, A., and Kipfer, R., 2005. Upward flux of methane in the Black Sea: does it reach the atmosphere? In Lee, J. H. W., and Lam, K. M. (eds.), *Environmental Hydraulics and Sustainable Water Management*. London: Taylor & Francis Group, pp. 423–429.
- Matthews, E., and Fung, I., 1987. Methane emission from natural wetlands: global distribution, area, and environmental characteristics of sources. *Global Biogeochemical Cycles*, **1**(1), 61–86.
- Megonigal, J. P., Hines, M. E., and Visscher, P. T., 2004. Anaerobic metabolism: linkages to trace gases and aerobic processes. In Schlesinger, W. H. (ed.), *Biogeochemistry*. Oxford: Elsevier-Pergamon, pp. 317–424.
- Milkov, A. V., 2004. Global estimates of hydrate-bound gas in marine sediments: how much is really out there? *Earth-Science Reviews*, **66**(3–4), 183–97.
- Peckmann, J., and Thiel, V., 2004. Carbon cycling at ancient methane-seeps. *Chemical Geology*, **205**(3–4), 443–467.
- Rasmussen, R. A., and Khalil, M. A. K., 1984. Atmospheric methane in the recent and ancient atmospheres: concentrations, trends, and interhemispheric gradient. *Journal of Geophysical Research*, **89**, 11599–11605.
- Schubert, C. J. et al., 2006. Aerobic and anaerobic methanotrophs in the Black Sea water column. *Environmental Microbiology*, **8**(10), 1844–1856.
- St. Louis, V. L., Kelly, C. A., Duchemin, E., Rudd, J. W. M., and Rosenberg, D. M., 2000. Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. *Bioscience*, **50**(9), 766–775.
- Sloan, E. D., 1998. *Clathrate Hydrates of Natural Gas*, New York: Marcel Dekker.
- Stevens, C. M., and Wahlen, M., 2000. The isotopic composition of atmospheric methane and its sources. In Khalil, M. A. K. (ed.), *Atmospheric Methane*. Berlin, Heidelberg: Springer, pp. 25–41.
- Tyler, S. C. et al., 1988. Measurements and interpretation of $\delta^{13}\text{C}$ of methane from termites, rice paddies, and wetlands in Kenya. *Global Biogeochemical Cycles*, **2**(4), 341–355.
- Tyler, S. C., 1991. The global methane budget. In Rogers, J. E., and Whitman, W. B. (eds.), *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes*. Washington, DC: American Society for Microbiology, pp. 7–38.
- Whiticar, M. J., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 219–314.
- Whiticar, M. J., Faber, E., and Schoell, M., 1986. Biogenic methane formation in marine and freshwater environments: CO_2 reduction vs. acetate fermentation - Isotope evidence. *Geochimica et Cosmochimica Acta*, **50**, 693–709.
- Wuebbles, D. J., and Hayhoe, K., 2002. Atmospheric methane and global change. *Earth-Science Reviews*, **57**(3–4), 177–210.
- Zinder, S. H., 1993. Physiological ecology of methanogens. In Jerry, J. G., (ed.), *Methanogenesis: Ecology, Physiology, Biochemistry and Genetics*, New York: Chapman Hall, pp. 128–206.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Cap Carbonates](#)
[Carbonates](#)
[Cold Seeps](#)
[Methane Oxidation \(Aerobic\)](#)

METHANOGENS

Please refer to "[Methane, Origin](#)" and "[Archaea](#)."

MICROBIAL BIOMINERALIZATION

Christine Heim
 University of Göttingen, Göttingen, Germany

Synonyms

Microbial biomineral formation

Definition

Microbial biomineralization describes the formation and deposition of minerals directly mediated or indirectly influenced by microorganisms (Mann, 2001; Weiner and Dove, 2003; Ehrlich, 1999). A huge variety of minerals results from individual biomineralization pathways linked to the phylogeny and metabolic activity of the microorganisms involved (Weiner and Dove, 2003;

MICROBIAL DEGRADATION

Erika Kothe
Friedrich Schiller University Jena, Jena, Germany

Synonyms

Mineralization; Nutrient cycling

Definition

Microbial. Processes driven by microorganisms.

Degradation. Use of chemical substances as carbon and energy source for metabolism, thereby breaking down larger molecules to smaller ones.

Introduction

Microorganisms are the basis for the process of mineralization by utilizing organic molecules for their catabolism, thereby returning the elements to the geobiological cycles. In catabolism, or energy conservation, energy-rich adenosine triphosphate (ATP) is formed (for general reading on microbial physiology: Madigan and Martinko, 2006). This is the basis for metabolic degradation performed by microorganisms in a great diversity. The degradation of organic material in terrestrial, limnological, and marine environments is thus based on the growth of microorganisms on organic compounds, which is termed chemoheterotrophic growth style, depending on the availability of organic nutrients for growth. The processes can be dependent on aerobic respiration, but many degradative processes, e.g., in sediments, are performed in the absence of oxygen under anaerobic conditions (Schink, 2002).

Aerobic and anaerobic respiration

The respiration with oxygen as the terminal electron acceptor is the process yielding the most ATP per mol substance oxidized and thus, aerobic respiration is the preferred route for degradation whenever enough oxygen is available. Under aerobic conditions, the degradation of organic matter is performed following general and ubiquitous pathways such as glycolysis; the pyruvate produced in the glycolytic chain enters the citrate cycle; and the reduction equivalents produced are then reoxidized by the respiratory chain coupled to the reduction of oxygen to water. All building blocks of living cells can be degraded that way, allowing growth of microorganisms. The carbon moieties are released as CO₂ into the atmosphere.

Under anaerobic conditions, anaerobic respiratory chains using alternative electron acceptors can ensue. For example, nitrate, nitrite, sulfate, sulfite, sulfur, iron, manganese or fumarate, or carbon dioxide in methanogenesis is then used to accept the electrons, thereby reducing these substances. Halogenated compounds can serve as electron acceptors in dehalorespiration.

All these respiratory chains depend on membrane-bound electron transport, and the reactions of electron transport are coupled to proton export across the cellular membrane to form a proton motive force. The proton motive force is then used to drive ATP formation *via* the enzyme ATP synthase, which can form 1 mol of ATP per 3 mol of protons carried back into the cytoplasm. The electrons for electron transport chains are derived from oxidation of the organic molecules of the nutrient source. If possible, the organic matter is fully oxidized to carbon dioxide. In this way, the organic matter is fully decomposed.

Fermentation

Another route, independent of respiratory chains, is possible for the fermentative degradation of organic matter. The fermentations performed by microorganisms can use oxidation of substrates to produce ATP and then reduce organic molecules for reoxidation of the reducing equivalents. While ATP yield in these systems is limited when compared to respiration, it still allows growth under conditions when no inorganic electron acceptor is available in sufficient amounts, or when iron as part of the electron carriers in the respiratory chain is not available and therefore, an electron transport chain cannot be synthesized.

In contrast to respiration, both aerobic and anaerobic, during fermentation the organic substrate is not fully oxidized to CO₂. Rather, energy conservation is dependent on the oxidation of an organic molecule in which an aldehyde may be formed as intermediate, which subsequently can be oxidized to form an acid. This exergonic reaction is coupled to the activation of an inorganic phosphate moiety which then can be transferred to ADP, thereby forming ATP. This process is called substrate-level phosphorylation in contrast to the electron transport phosphorylation occurring in respiratory chains. The oxidative reaction releases reduction equivalents, which cannot be transferred to an inorganic electron acceptor due to the lack of respiratory chains in fermenting microbes. Thus, the reduction of an organic compound is necessary to release the carrier for a new cycle of oxidation. The reduced organic substance is released into the environment. An alternative is to release the reduction equivalents as H₂.

The fermentation of sugars, which are present in large amounts due to photosynthesis products and constituents of plant cell wall material, can be performed using the Embden–Meyerhof pathway of glycolysis, the Entner–Doudoroff pathway, or modifications or parts thereof. Sugars with 5 C moieties, sugar acids, or carbonic acids can be converted by different pathways. Examples for sugar fermentation are homo- or hetero-fermentative lactic acid fermentation, alcoholic fermentation, propionate fermentation, butyrate or butanole fermentation, mixed acid fermentation, solvent fermentation, and many others. The names of the pathways indicate the products released in these different fermentative pathways.

Many bacteria can switch between fermentative and respiratory metabolism, depending on the availability of

electron acceptors for respiratory chains. If they can switch between anaerobic and aerobic metabolism, they are called facultative aerobes, while anaerobes are usually oxygen sensitive and grow exclusively under anaerobic conditions. Within this group, the Gram-positive *Clostridia* have evolved many fermentation pathways including those to degrade fatty acids, lipids, amino acids and proteins, or nucleic acids under anaerobic conditions. Thus, degradation of all constituents of the cytosol of living organisms under anaerobic conditions can be achieved.

As no oxygen is necessary for the fermentative metabolism, most fermentations will proceed under anoxic conditions. However, aerotolerant microorganisms such as some lactic acid bacteria that are active in decomposition of plant litter can perform this reaction also under oxic conditions, although these anaerobes do not use oxygen as electron acceptor.

Syntrophic degradation

The reduced compounds released by fermenting microorganisms constitute good nutritional sources for organisms that can use respiratory systems. In turn, the use of the product will allow for the continuous production of this compound, as no restrictions for the fermenting bacteria will inhibit their metabolism. The concomitant occurrence of bacteria in an ecological niche in which both partners are dependent on each other and degradation of the substrate is only possible when both partners are present is termed syntrophy.

The basis is the metabolic activity of one organism which forms a substrate for the other. This constant removal of a metabolic end product is exemplified by the release of H_2 which is often a link in such syntrophic systems. Use of hydrogen by a second organism allows the first organism to constantly produce this compound. In pure culture, the partial pressure of H_2 reaches levels inhibitory to the metabolism of the producing organism which subsequently ceases growth. Since the syntrophic partner constantly removes hydrogen, no cessation of growth is observed due to product inhibition in this coculture. This syntrophy, depending on hydrogen transfer between two microbes present in the same environment, is of special importance, and the term interspecies hydrogen transfer has been coined for this syntrophic relationship. However, many different examples can be given for such syntrophic associations of microorganisms (e.g., Ueda and Beppu, 2007). Degradation of recalcitrant substrates, including, e.g., halogenated compounds, is often seen in syntrophic associations.

Cometabolic degradation

Recalcitrant substrates are not, or very slowly, degraded because their conversion is not easily or not at all coupled with energy conservation. Thus, the degradation of these compounds is costly for the cell, and the microorganisms do not profit directly from degradation. Instead, the

bacteria have to use energy from a different, catabolic reaction to drive the unfavorable degradation process.

An example of immediate importance is lignin degradation, which is performed by white rot fungi. The brown rot fungi degrade lignin mainly in order to get access to the cellulose of plant cell wall which is a good nutrient source but not directly accessible in wood and timber. The recalcitrant nature of lignin is seen under anoxic conditions, where the degradation is not possible and lignin of plant origin is thus the basis for coal formation.

As cometabolic systems are costly, they are mostly linked to respiration. Degradation of plant alkaloids, which are also recalcitrant substrates, often occurs at higher rates in oxic conditions.

Degradation of plant litter and soil formation

Plant material is composed of the contents of the living cells and the cell wall materials. Within the cell, secondary plant metabolites are stored in the vacuole. Upon leaf fall or death of a plant, especially the secondary metabolites and the wall materials persist, while proteins and lipids are easily degradable in the unsaturated oxygen containing upper soil layers.

Plant cell walls are composed of cellulose, hemicellulose, pectin, polygalacturonans, and lignin. Even in herbaceous plants, a quarter of the secondary cell wall is lignin. This macromolecule can be degraded aerobically, while anoxic conditions lead to the formation of coal, since under these conditions lignin cannot be degraded. Wood rotting fungi, both brown rot and white rot fungi, are able to degrade lignin (Crawford, 1981). The enzymes involved in lignin degradation, peroxidases and laccases, are able to attack the bonds in the macromolecule linking the phenolic monomers to form the high molecular weight lignin. The monomers can then be degraded further and are metabolized to carbon dioxide. These processes have been studied in some detail at the molecular level (Gold and Alic, 1993). Whether bacteria are also able to degrade wood is still under discussion. Streptomycetes are able to perform some partial degradation. However, complete decomposition has yet to be shown with bacterial systems.

An interesting question in wood and lignin degradation is regarding the possible symbiosis of wood degrading fungi and bacterial populations at an oxic/anoxic border. Anaerobic bacteria able to convert lignin decomposition products and partially degrade these compounds have been described. The fermentation products of these organisms may serve as growth substrates for the lignin degrading fungi. Secondary metabolites of plants and microorganisms are often recalcitrant because they contain aromatic rings, heterocycles, and polyketides or are halogenated. Such compounds are not easily degraded, especially under anoxic conditions. If degradation does not lead to complete oxidation of carbon moieties to CO_2 , the formation of humic acids and fulvic acids and thus the production of humus ensues. This process is the driving force for soil genesis. Under aerobic conditions,

enzymatic reactions known from lignin oxidation are often involved (Bumpus and Aust, 1987; Hammel, 1989).

Degradation of aromatic and halogenated compounds

Under aerobic conditions, the chemically very stable aromatic ring can be broken by activities of mono- and di-oxygenases. While monooxygenases introduce one of the oxygen atoms of molecular oxygen into the organic molecule forming a hydroxyl group at one site of the ring, dioxygenases incorporate both oxygen atoms which leads to decyclization and ring opening. The linear compound can then be further oxidized after activation and fueled into general metabolic pathways. The aerobic degradation of aromatic compounds can be used to clean oil spills or other anthropogenic contaminations, especially applicable for the residual contamination after a first mechanical cleansing has been performed. Inoculation of a beach after an oil spill could show visible removal of the remaining oil within 1 year.

Under anoxic conditions, the ring opening seems more difficult and it had long been argued that this would not be possible. However, bacterial enrichment cultures could show that an anaerobic degradation of aromatic compounds is possible usually via activation and conversion to benzoyl CoA followed by ring reduction. These energetically unfavorable reactions are feasible with a thermodynamically favorable electron acceptor, e.g., when nitrate is available in an anaerobic respiratory chain. Thus, bacteria related to the nitrogen fixing genus *Azoarcus* link the degradation of aromatic compounds with denitrification. Many gene clusters with different substrate specificities have been identified to perform anaerobic degradation of different organic molecules including aromatic amino acids, benzene and its derivatives, phenols, and heterocyclic compounds. The linearized structures again can be fueled into metabolic pathways via acetyl CoA or propionyl CoA. Anthropogenic aromatic compounds can be degraded by these same processes, thus allowing complete decomposition and natural attenuation. The introduction of bacteria able to anaerobically degrade aromatic compounds into aquifers with (anthropogenic) contaminations opens the road to bioremediation of contaminated groundwater resources. In tertiary oil recovery, introduction of bacteria into the well to partially degrade the high molecular crude oil fraction uses bacterial enzymatic activities to form molecules of lower molecular weight from the tar fraction that then can be recovered.

Many anthropogenic contaminations contain halogen atoms which generally decrease metabolic degradation of the compounds. However, halogenated compounds are also known from natural sources. These include antibiotics with chloride or bromide atoms, plant-derived alkyl halides, or deterrents incorporated into, e.g., marine slugs or tropical frogs. Since nature already has learned to cope with these halogenated compounds, anthropogenic

halogenated substances can also be degraded by bacterial consortia, both aerobically and anaerobically (Abraham et al., 2002; Smidt and de Vos, 2004). Not only bacteria are known to be able to perform dehalogenation reactions, but also basidiomycete fungi can also degrade organohalogens (de Jong and Field, 1997). While aerobic dehalogenation is a simple enzymatic reaction, anaerobic dehalorespiration is an interesting field of research. In this case, the dehalogenation is coupled with electron transfer and directly coupled to ATP formation in an anaerobic respiratory process.

Use of microbes in bioremediation

The properties discussed above make the use of microbia or microbial consortia useful for bioremediation of different contamination scenarios. These include aerobic conditions such as in soil, anoxic condition such as in aquifers or sediments, and all imaginable sources of contamination from oil spill to xenobiotics such as plant protection chemicals or heavy metals. Some recent reviews are included to facilitate further reading on microbial degradation (Holtze et al., 2008; Johnsen and Karlson, 2007; Demnerova et al., 2005; Pieper, 2005; Kim and Rhee, 2003) and bioremediation (Watanabe et al., 2002; R  ling and van Versefeld, 2002; Schwitzguebel et al., 2002).

Summary

The biogeochemical cycles of the macronutrients carbon, nitrogen, sulfur, and phosphorus are dependent on microbial degradation of organic matter. Organic matter originating from carbon dioxide fixation, which is in terrestrial system usually performed by photosynthetically active bacteria and plant, is degraded to form carbon dioxide, either directly in one metabolic reaction or by cocultures in which the intermediate is released and taken up by another microbe. In addition, the formation of methane from CO₂ or acetate is possible. Thus, the carbon cycling involves CO₂, CH₄, and organic molecules. Nitrogen fixation of N₂ to NH₃ followed by fixation in organic amines constitutes one side of the cycle, while nitrification/denitrification releases the bound nitrogen into the atmosphere. The latter reaction sequences, i.e., oxidation of NH₃ to NO₂[−] or NO₃[−] and nitrate or nitrite respiration leading to N₂ formation, are dominant processes in the cycling of nitrogen. For the sulfur cycle, assimilation of sulfates and fixation into organic biomass, the oxidation of sulfur and reduction of sulfate or sulfite to elemental sulfur, or reduction of sulfur to H₂S are the main processes. Sulfur is a constituent of the amino acids cysteine and methionine and of Fe/S clusters in redox enzymes. Phosphate is generally not altered; however, there have recently been reports on the use of phosphite in an unusual energy fixation process.

These elemental cycles in the biogeosphere all involve steps of fixation within living biomass, and the degradation of dead biomass is an essential part in releasing the mineralized elements back into the cycle. Higher

organisms are not capable of using plant litter, e.g., directly and thus entirely dependent on microbial degradation processes to have sufficient minerals available for their nutrition. Properties of microbes for degradation can be useful in bioremediation approaches.

Bibliography

- Abraham, W. R., Nogales, B., Golyshin, P. N., Pieper, D. H., and Timmis, K. N., 2002. Polychlorinated biphenyl-degrading microbial communities in soils and sediments. *Current Opinion in Microbiology*, **5**, 246–253.
- Bumpus, J. A., and Aust, D., 1987. Biodegradation of environmental pollutants by the white rot fungus *Phaenorachete chrysosporium*: involvement of the lignin-degrading system. *Bioessays*, **6**, 166–170.
- Crawford, R. L., 1981. *Lignin Biodegradation and Transformation*. New York: Wiley.
- de Jong, F., and Field, D. A., 1997. Sulfur tuft and turkey tail: biosynthesis and biodegradation of organohalogenes by basidiomycetes. *Annual Review of Microbiology*, **51**, 375–414.
- Demnerova, K., Mackova, M., Specakova, V., Beranova, K., Kochankova, L., Lovecka, P., and Rylslava, E., 2005. Two approaches to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial populations. *International Microbiology*, **8**, 205–211.
- Gold, M. H., and Alic, M., 1993. Molecular biology of the lignin-degrading basidiomycete *Phaenorachete chrysosporium*. *Microbiology Reviews*, **57**, 605–622.
- Hammel, K. E., 1989. Organopollutant degradation by ligninolytic fungi. *Enzyme and Microbial Technology*, **11**, 776–777.
- Holtze, M. S., Sorensen, S. R., Sorensen, J., and Aamand, J., 2008. Microbial degradation of the benzonitrile herbicides dichlobenil, bromoxynil and ioxynil in soil and subsurface environments – insights into degradation pathways, persistent metabolites and involved degrader organisms. *Environmental Pollution*, **154**, 155–168.
- Johnsen, A. R., and Karlson, U., 2007. Diffuse PAH contamination of surface soils: environmental occurrence, bioavailability, and microbial degradation. *Applied Microbiology and Biotechnology*, **76**, 533–543.
- Kim, D. Y., and Rhee, Y. H., 2003. Biodegradation of microbial and synthetic polyesters by fungi. *Applied Microbiology and Biotechnology*, **61**, 300–308.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock: Biology of Microorganisms*. Upper Saddle River: Prentice-Hall.
- Pieper, D. H., 2005. Aerobic degradation of polychlorinated biphenyls. *Applied Microbiology and Biotechnology*, **67**, 170–191.
- Röling, W. F., and van Verseveld, H. W., 2002. Natural attenuation: what does the subsurface have in store? *Biodegradation*, **13**, 53–64.
- Schink, B., 2002. Anaerobic digestion: concepts, limits and perspectives. *Water Science and Technology*, **45**, 1–8.
- Schwitzguebel, J. P., Aubert, S., Grosse, W., and Laturmus, F., 2002. Sulphonated aromatic pollutants. Limits of microbial degradability and potential of phytoremediation. *Environmental Science and Pollution Research International*, **9**, 62–72.
- Smidt, H., and de Vos, W. M., 2004. Anaerobic microbial dehalogenation. *Annual Review of Microbiology*, **58**, 43–73.
- Ueda, K., and Beppu, T., 2007. Lessons from studies of *Symbiobacterium thermophilum*, a unique syntrophic bacterium. *Bioscience Biotechnology and Biochemistry*, **71**, 1115–1121.
- Watanabe, K., Futamata, H., and Harayama, S., 2002. Understanding the diversity in catabolic potential of microorganisms for the development of bioremediation strategies. *Antonie van Leeuwenhoek*, **81**, 655–663.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Transformation Processes, Microbiology](#)
[Phosphorus, Phosphorites](#)
[Soils](#)

MICROBIAL ECOLOGY OF SUBMARINE CAVES

Francesco Canganella, Giovanna Bianconi
 University of Tuscia, Viterbo, Italy

Definition

Essential concepts, current investigations, and discussion.

In recent years, the search for the limit of biodiversity in extreme and/or uncommon environments led to the investigation of peculiar ecosystems such as deep-sea, subterranean niches, Antarctic cores, hydrothermal vents, and extraterrestrial sites.

Submarine caves and cavities have also been explored, some were mainly exploited as popular attractions for scuba divers, but some were shown to be of great interest for geologists, biologists, and microbiologists.

Available scientific data on these ecosystems are not abundant, particularly as far as biological disciplines are concerned. Most of the published works have been related to specific submarine cavities known as Blue Holes and Black Holes, as well as to submarine caves showing inner sulfidic emissions. A larger number of data are currently available regarding the microbiology of terrestrial caves, particularly on the microbial ecology of carbonate layers, sediments, bat guano, and flooding waters (Vlasceanu et al., 1997, 2000; Holmes et al., 2001; Engel et al., 2003; Northup et al., 2003; Sugita et al., 2005; Zhou et al., 2007), but these latter ecosystems are not part of the current review.

Blue holes and black holes

The so-called blue holes are underwater karst systems which can develop horizontally in a very extensive way and appear blue due to a combination of blue sky reflection with the white carbonate sand deposited in the cave (Smart et al., 1988; Mylroie et al., 1995; Schwabe et al., 1997; Marano-Briggs, 2000; Colantoni et al., 2003; Canganella et al., 2004). Most of these sites were found and described in Bahamas and Hawaii.

Other cavern systems are known as “black holes,” which are cave systems without known lateral passages and located in the interior regions of the larger Bahamian islands. Most black holes are located in the central to western side of the island of South Andros in the Bahamas (Figure 1), although one has been found on the northern transitional shore of the Grand Bahama Island. They are vertical cave systems which

ANAEROBIC TRANSFORMATION PROCESSES, MICROBIOLOGY

Bernhard Schink

University of Konstanz, Konstanz, Germany

Definition and Introduction

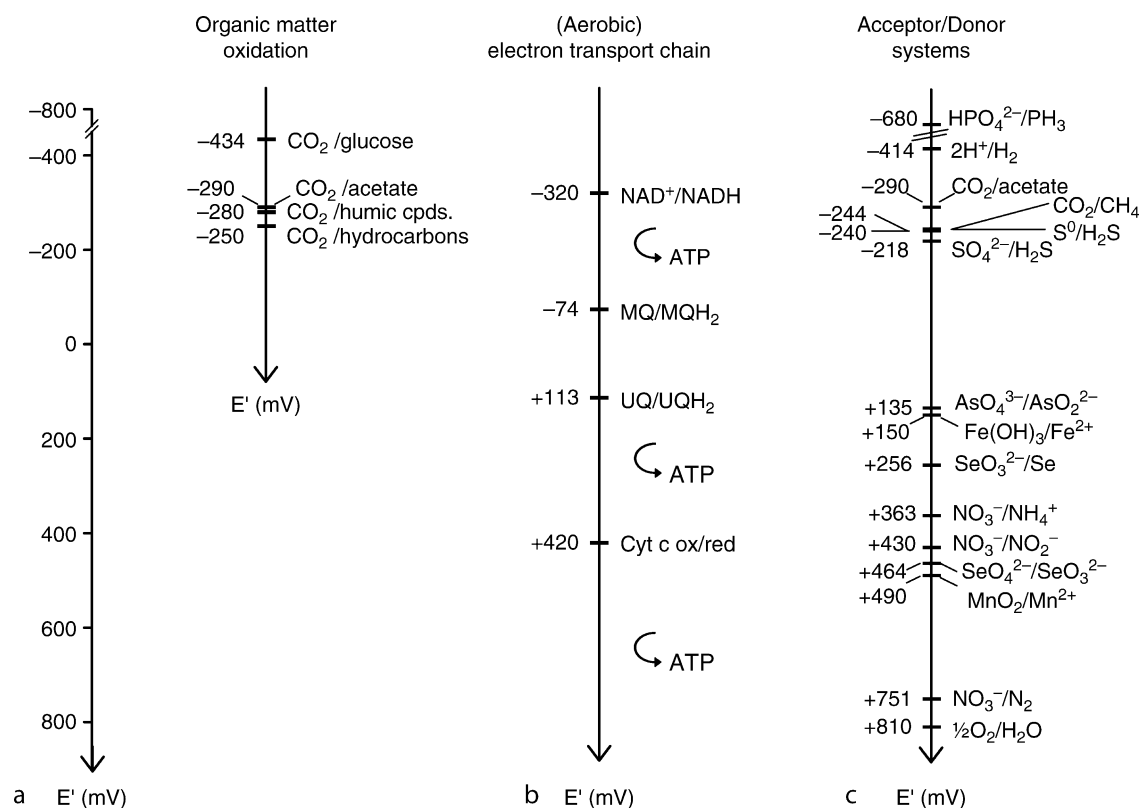
Microbial activities in geochemical processes are most often redox reactions, either between organic matter and inorganic compounds or between different inorganic compounds. In surface soils and lake sediments, organic matter typically derives from primary production by land plants or aquatic plants including algae, provided either directly or after primary digestion by animals. Underground soils and groundwater may receive major amounts of comparably stable organic matter, most often petroleum and its derivatives, through spills and accidents. Microbial oxidation helps to eliminate such contaminants, with concomitant reduction of inorganic electron acceptors. The surprising abundance of bacteria-like structures and even living bacteria in Deep Sea sediments and terrestrial rock material down to several 100 m below surface raised the question of how microbial life can be fuelled in the apparent absence of perceptible external substrate input. The

present overview will try to provide access for geoscientists and other nonbiologists to the concepts of microbial life in the anaerobic world in general.

The terms “anoxic” and “anaerobic” are often used simultaneously with more or less the same meaning. Anoxic means “oxygen-free” and refers to habitats, incubation conditions, etc. The term anaerobic contains the Greek root “bios” (life) and refers to microbes, microbial activities, enzymes, etc.

Organic matter as electron donor in geochemical processes

Organic matter as supplied by plant primary production consists mainly of carbohydrates, proteins, fats, and nucleic acids. Digestion by soil animals decreases the carbohydrate and protein fraction, and increases the content of polyanellated phenolic residues (humic compounds, Stevenson, 1994). The complete oxidation of carbohydrates at pH 7.0, using glucose as a representative molecule, yields electrons at $E_0' = -434$ mV on average (Figure 1a). These electrons are transferred inside microbial cells via specific electron carrier systems, most often via NAD^+/NADH , at $E_0' = -320$ mV. Other carriers operate at lower (e.g., ferredoxins around -420 mV) or higher (flavins, quinones, cytochromes; Figure 1b) redox



Anaerobic Transformation Processes, Microbiology, Figure 1 Redox potentials of (a) organic substrates, (b) biochemical electron carriers, and (c) natural electron acceptor/donor systems. Values taken or calculated from Thauer et al. (1977) and Zehnder and Stumm (1988). MQ menaquinone; UQ ubiquinone.

potentials, and finally all electrons may end up at the O_2/H_2O -couple at $E_0' = +810$ mV (Figure 1c). Electron transport from low to high redox potentials provides chemical energy, which the cell conserves in energy-rich phosphoric acid anhydride linkages in the form of ATP which can be formed in the cell basically by two alternative processes (Thauer et al., 1977): In substrate-level phosphorylation, the transformation of a substrate molecule leads directly to an energy-rich intermediate from which ATP is formed in a stoichiometrically linked reaction. In electron transport phosphorylation, electron transport over a sufficient redox potential difference is coupled to the establishment of a proton (sometimes Na^+ ion) gradient across the cytoplasmic membrane which secondarily drives ATP synthesis via a membrane-bound ATP synthase reaction. Major steps in electron transport phosphorylation are the electron transports between NADH and the quinones, between quinones and cytochrome c, and between cytochrome c and oxygen (Figure 1b and c). In total, oxidation of glucose to $6CO_2$ with oxygen as electron acceptor can yield up to 38 mol ATP per mol glucose (Fuchs, 2006).

Partial digestion of biomass leads to less energy-rich derivatives that are less valuable as electron donors. Incomplete oxidation and polymerization of aromatic and aliphatic plant matter residues forms humic compounds with a comparably long residence time of up to 100 years in soils. An average redox potential of humic compounds can be calculated with hydroquinone as a representative constituent, yielding a value at -280 to -300 mV. Fermentation of sugars and proteins produces acetate as the most important primary product; its oxidation yields electrons at -290 mV on average. Oxidation of aliphatic and aromatic hydrocarbons releases electrons at -240 to -270 mV. These examples illustrate that the typical constituents of partly digested or stabilized organic matter are less valuable electron donors in oxidation processes. Moreover, especially humic compounds and hydrocarbons are already comparably inert derivatives. Efficient degradation of these compounds requires molecular oxygen as a co-substrate in the initial oxygenase reactions for activation. Since oxygen-independent alternative reactions of activation of hydrocarbons (Widdel et al., 1993) or polyanellated aromatic compounds (humic compounds) are slow compared to the known oxygen-dependent ones, a major amount of organic matter (e.g., peat) is conserved in the absence of oxygen ("latch hypothesis"; Freeman et al., 2001).

The biogeochemical redox chain

Due to the low solubility of oxygen in water (about $300 \mu M$ O_2 in freshwater at air saturation and $10^\circ C$), aerobic oxidation of organic matter depends on a continuous supply of oxygen, either by plant photosynthesis or by convective transport from the atmosphere. Therefore, this oxygen supply is restricted to only few millimeters below the top surface in sediments or soils where only diffusive transport is possible. Under such conditions, other

electron acceptors can substitute for oxygen in its function as an electron acceptor, and the sequence of these subsequent redox processes is determined by the standard redox potentials of the individual redox processes (Zehnder and Stumm, 1988; Figure 1c). After oxygen consumption, nitrate is reduced first to nitrite, and afterwards either to N_2 via NO and N_2O , or to ammonia. In any case, nitrite is an important intermediate which may accumulate in laboratory experiments to toxic concentrations. In nature, nitrate concentrations rarely exceed $50 \mu M$ and nitrite never becomes toxic, therefore. As the redox potentials listed in Figure 1 indicate, nitrate reduction to N_2 (denitrification) appears to be energetically more feasible than nitrate reduction to ammonia (nitrate ammonification). However, some redox steps in denitrification are rather inefficiently coupled to ATP synthesis, thus wasting a considerable amount of energy and rendering nitrate ammonification the more efficient way of nitrate-dependent energy conservation (Strohm et al., 2007).

Subsequently, manganese(IV) oxides, e.g., MnO_2 (Braunstein), may be reduced to Mn^{2+} ions. The distribution of manganese oxides in soils is rather patchy; in lake sediments, it depends primarily on the chemistry of the catchment area. In Lake Constance, the eastern part of the lake receives ample supply of manganese oxides through the Alpine Rhine river whereas the western part of the lake is nearly devoid of manganese. Different from manganese, iron is a very dominant constituent of rocks and sediments, and can make up up to 1% of the total dry mass. Iron oxides, similar to manganese oxides, are nearly insoluble at neutral pH, and the redox potentials of Fe(III)-oxide reduction to Fe^{2+} -ions depends even on the individual iron(III) mineral involved ($+100$ mV with ferrihydrite, -287 mV with hematite; Widdel et al., 1993; Straub et al., 2005). The main problem in reduction of iron and manganese oxides is their apparent insolubility (Cornell and Schwertmann, 1996) and, with this, the necessity for bacteria to transport electrons to an acceptor outside the cell (see below).

Reduction of sulfate to sulfide is the dominant process of organic matter oxidation in marine sediments because seawater contains sulfate at high concentration (28 mM). Freshwaters contain far less sulfate (50 – $200 \mu M$) rendering this process substantially less important at first sight. Nonetheless, sulfide can be reoxidized, e.g., with Fe(III)-oxides (see below) thus making reduction of sulfur compounds probably far more important in freshwater systems than assumed so far. The key problem in sulfate reduction is the initial reduction of the comparably stable sulfate molecule to sulfite which corresponds to a very low redox potential ($E_0' = -527$ mV) that cannot be fed directly with electrons from organic carbon oxidation. The sulfate reducers overcome this problem by ATP investment into this step, thus shifting the resulting redox potential to -60 mV (for details, see Cypionka, *Aerobic Metabolism*). The ATP required for this initial activation step is recovered in the further reduction of sulfite to sulfide.

Elemental sulfur is reduced by specific sulfur-reducing bacteria, but also by sulfate reducers. Elemental sulfur is easy to reduce and does not require prior activation. The problem of sulfur metabolism is again its low solubility in water which may be circumvented by the intermediate formation of water-soluble polysulfides in the presence of the formed hydrogen sulfide.

The terminal process in organic matter degradation in freshwater environments is the formation of methane, either by reduction of CO_2 or by cleavage of acetate (Wolfe, 1992; Thauer, 1998); alternatively, acetate may be formed first by CO_2 reduction, and secondarily be converted to methane (Drake et al., 2002; K. Kuesel, this series). Since the methanogenic archaeobacteria are restricted to the use of only very few substrates (H_2 , C_1 compounds, and acetate) complex organic matter has to go through fermentations by several groups of specialized bacteria (Schink, 1997) before it finally ends up in a mix of CH_4 and CO_2 , i.e., in complete dismutation of the carbon atoms.

Hydrogen is an important product of microbial fermentations of organic matter. It can be formed as well by geochemical processes and may be a source for microbial life in deep sediments and rocks.

The middle axis in Figure 1 (b) positions the key elements of the biochemical electron transport chain between organic matter on the left and the various acceptor systems on the right. This arrangement illustrates the major paths of electron flow in microbial transformation processes. One should emphasize, however, that biological systems are able to shift electrons also against the “normal” electron flow by “reversed electron transport” at the expense of energy supplied by transport of other electrons in the “normal” direction from negative to positive potentials. The scheme illustrates that glucose is comparably a valuable electron donor whereas the electrons derived in degradation of acetate, humic compounds, or hydrocarbons cannot all be transported via NADH, rather, many of them have to enter the transport chain at the level of quinones (either menaquinone or ubiquinone) with the consequence that substantially less ATP is formed. The scheme also illustrates that, e.g., reduction of ferrihydrite can be fed with electrons only at the potential of quinones, and that necessarily the ATP yield of this process is much smaller than, e.g., that of the reduction of nitrate or oxygen. It also becomes evident that phosphate can hardly be reduced to phosphine in a respiratory process, analogous to sulfate or nitrate reduction, but would require substantial amounts of energy rather than yield energy. The origin of traces of phosphine in certain anoxic environments is still unclear; it probably derives from corrosion of iron phosphides in scrap metals (Ding et al., 2005).

Lithotrophic reoxidations

The reduced derivatives formed in the processes above during oxidation of organic matter can act again as electron donors in microbial energy metabolism, e.g., if H_2S ,

reduced Fe^{2+} , or Mn^{2+} diffuses upwards and meets electron acceptors of higher redox potential, thus again creating a redox couple that can yield energy. Such metabolic activities are called “lithotrophic” (Greek *lithos* = rock) because in this metabolism inorganic electron donors serve as electron source. A lithotrophic metabolism is most often, but not necessarily, combined with autotrophic fixation of CO_2 into cell material, i.e., bacterial growth. Obviously, synthesis of organic matter from inorganic sources has to be fueled by energy (ATP) that is derived, in this case, from inorganic redox processes. Therefore, the yield of organic matter, i.e., of microbial cell mass, in these processes is usually small. As the redox chain in Figure 1c illustrates, H_2 , H_2S , and Fe^{2+} are good electron donors whereas nitrite, selenite, and Mn^{2+} are not. Behind every such redox process stand the activities of specialized bacteria and archaea which try to exploit every fraction of energy that can be derived from these redox processes.

An exceptional case is methane oxidation which can proceed either with oxygen as a co-substrate and an electron acceptor, yielding a huge amount of energy or with sulfate as electron acceptor (anaerobic methane oxidation; see Boetius, *Anaerobic Oxidation of Methane with Sulfate*) yielding extremely little energy. Similar problems apply to higher aliphatic or aromatic hydrocarbons (Spormann and Widdel, 2000).

Oxidation of sulfide with ferric oxides should yield energy as well. Nonetheless, this reaction proceeds spontaneously as a chemical reaction (see below), and so far there is no indication for microbial activity exploiting this redox reaction.

Energetics

Bacterial growth requires energy which is derived from substrate conversions in the form of ATP. Irreversible synthesis of the energy-rich acid anhydride linkage of ATP by condensation of ADP with a phosphoryl residue under the conditions prevailing in living cells requires energy (ΔG^0 or Gibbs free energy) in the range of 60–70 kJ per mol (Thauer et al., 1977; Schink, 1997). Nonetheless, metabolic energy can also be converted into ATP at smaller increments. The membrane-bound ATPase enzyme mentioned above catalyzes the synthesis of ATP reversibly with a translocation of protons or Na^+ -ions; the stoichiometry is 3–4 H^+ or Na^+ ions per ATP. Thus, any reaction that can be coupled to the translocation of protons or Na^+ -ions across the membrane can be associated with ATP synthesis, down to reactions transferring only 1 H^+/Na^+ , equivalent to an ΔG^0 in the range of 15–20 kJ per mol. Of course, reactions can proceed also with lower energy yields, but then they cannot feed ATP synthesis and bacterial growth. Growth, on the other hand, is a very important means to maintain a degradative capacity in a certain environment and to adjust its activity to the local needs (different from so-called “co-metabolic” activities which are not positively correlated with bacterial growth; McCarty, 1988).

Extracellular electron transfer

Electron transfer from or into the electron transport chain (Figure 1b) is easy with substrates that can be taken up into the bacterium in a dissolved form. Non-dissolved substrates have to be oxidized or reduced outside the cell, either at the immediate cell surface or via dissolved electron carriers. The reduction of ferric iron oxides can be accomplished in direct contact with the bacterial cells; perhaps, even small cellular appendages can act as “nanowires” for electron transfer (Reguera et al., 2005). Alternatively, either dissolved humic compounds (Lovley et al., 1996) or low-molecular weight quinoid or phenazine compounds may act as electron carriers (Hernandez et al., 2004). As a further alternative, sulfur-reducing bacteria can indirectly reduce iron via H_2S at low (<0.1 mM) concentration (Straub and Schink, 2004). At low H_2S concentration, H_2S delivers electrons to ferric oxides and is converted to a more oxidized species, perhaps thiosulfate or polysulfide, which is reduced again by the bacterium. In this case, a small sulfur cycle acts as an electron delivery system. One should emphasize that in the latter cases, the bacteria do not actually reduce the ferric oxide. They reduce quinoid or sulfur compounds, and the ferric oxides are reduced indirectly in a subsequent unspecific reaction. Such secondary electron transfer reactions may be important also with respect to other reductive transformations such as nitro group reduction, dehalogenation, etc. (Holliger et al., 1997).

The example of indirect ferric iron reduction via sulfur compounds appears to contradict the generally accepted preference of biochemical redox processes according to the sequence mentioned above: since the redox potential of the iron(III)/ Fe^{2+} -couple is higher than that of the sulfate/sulfide system, iron should be reduced before the sulfur compounds. Nonetheless, for bacteria not only the redox potentials but also the bioavailability aspect counts: since the sulfur compounds are easier to access they may be preferred over the insoluble iron oxides.

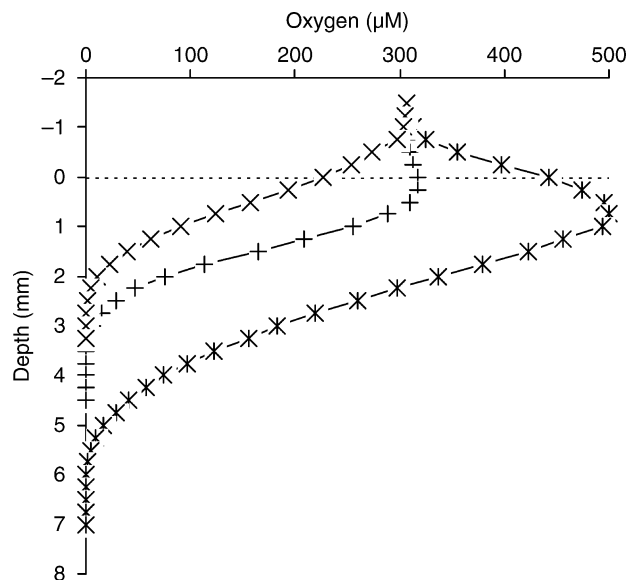
Phototrophic activities

Photosynthesis as performed by green plants and algae is called oxygenic because molecular oxygen is released from water through the activity of two combined light reactions. This biochemical potential was evolved by cyanobacteria (formerly called blue-green algae) and the evolution of oxygenic photosynthesis has changed the chemistry of our planet around 3.5 billion years ago in a most dramatic way from a reducing to an oxygenated atmosphere. The massive precipitation of iron oxides in the so-called “Banded Iron Formations” has for long times been considered to witness the evolution of molecular oxygen in the Earth’s atmosphere, but the oxygen thus evolved would have been consumed to a large extent over several hundred million years in the oxidation of Fe^{2+} to ferric oxides. Anoxygenic phototrophic bacteria perform a simpler, older type of photosynthesis from which oxygenic photosynthesis was later derived. The discovery of

Fe^{2+} -oxidation by anoxygenic phototrophs (Widdel et al., 1993) changed also our understanding of the development of Banded Iron Formations; this process became possible also without the involvement of molecular oxygen, and thus formation of Banded Iron Formations could now testify only the evolution of anoxygenic, rather than of oxygenic photosynthesis (Kappler et al., 2005).

Spatial/seasonal oscillations

Many microbial habitats such as the Deep Sea and its sediments, or rocks in the deep underground are highly stable systems which do not change their properties over many thousands or millions of years. On the other hand, microbial life conditions in a top soil may change dramatically with every rainfall, and bacteria inhabiting the sediment surface in a shallow, light-exposed lake may undergo dramatic life changes in diurnal cycles of light and oxygen supply which are probably the most dramatic changes for living systems at all. As shown in Figure 2, oxygen profiles during light exposure versus darkness show dramatic differences with respect to the oxygen concentration and to oxygen penetration depth into the sediment. Other parameters change over longer time scales, e.g., with seasons. Figure 3 shows a section of the littoral sediment of Lake Constance in January when the water level is low.



Anaerobic Transformation Processes, Microbiology, Figure 2 Distribution profiles of oxygen in littoral sediment of Lake Constance under the influence of different light conditions. (x) 12 h dark, (+) 12 h incubation at low light, equivalent to an overcast day, (*) 12 h incubation at high light intensity, equivalent to a sunny day. Incubation basins were exposed to light under a 12 h-light-dark cycle with three Osram lamps Biolux (L30W/72), Fluora (L30W/77), and Haloline (200 W/R7s) to generate high light intensity of 15 kLux ($250 \text{ E m}^{-2} \text{ s}^{-1}$) at the water surface.



Anaerobic Transformation Processes, Microbiology,

Figure 3 Leaching of ferrous iron from littoral sediment of Lake Constance in Winter 2002. Fe^{2+} ions leach from the upper right part to the left, with continuous chemical/microbial oxidation of Fe(II) to Fe(III) oxides.

The higher regions of the sediment (which are under water through the major part of the year, with annual water level changes of 2.5 m) are exposed to air in wintertime, and the reduced Fe^{2+} leaches out of the sediment to be oxidized to Fe(III) oxides. Of course, such water level fluctuations influence all life forms in the littoral sediment, beyond the normal fluctuations of light and dark in the day cycle, and of temperature through the annual cycle. The microbial community within the sediment either has to adapt to these changes as far as possible or has to migrate within the sediment to maintain a stable life situation.

Conclusions

Microbes are magnificent chemists. They catalyze reactions that are thermodynamically feasible, but enhance them kinetically as any good catalyst does. Beyond that, they can couple exergonic reactions in their energy metabolism with endergonic processes in their assimilatory metabolism in a way that chemical processes could never accomplish. Through bacterial growth, the catalytic potential is adjusted at a certain site to the local needs. Many processes are strongly accelerated by the action of microbes, and the total extent of their activities in Deep Sea sediments and in the deep underground can so far only roughly be estimated. Nonetheless, there is little doubt that microbes in the underground make up a significant part of living protoplasm on Earth in general, ranging in the same order of magnitude as that of plants and animals together (Whitman et al., 1998). The fascinating efficiency with which microbes catalyze chemical processes into impressive dimensions (as, e.g., in the precipitation of the Banded Iron Formations) gives us only a vague idea of the importance of microbial catalysis in the geochemistry of our planet. Geochemists and geomicrobiologists have to join their forces to understand the action of microbes in highly structured environments that appear extremely

poor with respect to utilizable energy sources, in order to assess the contributions of life to geochemistry correctly.

Acknowledgment

The author thanks Dr. Heike Freese, Konstanz, for helping with the design of Figure 1. Experimental work in the author's lab was supported by Deutsche Forschungsgemeinschaft, Bonn, Fonds der Chemischen Industrie, Frankfurt/M., and research funds of Universität Konstanz.

Bibliography

- Cornell, R. M., and Schwertmann, U., 1996. *The Iron Oxides – Structure, Properties, Reactions, Occurrence and Uses*. Weinheim: VCH.
- Ding, L., Wang, X., Zhu, Y., Edwards, M., Glindemann, D., and Ren, H., 2005. Effect of pH on phosphine production and the fate of phosphorus during anaerobic process with granular sludge. *Chemosphere*, **59**, 49.
- Drake, H. L., Küsel, K., and Matthies, C., 2002. Ecological consequences of the phylogenetic and physiological diversities of acetogens. *Antonie Van Leeuwenhoek*, **81**, 203.
- Freeman, C., Ostle, N., and Kang, H., 2001. An enzymic 'latch' on a global carbon store. *Nature (London)*, **409**, 149.
- Fuchs, G. (ed.), 2006. *Allgemeine Mikrobiologie*, begr. von H. G. Schlegel, 8. Aufl. Stuttgart: Thieme, 650 p.
- Hernandez, M. E., Kappler, A., and Newman, D. K., 2004. Phenazines and other redox-active antibiotics promote microbial mineral reduction. *Applied Environmental Microbiology*, **70**, 921.
- Holliger, C., Gaspard, S., Glod, G., Heijman, C., Schumacher, W., Schwarzenbach, R. P., and Vazquez, F., 1997. Contaminated environments in the subsurface and bioremediation: organic contaminants. *FEMS Microbiology Reviews*, **20**, 517.
- Kappler, A., Pasquero, C., Konhauser, K. O., and Newman, D. K., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II) -oxidizing bacteria. *Geology*, **33**, 865.
- Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., and Woodward, J. C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature*, **382**, 445.
- McCarty, P. L., 1988. Bioengineering issues related to in situ remediation of contaminated soils and groundwater. *Basic Life Science*, **45**, 143.
- Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., and Lovley, D. R., 2005. Extracellular electron transfer via microbial nanowires. *Nature*, **435**, 1098.
- Schink, B., 1997. Energetics of syntrophic cooperations in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, **61**, 262.
- Spormann, A. M., and Widdel, F., 2000. Metabolism of alkylbenzenes, alkanes, and other hydrocarbons in anaerobic bacteria. *Biodegradation*, **11**, 85.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: John Wiley & Sons.
- Straub, K. L., and Schink, B., 2004. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* through electron transfer via sulfur cycling. *Applied and Environmental Microbiology*, **70**, 5744.
- Straub, K. L., Kappler, A., and Schink, B., 2005. Enrichment and isolation of ferric iron- and humic acid reducing bacteria. *Methods in Enzymology*, **397**, 58.
- Strohm, T. O., Griffin, B., Zumft, W., and Schink, B., 2007. Growth yields in bacterial denitrification and nitrate ammonification. *Applied Environmental Microbiology*, **73**, 1420.
- Thauer, R. K., 1998. Biochemistry of methanogenesis: a tribute to Marjorie Stephenson. 1998 Marjorie Stephenson prize lecture. *Microbiology*, **144**, 2377.

- Thauer, R. K., Jungermann, K., and Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews*, **41**, 100.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Aßmus, B., and Schink B., 1993. Anaerobic ferrous iron oxidation by anoxygenic phototrophs. *Nature (London)*, **362**, 834.
- Wolfe, R. S., 1992. Biochemistry of methanogenesis. *Biochemical Society Symposia*, **58**, 41.
- Zehnder, A. J. B., and Stumm, W., 1988. Geochemistry and biogeochemistry of anaerobic habitats. In Zehnder, A. J. B. (ed.), *Biology of anaerobic microorganisms*. New York: John Wiley & Sons, pp. 1–38.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Hydrogen](#)
[Iron Sulfide Formation](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Nitrogen](#)
[Photosynthesis](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Terrestrial Deep Biosphere](#)

ANAMMOX

Anammox refers to the microbial **anaerobic oxidation** of **ammonium** by nitrite (NO₂⁻), which forms N₂. See entry “[Nitrogen](#)” for further reading.

ANIMAL BIOCALCIFICATION, EVOLUTION

Gert Wörheide¹, Daniel J. Jackson²

¹Ludwig-Maximilians-Universität München, München, Germany

²University of Göttingen, Göttingen, Germany

Definition

SOM. Soluble organic matrix

IOM. Insoluble organic matrix

GRN. Gene regulatory network

Introduction

One of the major events in the evolution of multicellular animals was the transition from soft-bodied organisms to those that possessed mineralized hard parts for protection

and support. This major evolutionary hallmark supported the rapid diversification of animals and their occupation of a diverse range of novel ecological niches at the dawn of the Phanerozoic, between 560 and 530 million years ago, when mineralized skeletons appeared relatively synchronous in a variety of Phyla during the so-called Cambrian Explosion (Knoll, 2003; Conway Morris, 2006). However, it is still unclear exactly what drove this sudden capacity to construct mineralized structures, be it changes in ocean chemistry (Brennan et al., 2004) or the evolution of more diverse ecologies (Cohen, 2005), including predators (Vermeij, 1989). Investigation of biomineralization processes will provide insight into the molecular and genetic mechanisms that accompanied the appearance of mineralized body parts during the early Cambrian, shedding light on the processes that generate the diversity of life we see around us today.

Until recently, studies of biomineralization processes were mainly restricted to describing the (ultra)structure of the minerals and their interaction with the organic material provided by the organism (Lowenstam and Weiner, 1989; Mann and Webb, 1989). However, the generation and elaboration of biominerals is ultimately controlled by genes (Jackson et al., 2006, 2007a, b), which must in turn be expressed in the appropriate tissue and at an appropriate stage of development. Understanding the evolution of such biomineralizing genes and the gene regulatory networks (GRNs) in which they act is, therefore, vital to the understanding of how animals evolved and will also provide clues for the fabrication of biomimetic materials.

Biomineralization

Biomineralization refers to the processes by which organisms form minerals (e.g., calcium carbonate, calcium phosphate, silica, iron oxides) under strict biological control. These processes are, therefore, defined as *biologically controlled mineralization*, with the skeletons formed being integral, functional parts of the organisms (Lowenstam and Weiner, 1989). There are, however, several other serendipitous processes linked to living organisms (e.g., microbes) or organic substances that result in mineral (mainly calcium carbonate) formation that are not under strict control of organisms. These processes are termed either *biologically induced mineralization*, where inorganic minerals are precipitated by adventitious precipitation resulting from secondary interactions between various metabolic processes and the surrounding environment (Mann, 2001), or *organomineralization*, a term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not (e.g., degrading organic matter) (Trichet and Defarge, 1995).

While both *biologically induced* and *organomineralization* play an important role in the formation of carbonate systems in the geological record (e.g., microbialites, stromatolites, ooids) (Reitner and Neuweiler, 1995; Arp,

- Jeanthon, C., 2000. Molecular ecology of hydrothermal vent microbial communities. *Antonie van Leeuwen.*, **77**, 117–133.
- Marano-Briggs, K., 2000. *Water Chemistry, Bacterial Abundance and Anoxygenic Photosynthetic Bacteria in an Estuarine Blue Hole, Andros Island, The Bahamas*. PhD Thesis, George Mason University, Fairfax, VA.
- Mattison, R. G., Abbiati, M., Dando, P. R., Fitzsimons, M. F., Pratt, S. M., Southward, A. J., and Southward, E. C., 1998. Chemoautotrophic microbial mats in submarine caves with hydrothermal sulphidic springs at Cape Palinuro, Italy. *Microbial Ecology*, **35**, 58–71.
- Myroie, J. E., Carew, J. L., and Moore, A. I., 1995. Blue holes: definition and genesis. *Carbonate and Evaporites*, **10**, 225–233.
- Northup, D. E., Barns, S. M., Yu, L. E., Spilde, M. N., Schelble, R. T., Dano, K. E., Crossey, L. J., Connolly, C. A., Boston, P. J., Natvig, D. O., and Dahm, C. N., 2003. Diverse microbial communities inhabiting ferromanganese deposits in Lechuguilla and Spider Caves. *Environmental Microbiology*, **5**, 1071–1086.
- Paine, S. G., Lingood, F. V., Schimmer, F., and Thrupp, T. C., 1993. *The Relationship of Microorganisms to the Decay of Stone*. London: Philosophical Transactions of the Royal Society, pp. 97–127.
- Schwabe, S. J., 1998. The Black Hole. Caves and caving. *British Cave Research Association*, **80**, 1–22.
- Schwabe, S. J., and Herbert, R. A., 2004. Black Holes of the Bahamas: what they are and why they are black. *Quaternary International*, **121**, 3–11.
- Schwabe, S. J., Parkes, R. J., and Smart, P. L., 1997. Biogeochemical investigation of submerged cave systems (Blue Holes) within Bahamian carbonate platforms. In *97th Applied and Environmental Microbiology General Meeting*, May 4–7, 1997, p. 398. Miami, FL.
- Smart, P. L., Dawans, J. M., Whitaker, F., 1988. Carbonate dissolution in a modern mixing zone. *Nature*, **335**, 811–813.
- Sugita, T., Kikuchi, K., Makimura, K., Urata, K., Someya, T., Kamei, K., Niimi, M., and Uehara, Y., 2005. Trichosporon species isolated from guano samples obtained from bat-inhabited caves in Japan. *Applied and Environmental Microbiology*, **71**, 7626–7629.
- Vlasceanu, L., Popa, R., and Kinkle, B., 1997. Characterization of *Thiobacillus thioparus* LV43 and its distribution in a chemoautotrophically based ecosystem. *Applied and Environmental Microbiology*, **63**, 33123–33127.
- Vlasceanu, L., Sarbu, S., Engel, A. S., and Kinkle, B., 2000. Acidic, cave wall biofilms located in the Frasassi George, Italy. *Geomicrobiology*, **17**, 125–139.
- Zhou, J., Gu, Y., Zou, C., and Mo, M., 2007. Phylogenetic diversity of bacteria in an earth-cave in Guizhou province, southwest of China. *Journal of Microbiology*, **45**, 105–112.

Cross-references

Bacteria
 Beggiatoa
 Biofilms
 Hydrothermal Environments, Marine
 Karst Ecosystems
 Metals, Acquisition by Marine Bacteria
 Microbial Communities, Structure, and Function
 Microbial Mats
 Nanocrystals, Microbially Induced
 Siderophores
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Sulfur Cycle

MICROBIAL MATS

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Algal mats; Bacterial mats; Biomats; Thick biofilms

Definition

Microbial mats are often centimeter-thick multilayered structures of microorganisms, mainly bacteria, archaea, fungi, and sometimes these mats are enriched with protozoans. Microbial biofilms are in contrast much thinner (10–100 μm) than microbial mats and have a different architecture (Characklis and Wilderer, 1989). Microbial mats grow at interfaces between different types of material, mostly on moist surfaces, but some of them are also found in dry environments like deserts (e.g., Stal and Caumette, 1994; Characklis and Wilderer, 1989). They colonize environments located in high altitude, they are common in the Deep Biosphere, in subterranean environments with temperatures from -40°C to $+120^{\circ}\text{C}$. There are also common as endosymbiotic structures, for example, in animals. They are very common in extreme environments like hydrothermal vents, cold seeps, and alkaline and hypersaline lakes (Van Dover, 2000; Pederesen, 2006; Arp et al., 2003; Riding and Awramik, 2000).

Geobiological implications

A microbial mat consists of several layers dominated by specific types of microorganism (Figures 1 and 2). Although the composition of individual mats varies depending on the environment, as a rule the by-products of each group of microorganisms serve as energy source for other groups. Different types of microorganisms dominate different layers based on their comparative advantage (Canfield and Des Marais, 1993; Allison et al., 2000). Ecological relationships between different groups are a combination of competition and cooperation. The metabolic capabilities of bacteria and archaea are generally depend on their phylogeny. In a wet environment where sunlight is available, the upper layers are generally dominated by aerobic consortia like cyanobacteria (Figure 1), while the lowest layers are normally dominated by anaerobic microorganisms such as sulfate-reducing bacteria, methanogenic and/or methanotrophic archaea, or general archaea. Marine mats may grow to a few centimeters in thickness, of which only the top few millimeters are oxygenated. Microbial mats are held together and bound to their substrates by sugar- and protein-rich extracellular polymeric substances (EPS), which they secrete (Decho et al., 2005; Wingender et al., 1999). In many cases, some of these microorganisms form filaments (threads), which tangle and thus increase the microbial



Microbial Mats, Figure 1 Phototrophic 5-cm-thick microbial mat from a hypersaline lake (Lake 21) on Kiritimati Island (Kiribati, Line Islands). 1 – Orange layer with cyanobacteria; 2 – Green layer with green bacteria, zone with the highest diversity of prokaryotes; 3 – Purple layer, zone with photosynthetic non-sulfur purple bacteria (alpha-, and beta proteobacteria); 4 – Gray layer with sulfate-reducing bacteria and archaea, zone of heavy carbonate and gypsum mineralization within degrading extracellular polymeric substances (EPS). The entire mat is interspersed with authigenic, spherulitic aragonite, and gypsum crystals that are growing within EPS.

colonies structural strength, especially if the filaments have sheaths.

Microbial mats are the earliest complex form of life on Earth. There is a fossil record from 3,500 million years ago, and they have always been the most important members and maintainers of earth ecosystems (Allwood et al., 2006; van Kranendonk, 2007). Early Precambrian microbial mats are the first evolved highly complexly organized multicellular organisms. Microbial mats occasionally mineralize if the ionic strength in the aquatic environment allows it. The mineralized structures are known as stromatolites and thrombolites and they represent the most important organo-sedimentary fabrics since the early beginning of microbial life on earth (Burne and Moore, 1987; Riding, 2000). Originally, microbial mats first occurred at hydrothermal vents for energy. Since the late Precambrian microbial mats have also played an important role at cold hydrocarbon seeps, so-called Cold Seeps. Most of the Cold Seeps are methane vents coupled with hydrogen sulfur microbial metabolism (Reitner et al., 2005) (Figure 2). However, phototrophic microorganisms such as cyanobacteria, unicellular algae (e.g., diatoms), and related consortia are the most abundant microorganisms in the formation of microbial mats (e.g., Arp et al., 2010). Within subterranean environments iron-oxidizing microbial consortia show a strong ability to form thick microbial mats (Kurz et al., 2010).

Microbial mats are the context within which the first multicellular organisms evolved. Sponges are, for



Microbial Mats, Figure 2 Anaerobic microbial mat from a cold seep environment of the Black Sea. Black mat represents the outer margin of the microbial mat. This mat is dominated by ANME 2 archaea and greigite-bearing sulfate reducing bacteria (GHOSTDABS field, for details see Reitner et al., 2005; Reitner, 2010). Orange-pink mat represents an archaea-(ANME1)-dominated mat portion. The green mat represents the inner most area close to the methane vent. This mat is also dominated by ANME archaea, Crenarcheota, and anaerobic bacteria. The entire mat is stabilized by authigenic methane-related aragonite and calcite crystal aggregates, which are also growing within EPS.

example, highly evolved microbial mats with a mutual relationship with eukaryote choanoflagellates. May be the Vendobionta, large Ediacaran organisms with characteristic quilt bauplan are part of well-developed microbial mats and may themselves be large colonies of various microbial communities (Seilacher, 1999; Schieber et al., 2007; Steiner and Reitner, 2001).

Conclusion

Microbial mats are normally cm-thick microbial communities often related with microbially induced mineral precipitates (calcite, aragonite, iron-oxides etc.). Characteristic for these mats is a multilayered structure which represents various microbial communities with different metabolic activities. In phototrophic microbial mats (e.g., cyanobacteria) dominate the uppermost layers whereas within the deep layers of the mats anaerobic microbes dominate like sulphate reducing bacteria and archaea. Microbial mats are complex multicellular systems which exhibit some accordance to basic metazoans like sponges. It is speculated that microbial mats are a model for the basic metazoan bauplan.

Bibliography

- Allison, D. G., Gilbert, P., Lappin-Scott, H. M., and Wilson, M., 2000. *Community Structure and Co-operation in Biofilms*. Cambridge: Cambridge University Press, 349 p.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshal, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–717.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.

- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., de Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. Tufa-forming biofilms of German karstwater streams: microorganisms, exopolymers, hydrochemistry and calcification. In Rogerson, M., and Pedley, M. M. (eds.), *Tufas and Speleothems: Unravelling the Microbial and Physical Controls*. London: Geological Society Special Publication 336, pp. 83–118.
- Burne, R. V., and Moore, L., 1987. Microbialites; organosedimentary deposits of benthic microbial communities. *Palaaios*, **2**, 241–254.
- Canfield, D. E., and Des Marais, D. J., 1993. Biogeochemical cycles of carbon, sulfur, and free oxygen in a microbial mat. *Geochimica et Cosmochimica Acta*, **57**, 3971–3984.
- Characklis, W. G., and Wilderer, P. A., 1989. Structure and function of Biofilms. *Life Sciences Research Report*, **46**, 1–387 (Wiley).
- Decho, A. W., Visscher, P. T., and Reid, P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 71–86.
- Kurz, J., Simon, K., Heim, C., Reitner, J., Nadia-Valérie Quéric, N.-V., and Volker Thiel, V., 2010. Trace element and biomarker signatures in iron-precipitating microbial mats from the tunnel of Åspö (Sweden). In Reitner, J., Quéric, N.-V., and Arp, G. (eds.), *Advances in Geobiology of Stromatolite Formation, Proceedings of the Kalkowsky Symposium held in Göttingen 2008*. Lecture Notes of Earth Sciences (Springer).
- Pedersen, K., 2006. Microbial life in deep granitic rock. *FEMS Microbiology Reviews*, **20**, 399–414.
- Reitner, J., 2010. Architecture of archaeal-dominated microbial mats from Cold Seeps in the Black Sea (Dnjepr Canyon, Lower Crimean Shelf). In Seckbach, J., and Oren, A. (eds.), *Microbial Mats, Modern and Ancient Microorganisms in Stratified Systems. - Cellular Origin, Life in Extreme Habitats and Astrobiology*, 14 (Springer).
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 66–79.
- Riding, R., and Awramik, S. M. (eds.), 2000. *Microbial Sediments*. Berlin: Springer, 331 pp.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**(Supplement 1), 179–214.
- Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu, O., 2007. *An Atlas of Microbial Mat Features Preserved Within the Siliciclastic Rock Record*. Amsterdam, The Netherlands: Elsevier.
- Seilacher, A., 1999. Biomat-related lifestyles in the Precambrian. *Palaaios*, **14**, 86–93.
- Stal, L., and Caumette, P., 1994. Microbial mats. NATO ASI Series, Series G. *Ecological Sciences*—. Berlin: Springer, Vol. 35, pp. 1–463.
- Steiner, M., and Reitner, J., 2001. Evidence of organic structures in Ediacara-type fossils and associated microbial mats. *Geology*, **29**, 1119–1122.
- Van Dover, C. L., 2000. *The Ecology of Deep-Sea Hydrothermal Vents*. Princeton: Princeton University Press, 424 p.
- Van Kranendonk, M. J., 2007. A review of the evidence for putative Paleoarchean life in the Pilbara Craton. In Van Kranendonk, M. J., Smithies, R. H., and Bennet, V. (eds.), *Earth's Oldest Rocks – Developments in Precambrian Geology*. Amsterdam, The Netherlands: Elsevier, pp. 855–896.
- Wingender, J., Neu, T. R., and Flemming, H.-C., 1999. Microbial Extracellular Polymeric Substances; Characterization, Structure, and Function. Berlin: Springer, 258 p.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biofilms and Fossilization](#)
[Calcite Precipitation, Microbially Induced](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Cyanobacteria](#)
[Diatoms](#)
[Ediacaran Biota](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Degradation](#)
[Microbial-Metal Binding](#)
[Microbialites, Modern](#)
[Mud Mounds](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Protozoa \(Heterotroph, Eukaryotic\)](#)

MICROBIAL SILICIFICATION – BACTERIA (OR PASSIVE)

Kurt O. Konhauser, Brian Jones
 University of Alberta, Edmonton, Alberta, Canada

Definition

The encrustation, impregnation, and/or replacement of microbial tissue by silica minerals, usually opal-A.

Overview

Silica precipitation is an important geological process in many modern geothermal systems where venting of super-saturated solutions leads to the formation of finely laminated siliceous sinters around geyser and hot spring vents and on their discharge aprons. The spring waters, which typically originate from deep, hot reservoirs, at equilibrium with quartz, commonly contain dissolved silica concentrations significantly higher than the solubility of amorphous silica at 100°C (Fournier, 1985). Therefore, when these fluids are discharged at the surface, decompressional degassing, rapid cooling to ambient temperatures, evaporation, mixing, and changes in solution pH collectively cause the solution to suddenly become supersaturated with respect to amorphous silica (also known as opal-A). In such supersaturated solutions, the discharged monomeric silica, Si(OH)₄, polymerizes, initially to oligomers (e.g., dimers, trimers, and tetramers), and eventually to polymeric species with spherical diameters of 1–5 nm, as the silanol groups (–Si–OH–) of each oligomer condense and dehydrate to produce the siloxane (–Si–O–Si–) cores of larger polymers. The polymers grow in size through Ostwald ripening such that a bimodal

- Semikhatov, M. A., Gebelein, C. D., Cloud, P., Awramik, S. M., and Benmore, W. C., 1979. Stromatolite morphogenesis - progress and problems. *Canadian Journal of Earth Science*, **16**, 992–1015.
- Serebryakov, S. N., (1976) Biotic and abiotic factors controlling the morphology of Riphean stromatolites. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology* 20, Amsterdam: Elsevier, pp. 321–336.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaaios*, **15**, 166–169.
- Shapiro, R. S., and Awramik, S. M., 2006. *Favosamaceria cooperi* new group and form: a widely dispersed, time-restricted thrombolite. *Journal of Paleontology*, **80**, 411–422.
- Sheldon, N. D., 2006. Precambrian paleosols and atmospheric CO₂ levels. *Precambrian Research*, **147**, 148–155.
- Sherman, A. G., James, N. P., and Narbonne, G. M., 2000. Sedimentology of a late Mesoproterozoic muddy carbonate ramp, northern Baffin Island, Arctic Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 275–294.
- Stal, L. J., van Gernerden, H., and Krumbein, W. E., 1985. Structure and development of a benthic marine microbial mat. *FEMS Microbiology Ecology*, **31**, 111–125.
- Steele, J. H., 1825. A description of the Oolitic Formation lately discovered in the county of Saratoga, and state of New-York. *American Journal of Science*, **9**, 16–19, part of pl. 2.
- Sumner, D. Y., 1997a. Carbonate precipitation and oxygen stratification in late Archean seawater as deduced from facies and stratigraphy of the Gamohaan and Frisco formations, Transvaal Supergroup, South Africa. *American Journal of Science*, **297**, 455–487.
- Sumner, D. Y., 1997b. Late Archean calcite-microbe interactions: two morphologically distinct microbial communities that affected calcite nucleation differently. *Palaaios*, **12**, 302–318.
- Sumner, D. Y., and Grotzinger, J. P., 2004. Implications for Neoproterozoic ocean chemistry from primary carbonate mineralogy of the Campbellrand-Malmani platform, South Africa. *Sedimentology*, **51**, 1–27.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., James, N. P., and Narbonne, G. M., 1997. Growth dynamics of Neoproterozoic calcimicrobial reefs, Mackenzie mountains, northwest Canada. *Journal of Sedimentary Research*, **67**, 437–450.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N.W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaaios*, **15**, 87–111.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Walcott, C. D., 1914. Cambrian geology and paleontology III: Precambrian Algonkian algal flora. *Smithsonian Miscellaneous Collection*, **64**, 77–156.
- Walter, M. R., 1972. Stromatolites and the biostratigraphy of the Australian Precambrian and Cambrian. *Special Papers in Palaeontology*, **11**, 190 pp, 33 pls.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of the Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research*, **100**, 371–433.
- Webb, G. E., 1987. Late Mississippian thrombolite bioherms from the Pitkin Formation of northern Arkansas. *Geological Society of America, Bulletin*, **99**, 686–698.
- Webb, G. E., 2005. Quantitative analysis and paleoecology of earliest Mississippian microbial reefs, lowermost Gudman formation, Queensland, Australia: not just post-disaster phenomena. *Journal of Sedimentary Research*, **75**, 875–894.
- Whittle, G. L., Kendall, C. G. St. C., Dill, R. F., and Rouch, L., 1993. Carbonate cement fabrics displayed: a traverse across the margin of the Bahama Platform near Lee Stocking Island in the Exuma Cays. *Marine Geology*, **110**, 213–243.

Cross-references

Biofilms
 Calcified Cyanobacteria
 Cap Carbonates
 Microbial Biomineralization
 Microbial Communities, Structure, and Function
 Microbial Mats
 Microbialites, Modern
 Organomineralization
 Snowball Earth

MICROBIAL-METAL BINDING

Kurt O. Konhauser, David A. Fowle
 University of Alberta, Edmonton, Alberta, Canada

Definition

The accumulation of metal cations to microbes, largely through adsorptive processes to the outer cellular surfaces.

Overview

All bacteria have low isoelectric points (below pH 2 in most cases), and consequently, they interact with soluble metal cations and have them intimately associated with their surfaces (Harden and Harris, 1953). Considering their ubiquity in the near-surface environment and their characteristically large surface area-to-volume ratios, bacteria can have a significant influence on metal mobility and speciation in these settings.

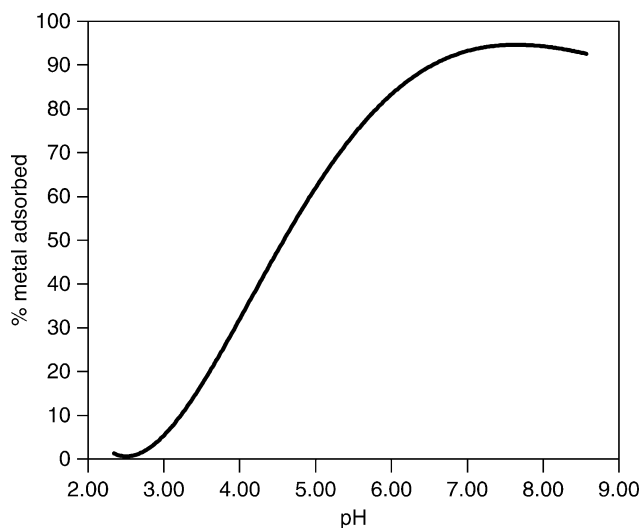
Some bound metals (e.g., Ca and Mg) serve the purpose of stabilizing the negative charges of the anionic functional groups, and thus are relatively “fixed” into place, while other metals are much more exchangeable and provide a temporary positive charge to counter the negative charge induced by the deprotonation of the cell’s surface functional groups (Carstensen and Marquis, 1968). The strength of the metal–microbial bond is quantified by the surface complexation/binding constant (K_M), where M refers to the specific metal of interest. The greater its surface complex formation constant, the less likely

a metal cation will be desorbed into solution. Metal cation sorption is also directly affected by pH, which dictates metal partitioning (or speciation) between solid and soluble phases, and hence, controls its mobility, reactivity, and toxicity in aquatic environments. Of particular importance here is the hydrolysis constant, which measures the tendency of proton release from the hydration sphere of the cation. Figure 1 depicts a generalized adsorption edge curve for a metal binding to a bacterial surface. As pH increases, the cell wall deprotonates and adsorption of the positively charged metal cation increases, but in some instances, adsorption may actually decrease at high pH values as metal hydrolysis is enhanced.

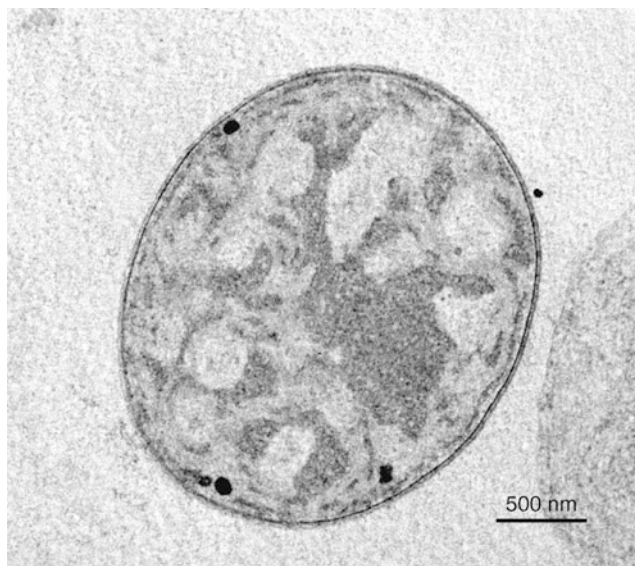
Much of our understanding about how bacteria bind metals stems from the pioneering work of Terry Beveridge on metal accumulation by the Gram-positive bacterium, *Bacillus subtilis* (Beveridge and Murray, 1976). His research demonstrated that it was the carboxyl groups within the peptidoglycan that were the most electronegative sites, and that the bulk of the binding capacity was associated with the cell wall. In similar studies with Gram-negative cells, such as *Escherichia coli*, it was demonstrated that they did not bind as much metal from solution as did their Gram-positive counterparts, a pattern stemming simply from the smaller amounts of peptidoglycan associated with the *E. coli* cell walls (Beveridge and Koval, 1981). Many Gram-positive and Gram-negative bacteria also produce extracellular polymers (EPS), and due to their hydrated nature, dissolved metals can freely diffuse throughout the structure, binding to the anionic carboxyl groups of uronic acids and the neutrally charged hydroxy groups of sugars (Geesey and Jang, 1989). By possessing a large and reactive surface area, it is thus not

unexpected that a number of studies have also documented that encapsulated bacteria bind more metals than nonencapsulated varieties (Rudd et al., 1983). Indeed, species producing EPS can tolerate higher metal concentrations than those that do not, and it has been shown that the proportion of encapsulated bacteria increases in metal-polluted sediment, whereas mutants that cannot produce capsules die off (Aislabie and Loutit, 1986).

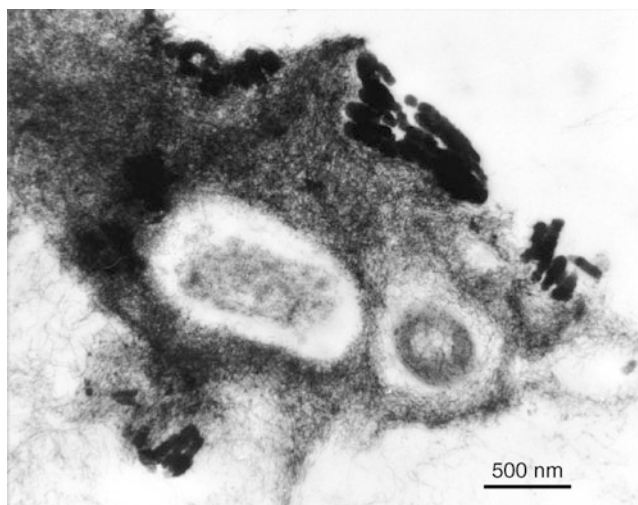
Irrespective of the bacteria studied, what was repeatedly observed was that considerable variations in metal immobilization could be displayed by a single species. In some instances, cell walls were diffusely stained (Figure 2), while at other times so much metal was fixed to the cell surface that it formed a distinct mineral phase (Figure 3). This led Beveridge and Murray (1976) to originally propose a two-step mechanism for the metal adsorption process; the first step in time is an electrostatic interaction between the metal cations and the anionic sites in the cell wall/EPS. This interaction then acts as a nucleation site for the deposition of more metal cations (and anions) from solution, potentially leading to biomineralization. The size of the deposit depends on a number of variables, including the concentration of ions and the amount of time through which the reactions proceed. If sufficient time exists, then the mineral product grows in size within the intermolecular spaces until it is physically constrained by the organic polymers. The end result is a bacterial wall/EPS that contains copious amounts of metal, often approaching the mass of the bacterial cell itself (Beveridge, 1984).



Microbial-Metal Binding, Figure 1 Generic adsorption curve for a metal cation binding to a bacterial surface. Metal adsorption increases as pH increases and cell wall functional groups deprotonate.



Microbial-Metal Binding, Figure 2 Transmission electron micrograph (TEM) of the lab-cultured cyanobacterium *Anabena* sp. that was stained with uranium acetate and lead citrate to reveal cell ultrastructure. Note the dark staining of the cell wall and some of the cytoplasmic constituents.



Microbial-Metal Binding, Figure 3 TEM image of two unidentified bacteria surrounded by iron-stained extracellular polymers. The dark grains at the top right are ferric hydroxide, $\text{Fe}(\text{OH})_3$. These cells grew naturally in Fe-rich hydrothermal fluids at the Lysuholl hot spring in Iceland. From Konhauser (2007).

Although binding of cations to a microbial surface is largely an electrostatic phenomenon, the structural and compositional complexity of the wall or EPS, as well as the unique physicochemical properties of each element, adds a level of complexity to the overall process. In other words, protons and each different cation should be capable of interacting in a distinctive way with surface ligands, such that a cell's surface will display varying affinities for binding different cations. Moreover, in natural systems, a multitude of cations (and anions) exist that should compete with one another for complex formation. This realization has led to a number of metal binding studies that have compared the relative affinities of protons and various cations for the anionic ligands on the cell surfaces of different microorganisms, by techniques that involve displacing one by another. Those studies have highlighted two very important points, the first being that metals and protons compete for the same surface sites, and as solution pH decreases, the functional groups become protonated, displacing loosely bound metal cations (Fowle and Fein, 2000). The second finding is that microbial-metal interactions are largely abiotic, nonspecific, and reliant upon thermodynamics (Warren and Haack, 2001). Some of the most important factors that influence metal binding to cells are (1) the ionic potential of the solution, (2) cell wall/EPS ligand spacing and their stereochemistry, (3) the ligand composition, and (4) the balance between the initial electrostatic attractions between a soluble metal cation and the organic ligands, and the subsequent covalent forces that arise from electron sharing across a metal cation-ligand molecular orbital (Williams, 1981). These properties are largely understood, and given

sufficient information about the environment in which a microorganism is growing, it is possible to extrapolate and predict metal binding patterns on a cell surface.

In the past decade, metal binding experiments have begun to emphasize the stability constants for metal-organic ligand interactions and elucidate how metal binding correlates with cell surface reactivity during changes in solution chemistry. Moreover, unlike many of the early biosorption studies that were carried out in supersaturated conditions with respect to the metal of interest, more recent studies have focused on describing metal-microbe interactions at more realistic undersaturated conditions. The goal for much of this research is to develop geochemical speciation models that describe how microorganisms interact with metals and mineral surfaces under varying geochemical conditions, thus enabling their use in reactive transport models (Fein, 2000 for review). The reactions can be quantified using two different approaches: (1) bulk partitioning relationships or (2) surface complexation models (SCM). In the first instance, partitioning models, such as K_d , Freundlich and Langmuir isotherms, can easily be applied to complex systems because they do not require a detailed understanding of the nature of the surfaces or the adsorption/desorption mechanisms involved. However, they are system-specific, meaning that the results from a set of experiments can be inapplicable to different systems. In contrast, the SCM takes into account the effects of changing pH, solution composition and ionic strength, the acid-base properties of surface functional groups, competitive sorption with other solutes, and solid-phase reactivity and mineralogy. It then draws upon that information to extrapolate to conditions beyond those tested in the laboratory. However, in order to utilize these models in more complex settings, more detailed understanding is required of not only surface and aqueous speciation but also the adsorption and desorption mechanisms.

New experimental techniques are helping to elucidate both the mechanisms and stoichiometry of metal-microbe interactions. Spectroscopic data provide more direct evidence of the metal coordination environment than traditional SCM and partitioning models. For example, metal binding onto *B. subtilis* has been examined by X-ray absorption spectroscopy (XAS) and provided estimates of metal:functional group stoichiometry (Boyanov et al., 2003; Kelly et al., 2002). Mechanisms of metal binding have been directly probed via time-resolved laser-induced fluorescence spectroscopy (TRLFS) and have shown that some metals preferentially bond to one functionality in the cell wall (Panak et al., 2000), while others are less specific (Texier et al., 2000; Markai et al., 2003). A notable recent advance in constraining the thermodynamics of metal-microbe adsorption reactions was the combination of calorimetric data with SCM to generate site-specific enthalpies and entropies of metal adsorption onto the cell wall of *B. subtilis*. This work indicated that heavy metals bind to the cell wall via inner sphere complexation (e.g., no interlayer water molecules) with multiple anionic

oxygen ligands. Stoichiometry and temperature dependence of the metal–bacteria adsorption reactions can also be extracted using this approach (Gorman-Lewis et al., 2006).

Summary

Microbial–metal adsorption includes the electrostatic and chemical association of metals with the organic functional groups of microbial cell surfaces. This can be considered a passive process driven by the electronegativity of the microbial cell wall/EPS and the Gibbs free energy of the adsorption reactions. These adsorption reactions seem to be dominated by binding of cations to the oxygen containing moieties on the cell surface. Future advances in elucidating the mechanisms of metal adsorption to microbes will come from combining novel analytical techniques, such as colorimetry and spectroscopy, with bulk partitioning studies and site-specific adsorption models.

Bibliography

- Aislabie, J., and Loutit, M. W., 1986. Accumulation of Cr(III) by bacteria isolated from polluted sediment. *Marine Environmental Research*, **20**, 221–232.
- Beveridge, T. J., 1984. Mechanisms of the binding of metallic ions to bacterial walls and the possible impact on microbial ecology. In Reddy, C. A., and Klug, M. J. (eds.), *Current Perspectives in Microbial Ecology*. Washington, DC: American Society for Microbiology, pp. 601–607.
- Beveridge, T. J., and Koval, S. F., 1981. Binding of metals to cell envelopes of *Escherichia coli* K-12. *Applied and Environmental Microbiology*, **42**, 325–335.
- Beveridge, T. J., and Murray, R. G. E., 1976. Uptake and retention of metals by cell walls of *Bacillus subtilis*. *Journal of Bacteriology*, **127**, 1502–1518.
- Boyanov, M. L., Kelly, S. D., Kemner, K. M., Bunker, B. A., Fein, J. B., and Fowle, D. A., 2003. Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: a pH dependent X-ray absorption fine structure spectroscopy study. *Geochimica et Cosmochimica Acta*, **67**, 3299–3311.
- Carstensen, E. L., and Marquis, R. E., 1968. Passive electrical properties of microorganisms. III. Conductivity of isolated bacterial cell walls. *Biophysics Journal*, **8**, 536–548.
- Fein, J. B., 2000. Quantifying the effects of bacteria on adsorption reactions in water-rock systems. *Chemical Geology*, **169**, 265–280.
- Fowle, D. A., and Fein, J. B., 2000. Experimental measurements of the reversibility of metal-bacteria adsorption reactions. *Chemical Geology*, **168**, 27–36.
- Geesey, G. G., and Jang, L., 1989. Interactions between metal ions and capsular polymers. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: John Wiley, pp. 325–357.
- Gorman-Lewis, D., Fein, J. B., and Jensen, M. P., 2006. Enthalpies and entropies of proton and cadmium adsorption onto *Bacillus subtilis* bacterial cells from calorimetric measurements. *Geochimica et Cosmochimica Acta*, **70**, 4862–4873.
- Harden, V. P., and Harris, J. O., 1953. The isoelectric point of bacterial cells. *Journal of Bacteriology*, **65**, 198–202.
- Kelly, S. D., Boyanov, M. I., Fein, J. B., Fowle, D. A., Yee, N., and Kemner, K. M., 2002. X-ray absorption fine structure determination of pH-dependent U-bacterial cell wall interactions. *Geochimica et Cosmochimica Acta*, **66**, 3855–3871.
- Markai, S., Andrés, Y., Montavon, G., and Grambow, B., 2003. Study of the interaction between europium (III) and *Bacillus subtilis*: fixation sites, biosorption modelling and reversibility. *Journal of Interface and Colloid Science*, **243**, 73–80.
- Panak, P., Raff, J., Selenska-Pobell, S., Geipel, G., Bernhard, G., and Nitsche, H., 2000. *Radiochimica Acta*, **88**, 71–76.
- Rudd, T., Sterritt, R. M., and Lester, J. N., 1983. Mass balance of heavy metal uptake by encapsulated cultures of *Klebsiella aerogenes*. *Microbial Ecology*, **9**, 261–272.
- Texier, A. C., Andrés, Y., Illemassene, M., and Le Cloirec, P., 2000. Characterization of lanthanide ions binding sites in the cell wall of *Pseudomonas aeruginosa*. *Environmental Science and Technology*, **34**, 610–615.
- Warren, L. A., and Haack, E. A., 2001. Biogeochemical controls on metal behaviour in freshwater environments. *Earth-Science Reviews*, **54**, 261–320.
- Williams, R. J. P., 1981. Physico-chemical aspects of inorganic element transfer through membranes. *Philosophical Transactions of the Royal Society of London*, **B294**, 57–74.

Cross-references

Carbonates
Clay Authigenesis, Bacterial
Dolomite, Microbial
Metalloenzymes
Microbial Biomineralization
Microbial Surface Reactivity
Nanocrystals, Microbially Induced
Organomineralization

MICROBIOCORROSION

Aline Tribollet¹, Stjepko Golubic², Gudrun Radtke³,
Joachim Reitner⁴
¹Institut de Recherche pour le Développement, Nouméa,
Nouvelle-Calédonie, France
²Boston University, Boston, MA, USA
³Hessisches Landesamt für Umwelt und Geologie,
Wiesbaden, Germany
⁴University of Göttingen, Göttingen, Germany

Definition

Destruction of rocks and minerals by biological activities has been termed bioerosion (Neumann, 1966). It includes mechanical as well as chemical effects, that is, bioabrasion and biocorrosion (Schneider, 1976; Golubic and Schneider, 1979). However, both the processes often co-occur; they are functionally interconnected and mutually supportive. Biocorrosion can result from the activity of macro- or microorganisms and, thus, is called macrobiocorrosion and microbiocorrosion. Microbiocorrosion can also be closely associated with microbial rock formation and consolidation in stromatolitic structures (Reid et al., 2000; Macintyre et al., 2000; Garcia-Pichel et al., 2004; Dupraz and Visscher, 2005). In fact, the oldest known fossils of microboring organisms were located in lithified horizons of silicified stromatolites (Zhang and Golubic, 1987).

- Joachimski, M., Ostertag-Henning, C., Pancost, R., Strauss, H., Freeman, K., Littke, R., Sinninghe Damste, J. S., and Racki, G., 2001. Water column anoxia, enhanced productivity and concomitant changes in $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ across the Frasnian-Famennian boundary (Kowala-Holt' Cross Mountains/Poland). *Chemical Geology*, **175**, 109–131.
- KieSSLing, W., and Simpson, C., 2010. On the potential for ocean acidification to be a general cause of ancient reef crises. *Global Change Biology*, doi:10.1111/j.1365-2486.2010.02204
- Krug, A. Z., and Patzkowsky, M. E., 2004. Rapid recovery from the Late Ordovician mass extinction. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 17605–17610.
- Kump, L. R., Pavlov, A., and Arthur, M. A., 2005. Massive release of hydrogen sulfide to the surface ocean and atmosphere during intervals of oceanic anoxia. *Geology*, **33**, 397–400.
- Le Guerroué, E., Allen, P. A., and Cozzi, A., 2006a. Chemostratigraphic and sedimentological framework of the largest negative carbon isotopic excursion in Earth history: the Neoproterozoic Shuram formation (Nafun Group, Oman). *Precambrian Research*, **146**(1–2), 68–92.
- Le Guerroué, E., Allen, P. A., Cozzi, A., Etienne, J. L., and Fanning, C. M., 2006b. 50 Myr recovery from the largest negative $\delta^{13}\text{C}$ excursion in the Ediacaran ocean. *Terra Nova*, **18**(2), 147–153.
- McGhee, G. R. Jr., 1996. *The Late Devonian Mass Extinction: the Frasnian/Famennian Crisis*. New York: Columbia University Press.
- Melott, A. L., Lieberman, B. S., Laird, C. M., Martin, L. D., Medvedev, M. V., Thomas, B. C., Cannizzo, J. K., Gehrels, N., and Jackman, C. H., 2004. Did a gamma-ray burst initiate the late Ordovician mass extinction? *International Journal of Astrobiology*, **3**, arXiv:astro-ph/0309415v3.
- Moczydlowska, M., 2008. The Ediacaran microbiota and the survival of Snowball Earth Snowball conditions. *Precambrian Research*, **167**, 1–15.
- Palfy, J., Demeny, A., Haas, J., Htenyi, M., Orchard, M. J., and Veto, I., 2001. Carbon isotope anomaly at the Triassic–Jurassic boundary from a marine section in Hungary. *Geology*, **29**, 1047–1050.
- Payne, J. L., Lehmann, D. J., Wei, J., Orchard, M. J., Schrag, D. P., and Knoll, A. H., 2004. Large perturbations of the carbon cycle during recovery from the End-Permian Extinction. *Science*, **305**, 506.
- Raup, D. M., and Sepkoski, J. J., 1982. Mass extinctions in the marine fossil record. *Science*, **215**(4539), 1501–1503.
- Reichow, M. K., Pringle, M. S., Al'Mukhamedov, A. I., Allen, M. B., Andreichev, V. L., Buslov, M. M., Davies, C. E., Fedoseev, G. S., Fitton, J. G., Inger, S., Medvedev, A. Ya., Mitchell, C., Puchkov, V. N., Safonova, I. Yu., Scott, R. A., and Saunders, A. D., 2009. The timing and extent of the eruption of the Siberian Traps large igneous province: implications for the end-Permian environmental crisis. *Earth and Planetary Science Letters*, **277**, 9–20.
- Retallack, G. J., Seyedolali, A., Krull, E. S., Holser, W. T., Ambers, C. P., and Kyte, F. T., 1998. Search for evidence of impact at the Permian–Triassic boundary in Antarctica and Australia. *Geology*, **26**, 979–982.
- Ruhl, M., Kuerschner, W. M., and Krystyn, L., 2009. Triassic–Jurassic organic carbon isotope stratigraphy of key sections in the western Tethys realm (Austria). *Earth and Planetary Science Letters*, **281**, 169–187.
- Sandberg, C. A., Morrow, J. R., and Ziegler, W., 2002. Late Devonian sea-level changes, catastrophic events, and mass extinctions. In Koeberl, C., and MacLeod, K. G. (eds.), *Catastrophic Events and Mass Extinctions: Impacts and Beyond*. Boulder: Geological Society of America. GSA Special Paper 356, pp. 473–487.
- Saunders, A., and Reichow, M. K., 2009. The Siberian traps and the End-Permian mass extinction: a critical review. *Chinese Science Bulletin*, **54**, 20–37.
- Sepkoski, J. J. Jr., 1984. A kinetic model of Phanerozoic taxonomic diversity. III. post-Paleozoic families and mass extinctions. *Paleobiology*, **10**, 246–267.
- Sepkoski, J. J. Jr., 1994. Extinction and the fossil record. *Geotimes*, **39**, 15–17.
- Sheehan, P., 2001. The late Ordovician mass extinction. *Annual Review of Earth and Planetary Sciences*, **29**, 331–364.
- Sole, R. V., and Newman, M., 2002. Extinctions and Biodiversity in the Fossil Record - Volume Two, The earth system: biological and ecological dimensions of global environment change. *Encyclopedia of Global Environmental Change*, New York: Wiley, pp. 297–391.
- Stanton, M. S., 2002. http://www.aapg.org/explorer/2002/12dec/gom_impact.pdf
- Twitchett, R. J., Looy, C. V., Morante, R., Visscher, H., and Wignall, P. B., 2001. Rapid and synchronous collapse of marine and terrestrial ecosystems during the end-Permian biotic crisis. *Geology*, **29**, 351–354.
- Wallsier, O. H. (ed.), 1995. *Global Events and Event Stratigraphy*. Berlin: Springer.
- Ward, P. D., Haggart, J. W., Carter, E. S., Wilbur, D., Tipper H. W., and Evans, T., 2001. Sudden productivity collapse associated with the Triassic–Jurassic boundary mass extinction. *Science*, **292**, 1148–1151.
- Webby, B. D., and Droser, M. L. (eds.), 2004. *The Great Ordovician Biodiversification Event*. New York: Columbia University Press.
- White, R. V., 2002. Earth's biggest “whodunnit”: unravelling the clues in the case of the end-Permian mass extinction. *Philosophical Transactions of the Royal Society of London*, **360**, 2963–2985.
- Whiteside, J. H., Olsen, P. E., Eglinton, T., Brookfield, M. E., and Sambrotto, R. N., 2010. Compound-specific carbon isotopes from Earth's largest flood basalt eruptions directly linked to the end-Triassic mass extinction. *Proceedings of the National Academy of Sciences*, **107**(15), 6721–6725.
- Young, S. A., Saltzman, M. R., Foland, K. A., Linder, J. J., and Kump, L. R., 2009. A major drop in seawater $87\text{Sr}/86\text{Sr}$ during the Middle Ordovician (Darriwilian): links to volcanism and climate? *Geology*, **37**, 951–954.

Cross-references

[Asteroid and Comet Impacts](#)
[Biological Volcanic Rock Weathering](#)
[Comets](#)
[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Snowball Earth](#)

MAT-RELATED SEDIMENTARY STRUCTURES

Hubertus Porada
 University of Göttingen, Göttingen, Germany

Synonyms

Microbially induced sedimentary structures (MISS) (The acronym MISS was introduced by Noffke et al. (2001) for “microbially induced sedimentary structures.” Many of the structures addressed, however, are not truly “induced” by microbes, but rather by physical forces

acting on a biostabilized sediment surface or a microbial mat. Since, in this scenario, the biological component significantly influences the shape of evolving structures, it is suggested to write out the acronym MISS as “microbially influenced sedimentary structures.”).

Definition

Mat-related sedimentary structures are small- to medium-scale sedimentary structures resulting from growth and extension of microbial populations and communities on a sediment surface, stabilization of surface sediments, trapping and binding of sediment particles, and from the impact of environmental factors, such as inundation, sedimentary deposition, subaerial desiccation, wind- or current-induced traction, on epibenthic microbial mats.

Introduction

Originally described from and applied to modern microbial mats (Gerdes et al., 2000; Noffke et al., 2001), mat-related sedimentary structures preferentially serve as proxies of the former existence of microbial mats in ancient siliciclastic sediments. Mat-related structures are in a way analogous to trace fossils (Schieber, 2004), whereby the former presence of mats can be inferred, e.g., from observations suggesting “unusual” sediment properties like original cohesiveness, tensile strength, and erosion-resistance of sand (Eriksson et al., 2007); uncharacteristic grain fabrics; and steep geochemical gradients on a mm-scale. Genetic explanation of the structures relies on an actualistic approach; preservation depends on intrinsic biological, and favorable environmental conditions.

In this chapter, the term “mat-related sedimentary structures” is applied to structures that form on, in, or below microbial mats with distinct properties, e.g., coherence, cohesiveness, minor thickness, and adaptation to periodic subaerial exposure. Further conditions include a fine-grained siliciclastic substrate (sand to silt) and lacking dominance of carbonate precipitation. The term also encompasses primary physical structures that have subsequently been microbially influenced.

Today, well-developed (cyanobacterial) microbial mats that meet the above conditions, are observed mainly in the intertidal to lower supratidal zones of low-gradient tidal flats where they grow in a wide range of climate zones from temperate humid to subtropical/tropical semi-arid and arid, at salinities ranging from brackish to hypersaline. Similar mats may also form in other environments, e.g., lacustrine, fluvial, where similar mat-related structures may partly form. Due to lacking vertical bioturbation in the Precambrian, the distribution of microbial mats may previously have been much wider, extending far into the subtidal zone.

Microbial mats are epibenthic, multilayered microbial communities including different functional groups of microorganisms (Stal, 2000) which act together in a kind of “joint venture” widely balancing organic matter production and destruction (van Gernerden, 1993). Thus, microbial mats in coastal environments normally do not

exceed a thickness of a few centimeters. The “biological stratification” of microorganisms is superimposed by a biosedimentary lamination resulting from the interplay of microbial growth and seasonal, periodic, or episodic depositional events related, e.g., to tides or storms.

The top layer of the mat system, which periodically is exposed to the atmosphere, usually consists of oxygenic, filamentous cyanobacteria, frequently *Microcoleus* sp., which form a “felty” fabric of interwoven filaments (Gerdes et al., 2000), including also sediment grains incorporated by “trapping and binding” processes (Noffke et al., 2003a, with references). The felty layer may be overlain, or in some cases replaced, by a resistant (“leathery”) layer of colloid extracellular polymeric substances (EPS) produced mainly by coccoid cyanobacteria. This top portion of the mat combines high mechanical resistance with low permeability and is the locus at which most of the mat-related sedimentary structures form. Deeper levels of the mat system are much less cohesive. They contain anaerobic bacteria among which sulfate-reducing bacteria produce sulfide leading to precipitation of iron sulfides (FeS) which gradually transform into pyrite. In the ancient record, the stratiform occurrence of pyrite, in some cases siderite or ferroan carbonate, within otherwise well oxidized sediments may serve as a proxy of the former existence of microbial mats.

Formation and types of structures

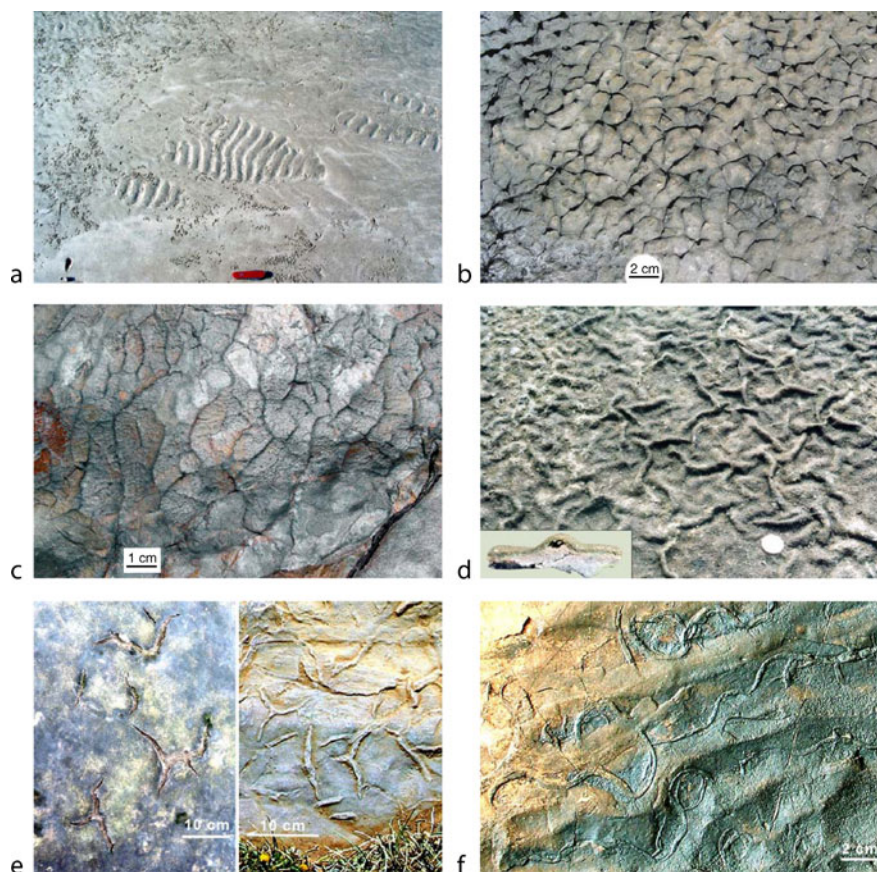
Mat-related sedimentary structures may form during all the stages from first microbial colonization of a sediment surface, through establishment and sustainment of a fully developed mat, to its destruction and final erosion.

Structures related to early microbial colonization

Microbial colonization leads to “biostabilization” (Krumbein et al., 1994) of a sediment surface which then can resist erosion to some degree. This property may lead to “palimpsest ripples” when new sediment is deposited on top a biostabilized rippled surface (Pflüger, 1999); to surfaces with “multi-directional ripple marks” (Noffke, 1998); and to “ripple patches” (Figure 1a) or “erosional pockets” when a biostabilized flat surface or mat is locally eroded (for ancient example, Noffke, 1999; McKenzie, 1972).

Structures related to microbial growth and mat growth

Depending on availability of water for some time, specific filamentous cyanobacteria (e.g., *Lyngbya aestuarii*) start to produce on the mat surface, a characteristic “reticulate growth pattern” of small, sharp-crested ridges stabilized by EPS (Figure 1b). Ancient structures of this type are named “elephant skin” texture (Gehling, 1999) (Figure 1c). There is considerable variability in respect of polygon size (few mm to few cm) and shape varying from nearly circular to narrow rectangular with almost linear crest orientation. A unifying feature, however, are the



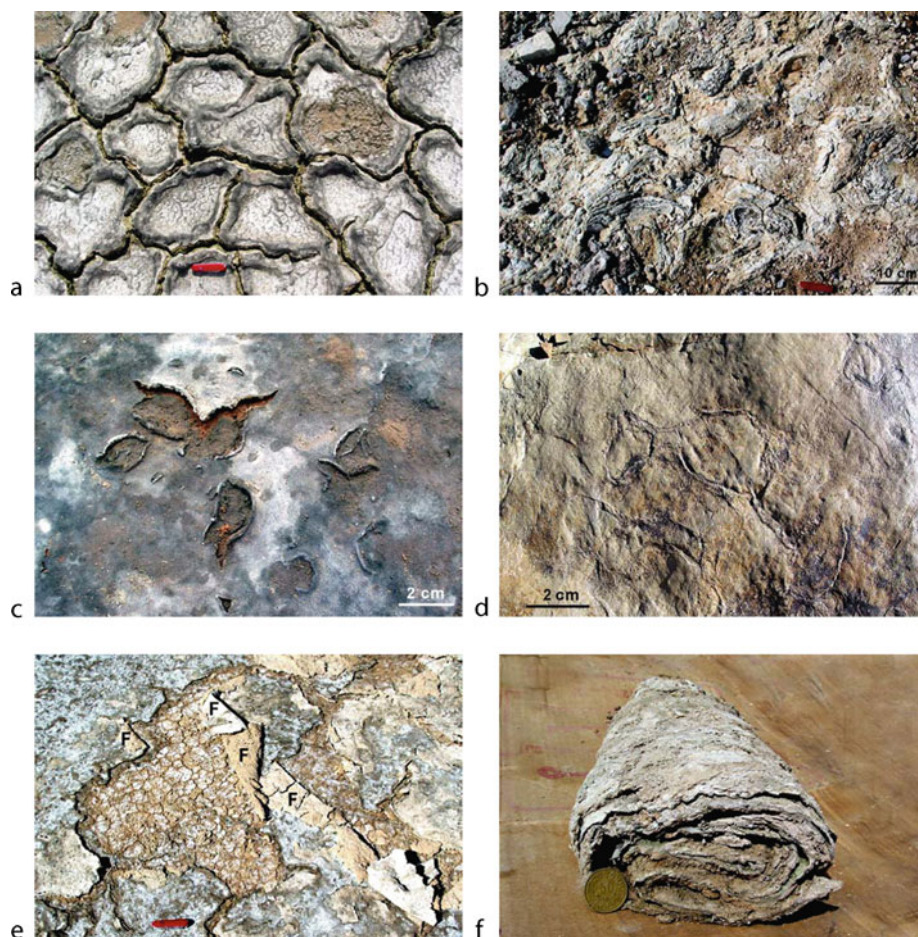
Mat-Related Sedimentary Structures, Figure 1 Structures related to biostabilization, microbial and mat growth, and desiccation. (a) Biostabilized sediment surface with isolated “ripples patches.” Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (b) Reticulate growth pattern produced by *Lyngbya aestuarii*. Locality: Salins du Midi, Réserve Nationale Camargue, southern France. (c) Upper surface of sericitic siltstone with “elephant skin” texture representing “reticulate growth pattern” of a previous microbial mat. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (d) “Petees” originating from lateral mat growth and deforming microbial mat into round-crested bulges: *Inset*: Cross-section showing that upper, cohesive part of the mat is deformed and separated from deeper, less cohesive mat layers. Scale (coin) is 24 mm. Locality: Amrum Island, southern North Sea, Germany. (e) Modern and ancient shrinkage cracks. *Left photograph*: modern microbial mat with isolated, sigmoidal, and tri-radiate cracks. Locality: Amrum Island, southern North Sea, Germany. *Right photograph*: fillings of ancient, spindle-shaped, and tri-radiate cracks. Locality: Neoproterozoic Tizi n-Taghatine Group; Imi n’Tizi area, Anti-Atlas, Morocco. (f) *Manchuriophycus*-type shrinkage cracks meandering in ripple troughs. Locality: Neoproterozoic Tizi n-Taghatine Group; Taghdout area, Anti-Atlas, Morocco.

narrow, sharp-crested ridges preserved on upper bedding surfaces.

A further type of structures related to mat growth are “mat expansion structures,” collectively termed “petees” (Gavish et al., 1985; Bouougri et al., 2007), which deform the upper, cohesive part of the mat into bulges and domes, thus enlarging the mat surface (Figure 1d). Occasionally, the bulges may be arranged in a polygonal pattern. Provided, petees are filled by sediment rising up from below, the structures may be preserved. Ancient examples have been named “petee ridges” (Schieber, 2004).

Structures related to mat desiccation and shrinkage
Subaerial exposure causes dehydration and shrinkage of the EPS and eventually cracking of the mat. Incipient,

isolated “shrinkage cracks” are sigmoidal and spindle-shaped, or tri-radiate starting from triple junctions, or occasionally circular in shape (Figure 1e). Crack propagation at progressive shrinkage leads to more or less complete networks of cracks. In general, shrinkage cracks in microbial mats develop a higher degree of curving unlike normal mud cracks. Ancient shrinkage cracks are also termed “sand cracks” referring to their occurrence in sandy sediment without shrinkable mud present. A specific type of shrinkage cracks, characterized by sinuoidal or sub-circular trends and developed mainly in ripple troughs, is referred to as “*Manchuriophycus*”-type in the ancient record (Porada and Löffler, 2000) (Figure 1f). A characteristic property of many ancient shrinkage cracks is their easy separability from the layers above and below, which is ascribed to soon microbial



Mat-Related Sedimentary Structures, Figure 2 Structures related to mat desiccation and deformation. (a) Polygonal pattern of shrinkage cracks with upturned margins in thick microbial mat. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (b) Surface outcrop of Holocene microbial mat exhibiting relics of polygons with upturned margins. Scale (knife) is 8 cm. Locality: Trucial Coast, west of Abu Dhabi, U.A.E. (c) Irregular to subcircular openings with curled margins, formed in a thin microbial mat due to desiccation and shrinkage. Note newly overgrown margin in lower right of photograph. Locality: Sabkha El Gourine, Mediterranean coast of southern Tunisia. (d) Upper surface of siltstone layer exhibiting irregular to circular cracks. The structures are interpreted as openings in a previous thin mat that underwent desiccation. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (e) Microbial mat, torn and partly removed by current or wind action. Flip-overs of mat margins are indicated by "F." Note small-scale desiccation cracks in exposed mat substratum. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (f) "Roll-up" structure ("jelly-roll") consisting of rolled-up microbial mat and adhering sediment. Scale (coin) is 23 mm. Locality: Bhar Alouane, Mediterranean coast of southern Tunisia.

overgrowth of the crack margins and of the sediment trapped in the crack.

Thick mats, frequently dominated by *Microcoleus chthonoplastes*, tend to form polygonal networks of wide cracks with "upturned" or involute, "curled margins" (Figure 2a); individual polygons may range in size from 10 to 60 cm across, depending likely on the thickness of the mat. Ancient examples exhibit a "chaotic" upper surface with irregularly oriented bedding (Figure 2b) and resemble sedimentary structures ascribed to seismic events (Donaldson and Chiarenzelli, 2007).

Thin mats, frequently dominated by coccoid cyanobacteria and vested with a strong EPS top layer, tend

to form circular openings with curled margins around (Figure 2c). Ancient examples have been described from thin siltstone layers within heterolithic deposits (Bouougri and Porada, 2007) (Figure 2d).

Structures related to mat deformation, destruction, and erosion

Cohesive microbial mats may become torn, detached from the substratum and tightly folded, and eventually eroded by strong currents, e.g., during storm and flooding events. Mats are also destructed by complete desiccation after cessation of groundwater supply, and dried mat fragments

may be transported by wind over wide distances and be deposited in environments where mats usually do not grow.

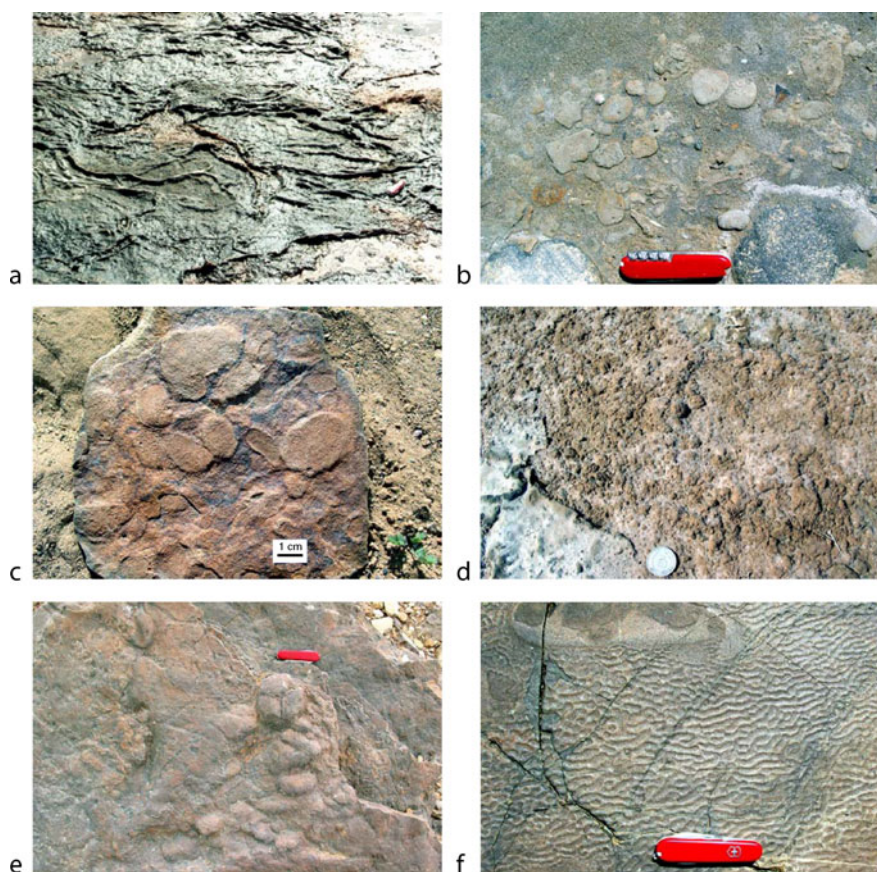
Mat destruction by currents leads to typical structures repeatedly observed in modern environments and in the ancient record: (1) “flip-over” structures (Figure 2e) result when a mat’s edge is flipped over; (2) cigar-shaped “roll-up” structures, also named roll-ups or “jelly-rolls” (Figure 2f), may develop when curled margins or flip-overs undergo additional rolling due to current action; and (3) irregular or arcuate belts of “mat deformation folds” form when a torn and detached mat is crumpled by tractional forces (Figure 3a); similar folds may also result from mat slumping on steep slopes, e.g., along tidal channels. Roll-up structures can withstand transport over some distance and may gather along trash lines, whereas

the other structures largely remain in situ. For ancient examples see Schieber et al. (2007).

“Mat chips” (Gerdes et al., 2000) are small fragments of eroded mats or biostabilized sediment. They primarily are irregular in shape with frayed edges, but may become pebble-shaped with transport (Figure 3b). Ancient examples have been named “microbial sand chips” (Pflüger and Gresse, 1996) or “sand clasts” in contrast to intraformational mud clasts (Figure 3c).

Structures formed beneath microbial mats

From the hydraulic perspective, the sediment beneath cohesive and coherent microbial mats may be addressed and treated as a confined aquifer in which, at sufficient hydraulic head, a potential for liquefaction may develop (Porada et al., 2007). In such case, hydraulic upward



Mat-Related Sedimentary Structures, Figure 3 Structures related to mat deformation and erosion, and subsurface structures. Scale (knife) in a, b, e, and f is 8 cm. (a) Detached, thin microbial mat, torn and strongly deformed by current action. Locality: Coastal sabkha between Gabes and Skhirat, Mediterranean coast of southern Tunisia. (b) Modern sandy-pebbly sediment surface with subrounded to rounded “mat chips”. Locality: Salins du Midi, Réserve Nationale Camargue, southern France. (c) Upper surface of sandstone bed carrying subrounded to rounded “sand clasts,” interpreted as previous “mat chips.”. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia. (d) Morphological details of a mat subsurface, exposed after removal of the mat. Scale (coin) is 24 mm. Locality: Sabkhet Mjasser, Mediterranean coast of southern Tunisia. (e) Upper surface of sandstone layer exhibiting irregular bulges and domes. The structure is considered to represent morphological features of a previous mat subsurface. Note tri-radiate crack in well-developed dome just below scale. Locality: Neoproterozoic Tizin-Taghatine Group; Taghdout area, Anti-Atlas, Morocco. (f) Upper surface of sandstone layer with “Kinneyia” structure. Locality: Terminal Proterozoic Vingerbreek Member, Nama Group; Farm Haruchas, Namibia.

pressure will lead to slow, upward movement of sediment grains. As a result, bulges and domes developed in the mat will gradually be filled from below; “petee ridges” have been quoted as an example above.

Thin microbial mats, particularly those dominated by coccoid cyanobacteria, may develop very irregular surfaces with numerous small domes and buckles which also appear as positive features on the subsurface (Figure 3d). Ancient examples of such “subsurface structures” strongly resemble load structures but are clearly distinguished from these by their occurrence as positive features on *upper* bedding surfaces (Figure 3e).

“Kinneyia” (Figure 3f) is a further structure developed in the sediment beneath a microbial mat. The structure is characterized by millimeter-scale, flat-topped, steeply sided, winding ridges separated by equally sized round-bottomed troughs and pits. It resembles small-scale interference ripples including crest-dominated linear and pit-dominated honeycomb-like patterns. The structure has been described from the Archean (Noffke et al., 2003b) to the Jurassic (Bloos, 1976), but not yet from the Recent. Genetic interpretation is thus difficult. Recent models suggest formation by trapping of gas underneath a sealing mat (Pflüger, 1999) or by reversals of groundwater flow in the liquefied mat substratum (Porada et al., 2007).

Wrinkle structures

The term “wrinkle structure” (Hagadorn and Bottjer, 1997, 1999) is currently used as a collective term for various small-scale irregularities developed on ancient, siliciclastic sediment surfaces. Application of the term implies that a microbial participation in the formation of the structure is suspected, at the least. Within this broad definition, wrinkle structures may originate from very different processes including microbial growth, mat deformation and subsurface processes (Porada and Bouougri, 2007). Included in the term are also structures like elephant skin and Kinneyia which are well defined and for which, if clearly identified, usage of the proper name is recommended.

Preservation of mat-related structures

As most of the organic matter produced by the cyanobacteria is soon decomposed and mineralized so that organic components or even carbon are rarely preserved in the ancient siliciclastic record, preservation of mat-related structures is largely dependent on the volume of inorganic material bound or produced by the mat or trapped by the structure. Microbially supported processes introducing inorganic material into the mat include “trapping and binding” of silt- to sand-sized grains and possibly clay minerals, formation of authigenic clay minerals during bacterial lysis, formation of iron sulfides and pyrite, and precipitation of carbonates. The mat-related structures themselves, being either positive or negative features on the mat surface, may act as sediments traps. Thus, shrinkage cracks may become filled with sediment by currents or

colian action, and involute (curled) margins may trap grains in their windings.

Another mechanism by which some mat-related structures, e.g., domes and bulges developed in the mat, may become preserved is “filling from below.” This process requires comparatively high hydraulic upward pressure enabling liquefaction of the sediment below the sealing mat so that grains are lifted and moved upwards. As a result, a previously flat surface of a sedimentary layer may be transformed into an irregular surface mimicking morphological features of the mat.

Conclusions

Simplistically subsumed under the main groups of “wrinkles,” “unusual cracks,” and mat chips in the fossil record, mat-related sedimentary structures exhibit a wide range of shapes and patterns resulting from various processes and interactions between conflicting biological and physical factors. Due to local microtopographic features and the intrinsic inhomogeneity of microbial mats on a small scale, even well-defined structures, like elephant skin or Kinneyia, may show a high degree of variability. Thus, one structure almost never is like the other and the paradigm of reproducibility is not strictly applicable to them. Nevertheless, the structures are distinctive as exemplified on Figures 1–3.

Mat-related sedimentary structures may serve as tools for detailed facies analysis in siliciclastic peritidal settings. Based on modern analogs, most of the structures form in the intertidal to supratidal zones, but mat extent may have been wider in the Precambrian. Structures requiring (at least temporarily) subaerial exposure for their formation, e.g., types of shrinkage cracks with or without upturned or curled margins, clearly indicate intertidal to supratidal positions, whereas others remain less unequivocal. Subsurface structures including Kinneyia may form down to the shallow subtidal zone, as long as coherent, cohesive mats are present.

In the fossil record, the significance of mat-related sedimentary structures increases greatly if several types are observed in association. Some mat-related structures, e.g., reticulate growth patterns, preferentially occur in heterolithic successions of thinly laminated siltstone and mudstone which, in some cases, may be addressed as “siliciclastic biolaminites” resulting from the interaction of microbial mat growth and sediment supply (Bouougri and Porada, 2007), whereas sand cracks are usually developed on sandstone layers intercalated in the succession. In contrast, Kinneyia structures exclusively occur on upper surfaces of sandstone beds which frequently represent event deposits after storms or floods.

Bibliography

Bloos, G., 1976. Untersuchungen über Bau und Entstehung der feinkörnigen Sandsteine des Schwarzen Jura α (Hettangium u. tiefstes Sinemurium) im schwäbischen Sedimentations-bereich. *Arb Inst Geol Paläont Univ Stuttgart*, **71**, 1–270.

- Bouougri, E. H., and Porada, H., 2007. Siliciclastic biolaminites indicative of widespread microbial mats in the Neoproterozoic Nama Group of Namibia. *Journal of African Earth Sciences*, **48**, 38–48.
- Bouougri, E., Gerdes, G., and Porada, H., 2007. Inherent problems of terminology: definition of terms frequently used in connection with microbial mats. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp.145–151.
- Eriksson, P. G., Schieber, J., Bouougri, E., Gerdes, G., Porada, H., Banerjee, S., Bose, P. K., and Sarkar, S., 2007. Classification of structures left by microbial mats in their host sediments. In Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O. (eds.), *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, pp. 39–52.
- Gehling, J. G., 1999. Microbial mats in terminal Proterozoic siliciclastics: Ediacaran death masks. *Palaaios*, **14**, 40–57.
- Gerdes, G., Klenke, Th., and Noffke, N., 2000. Microbial signatures in peritidal siliciclastic sediments: a catalogue. *Sedimentology*, **47**, 279–308.
- Hagadorn, J. W., and Bottjer, D. J., 1997. Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic-Phanerozoic transition. *Geology*, **25**, 1047–1050.
- Hagadorn, J. W., and Bottjer, D. J., 1999. Restriction of a late neoproterozoic biotope: suspect-microbial structures and trace fossils at the Vendian-Cambrian transition. *Palaaios*, **14**, 73–85.
- Krumbein, W. E., Paterson, D. M., and Stal, L. J. (eds.), 1994. *Biostabilization of Sediments*, Oldenburg: BIS Verlag, 526 p.
- McKenzie, D. B., 1972. Tidal sand flat deposits in Lower Cretaceous Dakota Group near Denver, Colorado. *The Mountain Geologist*, **9**, 269–277.
- Noffke, N., 1998. Multidirected ripple marks rising from biological and sedimentological processes in modern lower supratidal deposits (Mellum Island, southern North Sea). *Geology*, **26**, 879–882.
- Noffke, N., 1999. Erosional remnants and pockets evolving from biotic-physical interactions in a recent lower supratidal environment. *Sedimentary Geology*, **123**, 175–181.
- Noffke, N., Gerdes, G., Klenke, T., and Krumbein, W. E., 2001. Microbially induced sedimentary structures – a new category within the classification of primary sedimentary structures. *Journal of Sedimentary Research*, **71**, 649–656.
- Noffke, N., Gerdes, G., and Klenke, T., 2003a. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty, and evaporitic carbonatic). *Earth-science Reviews*, **62**, 163–176.
- Noffke, N., Hazen, R., and Nhlenko, N., 2003b. Earth's earliest microbial mats in a siliciclastic marine environment (2.9 Ga Mozaan Group, South Africa). *Geology*, **31**, 673–676.
- Pflüger, F., 1999. Matground structures and redox facies. *Palaaios*, **14**, 25–39.
- Porada, H., and Bouougri, E. H., 2007. Wrinkle structures – a critical review. *Earth-science Reviews*, **81**, 199–215.
- Porada, H., and Löffler, T., 2000. Microbial shrinkage cracks in siliciclastic rocks of the Neoproterozoic Nosib Group (Damara Supergroup) of central Namibia. *Communications of the Geological Survey of Namibia*, **12**, 63–72.
- Porada, H., Ghergut, J., and Bouougri, E. H., 2008. Kinneyia-type wrinkle structures –critical review and model of formation. *Palaaios*, **23**, 63–77.
- Schieber, J., 2004. Microbial mats in the siliciclastic rock record: a summary of diagnostic features. In Eriksson, P. G., Altermann, W., Nelson, D., Mueller, W. U., Catuneanu, O., and Strand, K. (eds.), *The Precambrian Earth: Tempos and Events. Developments in Precambrian Geology*. Amsterdam: Elsevier, pp. 663–672.
- Schieber, J., Bose, P. K., Eriksson, P. G., Banerjee, S., Sarkar, S., Altermann, W., and Catuneanu O., 2007. *Atlas of Microbial Mat Features Preserved within the Siliciclastic Rock Record*. Amsterdam: Elsevier, 311 p.
- Stal, L. J., 2000. Cyanobacterial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Amsterdam: Kluwer, pp. 61–120.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine Geology*, **113**, 3–25.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Iron Sulfide Formation](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Sulfate-Reducing Bacteria](#)
[Tidal Flats](#)

METAGENOMICS

Wolfgang Liebl
 Technische Universität München,
 Freising-Weihenstephan, Germany

Definition

Metagenomics is a research discipline that uses molecular biology methods to acquire, analyze, and interpret sequence data and/or functional data from the metagenomes (the collectivity of genomes) present in the environmental samples, without the need to cultivate the organisms in the samples. Related methodologies are so-called metatranscriptomics and metaproteomics that focus on the collective transcriptomes and proteomes, respectively, rather than on the genomes.

Introduction

Microorganisms constitute major life forms on earth with respect to cell numbers and total biomass, and fulfill essential functions in global element cycles. It has been estimated that the earth's biosphere harbors at least two to three orders of magnitude more prokaryotic cells ($4\text{--}6 \times 10^{30}$) than the number of plant and animal cells combined. The total carbon fixed in prokaryotic cells amounts to $3.5\text{--}5.5 \times 10^{17}$ g of C which is 60–100% of the estimated total carbon in plants (Whitman et al., 1998).

For many decades since the beginnings of microbiology, research in this field has depended on the isolation and cultivation of individual microorganisms (best illustrated for the area of medical microbiology with the work of Robert Koch on the isolation and characterization of pathogenic bacteria in monocultures, leading to the Koch's postulates) or well-defined syntrophic consortia, and the subsequent in-depth analysis of pure cultures. Until today, microbiological research with traditional cultivation and

- microbial mat from Lake Chiprana, Spain. *Environmental Microbiology*, **9**, 2007–2024.
- Revsbech, N. P., 1983. In situ measurement of oxygen profiles of sediments by use of oxygen microelectrodes. In Gnaiger, E., and Forstner, H. (eds.), *Polarographic Oxygen Sensors: Aquatic and Physiological Applications*. Berlin: Springer, pp. 265–273.
- Revsbech, N. P., and Jørgensen, B. B., 1986. Microelectrodes: their use in microbial ecology. *Advances in Microbial Ecology*, **9**, 293–352.
- Revsbech, N. P., and Ward, D. M., 1983. Oxygen microelectrode that is insensitive to medium chemical composition: use in an acid microbial mat dominated by *Cyanidium caldarium*. *Applied and Environmental Microbiology*, **45**, 755–759.
- Revsbech, N. P., Jørgensen, B. B., Blackburn, T. H., and Cohen, Y., 1983. Microelectrode studies of the photosynthesis and O₂, H₂S and pH profiles of a microbial mat. *Limnology and Oceanography*, **28**, 1062–1074.
- Revsbech, N. P., Madsen, B., and Jørgensen, B. B., 1986. Oxygen production and consumption in sediments determined at high spatial resolution by computer simulation of oxygen microelectrode data. *Limnology and Oceanography*, **31**, 293–304.
- Røy, H., Lee, J. S., Jansen, S., and de Beer, D., 2007. Tide-driven deep pore-water flow in intertidal sand flats. *Limnology and Oceanography*, **53**, 1521–1530.
- Santegoeds, C. M., Ferdelman, T. G., Muyzer, G., and de Beer, D., 1998. Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. *Applied and Environmental Microbiology*, **64**, 3731–3739.
- Schramm, A., Larsen, L. H., Revsbech, N. P., Ramsing, N. B., Amann, R., and Schleifer, K. H., 1996. Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. *Applied and Environmental Microbiology*, **62**, 4641–4647.
- Schramm, A., de Beer, D., van den Heuvel, J. C., Ottengraf, S. P. P., and Amann, R., 1999. Microscale distribution of populations and activities of *Nitrosospora* and *Nitrospira* spp. along a macroscale gradient in a nitrifying bioreactor: quantification by in situ hybridisation and the use of microsenors. *Applied and Environmental Microbiology*, **65**, 3690–3696.
- Schreiber, F., Polerecky, L., and de Beer, D., 2008. Nitric oxide microsensor for high spatial resolution measurements in biofilms and sediments. *Analytical Chemistry*, **80**, 1152–1158.
- Sorensen, J., Jørgensen, B. B., and Revsbech, N. P., 1981. A comparison of oxygen, nitrate, and sulfate respiration in coastal marine sediments. *Microbial Ecology*, **5**, 105–115.
- Sweerts, J.-P. R. A., 1990. Oxygen consumption processes, mineralization and nitrogen cycling at the sediment-water interface of North temperate lakes, University of Amsterdam, 136 pp.
- Sweerts, J.-P. R. A., and de Beer, D., 1989. Microelectrode measurements of nitrate gradients in the littoral and profundal sediments of a meso-eutrophic lake (lake Vechten, The Netherlands). *Applied and Environmental Microbiology*, **55**, 754–757.
- Sweerts, J.-P. R. A., de Beer, D., Nielsen, L. P., Verdouw, H., van den Heuvel, J. C., Cohen, Y., and Cappenberg, T. E., 1990. Denitrification by sulphur oxidizing *Beggiatoa* spp. mats on freshwater sediments. *Nature*, **344**, 762–763.
- Thomas, R. C., 1978. *Ion-Sensitive Intracellular Microelectrodes, How to Make and Use Them*. New York: Academic.
- Tsien, R. Y., and Rink, T. J., 1981. Ca²⁺-selective electrodes: a novel PVC-gelled neutral carrier mixture compared with other currently available sensors. *Journal of Neuroscience Methods*, **4**, 73–86.
- Weber, M., Faerber, P., Meyer, V., Lott, C., Eickert, G., Fabricius, K. E., and de Beer, D., 2007. In situ applications of a new diver-operated motorized microsensor profiler. *Environmental Science and Technology*, **41**, 6210–6215.
- Wenzhöfer, F., and Glud, R. N., 2002. Benthic carbon mineralization in the Atlantic: a synthesis based on in situ data from the last decade. *Deep-sea Research I*, **49**, 1255–1279.
- Wenzhöfer, F., Adler, M., Kohls, O., Hensen, C., Strotmann, B., Boehme, S., and Schulz, H. D., 2001a. Calcite dissolution driven by benthic mineralization in the deep-sea: in situ measurements of Ca²⁺, pH, pCO₂ and O₂. *Geochimica et Cosmochimica Acta*, **65**, 2677–2690.
- Wenzhöfer, F., Holby, O., and Kohls, O., 2001b. Deep penetrating oxygen profiles measured in situ by oxygen optodes. *Deep-sea Research I*, **48**, 1741–1755.
- Werner, U., Billerbeck, M., Polerecky, L., Franke, U., Huettel, M., van Beusekom, J. E. E., and de Beer, D., 2006a. Spatial and temporal patterns of mineralization rates and oxygen distribution in a permeable intertidal sand flat (Sylt, Germany). *Limnology and Oceanography*, **51**, 2549–2563.
- Werner, U., Bird, P., Wild, C., Ferdelman, T. G., Polerecky, L., Eickert, G., Jonstone, R., Hoegh-Guldberg, O., and de Beer, D., 2006b. Spatial patterns of aerobic and anaerobic mineralization rates and oxygen penetration dynamics in coral reef sediments (Heron Island, Australia). *Marine Ecology. Progress Series*, **309**, 93–105.
- Wieland, A., and Kuehl, M., 2000. Irradiance and temperature regulation of oxygenic photosynthesis and O₂ consumption in a hypersaline cyanobacterial mat (Solar Lake, Egypt). *Marine Biology*, **137**, 71–85.
- Wieland, A., Zopfi, J., Benthien, M., and Kühl, M., 2005. Biogeochemistry of an iron-rich hypersaline microbial mat (Camargue, France). *Microbial Ecology*, **49**, 34–49.
- Ziebis, W., Pillen, T., and Unger, B., 1998. A diver observatory for in situ studies in sublittoral sediments. *Journal of the Society for Underwater Technology*, **23**, 63–69.

Cross-references

[Biofilms](#)
[Biofilms and Fossilization](#)
[Cyanobacteria](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Hypersaline Environments](#)
[Microbial Biomineralization](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Photosynthesis](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Sulfur Cycle](#)

MOLAR-TOOTH STRUCTURE

Brian R. Pratt
 University of Saskatchewan, Saskatoon, SK, Canada

Definition

Molar-tooth structure is a synsedimentary, combined deformation and early diagenetic feature occurring in calcareous strata of mainly Precambrian age. It consists of arrays of closely spaced, sharply defined, upright veins, and subordinate horizontal sheets and spheroids composed of calcite microspar. Veins are vertically to obliquely oriented, discontinuous, typically strongly squashed or crumpled, and often brecciated.

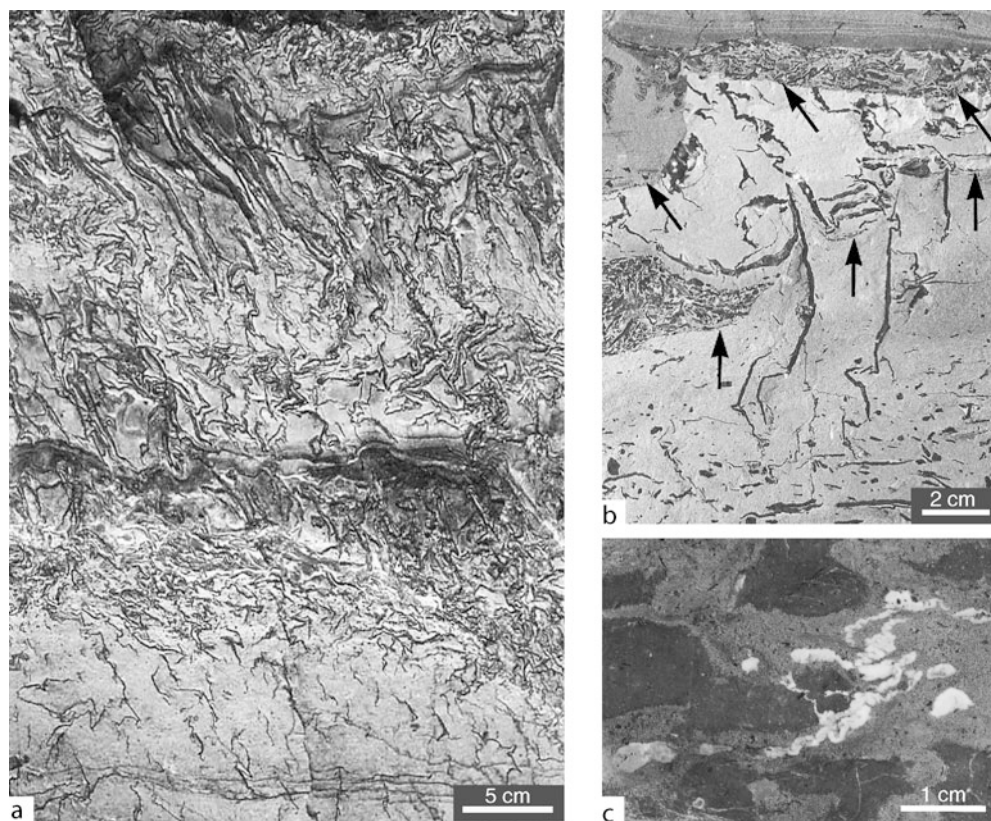
Geological age

Molar-tooth structure (MTS) was first named in 1885 from its appearance on limestone bedding planes in the Mesoproterozoic Purcell Supergroup (= Belt Supergroup in USA.) along the border between southwestern Canada and adjacent northwestern Montana. Almost all occurrences are in Mesoproterozoic to late Neoproterozoic (Ediacaran) strata, representing a 1 billion-year time span from about 1,600 to 600 Ma, but there are several examples noted from the Paleoproterozoic and one from the Neoarchean (James et al., 1998; Pratt, 1998b; Shields, 2002; Meng and Ge, 2002). Importantly, a few Phanerozoic cases have also been reported (Figure 1c; Pratt, 1982; Rossetti, 2000).

Geobiological implications

Organisms and organic matter have been alleged to be implicated in MTS formation in various ways. Its rarity

after the Neoproterozoic has been ascribed generally to substrate modification by animals. However, the drastic reduction of MTS occurrences took place before the known appearance of infaunal invertebrates and, instead, Shields (2002) saw the decline as due to changes in the chemistry of seawater, especially a decrease in CaCO_3 saturation caused by a drop in atmospheric pCO_2 . There may have been an evolutionary control on the shape and mineralogy of lime mud, leading to the precipitation of granular lime mud which was thus prone to fluidization (Pratt, 1998b, 2001). Pratt (1998b) postulated that the rheology of carbonate sediment altered with the changing nature of organic matter and the microbiota brought about by the “Cambrian explosion.” Biomarkers in late Neoproterozoic examples represent cyanobacterial, anaerobic bacterial, and eukaryotic algal precursors in the sediment (Kuang et al., 2004). According to some interpretations, MTS was originally void space created by CO_2 generated by the biodegradation of organic matter



Molar-tooth Structure, Figure 1 (a) Lime mudstone riddled with molar-tooth structure (MTS) veins (dark): initially sparse and upright, passing upward to dense and strongly oblique, thence, in the upper two thirds, to upright but markedly crumpled and tilted. George Formation, Muskwa Group (~1.6 Ga), northern British Columbia. (b) Mudstone with three generations of MTS and erosion surfaces. First-generation veins in lower part are fragmented and reworked while second-generation veins are upright. Lowest erosion surface (vertical arrows) is cusate around upright veins, with vein fragments collected in gutter at left. Overlying mudstone contains third-generation veins that are variably oriented. Second erosion surface (oblique arrows) truncates veins; vein fragments are collected in gutter at left and on horizontal surface at right. Overlying cross-laminated grainstone contains a fourth generation of rare upright veins. George Formation. (c) Wackestone (dolomitic areas light gray) with crumpled veins (whitish). Coronach Member, Herald Formation Red River Group (late Ordovician), 14-26-6-11W2 (2,565.5 m), southern Saskatchewan (see El Taki and Pratt, 2009).

(Furniss et al., 1998; Pollock et al., 2006) or by CO₂-clathrate destabilization (Marshall and Anglin, 2004). James et al. (1998) suggested that a surface microbial mat sealed the sediment. If not physicochemical, precipitation of CaCO₃ crystals in the veins (Pollock et al., 2006) or as sediment grains before being fluidized (Pratt, 1998b, 2001) may have been triggered by dissolved organic molecules, nucleated on organic particles, or induced by photosynthesis.

Shape

MTS occurs mostly in both clean and argillaceous subtidal lime mudstones (Figures 1a–c, 2a and b), but a few are in shales (Figure 2c). Individual vein arrays crosscut up to ~0.5 m of sedimentary bedding; locally, they can comprise more than 50% of the host rock. Veins are discontinuous, range from fairly uniform to variable in width up to ~5 mm along their length, and typically exhibit tapered terminations. Although more or less straight to gently curved in some beds, mostly veins are strongly squashed or crumpled. The whole array may be tilted in one direction. On bedding planes, veins describe variably continuous subparallel, crisscrossing, or irregularly reticulate lines. The upper and lower surfaces of horizontal veins match, indicating that they are dilatational sheet cracks. Crudely spheroidal blobs are up to ~1 cm across. Two crosscutting episodes of vein formation are common, and they typically show differing degrees of deformation. In some argillaceous and silty units, as many as four consecutive phases of vein generation can be delineated, with each containing progressively less calcite and more

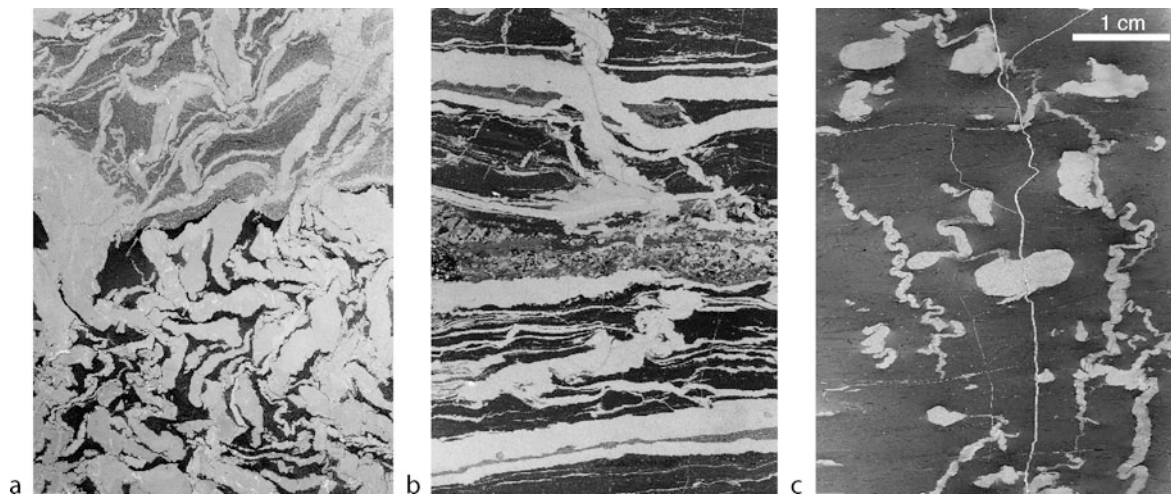
terrigenous particles, and consequently appearing less and less distinct from the matrix.

The matrix usually shows soft-sediment deformation especially in the form of small-scale folding. Typically, the matrix along with the enclosed MTS was deformed plastically together. Veins may be dislocated and the segments shingled in both vertical and horizontal directions, but they are also often stretched, boudinaged, or smeared. In many cases, a subsequent event caused the still plastic matrix to be deformed around veins that had become stiff due to calcite cementation, which also resulted in brecciation of the veins. After consolidation of the matrix, a further phase of deformation caused small mode I cracks in the veins but little displacement. These features are evidence for variably directed compressional, tensile and shear stresses that were imposed often repeatedly during the separate rheological evolution of both components.

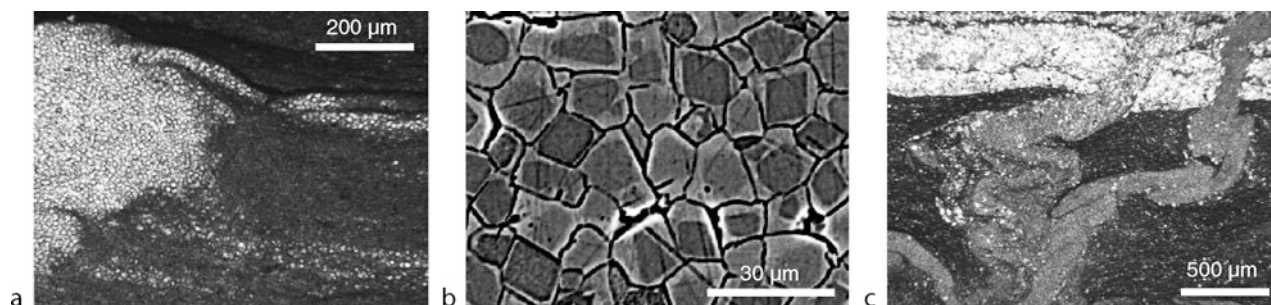
Seafloor erosion affected many beds with MTS. The more resistant veins commonly protruded above the substrate, and vein segments and fragments were winnowed out and concentrated into interbedded intraclastic grainstones (Figures 1b and 2b). In a so far unique case, cross-lamination in vein fills indicates that they were exhumed and reworked by waves before cementation (Bishop and Sumner, 2006).

Composition

Locally MTS consists of micrite. However, in most cases it is composed of interlocking, equant, inclusion-free microspar crystals 7–15 µm across (Figure 3a). Back-scattered electron microscopy and cathode-luminescence reveal that in some examples these crystals consist of



Molar-tooth Structure, Figure 2 Thin section photomicrographs of molar-tooth structure (MTS) (light gray); scale bar same for all. (a) C_{org}-rich mudstone with densely packed squashed and broken veins, overlain by distorted, laminated mudstone with tilted and crumpled upright and subhorizontal veins. George Formation. (b) Dolomitic mudstone with two crosscutting generations of horizontal and squashed upright veins. In middle, grainstone lamina (composed of micrite peloids and grains of MTS) is crossed by veins and part of its interparticle porosity is filled with microspar. I-5 unit, Atar Group (~1.1 Ga), Mauritania. (c) Slightly calcareous shale with spheroidal and crumpled upright veins. (Vertical crack in center is an artifact.) Chamberlain Formation, Belt Supergroup (1.45 Ga), central Montana.



Molar-tooth Structure, Figure 3 (a) Thin section photomicrograph of calcite microspar in pod-shaped molar-tooth structure (MTS) and smeared, variably distinct, horizontal veins, and stringers; isolated microspar grains are also dispersed in shale matrix. Chamberlain Formation. (b) Back-scattered electron photomicrograph showing microspar mosaic with euhedral mud-sized cores (dark gray). Cores are rhombs and less commonly multifaceted crystals. Monteville Formation, Transvaal Supergroup (~2.6 Ga), South Africa (see Bishop and Sumner, 2006, fig. 9C; photograph courtesy of J. W. Bishop). (c) Thin section photomicrograph of squashed and shingled veins crossing argillaceous lime mudstone overlain by sandstone. Veins contain quartz silt and very fine-grained sand (whitish grains near the sandstone and sourced from it; those elsewhere are authigenic quartz crystals). Helena Formation, Belt Supergroup, northwestern Montana.

well-sorted rhombs and multifaceted euhedra with calcite cement overgrowths (Figure 3b; Pollock et al., 2006; Bishop and Sumner, 2006). These cores may be the original mud particles or a product of syndimentary recrystallization. Similar grains occur in the matrix mudstone or shale. Microspar in MTS is typically uniform in size but locally there is variation that describes crude wall-parallel lamination; indistinct shear veins or cracks can be detected in some examples. In some units the MTS is recrystallized to pseudospar.

Where veins penetrate interbedded siltstone or sandstone layers the microspar may contain silt or fine sand plus minor amounts of clay (Figure 3c; Pratt, 1998b, 1999; Bishop and Sumner, 2006). MTS may grade into dikelets and thin sills of injected silt and sand of the kind that have been termed “syneresis” cracks (Pratt, 1998a; cf. Tanner, 1998; Shi et al., 2009).

The calcite in MTS exhibits $\delta^{13}\text{C}_{\text{PDB}}$ values in the range of about -1.5‰ to 3.5‰ , which are similar to those in co-occurring sedimentary carbonate particles (Frank and Lyons, 1998; Pratt, 1998b; Marshall and Anglin, 2004; Bishop et al., 2006; Kuang et al., 2007).

Formation

For more than a century, the enigmatic nature of MTS was tolerated but largely ignored. Because fragments of MTS comprise sedimentary particles, it was eventually recognized as having formed intrastratally just below the sediment–water interface. Some geologists presumed MTS to be a fossil algal or microbial object. Later it was accepted as a deformation feature involving substantial shrinkage of the host sediment – although not due to desiccation because there is no evidence for associated subaerial exposure.

Pore-lining cement fabrics are absent in MTS even though they are present in co-occurring grainstones. Nevertheless, it is still debated whether or not MTS was initially a system of cavities, in which case CaCO_3 precipitated later as tiny crystals inside them (Fairchild

et al., 1997; Furniss et al., 1998; Pollock et al., 2006; Bishop and Sumner, 2006; Bishop et al., 2006). The presence in MTS of sand, silt, and clay impurities as suspended particles, clusters of grains, or seams is difficult to reconcile with this interpretation.

The possibility that MTS began as voids forced open by bubbles of CO_2 passively generated by the oxidation of organic matter in the sediments is unlikely because of the extreme amount of organic matter required, the shallow-water, oxidizing depositional setting, the linear to reticulate shape of vein arrays, the evidence for concomitant sand and silt injection, and the complex nature of the stresses demonstrated by accompanying physical deformation (Pratt, 1999). Because of the tropical sedimentary environment of host limestones, the involvement of CO_2 -clathrates is implausible. In most units, the absence of closely associated high-energy features is further evidence against the possibility of void opening by wave-induced loading (Bishop et al., 2006).

Pratt (1998b) explained MTS as a seismically induced deformation feature of calcareous sediment possessing unique geotechnical properties for which modern analogues are lacking. By this model, cyclic loading during earthquakes caused elevated fluid pressure and loss of shear strength in the muddy sediment. During abrupt dewatering, veins, sheet cracks, and spheroidal pockets were jacked open by the injection of granular lime mud that was segregated from the sediment. Examples where more than 50% of limestones consist of MTS indicate that a substantial proportion of the lime mud was mobilized. However, shaly host rocks still contain poorly defined seams and residual disseminated calcite grains that did not find their way into veins. Incorporated sand and silt show that dewatering and injection were both downwardly and upwardly directed. Spheroids formed where dewatering was localized and fluids did not escape to the surface. All this caused a striking shrinkage of the matrix and compaction of upright veins. As shaking proceeded,

they were squeezed, sheared, and stretched in different directions. After cementation of the mud infill, subsequent events deformed the matrix again, generated more veins, and brecciated the first set of veins, until the sediment was too stiff to deform further. Indeed, in almost every case, distortion of MTS and the matrix testifies to differential rheologies and progressive evolution from liquefaction through plastic deformation to brittle failure, as would be expected if affected by repeated earthquakes during dewatering and early diagenesis. A similar seismic model has been invoked for sand and silt dykelets (syneresis cracks), except that synsedimentary cementation was not involved (Pratt, 1998a).

Bibliography

- Bishop, J. W., and Sumner, D. Y., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. I: constraints on microcrystalline CaCO_3 precipitation. *Sedimentology*, **53**, 1049–1058.
- Bishop, J. W., Sumner, D. Y., and Huerta, N. J., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. II: a wave-induced fluid flow model. *Sedimentology*, **53**, 1069–1082.
- El Taki, H., and Pratt, B. R., 2009. Synsedimentary deformation in laminated dolomites and evaporites of the Herald Formation (Red River Group): signature of Late Ordovician tectonic activity in southern Saskatchewan. In Summary of investigations 2009, Vol. 1. Saskatchewan Geological Survey, Saskatchewan Ministry of Energy and Resources, Miscellaneous Report 2009-4.1, Paper A-3, p. 10.
- Fairchild, I. J., Einsele, G., and Song, T., 1997. Possible seismic origin of molar tooth structures in Neoproterozoic carbonate ramp deposits, North China. *Sedimentology*, **44**, 611–636.
- Frank, T. D., and Lyons, T. W., 1998. “Molar-tooth” structures: a geochemical perspective on a Proterozoic enigma. *Geology*, **26**, 683–686.
- Furniss, G., Rittel, J. F., and Winston, D., 1998. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, Western Montana. *Journal of Sedimentary Research*, **68**, 104–114.
- James, N. P., Narbonne, G. M., and Sherman, A. G., 1998. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic. *Journal of Sedimentary Research*, **68**, 716–722.
- Kuang, H., Li, Y., Zeng, Y., Meng, X., and Ge, M., 2004. Biomarkers in the molar tooth (MT)-bearing limestones in the Jilin–Liaoning area of China. *Chinese Journal of Geochemistry*, **23**, 334–341.
- Kuang, H., Liu, Y., Meng, X., and Ge, M., 2007. A study on the environmental conditions of the microsparite (molar-tooth) carbonates. *Chinese Journal of Geochemistry*, **26**, 28–34.
- Marshall, D., and Anglin, C. D., 2004. CO_2 -clathrate destabilization: a new model of formation for molar tooth structures. *Precambrian Research*, **129**, 325–341.
- Meng, X., and Ge, M., 2002. The sedimentary features of Proterozoic microspar (molar-tooth) carbonates in China and their significance. *Episodes*, **25**, 185–195.
- Pollock, M. D., Kah, L. C., and Bartley, J. K., 2006. Morphology of molar-tooth structures in Precambrian carbonates: influence of substrate rheology and implications for diagenesis. *Journal of Sedimentary Research*, **76**, 310–323.
- Pratt, B. R., 1982. Limestone response to stress: pressure solution and dolomitization—discussion and examples of compaction in carbonate sediments. *Journal of Sedimentary Petrology*, **52**, 323–327.
- Pratt, B. R., 1998a. Syneresis cracks: subaqueous shrinkage in argillaceous sediments caused by earthquake-induced dewatering. *Sedimentary Geology*, **117**, 1–10.
- Pratt, B. R., 1998b. Molar-tooth structure in Proterozoic carbonate rocks: origin from synsedimentary earthquakes, and implications for the nature and evolution of basins and marine sediment. *Geological Society of America Bulletin*, **110**, 1028–1045.
- Pratt, B. R., 1999. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, western Montana—Discussion. *Journal of Sedimentary Research*, **68**, 1136–1140.
- Pratt, B. R., 2001. Oceanography, bathymetry and syndepositional tectonics of a Precambrian intracratonic basin: integrating sediments, storms, earthquakes and tsunamis in the Belt Supergroup (Helena Formation, ca. 145 Ga), Western North America. *Sedimentary Geology*, **141–142**, 371–394.
- Rossetti, D. F., 2000. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic—Discussion. *Journal of Sedimentary Research*, **70**, 1246–1248.
- Shi, X. Y., Jiang, G. Q., Zhang, C. H., Gao, L. Z., and Liu, J., 2009. Sand veins and MISS from the Mesoproterozoic black shale (ca. 1.7 Ga) in North China: implications for methane degassing from microbial mats. *Science in China Series D: Earth Sciences*, **51**, 1525–1536.
- Shields, G. A., 2002. ‘Molar-tooth microspar’: a chemical explanation for its disappearance ~750 Ma. *Terra Nova*, **14**, 108–113.
- Tanner, P. W. G., 1998. Interstratal dewatering origin for polygonal patterns of sand-filled cracks: a case study of late Proterozoic metasediments of Islay, Scotland. *Sedimentology*, **45**, 71–89.

Cross-references

Calcite Precipitation, Microbially Induced
Carbonate Environments
Carbonates
Deep Fluids
Microbialites, Modern

MOONMILK

Joachim Reitner
University of Göttingen, Göttingen, Germany

Synonyms

Bergmilch; Lac lunae; Lac montanum; Mannmilch; Mondmilch; Montmilch; Mundmilch

Definition

Moonmilk is a carbonate deposit that occurs within various subterranean systems. Moonmilk has a white to gray color and, in contrast to rigid cave deposits (*speleothems*) such as stalactites and stalagmites, exhibits a soft, muddy texture of microcrystalline aggregates. These aggregates are mainly composed of calcite, and to a lesser extent of aragonite, monohydrocalcite, hydromagnesite, sulfates, and nitrates (Martínez-Arkarazo et al., 2007; Richter et al., 2008; Cañaveras et al., 2006; Borsato et al., 2000). The calcite shows an aragonite-like needle form (“lublinite”), which is normally associated

they were squeezed, sheared, and stretched in different directions. After cementation of the mud infill, subsequent events deformed the matrix again, generated more veins, and brecciated the first set of veins, until the sediment was too stiff to deform further. Indeed, in almost every case, distortion of MTS and the matrix testifies to differential rheologies and progressive evolution from liquefaction through plastic deformation to brittle failure, as would be expected if affected by repeated earthquakes during dewatering and early diagenesis. A similar seismic model has been invoked for sand and silt dykelets (syneresis cracks), except that synsedimentary cementation was not involved (Pratt, 1998a).

Bibliography

- Bishop, J. W., and Sumner, D. Y., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. I: constraints on microcrystalline CaCO_3 precipitation. *Sedimentology*, **53**, 1049–1058.
- Bishop, J. W., Sumner, D. Y., and Huerta, N. J., 2006. Molar tooth structures of the Neoproterozoic Monteville Formation, Transvaal Supergroup, South Africa. II: a wave-induced fluid flow model. *Sedimentology*, **53**, 1069–1082.
- El Taki, H., and Pratt, B. R., 2009. Synsedimentary deformation in laminated dolomites and evaporites of the Herald Formation (Red River Group): signature of Late Ordovician tectonic activity in southern Saskatchewan. In Summary of investigations 2009, Vol. 1. Saskatchewan Geological Survey, Saskatchewan Ministry of Energy and Resources, Miscellaneous Report 2009-4.1, Paper A-3, p. 10.
- Fairchild, I. J., Einsele, G., and Song, T., 1997. Possible seismic origin of molar tooth structures in Neoproterozoic carbonate ramp deposits, North China. *Sedimentology*, **44**, 611–636.
- Frank, T. D., and Lyons, T. W., 1998. “Molar-tooth” structures: a geochemical perspective on a Proterozoic enigma. *Geology*, **26**, 683–686.
- Furniss, G., Rittel, J. F., and Winston, D., 1998. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, Western Montana. *Journal of Sedimentary Research*, **68**, 104–114.
- James, N. P., Narbonne, G. M., and Sherman, A. G., 1998. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic. *Journal of Sedimentary Research*, **68**, 716–722.
- Kuang, H., Li, Y., Zeng, Y., Meng, X., and Ge, M., 2004. Biomarkers in the molar tooth (MT)-bearing limestones in the Jilin–Liaoning area of China. *Chinese Journal of Geochemistry*, **23**, 334–341.
- Kuang, H., Liu, Y., Meng, X., and Ge, M., 2007. A study on the environmental conditions of the microsparite (molar-tooth) carbonates. *Chinese Journal of Geochemistry*, **26**, 28–34.
- Marshall, D., and Anglin, C. D., 2004. CO_2 -clathrate destabilization: a new model of formation for molar tooth structures. *Precambrian Research*, **129**, 325–341.
- Meng, X., and Ge, M., 2002. The sedimentary features of Proterozoic microspar (molar-tooth) carbonates in China and their significance. *Episodes*, **25**, 185–195.
- Pollock, M. D., Kah, L. C., and Bartley, J. K., 2006. Morphology of molar-tooth structures in Precambrian carbonates: influence of substrate rheology and implications for diagenesis. *Journal of Sedimentary Research*, **76**, 310–323.
- Pratt, B. R., 1982. Limestone response to stress: pressure solution and dolomitization—discussion and examples of compaction in carbonate sediments. *Journal of Sedimentary Petrology*, **52**, 323–327.
- Pratt, B. R., 1998a. Syneresis cracks: subaqueous shrinkage in argillaceous sediments caused by earthquake-induced dewatering. *Sedimentary Geology*, **117**, 1–10.
- Pratt, B. R., 1998b. Molar-tooth structure in Proterozoic carbonate rocks: origin from synsedimentary earthquakes, and implications for the nature and evolution of basins and marine sediment. *Geological Society of America Bulletin*, **110**, 1028–1045.
- Pratt, B. R., 1999. Gas bubble and expansion crack origin of “molar-tooth” calcite structures in the middle Proterozoic Belt Supergroup, western Montana—Discussion. *Journal of Sedimentary Research*, **68**, 1136–1140.
- Pratt, B. R., 2001. Oceanography, bathymetry and syndepositional tectonics of a Precambrian intracratonic basin: integrating sediments, storms, earthquakes and tsunamis in the Belt Supergroup (Helena Formation, ca. 145 Ga), Western North America. *Sedimentary Geology*, **141–142**, 371–394.
- Rossetti, D. F., 2000. Molar-tooth carbonates: shallow subtidal facies of the mid- to late Proterozoic—Discussion. *Journal of Sedimentary Research*, **70**, 1246–1248.
- Shi, X. Y., Jiang, G. Q., Zhang, C. H., Gao, L. Z., and Liu, J., 2009. Sand veins and MISS from the Mesoproterozoic black shale (ca. 1.7 Ga) in North China: implications for methane degassing from microbial mats. *Science in China Series D: Earth Sciences*, **51**, 1525–1536.
- Shields, G. A., 2002. ‘Molar-tooth microspar’: a chemical explanation for its disappearance ~750 Ma. *Terra Nova*, **14**, 108–113.
- Tanner, P. W. G., 1998. Interstratal dewatering origin for polygonal patterns of sand-filled cracks: a case study of late Proterozoic metasediments of Islay, Scotland. *Sedimentology*, **45**, 71–89.

Cross-references

Calcite Precipitation, Microbially Induced
Carbonate Environments
Carbonates
Deep Fluids
Microbialites, Modern

MOONMILK

Joachim Reitner
University of Göttingen, Göttingen, Germany

Synonyms

Bergmilch; Lac lunae; Lac montanum; Mannmilch; Mondmilch; Montmilch; Mundmilch

Definition

Moonmilk is a carbonate deposit that occurs within various subterranean systems. Moonmilk has a white to gray color and, in contrast to rigid cave deposits (*speleothems*) such as stalactites and stalagmites, exhibits a soft, muddy texture of microcrystalline aggregates. These aggregates are mainly composed of calcite, and to a lesser extent of aragonite, monohydrocalcite, hydromagnesite, sulfates, and nitrates (Martínez-Arkarazo et al., 2007; Richter et al., 2008; Cañaveras et al., 2006; Borsato et al., 2000). The calcite shows an aragonite-like needle form (“lublinite”), which is normally associated

with soil bacteria. The crystal needles have a diameter of about 0.1 μm and a length of ca. 8–10 μm . The name “moonmilk – Mondmilch” is derived from the name of a cave, “Mondmilchloch,” located at the Pilatus mountain (Emmental Alps, Switzerland) (Fischer, 1988).

Geobiological implications

Moonmilk differs in many aspects from the traditional cave carbonates such as stalagmites and stalactites (Moore and Sullivan, 1997; Bögli, 1978). Its formation is strongly influenced by microbial activity, as suggested by the presence of microbial aggregates, biofilms, and/or fungal mycelia (Cañaveras et al., 1999, 2001, 2006; Borsato et al., 2000; Gadd, 2004; Burford et al., 2003). Moonmilk is a result of (1) microbially mediated weathering and *microbiocorrosion* of carbonate host rock under highly humid conditions and (2) microbially mediated reprecipitation of various carbonate minerals. After dissolution of the mineral components, the residue exhibits high amounts of organic remains. Besides many fungal and actinomycetes, the bacterium *Macromonas bipunctata* plays a major role in the formation of moonmilk. *M. bipunctata* is a lophotrichous, heterotrophic and strictly aerobic betaproteobacterium that has been classified as a colorless sulfur bacterium capable of oxidizing sulfide (Dubinia and Grabovich, 1984). Within *extracellular polymeric substances (EPS)* of *M. bipunctata* and further microbes, calcite seed nuclei are formed to precipitate “lubinite” moonmilk crystals. *M. bipunctata* was originally described as *Pseudomonas bipunctata* by Gicklhorn (1920) and renamed by Utermöhl and Koppe (1924).

Bibliography

- Bögli, A., 1978. *Karsthydrographie und physische Speläologie*. Springer, Berlin/Heidelberg/New York.
- Borsato, A., Frisia, S., Jones, B., and Van den Borg, K., 2000. Calcite moonmilk: crystal morphology and environment of formation in caves in Italian alps. *Journal of Sedimentary Research*, **70**, 1179–1190.
- Burford, E. P., Fomina, M., and Gadd, G., 2003. Fungal involvement in bioweathering and biotransformations of rocks and minerals. *Mineralogical Magazine*, **67**(6), 1172–1155.
- Cañaveras, J. C., Hoyos, M., Sanchez-Moral, S., Sanz-Rubio, E., Bedoya, J., Soler, V., Groth, I., Schumann, P., Laiz, L., Gonzalez, I., and Saiz-Jimenez, C., 1999. Microbial communities associated with hydromagnesite and needle-fiber aragonite deposits in a Karstic cave (Altamira, Northern Spain). *Geomicrobiology Journal*, **16**, 9–25.
- Cañaveras, J. C., Sanchez-Moral, S., Sloer, V., and Saiz-Jimenez, C., 2001. Microorganisms and microbially induced fabrics in cave walls. *Geomicrobiology Journal*, **18**, 223–240.
- Cañaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J. M., and Saiz-Jimenez, C., 2006. On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften*, **93**, 27–32.
- Dubinia, G. A., and Grabovich, M. Y., 1984. Isolation, cultivation and characteristics of *Macromonas bipunctata*. *Mikrobiologiya*, **53**, 748–755.
- Fischer, H., 1988. Etymologie von Mondmilch bzw. Mondmilchloch. *Zeitschrift der deutschen Geologischen Gesellschaft*, **139**, 155–159.

- Gadd, G. M., 2004. Mycotransformation of organic and inorganic substrates. *Mycologist*, **18**, 60–70.
- Gicklhorn, J., 1920. Über neue farblose Schwefelbakterien.-Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt.2, **50**, 415–427.
- Martínez-Arkarazo, I., Angulo, M., Zuloaga, O., Usobiaga, A., and Madariaga, J. M., 2007. Spectroscopic characterisation of moonmilk deposits in Pozalagua tourist cave (Karrantza, Basque Country, North of Spain). *Spectrochimica Acta Part A*, **68**, 1058–1064.
- Moore, G. W., and Sullivan, N., 1997. *Speleology: Caves and the Cave Environment*. Huntsville: National Speleological Society, p. 176.
- Richter, D., Immenhauser, A., and Neuser, R. D., 2008. Electron backscatter diffraction documents randomly orientated c-axes in moonmilk calcite fibers: evidence for biologically induced precipitation. *Sedimentology*, **55**, 487–497.
- Utermöhl, H., and Koppe, F., 1924. Genus *Macromonas*. In Koppe, F. (ed.), *Die Schlammflora der ostholsteinischen Seen und des Bodensees; Archiv für Hydrobiologie*, **14**, 619–672.

Cross-references

[Biofilms](#)
[Carbonate Environments](#)
[Carbonates](#)
[Karst Ecosystems](#)
[Microbial Mineralisation](#)
[Microbial Mats](#)

MUD MOUNDS

Marta Rodríguez-Martínez
 Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Synonyms

Biodebital mound; Carbonate mounds; Carbonate mud mound; Lime mud mound; Microbial mound; Mudbank; Reef mound; Stromatolite mounds

Definition

Mud mounds are biosedimentary buildups, part of the reef system and are dominated by fine-grained carbonates (up and more than 50% of rock volume), which form heterogeneous polygenetic matrix-supported fabrics (stromatolitic, thrombolitic, leiolitic, fenestral, laminar, reticulate, stromatolite, etc.). These fabrics are composed of both allomicrites and automicrites (abiogenetic, biogenetically induced, and biogenetically controlled). Production and accretion mechanisms in mud mounds are varied and not always clear, but are associated with the activity of microbial benthic communities (in different degrees of participation). Metazoans, being important colonizers, can or cannot occur but never produce a primary skeletal framework. Mud mounds colonized the oceans from the Proterozoic times, and they grow up from deep aphotic basinal settings to shallow water platforms.

with soil bacteria. The crystal needles have a diameter of about 0.1 μm and a length of ca. 8–10 μm . The name “moonmilk – Mondmilch” is derived from the name of a cave, “Mondmilchloch,” located at the Pilatus mountain (Emmental Alps, Switzerland) (Fischer, 1988).

Geobiological implications

Moonmilk differs in many aspects from the traditional cave carbonates such as stalagmites and stalactites (Moore and Sullivan, 1997; Bögli, 1978). Its formation is strongly influenced by microbial activity, as suggested by the presence of microbial aggregates, biofilms, and/or fungal mycelia (Cañaveras et al., 1999, 2001, 2006; Borsato et al., 2000; Gadd, 2004; Burford et al., 2003). Moonmilk is a result of (1) microbially mediated weathering and *microbiocorrosion* of carbonate host rock under highly humid conditions and (2) microbially mediated reprecipitation of various carbonate minerals. After dissolution of the mineral components, the residue exhibits high amounts of organic remains. Besides many fungal and actinomycetes, the bacterium *Macromonas bipunctata* plays a major role in the formation of moonmilk. *M. bipunctata* is a lophotrichous, heterotrophic and strictly aerobic betaproteobacterium that has been classified as a colorless sulfur bacterium capable of oxidizing sulfide (Dubinia and Grabovich, 1984). Within *extracellular polymeric substances (EPS)* of *M. bipunctata* and further microbes, calcite seed nuclei are formed to precipitate “lubinite” moonmilk crystals. *M. bipunctata* was originally described as *Pseudomonas bipunctata* by Gicklhorn (1920) and renamed by Utermöhl and Koppe (1924).

Bibliography

- Bögli, A., 1978. *Karsthydrographie und physische Speläologie*. Springer, Berlin/Heidelberg/New York.
- Borsato, A., Frisia, S., Jones, B., and Van den Borg, K., 2000. Calcite moonmilk: crystal morphology and environment of formation in caves in Italian alps. *Journal of Sedimentary Research*, **70**, 1179–1190.
- Burford, E. P., Fomina, M., and Gadd, G., 2003. Fungal involvement in bioweathering and biotransformations of rocks and minerals. *Mineralogical Magazine*, **67**(6), 1172–1155.
- Cañaveras, J. C., Hoyos, M., Sanchez-Moral, S., Sanz-Rubio, E., Bedoya, J., Soler, V., Groth, I., Schumann, P., Laiz, L., Gonzalez, I., and Saiz-Jimenez, C., 1999. Microbial communities associated with hydromagnesite and needle-fiber aragonite deposits in a Karstic cave (Altamira, Northern Spain). *Geomicrobiology Journal*, **16**, 9–25.
- Cañaveras, J. C., Sanchez-Moral, S., Sloer, V., and Saiz-Jimenez, C., 2001. Microorganisms and microbially induced fabrics in cave walls. *Geomicrobiology Journal*, **18**, 223–240.
- Cañaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzalez, J. M., and Saiz-Jimenez, C., 2006. On the origin of fiber calcite crystals in moonmilk deposits. *Naturwissenschaften*, **93**, 27–32.
- Dubinia, G. A., and Grabovich, M. Y., 1984. Isolation, cultivation and characteristics of *Macromonas bipunctata*. *Mikrobiologiya*, **53**, 748–755.
- Fischer, H., 1988. Etymologie von Mondmilch bzw. Mondmilchloch. *Zeitschrift der deutschen Geologischen Gesellschaft*, **139**, 155–159.

- Gadd, G. M., 2004. Mycotransformation of organic and inorganic substrates. *Mycologist*, **18**, 60–70.
- Gicklhorn, J., 1920. Über neue farblose Schwefelbakterien.-Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abt.2, **50**, 415–427.
- Martínez-Arkarazo, I., Angulo, M., Zuloaga, O., Usobiaga, A., and Madariaga, J. M., 2007. Spectroscopic characterisation of moonmilk deposits in Pozalagua tourist cave (Karrantza, Basque Country, North of Spain). *Spectrochimica Acta Part A*, **68**, 1058–1064.
- Moore, G. W., and Sullivan, N., 1997. *Speleology: Caves and the Cave Environment*. Huntsville: National Speleological Society, p. 176.
- Richter, D., Immenhauser, A., and Neuser, R. D., 2008. Electron backscatter diffraction documents randomly orientated c-axes in moonmilk calcite fibers: evidence for biologically induced precipitation. *Sedimentology*, **55**, 487–497.
- Utermöhl, H., and Koppe, F., 1924. Genus *Macromonas*. In Koppe, F. (ed.), *Die Schlammflora der ostholsteinischen Seen und des Bodensees; Archiv für Hydrobiologie*, **14**, 619–672.

Cross-references

[Biofilms](#)
[Carbonate Environments](#)
[Carbonates](#)
[Karst Ecosystems](#)
[Microbial Mineralisation](#)
[Microbial Mats](#)

MUD MOUNDS

Marta Rodríguez-Martínez
 Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Synonyms

Biodebital mound; Carbonate mounds; Carbonate mud mound; Lime mud mound; Microbial mound; Mudbank; Reef mound; Stromatactis mounds

Definition

Mud mounds are biosedimentary buildups, part of the reef system and are dominated by fine-grained carbonates (up and more than 50% of rock volume), which form heterogeneous polygenetic matrix-supported fabrics (stromatolitic, thrombolitic, leiolitic, fenestral, laminar, reticulate, stromatactoid, etc.). These fabrics are composed of both allomicrites and automicrites (abiogenetic, biogenetically induced, and biogenetically controlled). Production and accretion mechanisms in mud mounds are varied and not always clear, but are associated with the activity of microbial benthic communities (in different degrees of participation). Metazoans, being important colonizers, can or cannot occur but never produce a primary skeletal framework. Mud mounds colonized the oceans from the Proterozoic times, and they grow up from deep aphotic basinal settings to shallow water platforms.

Evolution of the term mud mound

Probably, the core of the Silurian Wabash buildup (Indiana, EEUU) was the first carbonate deposit referred to in the literature as a “carbonate mud mound” and was described as a mound of calcisiltite containing crinoid, bryozoan, ostracode bioclastics, and stromatactis, but essentially no stromatoporoids or corals (Textoris, 1966). The Silurian mud mound facies were compared by Textoris with similar buildups reported from other parts of the world (Devonian of Canada, Carboniferous of Europe and EEUU).

When reefs turned mud mounds

In the 1960s, the lack of a recognizable skeletal framework in these buildups pointed out their differences with the “classical” reef idea. During some years, most works on mud mounds were focused on the study of their scarce fauna and few were mentions to the origin of the carbonate mud, paradoxically the main component. Wilson (1975) extended in his book the definition and denomination of “lime mud mound” to many other Phanerozoic buildups, which were seen as dominated by detrital, bioclastic micrites with minor organic boundstone and *perceived to accumulate both through hydrodynamic processes and in situ organic production*. Thus, during the 1970s, the origin of the matrix was explained by the baffling theory: the carbonate mud, externally produced, was baffled by metazoans (crinoids, bryozoans, sponges, etc.). The role of early submarine cementation was also taken into consideration to explain the stabilization of mud mound depositional slopes. Baffled micrites and subsequent cementation was the proposed origin; thus, mud mounds were compared with very different types of Holocene buildups: shallow-water seagrass mudbanks and deep-water coral lithoherms.

When some mud mounds turned into microbial reefs

From the 1970s to the 1980s, some fabrics and textures present in the mud mounds were related with “cryptalgal” and other biosedimentary structures as stromatolites and thrombolites (Monty, 1976; Pratt, 1982). The mud mound matrix could be now bound, trapped, and precipitated by the activity of microorganisms (“blue-green algae” – cyanophytes and cyanobacteria).

From then, the source or origin of the carbonate mud, the matrix problem, will be the leitmotiv in the study of mud mounds. Bosence et al. (1985), from the recent carbonate mounds from Florida Keys, proposed three mud mound models based on the relation between the external and the internal sediment (carbonate mud) supplies in the mound: export, import, and self-sufficient models, respectively. From the 1970s and clearly in the 1990s, mud mounds began to be seen as “self-sufficient” mounds, and the role of microbial communities in their construction gained more importance (Tsien, 1985; Lees and Miller, 1985; Camoin and Maurin, 1988).

James and Bourque (1992) proposed a conceptual classification of reefs and “mounds,” where mounds are *those structures which were built by smaller, commonly delicate and/or solitary elements in tranquil settings*. They differentiated three types of mounds: “microbial” mounds, “skeletal” mounds, and “mud” mounds. Microbial and skeletal mounds are grouped into “biogenic mounds” because they are organically controlled, whereas the term “mud mound” was restricted by the authors to those *formed by inorganic accumulation of mud with variable amounts of fossils*. The authors pointed out that *the division between biogenic mounds and mud mounds depends on the nature of the accumulation/construction controls, not on the percentage of fossils*.

One of the main outstanding questions concerning the mud mounds is related to the modes of formation of the carbonate mud matrix and their recognition. The increase in the number of studies on microbial carbonates and their formation and fabric classifications (Kennard and James, 1986; Burne and Moore, 1987; Riding, 1991) led to new horizons in the matrix problem. Fabrics and geochemical signatures from automicrites of recent cryptic microbialites and controls in their formation (Reitner, 1993) were found to be similar to those recorded in Cretaceous and Jurassic mud mounds (Neuweiler, 1993; Keupp et al., 1993; Reitner et al., 1995; Leinfelder and Keupp, 1995). Thus, modern automicrites began to be used as modern counterparts of some mud mound end products.

Some authors began to use the term mud mound in a descriptive nongenetic way as *carbonate buildup consisting of more than 50% of mud and/or peloidal mud* (Reitner and Neuweiler, 1995), having depositional relief, and forming part of a polygenetic and continuum spectrum ranging from “biodepitril” to “microbial” mud mounds (Bosence and Bridges, 1995). Pratt (1995) considered mud mounds as ecologic reefs because they possess “rigid frameworks” produced by both microbial activity and syndimentary cementation.

Last formal definition of mud mound has been given by Riding (2002), as *carbonate mud-dominated (micrite and fine-silt) deposits with topographic relief and with few or no stromatolites, thrombolites, or in-place skeletons*. Riding added that *the deposits can be organic and/or inorganic in origin and it can be difficult to distinguish their origins*.

General features

Mud mounds have varied external forms; the term mud mound is used here not in a morphological way due to the fact that not all of them are mounds or dome-shaped; they can be tabular-shaped and can occur as isolated massive bodies or as stacked or amalgamated bodies, spreading over kilometers. The biggest mud mound complex is the Waulsortian mudbank from Ireland.

They could develop as a high syndimentary relief and show depositional dips from 10° up to 50°, can be or cannot be flanked by bioclastic limestones, and grow up from

basinal to platform settings. Mud mounds are composed mainly of fine-grained carbonate-matrix-supported fabrics, which are called, depending on the classification schemes followed, mudstones, wackestones, biomicrites, etc. These carbonate muds are texturally and genetically varied. The presence of cavities can be a characteristic feature in some mud mounds. Their macrobiota are diverse and can be important volumetrically in some mounds, although they never constitute a skeletal framework.

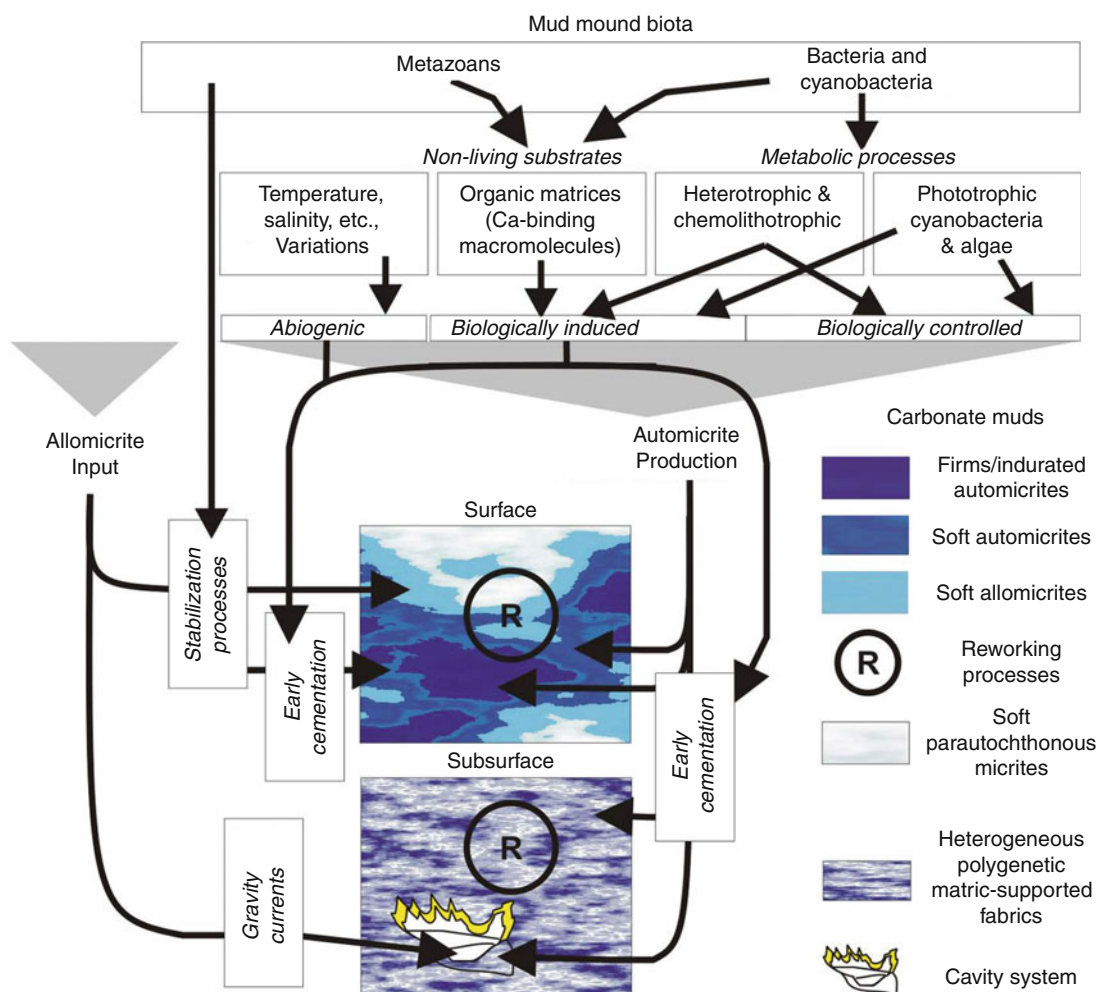
Types of carbonate muds

Carbonate muds are formed by pure aphanitic micrites and/or mixture of silt-sized calcite particles (up to 62 μm) as peloids. Then, they can be texturally grouped into aphanitic and peloidal micrites. Both types occur separately, or there is grading between them; they also occur as neomorphosed microspars. Microstructurally, they appear as dense and/or clotted/grumular/spongiostromic microfabrics, which constitute at mesoscale, stromatolitic,

thrombolitic, fenestral, and/or cryptalgal/cryptomicrobial/leiolitic fabrics (summarized in Riding, 2000).

The carbonate muds can be classified in relation to the *locus* of their production. The muds externally produced and later imported into the mound are named allomicrites (Figure 1). They are stabilized at mound surface by mound biota (eukaryotes and prokaryotes) through baffling, trapping, and/or binding mechanisms and early cementation.

The in situ produced carbonate muds are named automicrites. They are formed through abiogenic, biologically induced, and biologically controlled mechanisms (summarized in Flügel, 2004). The biologically related mechanisms are known as nonenzymatically and enzymatically controlled carbonates. Mud mounds seem to be specially dominated by the nonenzymatically carbonate production of automicrites via nonliving substrates (organomineralization of the organic matter derived from decaying organisms) and/or via metabolic processes (by heterotrophic, chemolithotrophic, and phototrophic bacteria and cyanobacteria). In the first case, the



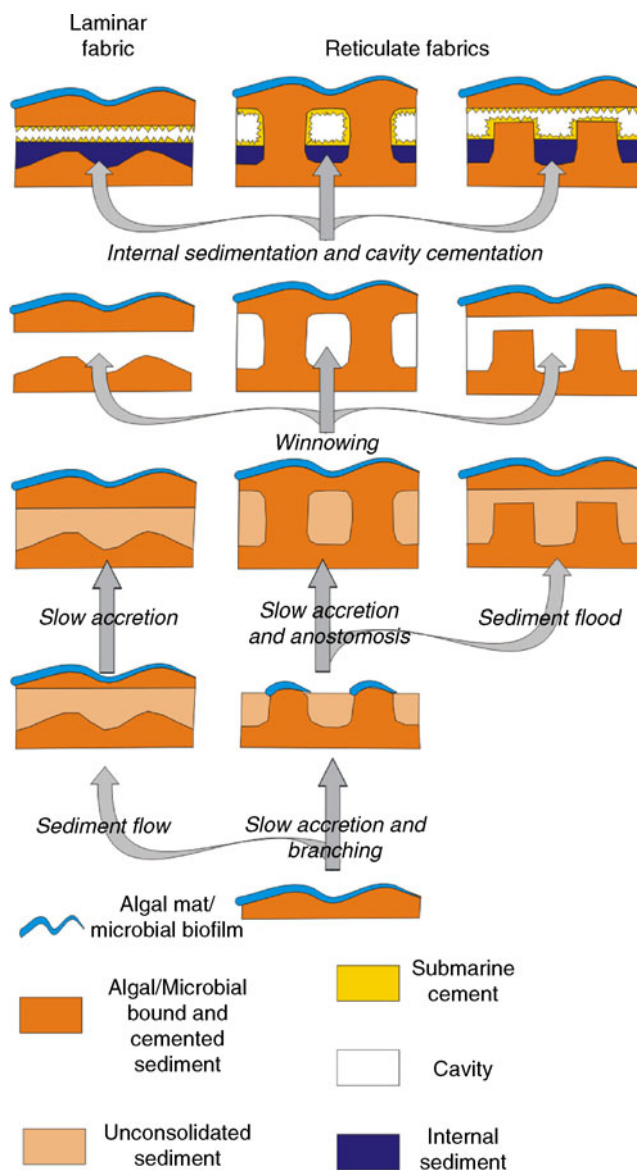
Mud Mounds, Figure 1 Relationships between the mud mound biota and carbonate mud stabilization and production processes.

resulting automicrites are known as organomicrites. The automicrite production occurs at mound surface as well as at subsurface.

The mud mound system comprises both soft (allomicrites and automicrites) as well as firm/early indurated carbonate muds (automicrites), which can suffer from reworking processes (chemical, physical, and/or biological ones) resulting in parautochthonous muds. These secondary muds or the next successive generations of in situ muds, therefore, resemble a mixture of previous ones. This system should be analyzed as a continuous series of processes of automicrite production, allomicrite input, and internal reworking.

The filling of mound cavity system records many of the last mud mound synsedimentary episodes: (1) the entry of allomicrite by gravity or currents, (2) the colonization by cryptic biota, (3) production of automicrites, (4) reworking processes, and (5) the early marine cementation phases as well as burial-related phases.

So mud mounds have polygenetic carbonate mud-dominated fabrics resulting from automicrites production, allomicrites input, and recycling processes (Figure 1). But some microfabrics have been related at the same time with different types of biogenic as well as abiogenic processes, and discriminating between some processes and their products is not always possible.



Mud Mounds, Figure 2 Microbial mat-based model for accretion of mud mounds and development of cavities. (Modified from Pratt, 1982.)

Biota

Dominant invertebrate macrofossils on mud mounds were suspension feeders as pelmatozoans, bryozoans, and sponges – siliceous and calcareous types, although others such as brachiopods, molluscs, and arthropods could be sporadically important. Solitary and colonial corals occasionally occurred on mud mound records as well as calcareous algae, but they were much less common (not as colonizers of the shallow-water mud mound phases). Others such as benthic foraminifera and polychaete worms also occurred as part of the mud mound biota from the Carboniferous times. In general, the biota of mud mounds was mainly dominated by heterozoan assemblages.

In mud mounds, excluding the evident microbial fabrics (stromatolitic, thrombolitic and others), the main signal or product of microbial activity is hidden in the carbonate mud (biogenic automicrites) and in some authigenic mineralizations (iron pigments, Fe/Mn surfaces, framboidal pyrites, etc.). There is also direct evidence of calcified microbes (*Girvanella*, *Renalcis*, *Epiphyton*, *Bacinella*, *Rivularia*-like forms, etc.), and most of them have been interpreted as cyanobacterial fossils. Other evidence of microbial activity is the presence of unidentified filament-rich microfibrils, filaments and spherulites as moulds or casts, and peloids.

Sponge–bacterial assemblages have also played an important role in the initiation and first stages of many mud mound developments (early cementation of sponge tissues is related with the activity of symbiotic bacteria as well as the organomicrite production through the decaying of their tissues).

Cavities

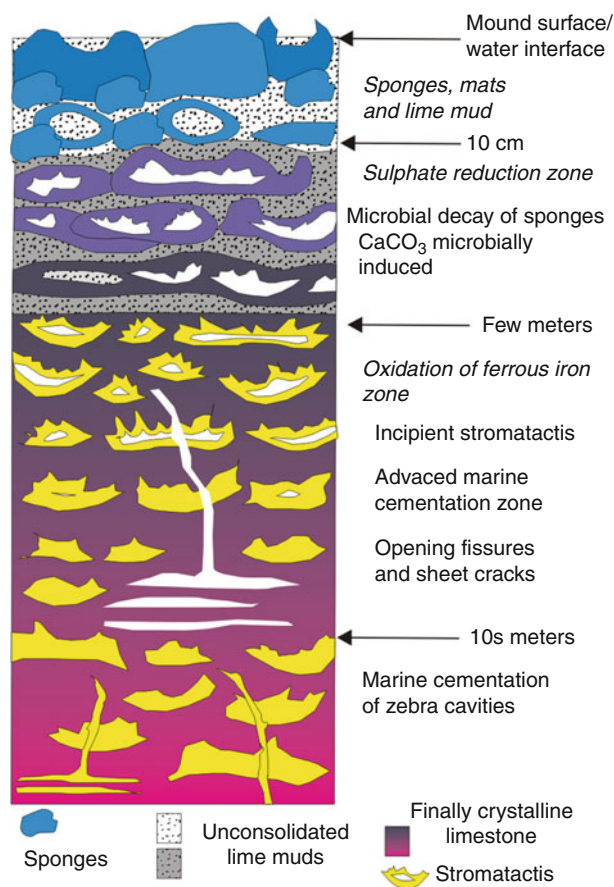
There is a wider spectrum of cavity types in mud mounds (stromatactis, zebra, irregular, and sheet-like shapes). Probably, the most famous are the stromatactis, with smooth, flat base and irregularly digitate roofs, matrix-supported in general, although some can be partially or totally sheltered by remains of fossils. Filled with centripetal marine fibrous cement crusts and varied internal sediments (allomicrites and automicrites), stromatactis were abundant in Paleozoic mud mounds, especially from the Ordovician–Mississippian interval.

The origin of stromatactis has been largely debated, and today it remains controversial. Several hypothesis has been used (summarized in Flügel, 2004, 194 p.), which can be grouped in “organic” (recrystallized fossils, related with decaying organism, burrowing, etc.) and “inorganic” theories (dissolution, collapse, internal erosion between submarine crusts or bioclasts, dewatering and compaction of the muds, recrystallization, enlargement of preexisting cavities, presence of gas hydrates, etc.). In the organic cases, there are two models: the cryptalgal/microbial mat model and the sponge model.

The microbial mat model was initially proposed by Pratt (1982) to explain the mud mound accretion and its

final observed fabrics (Figure 2). The fabrics and the resulting cavities were the result of the combination of three variables: (1) the distribution of the microbial mat, (2) the rate of sediment loading onto the mat, and (3) the degree of winnowing. The distribution of the microbial mat (laterally continuous or in patches) produces the resulting fabrics (laminar or reticulate, respectively). The winnowing of the unconsolidated sediment between the microbial bounds and cemented sediment produces the cavities.

In the sponge model (Bourque and Boulvain, 1993), the cavities are formed by the partial collapse of the sponge body as result of early diagenetic processes through four stages (Figure 3): (1) zone of the living community, (2) zone of sulphate reduction, (3) zone of oxidation of ferrous iron, and (4) zone of marine cementation. In the living surface, the mud is trapped by sponges and/or produced by microbial activity. Below the surface, the microbial decay of sponges occur; part of the sponge body and the mud are cemented, and other sponge parts are in collapsed state; the later internal erosion of the unconsolidated material produces the open cavities. A few meters



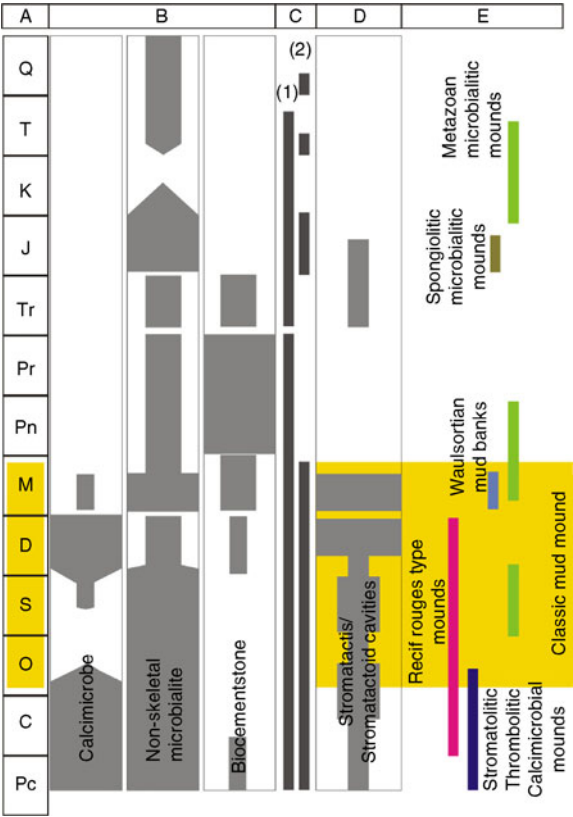
Mud Mounds, Figure 3 Sponge model for the Paleozoic red stromatolitic mud mounds. (Modified from Bourque and Boulvain, 1993.)

below, in the ferrous iron oxidation zone began the incipient marine cementation of these cavities forming the stromatactis.

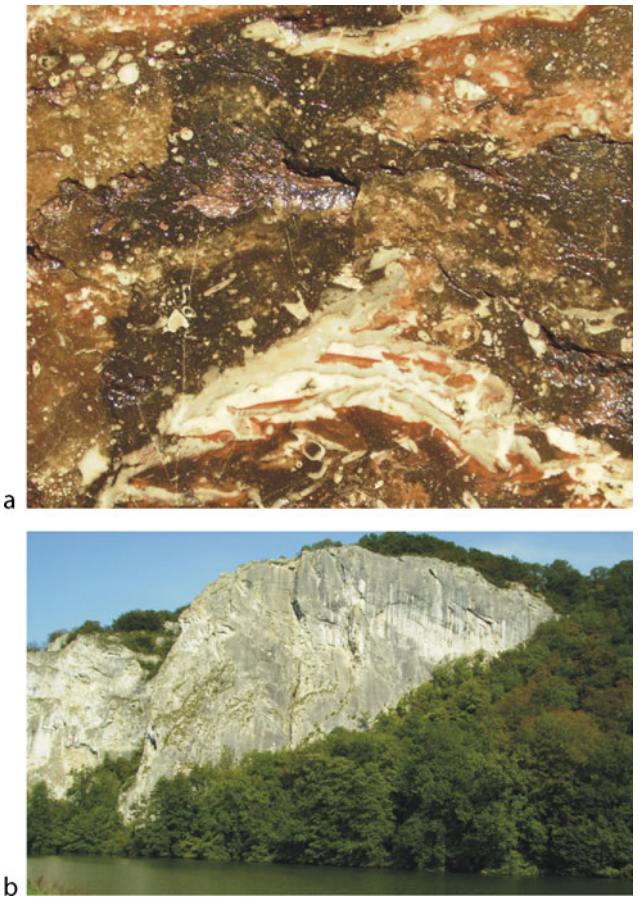
Types of mud mounds through the Phanerozoic record

The classification and distribution of mud mounds through the record depend on the “mud mound versus reef concept” of almost each researcher and thus remain controversial in several aspects. Mud mounds have existed from Proterozoic times and through the Phanerozoic record (see reviews by Monty, 1995; Pratt, 1995), and important growth episodes occurred in early Cambrian, late Devonian, and Mississippian times. Cambrian to Ordovician mud mounds (Figure 4) were characterized by different fabrics and assemblages (stromatolitic, thrombolitic, and massive fabrics with calcimicrobes, and cambrian archaeocyathid/sponge-calcimicrobial

assemblages). From the Ordovician to the Mississippian, some of the carbonate mud-rich buildups, which typify this interval, have been regarded as the classical massive mud mounds with abundant stromatactis (Figure 4) as the ‘red stromatactis mounds’ (Recif Rouges-type mound) and the Waulsortian mudbanks (Figure 5a and b). Clear microbilite-related fabrics (stromatolites and thrombolites) are extremely rare in classical mud mounds, although they show other nonskeletal reef frameworks (calcimicrobial and biocemenstones *sensu*, Webb, 1996), as well as filaments and some characteristic features (clotted, peloidal micrites, and fenestral microfabrics). The parallel diversification of skeletal mound macro- and micro-biota produced more varied metazoan–microbialitic assemblages. One of the most persistent through the mud mound record is the sponge–microbialitic association, very common in Jurassic mud mounds as well as in the deeper initial stages of many Paleozoic mounds. Early Cretaceous is considered the last significant moment of organomineralic mud mound development (Neuweiler et al., 1999), although



Mud Mounds, Figure 4 Distribution of mud mounds through the geologic record. (A) Precambrian to Quaternary time divisions. (B) Distribution and relative importance of nonenzymatic reef frameworks (from Webb, 1996), characteristic also in mud mounds. (C) Distribution of (1) microbial and (2) biotrital mud mounds. (Modified from Bosence and Bridges, 1995.) (D) Distribution of stromatactis and stromatactoid cavities, the size of the bar represents their relative importance in mud mounds. (E) Interval distribution of the main typologies of mud mounds.



Mud Mounds, Figure 5 Classical mud mounds. (a) Close view of a Devonian Recifs Rouges-type mound, Beauchateau Quarry, Belgium. (b) Carboniferous Waulsortian mudbank, Waulsort, Belgium.

other authors mentioned that the latest mud mounds are Miocene (Pratt, 2000).

Ecological zonation models

The Récifs Rouges-type mounds and the Waulsortian mudbanks are probably the most famous and are well known as “classical mud mounds” (Figures 4 and 5) Devonian (Frasnian) mounds from Belgium show a shallowing upward succession from deep basal greenish-grey shales through their mud mound facies: red lime mudstones to wackestones with stromatactis, pink wackestones with millimeter stromatactis to crudely bedded grey wackestones to packstones, and bindstones, and their associated biota. Similar facies (red stromatactis mounds) and assemblages have been found in other mud mounds through the Paleozoic record (Bourque, 1997). The carboniferous Waulsortian mudbanks of Belgium show a four grain-type assemblages related with the growth of these mounds from deep subtidal, aphotic to photic conditions. A depth-related phase model was recognized by Lees and coworkers (summarized in Lees and Miller, 1995) and applied to Waulsortian mudbanks of Europe and North America. Bourque (1997) compared Récifs Rouges-type mounds from Silurian of Canada and Devonian of France and Belgium with carboniferous mounds from Algeria and the Waulsortian mudbanks model and established an ecological zonation model formed by four benthic communities zones (Figure 6): (1) the sponge zone, (2) the fenestellid–sponge zone, (3) the delicate skeletons–sponge zone, and (4) the delicate skeletons–microbial encrusters zone.

Controlling factors

Today, many parameters about the initiation, accretion, and spread of many mud mounds seem to be enigmatic. However, some others begin to be more clear.

Locations of some mud mounds have been related with submarine cold seeps, vents, faults, halokinetic and argilokinetic settings, and local antecedent topographic irregularities, have been associated to nutrient-enriched areas (by endo-, down- and/or up-welling currents), and could be also related with stratified basin waters and fluctuating oxygen minimum zone. Many of them grew up into aphotic to disphotic depths (more than 200 m); however, they could reach and colonize the euphotic zone. Some grew at lower sedimentation rates and/or during sedimentary starvation during TST episodes, although this is not a general trend for all of them. Most of these factors are oceanographic and/or tectonically basin-related parameters and could co-occur together in many cases.

Those factors (biological and chemical parameters) associated with the carbonate mud precipitation related with eukaryotes and prokaryotes should be taken into consideration:

- Production of acidic organic macromolecules (AOM) and an increase in carbonate alkalinity via (1) bacterial heterotrophic pathways (ammonification and sulphate reduction), (2) carbonate, and (3) silicate weathering. AOM and high alkalinity favor the organomicrite production in microbialites and mud mounds (Reitner and Neuweiler, 1995).
- Extracellular polymeric substances (EPS)-biomineralization of microbial mats and biofilms.
- Calcification of cyanobacteria.

Cambrian-devonian Récifs rouges-type mounds			Carboniferous mud mounds			
Silurian (Canada)	Devonian (France)	Devonian (Belgium)	Viséan (Algerian)	Waulsortian mud banks phases	Depth-related communities	
<i>Fenestellids</i> <i>Stromatoporoids</i> <i>Calcareous algae</i> <i>Microbial encrusters</i>		<i>Corals</i> <i>Stromatoporoids</i> <i>Microbial encrusters</i>	<i>Rugose corals</i> <i>Microbial encrust.</i> <i>Calcareous algae</i>	Phase D: C + grain coating calcareous algae	<i>Delicate skeletons and microbial encrusters</i>	Photic
	<i>Fenestellid</i> <i>Sponge (spicules)</i> <i>Crinoids</i> + mud	<i>Corals</i> <i>Crinoids</i> <i>Bryozoans</i> + mud	<i>Crinoids</i> <i>Fenestellids</i> <i>Sponge (spicules)</i> <i>Red algae</i> + mud	Phase C: B + Plurilocular foraminifera	<i>Delicate skeletons and sponges + mud</i>	Aphotic
			<i>Sponges</i> <i>Fenestellids</i>	Phase B: A + Sponger spicules Phase A: fenestellids, crinoids, ostracods	<i>Fenestellids and sponges</i>	
<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i> (<i>Red stromatactis</i> limestone)	<i>Sponges</i>		<i>Sponges</i>	

Mud Mounds, Figure 6 Ecological zonation assemblages in some Paleozoic mud mounds. (Modified from Bourque, 1997.)

- (d) CO₂ depletion of the medium through autotrophic pathways by methanogenic archaea, sulfurous and nonsulfurous purple and green photosynthetic bacteria, and cyanobacteria (Castanier et al., 2000).

Conclusions

Mud mounds are considered mainly as nonenzymatic carbonate deposits that formed a polygenetic and very continuous record of buildups through the Phanerozoic and reached a maximum development during the Paleozoic times. They are of considerable importance not from an economic point of view (hydrocarbon reservoirs, major mineral host-rock and base deposits, ornamental rocks) alone. In fact, they represent an excellent record for the study and establishment of (1) the interactions between the marine microbial and nonmicrobial benthic communities (paleoecologic and paleoenvironmental parameters), (2) marine carbonate production models (mud mound factory), and (3) proxies in palaeoceanographic reconstructions (nonenzymatic carbonates are formed in equilibrium with ambient sea water).

Mud mounds are characterized by different modes of carbonate mud production (abiogenic, biologically induced, and biologically controlled automicrites) and sedimentation input (allomicrites). The key of mud mounds understanding lies behind here: to recognize and differentiate between the automicrite and allomicrite relationship and their contribution to the mud mound accretion.

Bibliography

- Bosence, D. W. J., and Bridges, P. H., 1995. A review of the origin and evolution of carbonate mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-mounds: Their Origin and Evolution*. Oxford: Blackwell Science for the International Association of Sedimentologists, Special Publication, **23**, pp. 3–9.
- Bosence, D. W. J., Rowlands, R. J., and Quine, M. L., 1985. Sedimentology and budget of a recent carbonate mound, Florida keys. *Sedimentology*, **32**, 317–343.
- Bourque, P. A., 1997. Paleozoic finely crystalline carbonate mounds: cryptic communities, petrogenesis and ecological zonation. *Facies*, **36**, 250–253.
- Bourque, P.-A., and Boulvain, F., 1993. A model for the origin and petrogenesis of the red stromatolite limestone of Paleozoic carbonate mounds. *Journal of Sedimentary Petrology*, **63**, 607–619.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaaios*, **2**(3), 241–254.
- Camoin, G., and Maurin, A.-F., 1988. Roles of micro-organisms (bacteria, cyanobacteria) in the formation of mud mounds: examples from the Turonian strata of Jebels Bireno and Mrhila (Tunisia). *Compte Rendu des Académie des Sciences de Paris, Série II*, **307**, 401–407.
- Castanier, S., Le Métyer-Levrel, G., and Perthuisot, J. P., 2000. Bacterial roles in the precipitation of carbonate minerals. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 32–39.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks: Analysis, Interpretation and Application*. Berlin: Springer, 976 p.
- James, N. P., and Bourque, P.-A., 1992. Reefs and mounds. In Walker, R. G., and James, N. P. (eds.), *Facies Models: Response to Sea Level Change*. St. John's, Newfoundland: Geological Association of Canada, pp. 323–347.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaaios*, **1**(5), 492–503.
- Keupp, H., Jenisch, A., Herrmann, R., Neuweiler, F., and Reitner, J., 1993. Microbial carbonate crusts – a key to the environmental analysis of fossil spongiolites? *Facies*, **29**, 41–54.
- Lees, A., and Miller, J., 1985. Facies variation in Waulsortian buildups. Part 2. Mid-Dinantian buildups from Europe and North America. *Geological Journal*, **20**, 159–180.
- Leinfelder, R. R., and Keupp, H., 1995. Part III Upper Jurassic mud mounds: allochthonous sedimentation versus autochthonous carbonate production. *Facies*, **32**, 17–26.
- Monty, C. L. V., 1976. The origin and development of cryptalgal fabrics. In Walter, M. R. (ed.), *Stromatolites: Developments in Sedimentology*. Amsterdam: Elsevier, Vol. 20, pp. 193–249.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, Their Origin and Evolution*. International Association of Sedimentologists. Special publication, **23**, pp. 11–48.
- Neuweiler, F., 1993. Development of Albian microbialites and microbialites reefs at marginal platform areas of the Vasco-Cantabrian Basin (Soba Reef, Cantabria, N. Spain). *Facies*, **29**, 231–250.
- Neuweiler, F., Gautret, P., Thiel, V., Langes, R., Michaelis, W., and Reitner, J., 1999. Petrology of lower cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Pratt, B. R., 1982. Stromatolitic framework of carbonate mud-mounds. *Journal of Sedimentary Petrology*, **52**(4), 1203–1227.
- Pratt, B. R., 1995. The origin, biota and evolution of deep water mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, Their Origin and Evolution*. Oxford: Blackwell Science for the International Association of Sedimentologists, Special Publication, **23**, pp. 49–123.
- Pratt, B. R., 2000. Microbial contribution to reefal mud-mounds in ancient deep-water settings: evidence from Cambrian. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 282–287.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan rich-facies from Lizard Island (Great Barrier Reef, Australia) – formation and concepts. *Facies*, **29**, 3–40.
- Reitner, J., and Neuweiler, F., 1995. Part I, mud mounds: recognizing a polygenetic spectrum of fine-grained carbonate buildups. *Facies*, **32**, 2–4.
- Reitner, J., Neuweiler, F., and Gautret, P., 1995. Part II modern and fossil automicrites: implications for mud mounds genesis. *Facies*, **32**, 4–17.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 21–51.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2002. Structure and composition of organic reef and carbonate mud mounds: concepts and categories. *Earth-Science Reviews*, **58**, 163–231.
- Textoris, D. A., 1966. Algal cap for a Niagaran (Silurian) carbonated mud mound of Indiana. *Journal of Sedimentary Petrology*, **36**(2), 455–461.

- Tsien, H. H., 1985. Algal-bacterial origin of micrites in mud mounds. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 290–296.
- Webb, G. E., 1996. Was Phanerozoic reef history controlled by the distribution of non-enzymatically secreted reef carbonates (microbial carbonate and biologically induced cement)? *Sedimentology*, **43**, 947–971.
- Wilson, J. L., 1975. *Carbonate Facies in Geologic History*. Berlin: Springer, 471 p.

Cross-references

[Bacteria](#)
[Biofilms](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Reefs](#)
[Waulsortian Mud Mounds](#)

MUTUALISM

Mutualism is a form of a symbiosis, in which both organisms involved benefit. See entry “[Symbiosis](#)” for further reading.

MYCORRHIZAE

A mycorrhiza (pl mycorrhizae, from the Greek “*mycos*” = fungus and “*rhiza*” = root) is a plant-fungal association that comprises the most widespread type of terrestrial symbiosis. See entry “[Symbiosis](#)” for further reading.

N

NAN(N)OBACTERIA

Muriel Pacton¹, Georges E. Gorin²

¹ETH Zurich, Zurich, Switzerland

²University of Geneva, Geneva, Switzerland

Synonyms

Living nanovesicles; Nanobes; Nanocalcifying particles (NCP); Nanoforms; Nanoglobules; Nanons; Nano-organisms; Nanoparticles

Biologists used the word *nanobacteria* to describe organisms that possibly enhance biomineralization such as that of kidney stones in humans, whereas *nannobacteria* is used by geologists for organisms responsible for mineral precipitation in terrestrial and extraterrestrial rocks.

Definition

Nan(n)obacteria are tiny globular objects, ranging in diameter from 20 to 200 nm, with the ability to nucleate minerals. They are the smallest known, self-replicating particles with slow growth rate. Because they are by definition down to 1/10 the size of bacteria, it raises the problem of whether or not an organism of this size is large enough to house necessary cell components such as DNA, RNA, and plasmids.

Introduction

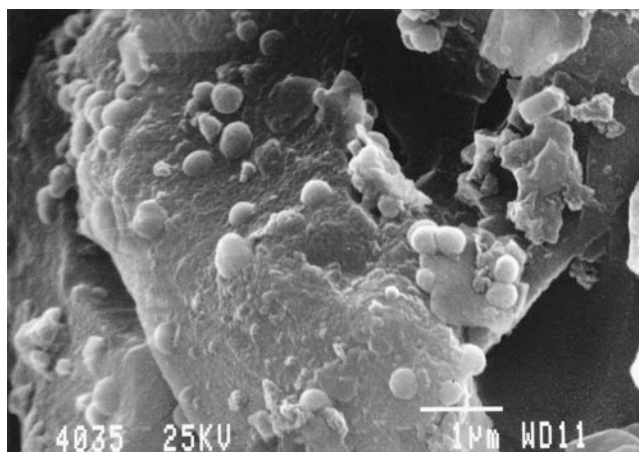
The term “nan(n)obacteria” has been originally proposed by Folk (1993) to describe 20–200 nm wide nanovesicles observed in carbonates using scanning electron microscopy (SEM). Kajander and Çiftçioglu (1998) defined the role of similar tiny particles in kidney stone formation. Based on their mineralogical properties, nan(n)obacteria have also been called calcifying nanoparticles (CNP) by

these authors. Nan(n)obacteria-like particles have been observed in Martian meteorites, thereby raising questions about a primitive form of life (Bradley et al., 1997). CNP are studied extensively in pathogenic diseases as infectious agents by nucleating crystals (hydroxyapatite). Therefore, some comparisons have tentatively been made with the geological record. Although they display some evidences of bacterial nature, their living status is subject to controversy because of their small size and the fact that DNA extraction remains unsuccessful. On one hand, biologists discuss their metabolic existence and on the other hand geologists argue for widespread organic fossilization in palaeoenvironments. When interrelations between geology and microbiology are possible, they are looked at only from the point of view of experimental procedures (e.g., one emphasizes the use of DNA probes in geological samples). This prohibits an objective comparison of these very small particles.

Reported nan(n)obacteria in the literature

In the geological record, nan(n)obacteria-like particles are encountered mainly in carbonates: in calcite (Dix et al., 1999; Chen et al., 2005; Mamet and Pr  at, 2006), microbialites (Camoin et al., 1999), dolomite (Figure 1; Vasconcelos et al., 1995; Gournay et al., 1999; Wright, 1999; Al  i  ek, 2009), hydrothermal springs (Casanova et al., 1999), and oncolite laminations (Cavalazzi et al., 2007). They have also been observed in sandstones without mineral precipitation (Uwins et al., 1998) and on quartz overgrowth in crude oil (Spark et al., 2000).

In medical biology, CNP have been the focus of numerous studies on various pathogenic diseases (see Urbano and Urbano (2007) for a review), for example, gallbladder (Wang et al., 2006), nasopharyngeal cancer (Zhou et al., 2006), and renal stone formations (e.g., Kajander and   ift  ioglu (1998) and Jones et al. (2009)).



Nan(n)obacteria, Figure 1 Nan(n)obacteria encrusted by nanocrystals of carbonate on a quartz substrate. (Picture courtesy of Crisogono Vasconcelos.)

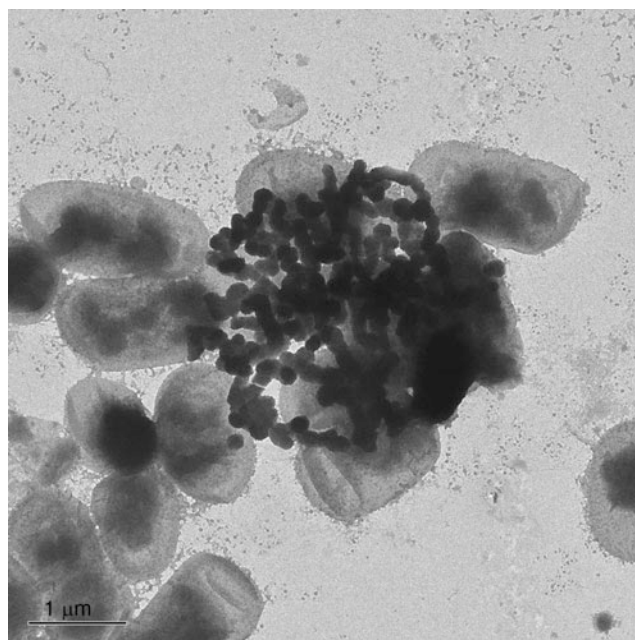
Characterization of purported nan(n)obacteria

Nan(n)obacteria exhibit some characteristics, which seem to correspond to the classical criteria indicating a biological origin: (1) a typical bacterial appearance (Kajander and Çiftçioglu, 1998; Uwins et al., 1998); (2) a slow growth in liquid cell culture media; (3) an expression of specific antigens (Hjelle et al., 2000; Miller et al., 2004) by using monoclonal antibodies commercially available through Nanobac Oy (Finland) as 8D10 and through Santa Cruz Biotechnology, USA, named GM-CSFR α ; (4) a staining with nucleic fluorochroms (Uwins et al., 1998; Miller et al., 2004) using 4',6-diamidino-2-phenylindole (DAPI), acridine orange, Hoechst 33258, Feulgen, and/or Picogreen; (5) a resistance to antibiotics such as tetracycline and citrate (Maniscalco and Taylor, 2004); (6) a sensitivity to calcium-chelating agents (Rawal and Pretorius, 2005); and (7) a DNA sequencing tentatively assigned to the α -2 subgroup of proteobacteria, which also includes *Brucella* and *Bartonella* species occurring within and outside of host cells (Kajander and Çiftçioglu, 1998; Çiftçioglu and Kajander, 1998). According to Cisar et al. (2000), they could come from PCR contaminant.

Possible origins for nan(n)obacteria

Due to the very small size of purported nan(n)obacteria, the hypothesis that they might represent living entities is very controversial. Several, alternative origins have been proposed:

Inorganic origin: Inorganic precipitates (Vecht and Ireland, 2000; Cisar et al., 2000) and artifacts due to sample preparation (e.g., coating for SEM, acid treatment, polishing artifacts) have been considered as an alternative origin for these nanostructures. However, nanostructures



Nan(n)obacteria, Figure 2 Chain of dolomite nanoglobules formed in *Halomonas meridiana* culture. (Picture courtesy of Monica Sanchez-Roman.)

can also be observed in samples that have not been exposed to these techniques (Folk and Lynch, 1997).

Extracellular polymeric substances (EPS): Reitner et al. (1995) have proposed that nanospheres were an initial calcification of EPS. They have been interpreted as early stages of dolomite precipitation in anaerobic cultures (Bontognali et al., 2008) and aerobic cultures (Figure 2; Sanchez-Roman et al., 2008). Similar observations have been made on aragonite nanocrystals that contained organic molecules (Benzerara et al., 2006).

Proteins: Tissue degradation caused by microbial activity can create nanobacteria-like particles of proteinous origin (Schieber and Arnott, 2003). In the case of kidney stones, it has been confirmed that nan(n)obacteria are in fact related to blood proteins (Martel and Young 2008), that is, fetuin (Raoult et al., 2008).

Small bacteria: Dwarf bacteria generally show a decrease in cell size. This is assumed to be the case of bacteria exposed to low nutrient or starvation conditions (Velimirov, 2001). Ultramicrobacteria exhibit a very low growth rate (Torrella and Morita, 1981). The distinction between ultramicrobacteria and starvation forms is based on their physiological properties (Velimirov, 2001).

Viruses: Stressed bacteria overproduce vesicles that are apparently used to selectively eliminate unwanted material, for example, unfolded proteins (Soler et al., 2008). All fluorescent small-size particles are assumed to correspond to a DNA virus (Soler et al., 2008). Membrane vesicles seem to be produced by most bacteria and have been

detected in natural environments, including biofilms (Schooling and Beveridge, 2006).

Minimal conditions for a living cell

Theoretical calculations have been made in order to define the smallest size needed for a cell to have metabolic processes and accommodate the smallest genome. According to these assumptions, the minimal cell size should fall within the range of 100 nm (Boal, 1999), 140 nm (Maniloff et al., 1997), and 170 nm (Adams, 1999). Assuming a cell genome housing 250 genes (which is considered the minimal number needed to support heterotrophic cell growth in a nutrient-rich environment with a minimal metabolic pathway), a minimum diameter of 50 nm has been established, which could contain eight genes (Adams, 1999). Nevertheless, a cell with a size of only 50 nm, a 5 nm cell wall, 2 ribosomes, 520 protein molecules, and a DNA strand containing 8 genes is considered too small to function like recent bacteria, based on the smallest known living organism, that is, *Nanoarchaeum equitans* (Huber et al., 2002).

Universal origin for nan(n)obacteria?

The analogy between nan(n)obacteria-like particles in pathogenic diseases and geological records is often made even though the methodology differs. Nan(n)obacteria have not been found in any clinical condition that does not involve calcification, which is not the case of some geological samples (Uwins et al., 1998; Schieber and Arnott, 2003). Recently, nan(n)obacteria-like particles associated with kidney stone formation have been assigned directly to blood proteins reacting with antibodies that were so far claimed to be specifically associated to nan(n)obacteria (Martel and Young, 2008; Raoult et al., 2008). The latter authors suggested that they are nucleic-acid free. An alternative origin has been proposed where nan(n)obacteria could be the by-product of the living activities of bona fide bacteria inhabiting kidney stones (Aloisi, 2008). If we consider that nan(n)obacteria could be EPS accumulative and knowing that EPS contain DNA (Whitchurch et al., 2002; Steinberg and Holden, 2005), it is unlikely that this hypothesis fits with nanobes observed by Uwins et al. (1998), where no other microorganisms were detected.

Summary

Nan(n)obacteria emerged as a new form of life during the last two decades, based mainly on their capability to trigger infectious diseases. Their interpretation as simple proteins in kidney stones has been recently ruled out, although similar nano-objects have been also observed in geological records. The controversy about their existence (too small to be biologically viable) is still ongoing today because no universal origin can be attributed to these nanoparticles. The EM imaging does not present

enough evidence alone, and molecular and physiological data are required in order to try to define their properties.

Bibliography

- Adams, M. W. W., 1999. The influence of environment and metabolic capacity on the size of a microorganism. In Steering Group, (eds.), *Size limits of very small microorganisms: Proceedings of a workshop*. Washington, DC: National Academy Press, National Research Council, Space Studies Board, 74–80.
- Alçıçek, H., 2009. Late Miocene nonmarine sedimentation and formation of magnesites in the Acigöl Basin, southwestern Anatolia, Turkey. *Sedimentary Geology*, **219**, 115–135.
- Aloisi, G., 2008. An alternative origin for nanobacteria in kidney stones. *Bioscience Hypotheses*, **1**, 138–141.
- Benzerara, K., Menguy, N., Lopez-Garcia, P., Yoon, T.-H., Kazmierczak, J., Tyliszczak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 9440–9445.
- Boal, D., 1999. Mechanical characteristics of very small cells. In Steering Group, (eds.), *Size limits of very small microorganisms: Proceedings of a workshop*. Washington, DC: National Academy Press, National Research Council, Space Studies Board, 26–31.
- Boal, D., 2001. How small can a microorganism be? In: *Size limits of very small microorganisms*. Washington, DC: National Academy of Sciences.
- Bontognali, T. R. R., Vasconcelos, C., Warthmann, R. J., Dupraz, C., Bernasconi, S. M., and McKenzie, J. A., 2008. Microbes produce nanobacteria-like structures, avoiding cell entombment. *Geology*, **36**, 663–666.
- Bradley, J. P., Harvey, R. P., McSween, H. Y., Gibson, E., Thomas-Keptra, K., and Vali, H., 1997. No “nanofossils” in Martian meteorite. *Nature*, **390**, 454–456.
- Camoin, G. F., Gautret, P., Montaggioni, L. F., and Cabioch, G., 1999. Nature and environmental significance of microbialites in Quaternary reefs: the Tahiti paradox. *Sedimentary Geology*, **126**, 271–304.
- Casanova, J., Bodéan, F., Négrel, P., and Azaroual, M., 1999. Microbial control on the precipitation of modern ferrihydrite and carbonate deposits from the Cézallier hydrothermal springs (Massif Central, France). *Sedimentary Geology*, **126**, 125–145.
- Cavalazzi, B., Barbieri, R., and Ori, G. G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chen, D. F., Huang, Y. Y., Yuan, X. L., and Cathles, L. M., 2005. Seep carbonates and preserved methane oxidizing archaea and sulphate reducing bacteria fossils suggest recent gas venting on the seafloor in the Northeastern South China Sea. *Marine and Petroleum Geology*, **22**, 613–621.
- Çiftçioglu, N., and Kajander, E. O., 1998. Interaction of nanobacteria with cultured mammalian cells. *Pathophysiology*, **4**, 259–270.
- Cisar, J., De-Qi, X., Thompson, J., Swaim, W., Hu, L., and Kopecko, D., 2000. An alternative interpretation of nanobacteria-induced biomineralization. *Microbiology*, **97**, 11511–11515.
- Dix, G. R., Patterson, T. R., and Park, L. E., 1999. Marine saline ponds as sedimentary archives of late Holocene climate and sea-level variation along a carbonate platform margin: Lee Stocking Island, Bahamas. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **150**, 223–246.

- Folk, R., 1993. SEM imaging of bacteria and nanobacteria in carbonate sediments and rocks. *Journal of Sedimentary Petrology*, **63**, 990–999.
- Folk, R., and Lynch, L., 1997. The possible role of nanobacteria in clay-mineral diagenesis and the importance of careful sample preparation in high-magnification SEM study. *Journal of Sedimentary Research*, **67**, 583–589.
- Gournay, J., Kirkland, B., Folk, R., and Lynch, F., 1999. Nanometer-scale features in dolomite from Pennsylvanian rocks, Paradox Basin, Utah. *Sedimentary Geology*, **126**, 243–252.
- Hjelle, J. T., Miller-Hjelle, M. A., Poxton, I. R., Kajander, E. O., Çiftçioglu, N., Jones, M. L., Caughey, R. C., Brown, R., Millikin, P. D., and Darras, F. S., 2000. Endotoxin and nanobacteria in polycystic kidney disease. *Kidney International*, **57**, 2360–2374.
- Huber, H., Hohn, M. J., Rachel, R., Fuchs, T., Wimmer, V. C., and Stetter, K. O., 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature*, **417**, 63–67.
- Jones, J. A., Çiftçioglu, N., Schmid, J. F., Barr, Y. R., and Griffith, D., 2009. Calcifying nanoparticles (nanobacteria): an additional potential factor for urolithiasis in space flight crews. *Urology*, **73**, e11–e13.
- Kajander, E. O., and Çiftçioglu, N., 1998. Nanobacteria: an alternative mechanism for pathogenic intra- and extracellular calcification and stone formation. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 8274–8279.
- Mamet, B., and Pr  at, A., 2006. Iron-bacterial mediation in Phanerozoic red limestones: State of the art. *Sedimentary Geology*, **185**, 147–157.
- Maniloff, J., Nealson, K. H., Psenner, R., Loferer, M., and Folk, R. L., 1997. Nanobacteria: size limits and evidence. Letters from: Jack Maniloff, Kenneth H. Nealson, Roland Psenner and Maria Loferer, and Robert L. Folk. *Science*, **276**, 1773–1776.
- Maniscalco, B. S., and Taylor, K. A., 2004. Calcification in coronary artery disease can be reversed by EDTA-tetracycline long-term chemotherapy. *Pathophysiology*, **11**, 95–101.
- Martel, J., and Young, J. D.-E., 2008. Purported nanobacteria in human blood as calcium carbonate nanoparticles. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 5549–5554.
- Miller, V. M., Rodgers, G., Charlesworth, J. A., Kirkland, B., Severson, S. R., Rasmussen, T. E., Yagubyan, M., Rodgers, J. C., Cockerill, F. R., III, Folk, R. L., Rzewuska-Lech, E., Kumar, V., Farrell-Baril, G., and Lieske, J. C., 2004. Evidence of nanobacterial-like structures in calcified human arteries and cardiac valves. *American Journal of Physiology – Heart and Circulatory Physiology*, **287**, H1115–1124.
- Raoult, D., Drancourt, M., Azza, S., Nappiez, C., Guieu, R., Rolain, J.-M., Fourquet, P., Campagna, B., La Scola, B., Mege, J.-L., Mansuelle, P., Lechevalier, E., Berland, Y., Gorvel, J.-P., and Renesto, P., 2008. Nanobacteria are mineralo fetuin complexes. *PLoS Pathogens*, **4**, e41.
- Rawal, B. D., and Pretorius, A.-M., 2005. “Nanobacterium sanguineum” Is it a new life-form in search of human ailment or commensal: overview of its transmissibility and chemical means of intervention. *Medical Hypotheses*, **65**, 1062–1066.
- Reitner, J., Gautret, P., Marin, F., and Neuweiler, F., 1995. Automicrites in a modern microbialite. Formation model via organic matrices (Lizard Island, Great Barrier Reef, Australia). *Bulletin de l’Institut Oc  anographique (Monaco)*, **14**, 237–263.
- Sanchez-Roman, M., Vasconcelos, C., Schmid, T., Dittrich, M., McKenzie, J. A., Zenobi, R., and Rivadeneyra, M. A., 2008. Aerobic microbial dolomite at the nanometer scale: implications for the geologic record. *Geology*, **36**, 879–882.
- Schieber, J., and Arnott, H. J., 2003. Nanobacteria as a by-product of enzyme-driven tissue decay. *Geology*, **31**, 717–720.
- Schooling, S. R., and Beveridge, T. J., 2006. Membrane vesicles: an overlooked component of the matrices of biofilms. *Journal of Bacteriology*, **188**, 5945–5957.
- Soler, N., Marguet, E., Verbavatz, J.-M., and Forterre, P., 2008. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. *Research in Microbiology*, **159**, 390–399.
- Spark, I., Patey, I., Duncan, B., Hamilton, A., Devine, C., and McGovern-Traa, C., 2000. The effects of indigenous and introduced microbes on deeply buried hydrocarbon reservoirs, North Sea. *Clay Minerals*, **35**, 5–12.
- Steinberg, R. E., and Holden, P. A., 2005. Extracellular DNA as single- and multiple-species unsaturated biofilms. *Applied and Environmental Microbiology*, **71**, 5404–5410.
- Torella, F., and Morita, R. Y., 1981. Microcultural study of bacteria size changes and microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. *Applied and Environmental Microbiology*, **41**, 518–527.
- Urbano, P., and Urbano, F., 2007. Nanobacteria: facts or fancies? *PLoS Pathogens*, **3**, e55.
- Uwins, P., Webb, R., and Taylor, A., 1998. Novel nano-organisms from Australian sandstones. *American Mineralogist*, **83**, 1541–1550.
- Vasconcelos, C., McKenzie, J. A., Bernasconi, S., Grujic, D., and Tien, A. J., 1995. Microbial mediation as a possible mechanism for natural dolomite formation at low temperatures. *Nature*, **377**, 220–222.
- Vecht, A., and Ireland, T. G., 2000. The role of vaterite and aragonite in the formation of pseudo-biogenic carbonate structures: implications for Martian exobiology. *Geochimica et Cosmochimica Acta*, **64**, 2719–2725.
- Velimirov, B., 2001. Nanobacteria, ultramicrobacteria and starvation forms: a search for the smallest metabolizing bacterium. *Microbes and Environments*, **16**, 67–77.
- Wang, L., Shen, W., Wen, J., An, X., Cao, L., and Wang, B., 2006. An animal model of black pigment gallstones caused by nanobacteria. *Digestive Diseases and Sciences*, **51**, 1126–1132.
- Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C., and Mattick, J. S., 2002. Extracellular DNA required for bacterial biofilm formation. *Science*, **295**, 1487.
- Wright, D. T., 1999. The role of sulphate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia. *Sedimentary Geology*, **126**, 147–157.
- Zhou, H.-D., Li, G.-Y., Yang, Y.-X., Li, X.-L., Sheng, S.-R., Zhang, W.-L., and Zhao, J., 2006. Intracellular co-localization of SPLUNC1 protein with nanobacteria in nasopharyngeal carcinoma epithelia HNE1 cells depended on the bactericidal permeability increasing protein domain. *Molecular Immunology*, **43**, 1864–1871.

Cross-references

Bacteria
 Biosignatures in Rocks
 Dolomite, Microbial
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Nanocrystals, Microbially Induced
 Origin of Life

NANOCRYSTALS, MICROBIALLY INDUCED

Susan Glasauer

University of Guelph, Guelph, Ontario, Canada

Definition

Mineral particles less than 0.1 μm are a vital component of geochemical cycling in the Earth surface systems. Particles in this size range are generally termed “nanoparticles”; nanocrystals are nanoparticles that exhibit long-range structural order. Their small size implies that they may be relatively mobile in natural systems, as well as more chemically labile than large crystals. They have recently come to the forefront of material science, with new evidence that the properties of some materials are a strong function of particle size, particularly at the nanometer scale.

Nanocrystals are characterized by higher surface strain and disorder, higher surface reactivity, and differences in reaction kinetics compared to larger crystals of the same composition. These properties make them important reactive agents in natural systems, especially given that minerals in surface and near-surface environments are typically smaller and more highly disordered than minerals crystallized under ideal conditions. In general, the interest in biological nanomaterials is focused on specific properties that a given material can offer. For example, the electronic structure of nanocrystals is of particular interest from the perspective of material science. For a review of chemical and physical properties related to nanomineral structure and composition, see Gilbert and Banfield (2005).

The crystallization of biominerals spans eukaryotic (“true nucleus”) and prokaryotic organisms (Lowenstam and Weiner, 1989). Eukaryotes form geologically important structures that may be based on nanoparticles, such as the silica-reinforced shells of diatoms or the calcite shells of coccolithophores and foraminifera (Weiner and Dove, 2003). Prokaryotes dominate, however, among microorganisms that mediate the development of nanoparticles, in terms of their ubiquity in surface and subsurface environments and the compositional and structural range of materials they can form (Gilbert and Banfield, 2005). This article focuses mainly on bacterial nanoparticles that contain metals because of their importance in Earth surface and subsurface environments as well as their relevance to other disciplines related to nanoscience.

Bacterial nanocrystals

Induced versus controlled crystallization

The development of biominerals or biocrystals may be either biologically induced or biologically controlled by the bacteria (Lowenstam and Weiner, 1989). In *biologically controlled mineralization*, microbes exert genetic

and chemical control over the formation of precipitates that are designed to serve a particular function. In contrast, *biologically induced mineralization* yields particles that are secondary to a chemical process controlled by the microorganism. In this case, the precipitates serve no physiological purpose.

Biologically induced nanominerals can result from a range of bacterial processes, including respiration, detoxification, and ion uptake or expulsion during metabolism. Although minerals with a range of sizes and crystal structures can be produced as a result of biologically induced mineralization (e.g., Fortin et al., 2008), the nanometer-sized minerals are of particular interest because of their chemical reactivity as well as the potential for transport in natural systems. Reactivity is a consequence of the high proportion of surface atoms, which are chemically uncoordinated at the solution interface and less ordered than fully coordinated atoms in the structure.

Nanomineral formation can be induced in the extracellular environment, as a result of changes in solution chemistry caused by uptake or expulsion of ions (e.g., H^+) from the cell (Fortin et al., 2008). In addition, bacterial surfaces carry negative and positive charges due to the dissociation of functional groups integral to the cell wall (Beveridge, 1989). Sorption of ions to charged bacterial surfaces or sheaths can lower the nucleation energy for crystal formation and induce thick mantles to develop based on nanoparticles. This process can be observed during the growth of silica sheaths around bacteria and cyanobacteria, which may also incorporate metal ions (Ferris et al., 1986; Konhauser et al., 2004). More examples of induced mineralization are discussed below.

Extracellular nanominerals

Bacterial metal respiration is a process in which electrons are removed or accepted by a metal ion to generate energy for the cell. Specific bacteria are capable of either oxidation or reduction; for example, *Gallionella* spp. and *Leptothrix* spp. are known iron oxidizers, whereas *Shewanella* and *Geobacter* are genera associated with metal reduction. Metal oxidation and reduction (“redox”) by bacteria may take place in chemically stratified zones within a sediment or water column, or may occur in microscale zones (Nealson and Stahl, 1997; Sobolov and Roden, 2002). Minerals form when a change in metal valence induced by respiration affects the solubility of a metal, in the presence of a suitable counterion to react with the reduced or oxidized metal species; the minerals form by an induced process rather than a controlled one.

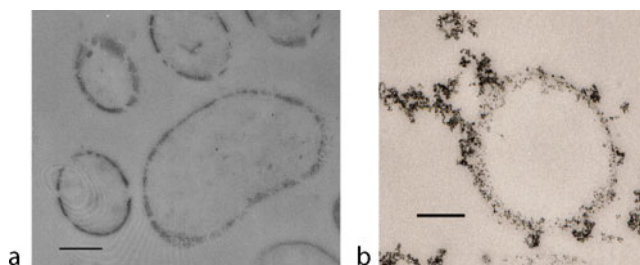
Iron- and manganese-oxidizing bacteria can produce copious quantities of nanoparticulate minerals via a respiratory process that strips electrons from lower valence states of the metal and transfers them to the electron transport chain (Tebo et al., 1997; Kappler and Straub, 2005). In the case of iron, particles of poorly crystalline ferric hydroxide result that are typically 3–5 nm in diameter;

these aggregate strongly and eventually form larger particles of more crystalline iron oxides (Kappler and Straub, 2005).

For metal-reducing bacteria, metals serve as the terminal electron acceptor during respiration rather than being assimilated by the bacteria. Metals transformed by bacteria during dissimilatory respiration that form nanoparticles include Fe, Mn, U, Tc, Cr, V, and Pu (Lloyd et al., 2002; Boukhalfa et al., 2007; Fortin et al., 2008). Of respirable metals, iron is ubiquitous and relatively abundant in most environments, and copious amounts of secondary ferrous or mixed valence precipitates may form. Reduction of Fe(III) by dissimilatory metal reducing bacteria such as *Shewanella putrefaciens* and *Geobacter metallireducens* can result in extracellular crystals of magnetite (Fe_3O_4) around 10 nm in diameter, which serve no known purpose for the organisms that produce them (Frankel and Bazylinski, 2003), in addition to larger crystals of minerals with different structures and compositions (Fortin et al., 2008).

Nanominerals associated with the bacterial cell wall

Soluble metals, such as species formed by V^{5+} and U^{6+} , can penetrate the outer membrane of Gram-negative bacteria and form nanometer-size precipitates via an induced process in the periplasm upon reduction during respiration, in addition to forming abundant extracellular precipitates (Figure 1) (Suzuki et al., 2002; Lloyd et al., 2002). Soluble cytochromes in the periplasm catalyze the reduction of the metals to lower valence, insoluble species, e.g., U(IV) and V(IV). These precipitates are several nanometers in diameter and may have considerable consequences for metal mobility in the environment (Suzuki et al., 2002). Recent evidence suggests, however, that extracellular proteins in biofilms inhibit the mobilization of nanoprecipitates by inducing aggregation (Moreau et al., 2007). Nanoparticles generally have a strong tendency to aggregate.



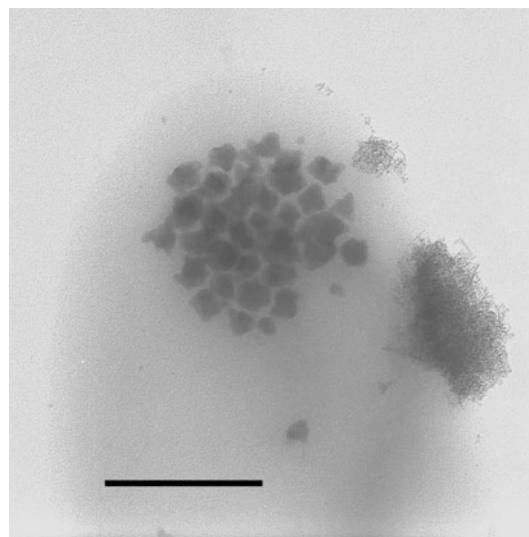
Nanocrystals, Microbially Induced, Figure 1 Precipitates containing V(IV) (a) or U(VI) (b) in *Shewanella putrefaciens* or *Geobacter sulfurreducens*, respectively, formed in the periplasmic space during the dissimilatory reduction of V^{5+} or U^{6+} . Transmission electron micrographs were obtained on thin sections prepared using conventional embedding techniques. Scale bar = 200 nm.

Intracellular nanominerals

Nanocrystalline precipitates of respired metals generally remain external to the cytoplasm or within the periplasm. It has been observed, however, that reduced iron is reoxidized and compartmentalized within the cytoplasm as clusters of 40–50 nm precipitates at the poles of *S. putrefaciens* CN32 during the dissimilatory reduction of iron (Figure 2) (Glasauer et al., 2002; Glasauer et al., 2007). The particles contain both ferric and ferrous iron, display a low degree of crystal order, and appear to be surrounded by a membrane. The function of the particles, if any, is still unknown.

Some soluble, oxidized forms of metals more scarce than Fe can penetrate the plasma membrane and precipitate as induced nanocrystals in the cytoplasm of bacteria, as well as form extracellular precipitates. Examples of these include gold, silver, tellurium, and cadmium; all are highly toxic, and chemical reduction may be part of a detoxification strategy (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001; Baesman et al., 2007). In the case of gold and silver, the intracellular precipitates are highly crystalline and have distinctive morphologies (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001). During detoxification of cadmium present in the extracellular environment, *Schizosaccharomyces pombe* produces intracellular nanocrystalline particles of cadmium sulfide, around 1.8 nm in size, which can act as quantum semiconductor crystallites (Klaus-Joerger et al., 2001).

The challenge in harnessing bacterial metabolism to produce nanoscale minerals lies in controlling the size



Nanocrystals, Microbially Induced, Figure 2 Transmission electron micrograph (TEM) showing intracellular Fe-particles at one end of a *Shewanella putrefaciens* CN32 cell, and ferric hydroxide sorbed to the cell exterior. *S. putrefaciens* produces the intracellular particles during dissimilatory reduction of the ferric hydroxide. Scale bar = 200 nm.

and crystal structure of the products. Extracellular precipitates are subject to the diverse range of chemical conditions that the cell encounters, resulting in amorphous or poorly crystalline materials with a high proportion of impurities (Gilbert and Banfield, 2005). In contrast, *biologically controlled mineralization* takes place in the tightly regulated intracellular environment and results in nanoscale minerals with architectures that are exquisitely defined (Bazylinski and Frankel, 2003). The only known example of bacteria that direct the formation of iron nanocrystals are the magnetotactic bacteria. The intracellular minerals they form consist of either magnetite or greigite (Fe_3S_4). Each crystal functions as a single domain magnet, termed a magnetosome. The magnetosomes are aligned in single or multiple chains within the cytoplasm (Figure 3), which increases the magnetic susceptibility. The function of the magnetosomes is unknown, although it has been widely speculated that the crystals help maintain the bacteria in their optimal environment within the freshwater or brackish aquatic systems where they are found (Bazylinski and Frankel, 2003). Magnetosome magnetite is of great interest because of its magnetic properties and crystal structures, and it is anticipated that understanding the molecular genetics of magnetosome formation will lead to the abiotic synthesis of nanocrystalline magnetite particles with the same morphologies and properties, as well as help explaining their occurrence (Bazylinski and Frankel, 2003).



Nanocrystals, Microbially Induced, Figure 3 Thin section of ARB-1, an uncultured magnetotactic bacterium, imaged by TEM. Magnetosomes are aligned in a short chain at the right of the cell. Large spheroid shapes within the cell are phosphorous-rich inclusions (Cox et al., 2002).

Future directions in microbial nanocrystal research

The use of living or intact bacteria to fabricate nanocrystals requires the cultivation of biomass on a large scale and selective extraction of the mineral particles that form, both of which present challenges. The greatest potential in harnessing bacterial activities for nanocrystal fabrication lies in identifying the genetic systems that control precipitation. The identified systems may then be applied using organisms that are more readily cultivated or to produce larger quantities of nanocrystals. Genetic engineering techniques may be used to exert greater control over the composition and crystal structures of the mineral products. An interesting new research direction in biomaterials considers the impact of biopolymers such as peptides and RNA on nanocrystal formation (Feldheim and Eaton, 2007). Future research may combine bacterial processes that produce abundant nanoparticles with exposure to biopolymers that mediate their formation to generate crystals that have been engineered to impart specific properties.

Summary

Bacterial nanocrystals are of great interest because of their importance in natural environments as well as their practical applications. Their broad relevance has joined scientists across disciplines from geology to biology and material science, which has rapidly advanced the understanding of nanoparticulate matter and its reactivity in natural settings. Controlled nanocrystal synthesis among bacteria is rare and yields relatively low amounts of solid relative to biomass. Induced crystallization processes can result in large volumes of poorly crystalline or morphologically heterogeneous minerals and span a wide range of elemental compositions. Harnessing biological reactions to control microbial nanocrystallization is an outstanding challenge and presents intriguing opportunities for new research paths. Finally, understanding the properties of nanocrystals as well as why some bacteria produce them is crucial to predicting how metals react and transform in natural environments.

Bibliography

- Baesman, S. M., Bullen, T. D., Dewald, J., Zhang, D., Curran, S., Islam, F. S., Beveridge, T. J., and Oremland, R. S., 2007. Formation of tellurium nanocrystals during anaerobic growth of bacteria that use Te oxyanions as respiratory electron acceptors. *Applied and Environmental Microbiology*, **73**, 2135–2143.
- Bazylinski, D. A., and Frankel, R. B., 2003. Biologically controlled mineralization in prokaryotes. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 217–247.
- Beveridge, T. J., 1989. Metal ions and bacteria. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley.
- Boukhalfa, H., Icopini, G. A., Reilly, S. D., and Neu, M. P., 2007. Plutonium (IV) reduction by the metal-reducing bacteria *Geobacter metallireducens* GS15 and *Shewanella oneidensis*.

- MR1. *Applied and Environmental Microbiology*, **73**, 5897–5903.
- Cox, B. L., Popa, R., Bazylnski, D. A., Lanoil, B., Douglas, S., Belz, A., Engler, D. L., and Nealson, K. H., 2002. Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci. *Geomicrobiology Journal*, **19**, 387–406.
- Feldheim, D. L., and Eaton, B. E., 2007. Selection of biomolecules capable of mediating the formation of nanocrystals. *ACA Nano*, **3**, 154–159.
- Ferris, F. G., Beveridge, T. J., and Fyfe, W. S., 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. *Nature*, **320**, 609–611.
- Fortin, D., Langley, S., and Glasauer, S., 2008. Biomineralization: from nature to application. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Metal Ions in Life Sciences*. Chichester: Wiley, Vol. 4, pp. 377–411.
- Frankel, R. B., and Bazylnski, D. A., 2003. Biologically induced mineralization by bacteria. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 95–114.
- Gilbert, B., and Banfield, J. F., 2005. Molecular-scale processes involving nanoparticulate minerals in biogeochemical systems. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 109–155.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2002. Intracellular Fe minerals in a dissimilatory iron-reducing bacterium. *Science*, **295**, 117–119.
- Glasauer, S., Langley, S., Boyanov, M., Lai, B., Kemner, K., and Beveridge, T. J., 2007. Mixed-valence cytoplasmic iron granules are linked to anaerobic respiration. *Applied and Environmental Microbiology*, **73**, 993–996.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 85–108.
- Klaus-Joerger, T., Joerger, R., Olsson, E., and Granqvist, C. G., 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. *Trends in Biotechnology*, **19**, 15–20.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Lloyd, J., Chesnes, J., Glasauer, S., Bunker, D., Livens, F. R., and Lovley, D. R., 2002. Reduction of actinides and fission products by Fe(III)-reducing bacteria. *Geomicrobiology Journal*, **19**, 103–120.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press.
- Moreau, J. W., Weber, P. K., Martin, M. C., Gilbert, B., Hutcheon, I. D., and Banfield, J. F., 2007. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science*, **316**, 1600–1603.
- Nealson, K. H., and Stahl, D. H., 1997. Microorganisms and biogeochemical cycles: What can we learn from stratified communities? In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Sobolov, D., and Roden, E., 2002. Evidence for rapid microscale bacterial redox cycling of iron in circumneutral environments. *Anton van Leeuw*, **181**, 587–597.
- Southam, G., and Beveridge, T. J., 1994. The in vitro formation of placer gold by bacteria. *Geochimica et Cosmochimica Acta*, **58**, 4527–4530.
- Suzuki, Y., Kelly, S. D., Kemner, K. M., and Banfield, J. F., 2002. Nanometre-size products of uranium bioreduction. *Nature*, **419**, 134.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: insights into manganese (II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 1–29.

Cross-references

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Geobacter](#)
[Leptothrix](#)
[Magnetotactic Bacteria](#)
[Microbial Biomineralization](#)
[Microbial Metal Binding](#)
[Nan\(n\)obacteria](#)
[Shewanella](#)

NICKEL, BIOLOGY

Martin Krüger
 Federal Institute for Geosciences and Natural Resources,
 Hannover, Germany

Definition

Nickel, atomic number 28, is a member of the same group in the periodic table as iron, cobalt, and copper. In biology, nickel is a major trace element and part of the catalytic centers of many important metabolic enzymes.

Nickel is one of the most important trace metals in biology. While its concentration in most rocks is only between 10 and 150 $\mu\text{g g}^{-1}$, it is highly enriched in ultramafic rocks, like peridotite and serpentine (Sigel et al., 2006). Nickel is a key component in the active center of a number of enzymes involved in different microbial pathways (Ragsdale, 2007; Ermler et al., 1998). Three nickel metalloenzymes play important roles in the carbon cycle: CO dehydrogenase (CODH), acetyl-CoA synthase (ACS), and methyl-coenzyme M reductase (MCR). The first two enzymes are involved in carbon fixation of anaerobic microorganisms via the Wood–Ljungdahl or reductive acetyl-CoA pathway. CODH allows microorganisms to use carbon monoxide as sole carbon and energy source. It has been suggested that carbon monoxide can be consumed and produced in a bioenergetic cycle

- MR1. *Applied and Environmental Microbiology*, **73**, 5897–5903.
- Cox, B. L., Popa, R., Bazylnski, D. A., Lanoil, B., Douglas, S., Belz, A., Engler, D. L., and Nealson, K. H., 2002. Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci. *Geomicrobiology Journal*, **19**, 387–406.
- Feldheim, D. L., and Eaton, B. E., 2007. Selection of biomolecules capable of mediating the formation of nanocrystals. *ACA Nano*, **3**, 154–159.
- Ferris, F. G., Beveridge, T. J., and Fyfe, W. S., 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. *Nature*, **320**, 609–611.
- Fortin, D., Langley, S., and Glasauer, S., 2008. Biomineralization: from nature to application. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Metal Ions in Life Sciences*. Chichester: Wiley, Vol. 4, pp. 377–411.
- Frankel, R. B., and Bazylnski, D. A., 2003. Biologically induced mineralization by bacteria. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 95–114.
- Gilbert, B., and Banfield, J. F., 2005. Molecular-scale processes involving nanoparticulate minerals in biogeochemical systems. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 109–155.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2002. Intracellular Fe minerals in a dissimilatory iron-reducing bacterium. *Science*, **295**, 117–119.
- Glasauer, S., Langley, S., Boyanov, M., Lai, B., Kemner, K., and Beveridge, T. J., 2007. Mixed-valence cytoplasmic iron granules are linked to anaerobic respiration. *Applied and Environmental Microbiology*, **73**, 993–996.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 85–108.
- Klaus-Joerger, T., Joerger, R., Olsson, E., and Granqvist, C. G., 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. *Trends in Biotechnology*, **19**, 15–20.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Lloyd, J., Chesnes, J., Glasauer, S., Bunker, D., Livens, F. R., and Lovley, D. R., 2002. Reduction of actinides and fission products by Fe(III)-reducing bacteria. *Geomicrobiology Journal*, **19**, 103–120.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press.
- Moreau, J. W., Weber, P. K., Martin, M. C., Gilbert, B., Hutcheon, I. D., and Banfield, J. F., 2007. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science*, **316**, 1600–1603.
- Nealson, K. H., and Stahl, D. H., 1997. Microorganisms and biogeochemical cycles: What can we learn from stratified communities? In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Sobolov, D., and Roden, E., 2002. Evidence for rapid microscale bacterial redox cycling of iron in circumneutral environments. *Anton van Leeuw*, **181**, 587–597.
- Southam, G., and Beveridge, T. J., 1994. The in vitro formation of placer gold by bacteria. *Geochimica et Cosmochimica Acta*, **58**, 4527–4530.
- Suzuki, Y., Kelly, S. D., Kemner, K. M., and Banfield, J. F., 2002. Nanometre-size products of uranium bioreduction. *Nature*, **419**, 134.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: insights into manganese (II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 1–29.

Cross-references

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Geobacter](#)
[Leptothrix](#)
[Magnetotactic Bacteria](#)
[Microbial Biomineralization](#)
[Microbial Metal Binding](#)
[Nan\(n\)obacteria](#)
[Shewanella](#)

NICKEL, BIOLOGY

Martin Krüger
 Federal Institute for Geosciences and Natural Resources,
 Hannover, Germany

Definition

Nickel, atomic number 28, is a member of the same group in the periodic table as iron, cobalt, and copper. In biology, nickel is a major trace element and part of the catalytic centers of many important metabolic enzymes.

Nickel is one of the most important trace metals in biology. While its concentration in most rocks is only between 10 and 150 $\mu\text{g g}^{-1}$, it is highly enriched in ultramafic rocks, like peridotite and serpentine (Sigel et al., 2006). Nickel is a key component in the active center of a number of enzymes involved in different microbial pathways (Ragsdale, 2007; Ermler et al., 1998). Three nickel metalloenzymes play important roles in the carbon cycle: CO dehydrogenase (CODH), acetyl-CoA synthase (ACS), and methyl-coenzyme M reductase (MCR). The first two enzymes are involved in carbon fixation of anaerobic microorganisms via the Wood–Ljungdahl or reductive acetyl-CoA pathway. CODH allows microorganisms to use carbon monoxide as sole carbon and energy source. It has been suggested that carbon monoxide can be consumed and produced in a bioenergetic cycle

(Dobbek et al., 2001; Voordouw, 2002). The Ni-containing CODH is involved in many reactions in anaerobic microorganisms: it catalyzes the oxidation of CO with H₂O to CO₂ and H₂ in carboxydophilic bacteria, and assists the oxidation of acetate to CO₂ in sulfate-reducing bacteria and archaea and the disproportionation of acetate to CH₄ and CO₂ in methanogenic archaea. In many groups of anaerobic microorganisms utilizing the reductive acetyl-CoA pathway, such as acetogenic bacteria or methanogenic archaea, CODH is often found in a complex with ACS (Strauss and Fuchs, 1993; Holo and Sirevag, 1986; Drennan et al., 2004). ACS catalyzes the formation of acetyl-CoA from CO, leading in combination with the CODH to the synthesis of acetate from two CO₂. These two enzymes play an important role in the global carbon cycle, since up to 10% of the organic carbon produced by photosynthesis is remineralized by anaerobic microorganisms involving CODH/ACS (Drake, 1994). MCR is the key enzyme of methanogenic archaea catalyzing the final step during the reduction of carbon dioxide to methane (Thauer, 1998). All biologically formed methane is produced by methanogenic organisms in the terminal step of anaerobic degradation. The nickel is located in a tetrapyrrole coenzyme named F₄₃₀, which is unique to methanogenic archaea. MCR catalyzes the reduction of methyl-coenzyme M with a reduced coenzyme B, leading to the release of methane and a heterodisulfide of the two coenzymes. The latter can be cleaved in an energy yielding reaction. Furthermore, in a process called “reverse methanogenesis,” methane is anaerobically oxidized with sulfate as electron acceptor. This process – known as AOM – is carried out by an uncultured group of archaea (ANME), which has been shown to contain high amounts of a modified MCR (Krüger et al., 2003; Hallam et al., 2003). Hydrogenases are a family of oxidoreductase enzymes that catalyze the reversible oxidation of molecular hydrogen. Nickel-containing hydrogenases have been isolated from bacteria and archaea (Voordouw, 1992). Finally, nickel is also present in a number of other enzymes, such as urease, a superoxide dismutase, and glyoxylases. The functions and biological roles of these metalloproteins have been described in detail (Sigel et al., 2006).

Recently, due to its strong involvement in geologically important microbial reactions, especially methanogenesis, AOM, and carbon fixation, nickel has raised interest as a potential biosignature for these processes and the involved microorganisms in recent or ancient environments (Reitner et al., 2005; Hausrath et al., 2007).

Bibliography

- Dobbek, H., Svetlitchnyi, V., Gremer, L., Huber, R., and Meyer, O., 2001. Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. *Science*, **293**, 1281.
- Drake, H. L., 1994. *Acetogenesis*. New York: Chapman & Hall, pp. 273–504.

- Drennan, C. L., Doukov, T. I., and Ragsdale, S. W., 2004. The metallocusters of carbon monoxide dehydrogenase/acetyl-CoA synthase: a story in pictures. *Journal of Biological Inorganic Chemistry*, **9**, 511–515.
- Ermiler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer, R. K., 1998. Active sites of transition metal enzymes with focus on nickel. *Current Opinion Structural Biology*, **8**, 749–756.
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F., 2003. Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing Archaea. *Applied and Environmental Microbiology*, **69**, 5483–5491.
- Hausrath, E. M., Liermann, L. J., House, C. H., Ferry, J. G., and Brantley, S. L., 2007. The effect of methanogen growth on mineral substrates: will Ni markers of methanogen-based communities be detectable in the rock record? *Geobiology*, **5**, 49–61.
- Holo, H., and Sirevag, R., 1986. Autotrophic growth and CO₂ fixation of *Chloroflexus aurantiacus*. *Archives Microbiology*, **145**, 173–180.
- Krüger, M., Meyerdierks, A., Glöckner, F. O., Amann, R., Widdel, F., et al., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature*, **426**, 878–881.
- Ragsdale, S. W., 2007. Nickel and the carbon cycle. *Journal of Inorganic Biochemistry*, **101**, 1657–1666.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 71–84.
- Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), 2006. *Nickel and its Surprising Role in Nature*. Weinheim, Germany: Wiley VCH.
- Strauss, G., and Fuchs, G., 1993. Enzymes of a novel autotrophic CO₂ fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *European Journal Biochemistry*, **215**, 633–643.
- Thauer, R. K., 1998. Biochemistry of methanogenesis, a tribute to Marjory Stephenson. *Microbiology*, **144**, 2377–2406.
- Voordouw, G., 1992. Evolution of hydrogenase genes. In Cammack, R., and Sykes, A. (eds.), *Advances in Inorganic Chemistry*. London: Academic Press, Vol. 38, pp. 397–422.
- Voordouw, G., 2002. Carbon monoxide cycling by *Desulfovibrio vulgaris* Hildenborough. *Journal Bacteriology*, **184**, 5903–5911.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Biosignatures in Rocks](#)
[Carbon Cycle](#)
[Hydrogen](#)
[Metalloenzymes](#)
[Methanogens](#)
[Sulfate-Reducing Bacteria](#)

NITRIFICATION

Nitrification is the two-step microbial process by which ammonia (NH₃) is first oxidized to nitrite (NO₂[−]) which is, in a second reaction, converted to nitrate (NO₃[−]). See entry “[Nitrogen](#)” for detailed reading.

N

NAN(N)OBACTERIA

Muriel Pacton¹, Georges E. Gorin²

¹ETH Zurich, Zurich, Switzerland

²University of Geneva, Geneva, Switzerland

Synonyms

Living nanovesicles; Nanobes; Nanocalcifying particles (NCP); Nanoforms; Nanoglobules; Nanons; Nano-organisms; Nanoparticles

Biologists used the word *nanobacteria* to describe organisms that possibly enhance biomineralization such as that of kidney stones in humans, whereas *nannobacteria* is used by geologists for organisms responsible for mineral precipitation in terrestrial and extraterrestrial rocks.

Definition

Nan(n)obacteria are tiny globular objects, ranging in diameter from 20 to 200 nm, with the ability to nucleate minerals. They are the smallest known, self-replicating particles with slow growth rate. Because they are by definition down to 1/10 the size of bacteria, it raises the problem of whether or not an organism of this size is large enough to house necessary cell components such as DNA, RNA, and plasmids.

Introduction

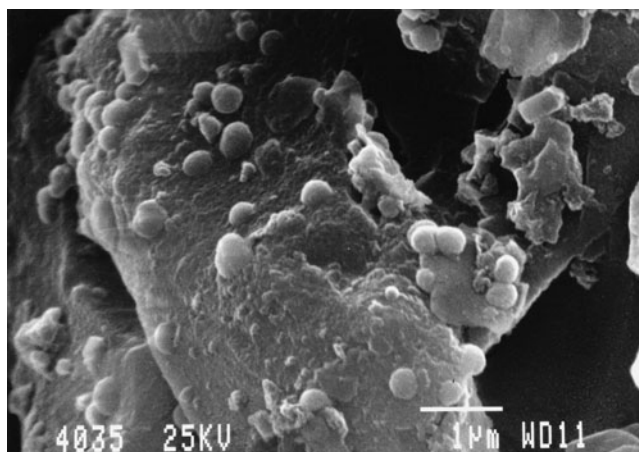
The term “nan(n)obacteria” has been originally proposed by Folk (1993) to describe 20–200 nm wide nanovesicles observed in carbonates using scanning electron microscopy (SEM). Kajander and Çiftçioglu (1998) defined the role of similar tiny particles in kidney stone formation. Based on their mineralogical properties, nan(n)obacteria have also been called calcifying nanoparticles (CNP) by

these authors. Nan(n)obacteria-like particles have been observed in Martian meteorites, thereby raising questions about a primitive form of life (Bradley et al., 1997). CNP are studied extensively in pathogenic diseases as infectious agents by nucleating crystals (hydroxyapatite). Therefore, some comparisons have tentatively been made with the geological record. Although they display some evidences of bacterial nature, their living status is subject to controversy because of their small size and the fact that DNA extraction remains unsuccessful. On one hand, biologists discuss their metabolic existence and on the other hand geologists argue for widespread organic fossilization in palaeoenvironments. When interrelations between geology and microbiology are possible, they are looked at only from the point of view of experimental procedures (e.g., one emphasizes the use of DNA probes in geological samples). This prohibits an objective comparison of these very small particles.

Reported nan(n)obacteria in the literature

In the geological record, nan(n)obacteria-like particles are encountered mainly in carbonates: in calcite (Dix et al., 1999; Chen et al., 2005; Mamet and Pr  at, 2006), microbialites (Camoin et al., 1999), dolomite (Figure 1; Vasconcelos et al., 1995; Gournay et al., 1999; Wright, 1999; Al  i  ek, 2009), hydrothermal springs (Casanova et al., 1999), and oncolite laminations (Cavalazzi et al., 2007). They have also been observed in sandstones without mineral precipitation (Uwins et al., 1998) and on quartz overgrowth in crude oil (Spark et al., 2000).

In medical biology, CNP have been the focus of numerous studies on various pathogenic diseases (see Urbano and Urbano (2007) for a review), for example, gallbladder (Wang et al., 2006), nasopharyngeal cancer (Zhou et al., 2006), and renal stone formations (e.g., Kajander and   ift  i  glu (1998) and Jones et al. (2009)).



Nan(n)obacteria, Figure 1 Nan(n)obacteria encrusted by nanocrystals of carbonate on a quartz substrate. (Picture courtesy of Crisogono Vasconcelos.)

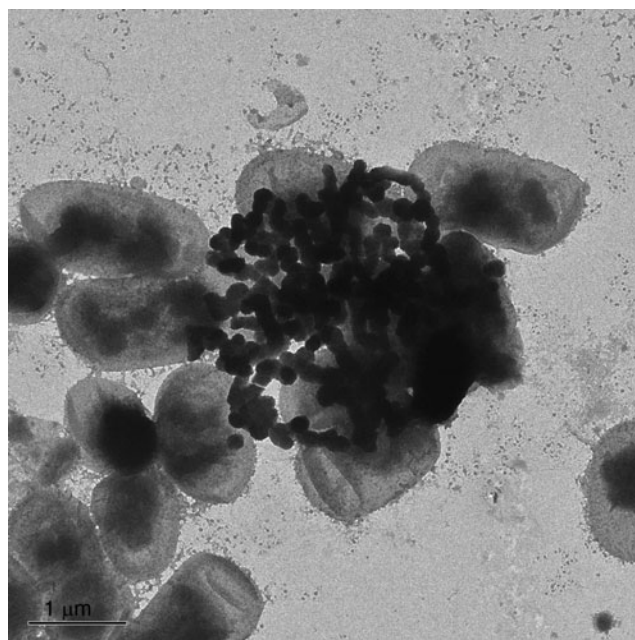
Characterization of purported nan(n)obacteria

Nan(n)obacteria exhibit some characteristics, which seem to correspond to the classical criteria indicating a biological origin: (1) a typical bacterial appearance (Kajander and Çiftçioglu, 1998; Uwins et al., 1998); (2) a slow growth in liquid cell culture media; (3) an expression of specific antigens (Hjelle et al., 2000; Miller et al., 2004) by using monoclonal antibodies commercially available through Nanobac Oy (Finland) as 8D10 and through Santa Cruz Biotechnology, USA, named GM-CSFR α ; (4) a staining with nucleic fluorochroms (Uwins et al., 1998; Miller et al., 2004) using 4',6-diamidino-2-phenylindole (DAPI), acridine orange, Hoechst 33258, Feulgen, and/or Picogreen; (5) a resistance to antibiotics such as tetracycline and citrate (Maniscalco and Taylor, 2004); (6) a sensitivity to calcium-chelating agents (Rawal and Pretorius, 2005); and (7) a DNA sequencing tentatively assigned to the α -2 subgroup of proteobacteria, which also includes *Brucella* and *Bartonella* species occurring within and outside of host cells (Kajander and Çiftçioglu, 1998; Çiftçioglu and Kajander, 1998). According to Cisar et al. (2000), they could come from PCR contaminant.

Possible origins for nan(n)obacteria

Due to the very small size of purported nan(n)obacteria, the hypothesis that they might represent living entities is very controversial. Several, alternative origins have been proposed:

Inorganic origin: Inorganic precipitates (Vecht and Ireland, 2000; Cisar et al., 2000) and artifacts due to sample preparation (e.g., coating for SEM, acid treatment, polishing artifacts) have been considered as an alternative origin for these nanostructures. However, nanostructures



Nan(n)obacteria, Figure 2 Chain of dolomite nanoglobules formed in *Halomonas meridiana* culture. (Picture courtesy of Monica Sanchez-Roman.)

can also be observed in samples that have not been exposed to these techniques (Folk and Lynch, 1997).

Extracellular polymeric substances (EPS): Reitner et al. (1995) have proposed that nanospheres were an initial calcification of EPS. They have been interpreted as early stages of dolomite precipitation in anaerobic cultures (Bontognali et al., 2008) and aerobic cultures (Figure 2; Sanchez-Roman et al., 2008). Similar observations have been made on aragonite nanocrystals that contained organic molecules (Benzerara et al., 2006).

Proteins: Tissue degradation caused by microbial activity can create nanobacteria-like particles of proteinous origin (Schieber and Arnott, 2003). In the case of kidney stones, it has been confirmed that nan(n)obacteria are in fact related to blood proteins (Martel and Young 2008), that is, fetuin (Raoult et al., 2008).

Small bacteria: Dwarf bacteria generally show a decrease in cell size. This is assumed to be the case of bacteria exposed to low nutrient or starvation conditions (Velimirov, 2001). Ultramicrobacteria exhibit a very low growth rate (Torella and Morita, 1981). The distinction between ultramicrobacteria and starvation forms is based on their physiological properties (Velimirov, 2001).

Viruses: Stressed bacteria overproduce vesicles that are apparently used to selectively eliminate unwanted material, for example, unfolded proteins (Soler et al., 2008). All fluorescent small-size particles are assumed to correspond to a DNA virus (Soler et al., 2008). Membrane vesicles seem to be produced by most bacteria and have been

detected in natural environments, including biofilms (Schooling and Beveridge, 2006).

Minimal conditions for a living cell

Theoretical calculations have been made in order to define the smallest size needed for a cell to have metabolic processes and accommodate the smallest genome. According to these assumptions, the minimal cell size should fall within the range of 100 nm (Boal, 1999), 140 nm (Maniloff et al., 1997), and 170 nm (Adams, 1999). Assuming a cell genome housing 250 genes (which is considered the minimal number needed to support heterotrophic cell growth in a nutrient-rich environment with a minimal metabolic pathway), a minimum diameter of 50 nm has been established, which could contain eight genes (Adams, 1999). Nevertheless, a cell with a size of only 50 nm, a 5 nm cell wall, 2 ribosomes, 520 protein molecules, and a DNA strand containing 8 genes is considered too small to function like recent bacteria, based on the smallest known living organism, that is, *Nanoarchaeum equitans* (Huber et al., 2002).

Universal origin for nan(n)obacteria?

The analogy between nan(n)obacteria-like particles in pathogenic diseases and geological records is often made even though the methodology differs. Nan(n)obacteria have not been found in any clinical condition that does not involve calcification, which is not the case of some geological samples (Uwins et al., 1998; Schieber and Arnott, 2003). Recently, nan(n)obacteria-like particles associated with kidney stone formation have been assigned directly to blood proteins reacting with antibodies that were so far claimed to be specifically associated to nan(n)obacteria (Martel and Young, 2008; Raoult et al., 2008). The latter authors suggested that they are nucleic-acid free. An alternative origin has been proposed where nan(n)obacteria could be the by-product of the living activities of bona fide bacteria inhabiting kidney stones (Aloisi, 2008). If we consider that nan(n)obacteria could be EPS accumulative and knowing that EPS contain DNA (Whitchurch et al., 2002; Steinberg and Holden, 2005), it is unlikely that this hypothesis fits with nanobes observed by Uwins et al. (1998), where no other microorganisms were detected.

Summary

Nan(n)obacteria emerged as a new form of life during the last two decades, based mainly on their capability to trigger infectious diseases. Their interpretation as simple proteins in kidney stones has been recently ruled out, although similar nano-objects have been also observed in geological records. The controversy about their existence (too small to be biologically viable) is still ongoing today because no universal origin can be attributed to these nanoparticles. The EM imaging does not present

enough evidence alone, and molecular and physiological data are required in order to try to define their properties.

Bibliography

- Adams, M. W. W., 1999. The influence of environment and metabolic capacity on the size of a microorganism. In Steering Group, (eds.), *Size limits of very small microorganisms: Proceedings of a workshop*. Washington, DC: National Academy Press, National Research Council, Space Studies Board, 74–80.
- Alçıçek, H., 2009. Late Miocene nonmarine sedimentation and formation of magnesites in the Acıgöl Basin, southwestern Anatolia, Turkey. *Sedimentary Geology*, **219**, 115–135.
- Aloisi, G., 2008. An alternative origin for nanobacteria in kidney stones. *Bioscience Hypotheses*, **1**, 138–141.
- Benzerara, K., Menguy, N., Lopez-Garcia, P., Yoon, T.-H., Kazmierczak, J., Tyliszczak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 9440–9445.
- Boal, D., 1999. Mechanical characteristics of very small cells. In Steering Group, (eds.), *Size limits of very small microorganisms: Proceedings of a workshop*. Washington, DC: National Academy Press, National Research Council, Space Studies Board, 26–31.
- Boal, D., 2001. How small can a microorganism be? In: *Size limits of very small microorganisms*. Washington, DC: National Academy of Sciences.
- Bontognali, T. R. R., Vasconcelos, C., Warthmann, R. J., Dupraz, C., Bernasconi, S. M., and McKenzie, J. A., 2008. Microbes produce nanobacteria-like structures, avoiding cell entombment. *Geology*, **36**, 663–666.
- Bradley, J. P., Harvey, R. P., McSween, H. Y., Gibson, E., Thomas-Keptra, K., and Vali, H., 1997. No “nanofossils” in Martian meteorite. *Nature*, **390**, 454–456.
- Camoin, G. F., Gautret, P., Montaggioni, L. F., and Cabioch, G., 1999. Nature and environmental significance of microbialites in Quaternary reefs: the Tahiti paradox. *Sedimentary Geology*, **126**, 271–304.
- Casanova, J., Bodéan, F., Négrel, P., and Azaroual, M., 1999. Microbial control on the precipitation of modern ferrihydrite and carbonate deposits from the Cézallier hydrothermal springs (Massif Central, France). *Sedimentary Geology*, **126**, 125–145.
- Cavalazzi, B., Barbieri, R., and Ori, G. G., 2007. Chemosynthetic microbialites in the Devonian carbonate mounds of Hamar Laghdad (Anti-Atlas, Morocco). *Sedimentary Geology*, **200**, 73–88.
- Chen, D. F., Huang, Y. Y., Yuan, X. L., and Cathles, L. M., 2005. Seep carbonates and preserved methane oxidizing archaea and sulphate reducing bacteria fossils suggest recent gas venting on the seafloor in the Northeastern South China Sea. *Marine and Petroleum Geology*, **22**, 613–621.
- Çiftçioglu, N., and Kajander, E. O., 1998. Interaction of nanobacteria with cultured mammalian cells. *Pathophysiology*, **4**, 259–270.
- Cisar, J., De-Qi, X., Thompson, J., Swaim, W., Hu, L., and Kopecko, D., 2000. An alternative interpretation of nanobacteria-induced biomineralization. *Microbiology*, **97**, 11511–11515.
- Dix, G. R., Patterson, T. R., and Park, L. E., 1999. Marine saline ponds as sedimentary archives of late Holocene climate and sea-level variation along a carbonate platform margin: Lee Stocking Island, Bahamas. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **150**, 223–246.

- Folk, R., 1993. SEM imaging of bacteria and nannobacteria in carbonate sediments and rocks. *Journal of Sedimentary Petrology*, **63**, 990–999.
- Folk, R., and Lynch, L., 1997. The possible role of nannobacteria in clay-mineral diagenesis and the importance of careful sample preparation in high-magnification SEM study. *Journal of Sedimentary Research*, **67**, 583–589.
- Gournay, J., Kirkland, B., Folk, R., and Lynch, F., 1999. Nanometer-scale features in dolomite from Pennsylvanian rocks, Paradox Basin, Utah. *Sedimentary Geology*, **126**, 243–252.
- Hjelle, J. T., Miller-Hjelle, M. A., Poxton, I. R., Kajander, E. O., Çiftçioglu, N., Jones, M. L., Caughey, R. C., Brown, R., Millikin, P. D., and Darras, F. S., 2000. Endotoxin and nanobacteria in polycystic kidney disease. *Kidney International*, **57**, 2360–2374.
- Huber, H., Hohn, M. J., Rachel, R., Fuchs, T., Wimmer, V. C., and Stetter, K. O., 2002. A new phylum of Archaea represented by a nanosized hyperthermophilic symbiont. *Nature*, **417**, 63–67.
- Jones, J. A., Çiftçioglu, N., Schmid, J. F., Barr, Y. R., and Griffith, D., 2009. Calcifying nanoparticles (nanobacteria): an additional potential factor for urolithiasis in space flight crews. *Urology*, **73**, e11–e13.
- Kajander, E. O., and Çiftçioglu, N., 1998. Nanobacteria: an alternative mechanism for pathogenic intra- and extracellular calcification and stone formation. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 8274–8279.
- Mamet, B., and Pr  at, A., 2006. Iron-bacterial mediation in Phanerozoic red limestones: State of the art. *Sedimentary Geology*, **185**, 147–157.
- Maniloff, J., Nealson, K. H., Psenner, R., Loferer, M., and Folk, R. L., 1997. Nannobacteria: size limits and evidence. Letters from: Jack Maniloff, Kenneth H. Nealson, Roland Psenner and Maria Loferer, and Robert L. Folk. *Science*, **276**, 1773–1776.
- Maniscalco, B. S., and Taylor, K. A., 2004. Calcification in coronary artery disease can be reversed by EDTA-tetracycline long-term chemotherapy. *Pathophysiology*, **11**, 95–101.
- Martel, J., and Young, J. D.-E., 2008. Purported nanobacteria in human blood as calcium carbonate nanoparticles. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 5549–5554.
- Miller, V. M., Rodgers, G., Charlesworth, J. A., Kirkland, B., Severson, S. R., Rasmussen, T. E., Yagubyan, M., Rodgers, J. C., Cockerill, F. R., III, Folk, R. L., Rzewuska-Lech, E., Kumar, V., Farrell-Baril, G., and Lieske, J. C., 2004. Evidence of nanobacterial-like structures in calcified human arteries and cardiac valves. *American Journal of Physiology – Heart and Circulatory Physiology*, **287**, H1115–1124.
- Raoult, D., Drancourt, M., Azza, S., Nappiez, C., Guieu, R., Rolain, J.-M., Fourquet, P., Campagna, B., La Scola, B., Mege, J.-L., Mansuelle, P., Lechevalier, E., Berland, Y., Gorvel, J.-P., and Renesto, P., 2008. Nanobacteria are mineralo fetuin complexes. *PLoS Pathogens*, **4**, e41.
- Rawal, B. D., and Pretorius, A.-M., 2005. “Nanobacterium sanguineum” Is it a new life-form in search of human ailment or commensal: overview of its transmissibility and chemical means of intervention. *Medical Hypotheses*, **65**, 1062–1066.
- Reitner, J., Gautret, P., Marin, F., and Neuweiler, F., 1995. Automicrites in a modern microbialite. Formation model via organic matrices (Lizard Island, Great Barrier Reef, Australia). *Bulletin de l’Institut Oc  anographique (Monaco)*, **14**, 237–263.
- Sanchez-Roman, M., Vasconcelos, C., Schmid, T., Dittrich, M., McKenzie, J. A., Zenobi, R., and Rivadeneyra, M. A., 2008. Aerobic microbial dolomite at the nanometer scale: implications for the geologic record. *Geology*, **36**, 879–882.
- Schieber, J., and Arnott, H. J., 2003. Nannobacteria as a by-product of enzyme-driven tissue decay. *Geology*, **31**, 717–720.
- Schooling, S. R., and Beveridge, T. J., 2006. Membrane vesicles: an overlooked component of the matrices of biofilms. *Journal of Bacteriology*, **188**, 5945–5957.
- Soler, N., Marguet, E., Verbavatz, J.-M., and Forterre, P., 2008. Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. *Research in Microbiology*, **159**, 390–399.
- Spark, I., Patey, I., Duncan, B., Hamilton, A., Devine, C., and McGovern-Traa, C., 2000. The effects of indigenous and introduced microbes on deeply buried hydrocarbon reservoirs, North Sea. *Clay Minerals*, **35**, 5–12.
- Steinberg, R. E., and Holden, P. A., 2005. Extracellular DNA as single- and multiple-species unsaturated biofilms. *Applied and Environmental Microbiology*, **71**, 5404–5410.
- Torella, F., and Morita, R. Y., 1981. Microcultural study of bacteria size changes and microcolony and ultramicrocolony formation by heterotrophic bacteria in seawater. *Applied and Environmental Microbiology*, **41**, 518–527.
- Urbano, P., and Urbano, F., 2007. Nanobacteria: facts or fancies? *PLoS Pathogens*, **3**, e55.
- Uwins, P., Webb, R., and Taylor, A., 1998. Novel nano-organisms from Australian sandstones. *American Mineralogist*, **83**, 1541–1550.
- Vasconcelos, C., McKenzie, J. A., Bernasconi, S., Grujic, D., and Tien, A. J., 1995. Microbial mediation as a possible mechanism for natural dolomite formation at low temperatures. *Nature*, **377**, 220–222.
- Vecht, A., and Ireland, T. G., 2000. The role of vaterite and aragonite in the formation of pseudo-biogenic carbonate structures: implications for Martian exobiology. *Geochimica et Cosmochimica Acta*, **64**, 2719–2725.
- Velimirov, B., 2001. Nanobacteria, ultramicrobacteria and starvation forms: a search for the smallest metabolizing bacterium. *Microbes and Environments*, **16**, 67–77.
- Wang, L., Shen, W., Wen, J., An, X., Cao, L., and Wang, B., 2006. An animal model of black pigment gallstones caused by nanobacteria. *Digestive Diseases and Sciences*, **51**, 1126–1132.
- Whitchurch, C. B., Tolker-Nielsen, T., Ragas, P. C., and Mattick, J. S., 2002. Extracellular DNA required for bacterial biofilm formation. *Science*, **295**, 1487.
- Wright, D. T., 1999. The role of sulphate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia. *Sedimentary Geology*, **126**, 147–157.
- Zhou, H.-D., Li, G.-Y., Yang, Y.-X., Li, X.-L., Sheng, S.-R., Zhang, W.-L., and Zhao, J., 2006. Intracellular co-localization of SPLUNC1 protein with nanobacteria in nasopharyngeal carcinoma epithelia HNE1 cells depended on the bactericidal permeability increasing protein domain. *Molecular Immunology*, **43**, 1864–1871.

Cross-references

Bacteria
 Biosignatures in Rocks
 Dolomite, Microbial
 Extracellular Polymeric Substances (EPS)
 Microbial Biomineralization
 Nanocrystals, Microbially Induced
 Origin of Life

NANOCRYSTALS, MICROBIALLY INDUCED

Susan Glasauer

University of Guelph, Guelph, Ontario, Canada

Definition

Mineral particles less than 0.1 μm are a vital component of geochemical cycling in the Earth surface systems. Particles in this size range are generally termed “nanoparticles”; nanocrystals are nanoparticles that exhibit long-range structural order. Their small size implies that they may be relatively mobile in natural systems, as well as more chemically labile than large crystals. They have recently come to the forefront of material science, with new evidence that the properties of some materials are a strong function of particle size, particularly at the nanometer scale.

Nanocrystals are characterized by higher surface strain and disorder, higher surface reactivity, and differences in reaction kinetics compared to larger crystals of the same composition. These properties make them important reactive agents in natural systems, especially given that minerals in surface and near-surface environments are typically smaller and more highly disordered than minerals crystallized under ideal conditions. In general, the interest in biological nanomaterials is focused on specific properties that a given material can offer. For example, the electronic structure of nanocrystals is of particular interest from the perspective of material science. For a review of chemical and physical properties related to nanomineral structure and composition, see Gilbert and Banfield (2005).

The crystallization of biominerals spans eukaryotic (“true nucleus”) and prokaryotic organisms (Lowenstam and Weiner, 1989). Eukaryotes form geologically important structures that may be based on nanoparticles, such as the silica-reinforced shells of diatoms or the calcite shells of coccolithophores and foraminifera (Weiner and Dove, 2003). Prokaryotes dominate, however, among microorganisms that mediate the development of nanoparticles, in terms of their ubiquity in surface and subsurface environments and the compositional and structural range of materials they can form (Gilbert and Banfield, 2005). This article focuses mainly on bacterial nanoparticles that contain metals because of their importance in Earth surface and subsurface environments as well as their relevance to other disciplines related to nanoscience.

Bacterial nanocrystals

Induced versus controlled crystallization

The development of biominerals or biocrystals may be either biologically induced or biologically controlled by the bacteria (Lowenstam and Weiner, 1989). In *biologically controlled mineralization*, microbes exert genetic

and chemical control over the formation of precipitates that are designed to serve a particular function. In contrast, *biologically induced mineralization* yields particles that are secondary to a chemical process controlled by the microorganism. In this case, the precipitates serve no physiological purpose.

Biologically induced nanominerals can result from a range of bacterial processes, including respiration, detoxification, and ion uptake or expulsion during metabolism. Although minerals with a range of sizes and crystal structures can be produced as a result of biologically induced mineralization (e.g., Fortin et al., 2008), the nanometer-sized minerals are of particular interest because of their chemical reactivity as well as the potential for transport in natural systems. Reactivity is a consequence of the high proportion of surface atoms, which are chemically uncoordinated at the solution interface and less ordered than fully coordinated atoms in the structure.

Nanomineral formation can be induced in the extracellular environment, as a result of changes in solution chemistry caused by uptake or expulsion of ions (e.g., H^+) from the cell (Fortin et al., 2008). In addition, bacterial surfaces carry negative and positive charges due to the dissociation of functional groups integral to the cell wall (Beveridge, 1989). Sorption of ions to charged bacterial surfaces or sheaths can lower the nucleation energy for crystal formation and induce thick mantles to develop based on nanoparticles. This process can be observed during the growth of silica sheaths around bacteria and cyanobacteria, which may also incorporate metal ions (Ferris et al., 1986; Konhauser et al., 2004). More examples of induced mineralization are discussed below.

Extracellular nanominerals

Bacterial metal respiration is a process in which electrons are removed or accepted by a metal ion to generate energy for the cell. Specific bacteria are capable of either oxidation or reduction; for example, *Gallionella* spp. and *Leptothrix* spp. are known iron oxidizers, whereas *Shewanella* and *Geobacter* are genera associated with metal reduction. Metal oxidation and reduction (“redox”) by bacteria may take place in chemically stratified zones within a sediment or water column, or may occur in microscale zones (Nealson and Stahl, 1997; Sobolov and Roden, 2002). Minerals form when a change in metal valence induced by respiration affects the solubility of a metal, in the presence of a suitable counterion to react with the reduced or oxidized metal species; the minerals form by an induced process rather than a controlled one.

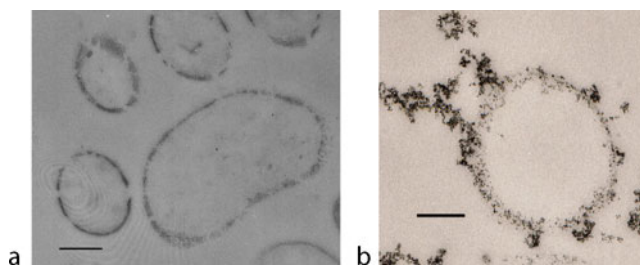
Iron- and manganese-oxidizing bacteria can produce copious quantities of nanoparticulate minerals via a respiratory process that strips electrons from lower valence states of the metal and transfers them to the electron transport chain (Tebo et al., 1997; Kappler and Straub, 2005). In the case of iron, particles of poorly crystalline ferric hydroxide result that are typically 3–5 nm in diameter;

these aggregate strongly and eventually form larger particles of more crystalline iron oxides (Kappler and Straub, 2005).

For metal-reducing bacteria, metals serve as the terminal electron acceptor during respiration rather than being assimilated by the bacteria. Metals transformed by bacteria during dissimilatory respiration that form nanoparticles include Fe, Mn, U, Tc, Cr, V, and Pu (Lloyd et al., 2002; Boukhalfa et al., 2007; Fortin et al., 2008). Of respirable metals, iron is ubiquitous and relatively abundant in most environments, and copious amounts of secondary ferrous or mixed valence precipitates may form. Reduction of Fe(III) by dissimilatory metal reducing bacteria such as *Shewanella putrefaciens* and *Geobacter metallireducens* can result in extracellular crystals of magnetite (Fe_3O_4) around 10 nm in diameter, which serve no known purpose for the organisms that produce them (Frankel and Bazylinski, 2003), in addition to larger crystals of minerals with different structures and compositions (Fortin et al., 2008).

Nanominerals associated with the bacterial cell wall

Soluble metals, such as species formed by V^{5+} and U^{6+} , can penetrate the outer membrane of Gram-negative bacteria and form nanometer-size precipitates via an induced process in the periplasm upon reduction during respiration, in addition to forming abundant extracellular precipitates (Figure 1) (Suzuki et al., 2002; Lloyd et al., 2002). Soluble cytochromes in the periplasm catalyze the reduction of the metals to lower valence, insoluble species, e.g., U(IV) and V(IV). These precipitates are several nanometers in diameter and may have considerable consequences for metal mobility in the environment (Suzuki et al., 2002). Recent evidence suggests, however, that extracellular proteins in biofilms inhibit the mobilization of nanoprecipitates by inducing aggregation (Moreau et al., 2007). Nanoparticles generally have a strong tendency to aggregate.



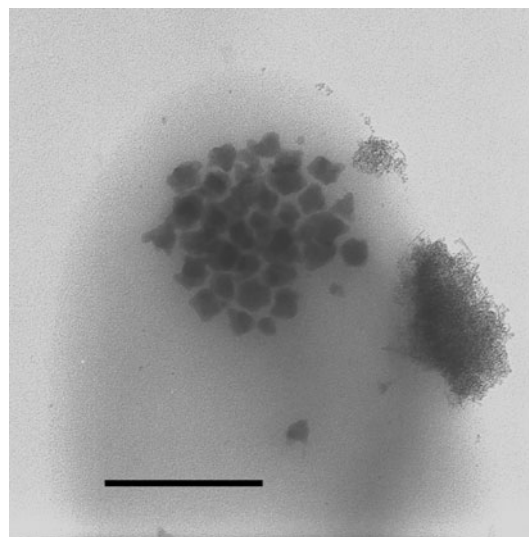
Nanocrystals, Microbially Induced, Figure 1 Precipitates containing V(IV) (a) or U(VI) (b) in *Shewanella putrefaciens* or *Geobacter sulfurreducens*, respectively, formed in the periplasmic space during the dissimilatory reduction of V^{5+} or U^{6+} . Transmission electron micrographs were obtained on thin sections prepared using conventional embedding techniques. Scale bar = 200 nm.

Intracellular nanominerals

Nanocrystalline precipitates of respired metals generally remain external to the cytoplasm or within the periplasm. It has been observed, however, that reduced iron is reoxidized and compartmentalized within the cytoplasm as clusters of 40–50 nm precipitates at the poles of *S. putrefaciens* CN32 during the dissimilatory reduction of iron (Figure 2) (Glasauer et al., 2002; Glasauer et al., 2007). The particles contain both ferric and ferrous iron, display a low degree of crystal order, and appear to be surrounded by a membrane. The function of the particles, if any, is still unknown.

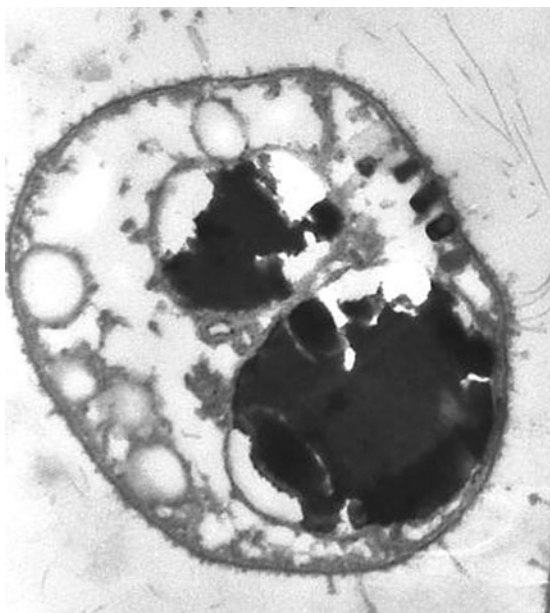
Some soluble, oxidized forms of metals more scarce than Fe can penetrate the plasma membrane and precipitate as induced nanocrystals in the cytoplasm of bacteria, as well as form extracellular precipitates. Examples of these include gold, silver, tellurium, and cadmium; all are highly toxic, and chemical reduction may be part of a detoxification strategy (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001; Baesman et al., 2007). In the case of gold and silver, the intracellular precipitates are highly crystalline and have distinctive morphologies (Southam and Beveridge, 1994; Klaus-Joerger et al., 2001). During detoxification of cadmium present in the extracellular environment, *Schizosaccharomyces pombe* produces intracellular nanocrystalline particles of cadmium sulfide, around 1.8 nm in size, which can act as quantum semiconductor crystallites (Klaus-Joerger et al., 2001).

The challenge in harnessing bacterial metabolism to produce nanoscale minerals lies in controlling the size



Nanocrystals, Microbially Induced, Figure 2 Transmission electron micrograph (TEM) showing intracellular Fe-particles at one end of a *Shewanella putrefaciens* CN32 cell, and ferric hydroxide sorbed to the cell exterior. *S. putrefaciens* produces the intracellular particles during dissimilatory reduction of the ferric hydroxide. Scale bar = 200 nm.

and crystal structure of the products. Extracellular precipitates are subject to the diverse range of chemical conditions that the cell encounters, resulting in amorphous or poorly crystalline materials with a high proportion of impurities (Gilbert and Banfield, 2005). In contrast, *biologically controlled mineralization* takes place in the tightly regulated intracellular environment and results in nanoscale minerals with architectures that are exquisitely defined (Bazylinski and Frankel, 2003). The only known example of bacteria that direct the formation of iron nanocrystals are the magnetotactic bacteria. The intracellular minerals they form consist of either magnetite or greigite (Fe_3S_4). Each crystal functions as a single domain magnet, termed a magnetosome. The magnetosomes are aligned in single or multiple chains within the cytoplasm (Figure 3), which increases the magnetic susceptibility. The function of the magnetosomes is unknown, although it has been widely speculated that the crystals help maintain the bacteria in their optimal environment within the freshwater or brackish aquatic systems where they are found (Bazylinski and Frankel, 2003). Magnetosome magnetite is of great interest because of its magnetic properties and crystal structures, and it is anticipated that understanding the molecular genetics of magnetosome formation will lead to the abiotic synthesis of nanocrystalline magnetite particles with the same morphologies and properties, as well as help explaining their occurrence (Bazylinski and Frankel, 2003).



Nanocrystals, Microbially Induced, Figure 3 Thin section of ARB-1, an uncultured magnetotactic bacterium, imaged by TEM. Magnetosomes are aligned in a short chain at the right of the cell. Large spheroid shapes within the cell are phosphorous-rich inclusions (Cox et al., 2002).

Future directions in microbial nanocrystal research

The use of living or intact bacteria to fabricate nanocrystals requires the cultivation of biomass on a large scale and selective extraction of the mineral particles that form, both of which present challenges. The greatest potential in harnessing bacterial activities for nanocrystal fabrication lies in identifying the genetic systems that control precipitation. The identified systems may then be applied using organisms that are more readily cultivated or to produce larger quantities of nanocrystals. Genetic engineering techniques may be used to exert greater control over the composition and crystal structures of the mineral products. An interesting new research direction in biomaterials considers the impact of biopolymers such as peptides and RNA on nanocrystal formation (Feldheim and Eaton, 2007). Future research may combine bacterial processes that produce abundant nanoparticles with exposure to biopolymers that mediate their formation to generate crystals that have been engineered to impart specific properties.

Summary

Bacterial nanocrystals are of great interest because of their importance in natural environments as well as their practical applications. Their broad relevance has joined scientists across disciplines from geology to biology and material science, which has rapidly advanced the understanding of nanoparticulate matter and its reactivity in natural settings. Controlled nanocrystal synthesis among bacteria is rare and yields relatively low amounts of solid relative to biomass. Induced crystallization processes can result in large volumes of poorly crystalline or morphologically heterogeneous minerals and span a wide range of elemental compositions. Harnessing biological reactions to control microbial nanocrystallization is an outstanding challenge and presents intriguing opportunities for new research paths. Finally, understanding the properties of nanocrystals as well as why some bacteria produce them is crucial to predicting how metals react and transform in natural environments.

Bibliography

- Baesman, S. M., Bullen, T. D., Dewald, J., Zhang, D., Curran, S., Islam, F. S., Beveridge, T. J., and Oremland, R. S., 2007. Formation of tellurium nanocrystals during anaerobic growth of bacteria that use Te oxyanions as respiratory electron acceptors. *Applied and Environmental Microbiology*, **73**, 2135–2143.
- Bazylinski, D. A., and Frankel, R. B., 2003. Biologically controlled mineralization in prokaryotes. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 217–247.
- Beveridge, T. J., 1989. Metal ions and bacteria. In Beveridge, T. J., and Doyle, R. J. (eds.), *Metal Ions and Bacteria*. New York: Wiley.
- Boukhalfa, H., Icopini, G. A., Reilly, S. D., and Neu, M. P., 2007. Plutonium (IV) reduction by the metal-reducing bacteria *Geobacter metallireducens* GS15 and *Shewanella oneidensis*.

- MR1. *Applied and Environmental Microbiology*, **73**, 5897–5903.
- Cox, B. L., Popa, R., Bazylnski, D. A., Lanoil, B., Douglas, S., Belz, A., Engler, D. L., and Nealson, K. H., 2002. Organization and elemental analysis of P-, S-, and Fe-rich inclusions in a population of freshwater magnetococci. *Geomicrobiology Journal*, **19**, 387–406.
- Feldheim, D. L., and Eaton, B. E., 2007. Selection of biomolecules capable of mediating the formation of nanocrystals. *ACA Nano*, **3**, 154–159.
- Ferris, F. G., Beveridge, T. J., and Fyfe, W. S., 1986. Iron-silica crystallite nucleation by bacteria in a geothermal sediment. *Nature*, **320**, 609–611.
- Fortin, D., Langley, S., and Glasauer, S., 2008. Biomineralization: from nature to application. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *Metal Ions in Life Sciences*. Chichester: Wiley, Vol. 4, pp. 377–411.
- Frankel, R. B., and Bazylnski, D. A., 2003. Biologically induced mineralization by bacteria. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization. Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 95–114.
- Gilbert, B., and Banfield, J. F., 2005. Molecular-scale processes involving nanoparticulate minerals in biogeochemical systems. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 109–155.
- Glasauer, S., Langley, S., and Beveridge, T. J., 2002. Intracellular Fe minerals in a dissimilatory iron-reducing bacterium. *Science*, **295**, 117–119.
- Glasauer, S., Langley, S., Boyanov, M., Lai, B., Kemner, K., and Beveridge, T. J., 2007. Mixed-valence cytoplasmic iron granules are linked to anaerobic respiration. *Applied and Environmental Microbiology*, **73**, 993–996.
- Kappler, A., and Straub, K. L., 2005. Geomicrobiological cycling of iron. In Banfield, J. F., Cervini-Silva, J., and Nealson, K. M. (eds.), *Molecular Geomicrobiology, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 59, pp. 85–108.
- Klaus-Joerger, T., Joerger, R., Olsson, E., and Granqvist, C. G., 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. *Trends in Biotechnology*, **19**, 15–20.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Lloyd, J., Chesnes, J., Glasauer, S., Bunker, D., Livens, F. R., and Lovley, D. R., 2002. Reduction of actinides and fission products by Fe(III)-reducing bacteria. *Geomicrobiology Journal*, **19**, 103–120.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press.
- Moreau, J. W., Weber, P. K., Martin, M. C., Gilbert, B., Hutcheon, I. D., and Banfield, J. F., 2007. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science*, **316**, 1600–1603.
- Nealson, K. H., and Stahl, D. H., 1997. Microorganisms and biogeochemical cycles: What can we learn from stratified communities? In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Sobolov, D., and Roden, E., 2002. Evidence for rapid microscale bacterial redox cycling of iron in circumneutral environments. *Anton van Leeuw*, **181**, 587–597.
- Southam, G., and Beveridge, T. J., 1994. The in vitro formation of placer gold by bacteria. *Geochimica et Cosmochimica Acta*, **58**, 4527–4530.
- Suzuki, Y., Kelly, S. D., Kemner, K. M., and Banfield, J. F., 2002. Nanometre-size products of uranium bioreduction. *Nature*, **419**, 134.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: insights into manganese (II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions Between Microbes and Minerals, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 35, pp. 225–260.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. In Dove, P. M., De Yoreo, J. J., and Weiner, S. (eds.), *Biomineralization, Reviews in Mineralogy*. Washington, DC: Mineralogical Society of America, Vol. 54, pp. 1–29.

Cross-references

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Gallionella](#)
[Geobacter](#)
[Leptothrix](#)
[Magnetotactic Bacteria](#)
[Microbial Biomineralization](#)
[Microbial Metal Binding](#)
[Nan\(n\)obacteria](#)
[Shewanella](#)

NICKEL, BIOLOGY

Martin Krüger
 Federal Institute for Geosciences and Natural Resources,
 Hannover, Germany

Definition

Nickel, atomic number 28, is a member of the same group in the periodic table as iron, cobalt, and copper. In biology, nickel is a major trace element and part of the catalytic centers of many important metabolic enzymes.

Nickel is one of the most important trace metals in biology. While its concentration in most rocks is only between 10 and 150 $\mu\text{g g}^{-1}$, it is highly enriched in ultramafic rocks, like peridotite and serpentine (Sigel et al., 2006). Nickel is a key component in the active center of a number of enzymes involved in different microbial pathways (Ragsdale, 2007; Ermler et al., 1998). Three nickel metalloenzymes play important roles in the carbon cycle: CO dehydrogenase (CODH), acetyl-CoA synthase (ACS), and methyl-coenzyme M reductase (MCR). The first two enzymes are involved in carbon fixation of anaerobic microorganisms via the Wood–Ljungdahl or reductive acetyl-CoA pathway. CODH allows microorganisms to use carbon monoxide as sole carbon and energy source. It has been suggested that carbon monoxide can be consumed and produced in a bioenergetic cycle

(Dobbek et al., 2001; Voordouw, 2002). The Ni-containing CODH is involved in many reactions in anaerobic microorganisms: it catalyzes the oxidation of CO with H₂O to CO₂ and H₂ in carboxydophilic bacteria, and assists the oxidation of acetate to CO₂ in sulfate-reducing bacteria and archaea and the disproportionation of acetate to CH₄ and CO₂ in methanogenic archaea. In many groups of anaerobic microorganisms utilizing the reductive acetyl-CoA pathway, such as acetogenic bacteria or methanogenic archaea, CODH is often found in a complex with ACS (Strauss and Fuchs, 1993; Holo and Sirevag, 1986; Drennan et al., 2004). ACS catalyzes the formation of acetyl-CoA from CO, leading in combination with the CODH to the synthesis of acetate from two CO₂. These two enzymes play an important role in the global carbon cycle, since up to 10% of the organic carbon produced by photosynthesis is remineralized by anaerobic microorganisms involving CODH/ACS (Drake, 1994). MCR is the key enzyme of methanogenic archaea catalyzing the final step during the reduction of carbon dioxide to methane (Thauer, 1998). All biologically formed methane is produced by methanogenic organisms in the terminal step of anaerobic degradation. The nickel is located in a tetrapyrrole coenzyme named F₄₃₀, which is unique to methanogenic archaea. MCR catalyzes the reduction of methyl-coenzyme M with a reduced coenzyme B, leading to the release of methane and a heterodisulfide of the two coenzymes. The latter can be cleaved in an energy yielding reaction. Furthermore, in a process called “reverse methanogenesis,” methane is anaerobically oxidized with sulfate as electron acceptor. This process – known as AOM – is carried out by an uncultured group of archaea (ANME), which has been shown to contain high amounts of a modified MCR (Krüger et al., 2003; Hallam et al., 2003). Hydrogenases are a family of oxidoreductase enzymes that catalyze the reversible oxidation of molecular hydrogen. Nickel-containing hydrogenases have been isolated from bacteria and archaea (Voordouw, 1992). Finally, nickel is also present in a number of other enzymes, such as urease, a superoxide dismutase, and glyoxylases. The functions and biological roles of these metalloproteins have been described in detail (Sigel et al., 2006).

Recently, due to its strong involvement in geologically important microbial reactions, especially methanogenesis, AOM, and carbon fixation, nickel has raised interest as a potential biosignature for these processes and the involved microorganisms in recent or ancient environments (Reitner et al., 2005; Hausrath et al., 2007).

Bibliography

- Dobbek, H., Svetlitchnyi, V., Gremer, L., Huber, R., and Meyer, O., 2001. Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. *Science*, **293**, 1281.
- Drake, H. L., 1994. *Acetogenesis*. New York: Chapman & Hall, pp. 273–504.

- Drennan, C. L., Doukov, T. I., and Ragsdale, S. W., 2004. The metallocusters of carbon monoxide dehydrogenase/acetyl-CoA synthase: a story in pictures. *Journal of Biological Inorganic Chemistry*, **9**, 511–515.
- Ermiler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer, R. K., 1998. Active sites of transition metal enzymes with focus on nickel. *Current Opinion Structural Biology*, **8**, 749–756.
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F., 2003. Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing Archaea. *Applied and Environmental Microbiology*, **69**, 5483–5491.
- Hausrath, E. M., Liermann, L. J., House, C. H., Ferry, J. G., and Brantley, S. L., 2007. The effect of methanogen growth on mineral substrates: will Ni markers of methanogen-based communities be detectable in the rock record? *Geobiology*, **5**, 49–61.
- Holo, H., and Sirevag, R., 1986. Autotrophic growth and CO₂ fixation of *Chloroflexus aurantiacus*. *Archives Microbiology*, **145**, 173–180.
- Krüger, M., Meyerdierks, A., Glöckner, F. O., Amann, R., Widdel, F., et al., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature*, **426**, 878–881.
- Ragsdale, S. W., 2007. Nickel and the carbon cycle. *Journal of Inorganic Biochemistry*, **101**, 1657–1666.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 71–84.
- Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), 2006. *Nickel and its Surprising Role in Nature*. Weinheim, Germany: Wiley VCH.
- Strauss, G., and Fuchs, G., 1993. Enzymes of a novel autotrophic CO₂ fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *European Journal Biochemistry*, **215**, 633–643.
- Thauer, R. K., 1998. Biochemistry of methanogenesis, a tribute to Marjory Stephenson. *Microbiology*, **144**, 2377–2406.
- Voordouw, G., 1992. Evolution of hydrogenase genes. In Cammack, R., and Sykes, A. (eds.), *Advances in Inorganic Chemistry*. London: Academic Press, Vol. 38, pp. 397–422.
- Voordouw, G., 2002. Carbon monoxide cycling by *Desulfovibrio vulgaris* Hildenborough. *Journal Bacteriology*, **184**, 5903–5911.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Biosignatures in Rocks](#)
[Carbon Cycle](#)
[Hydrogen](#)
[Metalloenzymes](#)
[Methanogens](#)
[Sulfate-Reducing Bacteria](#)

NITRIFICATION

Nitrification is the two-step microbial process by which ammonia (NH₃) is first oxidized to nitrite (NO₂[−]) which is, in a second reaction, converted to nitrate (NO₃[−]). See entry “[Nitrogen](#)” for detailed reading.

NITROGEN

Volker Thiel
University of Göttingen, Göttingen, Germany

Definition

Nitrogen is a nonmetal element with atomic number 7 and atomic mass of 14.00674 u. It was discovered in 1772 by Daniel Rutherford (1749–1819), a Scottish physician, botanist and chemist (Weeks, 1934).

Basic information

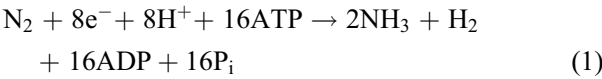
Nitrogen mainly occurs as dinitrogen (N₂), a diatomic, colorless, odorless, and inert gas at standard conditions. The atoms of N₂ are connected via an extremely stable triple bond, the strongest in nature (bond energy = 945 kJ mol⁻¹). N₂ constitutes 78.1 vol% of the atmosphere, making up the largest nitrogen reservoir on earth, followed by sediments and the oceans. Other, geobiologically relevant forms of inorganic nitrogen in fresh and marine waters, soils, and sediments include ammonia (NH₃), its ionic form ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), and gaseous nitrogen oxides (N₂O, NO_x) (Bashkin, 2002). An overview about major global nitrogen reservoirs is given in Table 1.

Nitrogen is one of the “biological elements” (beside C, H, O, P, and S), and is a major component of amino acids/proteins, nucleic acids (DNA, RNA), phospholipids, porphyrins, and secondary metabolites. According to the “Redfield–Richards Ratio,” the C:N:P ratio of phytoplanktonic biomass is, with some variation, 106:16:1 (C/N = 6.6). The C/N ratio of terrestrial plants is substantially more variable and also tends to be larger than that for marine phytoplankton, mainly due to a higher portion of nitrogen-free cellulose- and lignin-based wooden tissues. Although biomass plays only a minor role as a global nitrogen reservoir

(Table 1), nitrogen is, after carbon and oxygen, quantitatively the most abundant element in living organisms and its biogeochemical cycle is of great importance in the biosphere. The key processes involved in the nitrogen cycle are the following. For a schematic overview, see Figure 1).

Biological nitrogen fixation

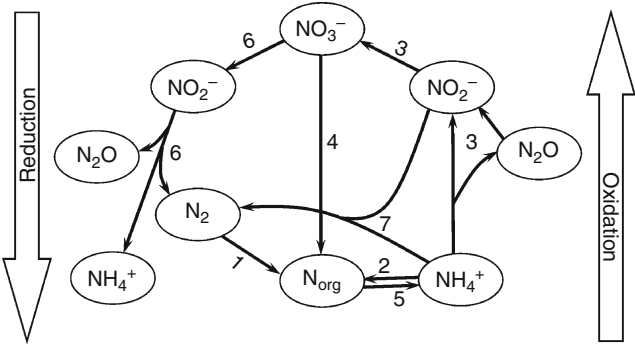
Biological nitrogen fixation refers to the biological conversion of dinitrogen (N₂) into ammonia (NH₃), ammonium (NH₄⁺), and various organic compounds, and is the ultimate source of nitrogen for all organisms. There are two major limitations for biological nitrogen fixation (Bashkin, 2002). First, because of the stability of the triple bond in N₂, nitrogen fixation requires a high energy input. Only some symbiotic and non-symbiotic bacteria, archaea, and algae, the so-called nitrogen fixers or diazotrophs, are able to perform the reduction of N₂, employing a specific Fe and Mo-bearing enzyme, nitrogenase, which catalyzes the following reaction:



Second, nitrogenase is rapidly, and irreversibly, deactivated by oxygen. Therefore, only those organisms that live in anoxic environments or that can locally create such an environment will fix nitrogen. In terrestrial habitats, symbiotic bacteria, of which the genus *Rhizobium* is the most well studied, are important nitrogen fixers. These bacteria are found on the roots of leguminous plants (peas, soybeans) and some woody shrubs and trees. In aquatic systems, including the oceans, cyanobacteria are considered as the most important players in nitrogen fixation (Stal and Zehr, 2008). In cyanobacteria, nitrogen fixation takes place in particular cell compartments, the heterocysts, which are well secluded from the sites of oxygen production (see entry *Cyanobacteria*). N₂ fixation in

Nitrogen, Table 1 Major global nitrogen reservoirs-(After Reeburgh, 1997; Purvaja et al., 2008.)

Reservoir	Tg N (1 Tg = 10 ¹² g)
Atmosphere	
N ₂	3.9 × 10 ⁹
Fixed N	1.3 × 10 ³
N ₂ O	1.3 × 10 ³
Ocean	
N ₂	2.2 × 10 ⁷
N ₂ O	1.7 × 10 ²
Inorganic N	6.0 × 10 ⁵
Organic N	2.0 × 10 ⁵
Biomass	
Marine (plants)	3.0 × 10 ²
Marine (animals)	1.7 × 10 ²
Terrestrial	3.5 × 10 ⁴
Soil	9.5 × 10 ⁴
Sediments	4.0 × 10 ⁷



Nitrogen, Figure 1 Major pathways in the biogeochemical nitrogen cycle. 1 = Biological nitrogen fixation, 2 = Ammonia assimilation, 3 = Nitrification, 4 = Assimilatory nitrate reduction, 5 = Ammonification, 6 = Denitrification (dissimilatory nitrate reduction), 7 = Anammox.

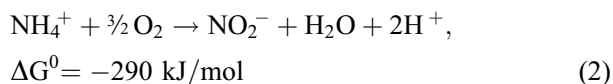
Archaea is exclusive of, but widespread within, methanogenic euryarchaeota (Cabello et al., 2004, see entry *Archaea*). In addition to the natural pathways, anthropogenic processes, namely fertilizer production and fossil fuel combustion, may fix N_2 , and greatly accelerate the global nitrogen cycle (Gruber and Galloway, 2008). In the 1990s, these sources of anthropogenic nitrogen to the environment amounted to more than 160 Tg N year⁻¹. On a global basis, this is more than that supplied by natural biological nitrogen fixation on land (110 Tg N year⁻¹) or in the ocean (140 Tg N year⁻¹) (Gruber and Galloway, 2008).

Ammonia assimilation

Ammonia assimilation (uptake) is the process by which NH_3/NH_4^+ is transferred to organic nitrogen-containing compounds (e.g., amino acids or nucleotides; Madigan and Martinko, 2006). Such direct assimilation of NH_3/NH_4^+ into biomass has significant energetic advantages for those organisms which are able to use this form of nitrogen. In oxic environments, however, ammonium ions have a very limited lifetime because they are easily consumed in the nitrification process (see below).

Nitrification

Nitrification is the process by which ammonium is oxidized to nitrite and further to nitrate. The process includes two conjugated stages both of which are performed by aerobic bacteria, mainly chemoautotrophs (Meiklejohn, 2006). At present, no organism able to perform both steps simultaneously is known. The first stage of nitrification is the oxidation of ammonium to nitrite, according to the following reaction:



Representative bacteria that oxidize ammonium to nitrite include *Nitrosomonas* (very common in soils), *Nitrospira*, and *Nitrocystis* species. Notably, it has recently been reported that some bacteria (*Thiocapsa* sp., *Rhodopseudomonas* sp.) oxidize nitrite to nitrate in the light, thus using nitrite as an electron donor for photosynthesis. So far, this is the only known photosynthetic oxidation in the nitrogen cycle (Schott et al., 2010).

The second step in nitrification is the oxidation of nitrite to nitrate.



Representative bacteria that oxidize nitrite to nitrate include *Nitrobacter* (very common in soils), *Nitrospina*, and *Nitrococcus* species.

Artificial fertilizers containing reduced forms of nitrogen, namely urea and ammonium sulfate ($(NH_4)_2SO_4$), may fuel the nitrification process in well-drained agricultural soils. This has considerable environmental consequences, because in the nitrification process, some

intermediates like hydroxylamine (NH_2OH), NO, and N_2O are produced in significant amounts. Particularly the release of N_2O to the atmosphere may cause considerable environmental problems with respect to ozone depletion and global warming effects (IPCC, 2007). Nitrate in the environment is subject to two major pathways, assimilatory nitrate reduction and denitrification (see below).

Assimilatory nitrate reduction

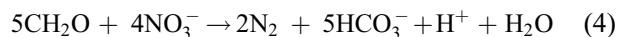
Assimilatory nitrate reduction is defined as the reduction of nitrate (NO_3^-) and assimilation (uptake) of the nitrate-derived nitrogen into biomass. This pathway is somewhat more energy-expensive than ammonia assimilation, and therefore, it is prevalent when reduced nitrogen, i.e., ammonium, is low in supply, such as in aerated soils or in the oxic water column of aquatic environments. Assimilatory nitrate reduction is a primary nitrogen input of many microorganisms. Plants can commonly assimilate both ammonium and nitrate.

Ammonification

Ammonification refers to the breakdown of organic nitrogen into ammonium (NH_4^+). Strictly speaking, it is a two-step process (Libes, 1992). In the initial stage, particular organic nitrogen (PON) compounds are broken down to smaller dissolved molecules, i.e., dissolved organic nitrogen (DON). The DON is then further processed by heterotrophic bacteria to form ammonium. This process is actually called “ammonification,” and involves the enzymatically driven hydrolysis of peptide bonds of proteins to form amino acids, and the subsequent cleavage of the amino groups from the molecules. The resulting ammonium can be assimilated or microbially oxidized in the nitrification process, as outlined above.

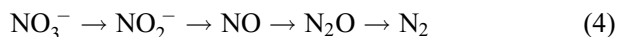
Denitrification

Denitrification is the biologically facilitated reduction of nitrate (NO_3^-) to N_2 and other gaseous intermediates, mostly N_2O , which may return to the atmosphere (Bashkin, 2002). In denitrification, heterotrophic bacteria, such as members of the genera *Bacillus*, *Paracoccus*, and *Pseudomonas* (Madigan and Martinko, 2006), and some archaea (Cabello et al., 2004) oxidize organic matter by using nitrate as their electron acceptor:

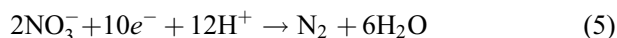


Recent work has also shown that nitrate reduction can be coupled to methane oxidation (Raghoebarsing et al., 2006). Furthermore, several reactions of nitrate with reduced inorganic species have been reported that may also lead to the reduction of nitrate, whether biologically facilitated (Fe(II); Muehe et al., 2009) or not (MnO, “chemodenitrification”; Brandes et al., 2007). Denitrification leads to an overall loss of organic nitrogen (Hulth et al., 2005). It is the most energetically favorable anaerobic respiration process, with an energy yield only slightly

less than that of aerobic (O_2) respiration. During denitrification, some of the nitrate is not assimilated into biomass but reduced sequentially involving the following reduction steps (canonical denitrification):



and the overall reaction

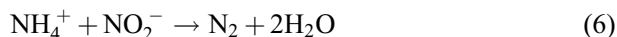


Denitrification is usually thought to prevail mainly in soils, waters, and sediments under suboxic or anoxic conditions (Libes, 1992), because O_2 acts as a competing electron acceptor for nitrate, and key enzymes of the denitrification pathways are inhibited by O_2 . However, it has recently been reported that denitrification is also proceeding in oxic marine sediments (Gao et al., 2010). Evidently, denitrification is not necessarily inhibited in the presence of substantial oxygen concentrations but rather the co-respiration of O_2 and nitrate occurs. It is as yet unknown, whether this co-metabolism takes place in a single organism, or in different populations within the microbial community.

Like in the nitrification process, denitrification produces gaseous intermediates that cause environmental problems with respect to ozone depletion and global warming effects (Bateman and Baggs, 2005). N_2O is the quantitatively most important of these intermediates. It becomes particularly abundant under acidic conditions of denitrification, and may account for 0–20% of the gaseous nitrogen released (N_2 : 80–100%; Bashkin, 2002). In agriculture, denitrification is an undesired process because it removes nitrate, a nitrogen source amenable for plants, from the soil, thus decreasing soil fertility.

Anammox

Anammox, the anaerobic oxidation of ammonium, has only recently been recognized as an important route in the marine nitrogen cycle (Ward, 2003; Dalsgaard et al., 2005). In oxygen-deficient systems, anammox bacteria remove ammonium by reacting it with nitrite (NO_2^-) to form N_2 :



The anammox bacteria are highly specialized and belong to the order Planctomycetales (Schmid et al., 2005). Anammox was recognized as a major sink for fixed inorganic nitrogen in coastal sediments, in anoxic waters of the Black Sea, and in oxygen minimum zones in upwelling systems (Kuyper et al., 2003, 2005). In isotope labeling studies on surface sediments, anammox was found to account for 4–79% of the total N_2 production (Engström et al., 2005). On a global scale, it was estimated that anammox is responsible for up to 50% of the removal of fixed nitrogen from the world's oceans (Ward, 2003; Dalsgaard et al., 2005).

Geological and evolutionary aspects

Being a major constituent of the precursor biomass, organic nitrogen is generally found in fossil organic matter, i.e., kerogen, coal, thermogenic natural gas, and crude oils. However, organic nitrogen tends to become more and more depleted over geological times, as a result of preferential loss of N-bearing functional groups from organic matter during burial (e.g., desamination).

Whereas nitrogen is abundant in the atmospheric and biological pools, nitrogen-containing rocks and minerals are relatively scarce. Nitrogen occurs naturally only in a few relatively rare minerals, of which the nitrate salts saltpeter (KNO_3) and Chile saltpeter ($NaNO_3$) are quantitatively most abundant. Due to the extremely high solubility, these salts are stable only in hyperarid (precipitation <5 mm/year) settings such as the Atacama Desert in Chile. Here, the Chile saltpeter ores, also known as “caliche,” are soil-hosted ($NaNO_3$ content 3% to >13%, reserves ca. 500,000,000 t). The sources and formation mechanisms of the Atacama nitrate ores have long been debated (Ericksen, 1983). Recent isotope studies support an abiotic origin (Böhlke et al., 1997; Michalski et al., 2004). According to these studies, the main source of the Chilean saltpeter deposits is the precipitation of nitrate produced by photochemical reactions in the atmosphere, and the long-term accumulation in the absence of leaching and biological activity. It has also been suggested that volcanic eruptions from the close-by 70,000 km² Altiplano-Puna volcanic plateau may have fueled the thermal and electric (lightning-induced) fixation of atmospheric nitrogen as nitrate.

Apart from the formation of the actual nitrogen minerals, the adsorption of ammonium and its exchange for alkali metals in clay minerals are important and ubiquitous processes that decrease the bioavailability of nitrogen in sediments and soils, with major consequences for nitrogen turnover rates and soil fertility (Rosenfeld, 1979; Mamo et al., 1993). Moreover, studies revealed evidence that exchange reactions between potassium and ammonium are the dominant control of N_2 generation at depth, and the storage, release, and migration of nitrogen gas in sedimentary rocks. As a consequence, nitrogen frequently occurs as an undesired component natural gas that may potentially reduce the quality of hydrocarbon gas reservoirs (Krooss et al., 2006; Mingram et al., 2005).

The modern biogeochemical cycle of nitrogen depends on two obligately aerobic steps related to nitrification, i.e., ammonium oxidation to nitrite, and nitrite oxidation to nitrate. Hence, closure of the nitrogen cycle, as it is proceeding today, could be achieved only when free oxygen became available after the “Great Oxidation Event” ca. 2.3 billion years ago and promoted the evolution of aerobic ammonia and nitrite oxidizers (see entry *Critical Intervals in Earth History*). It is unknown whether electron acceptors other than oxygen, such as Fe^{3+} , could have been used as electron acceptors fueling the nitrogen cycle prior to rise of oxygen (Bashkin, 2002).

Likewise, the different routes for nitrogen fixation and assimilation require a portfolio of specialized biochemical pathways operating under different environmental conditions. Because a number of metalloenzymes are necessary for nitrogen fixation and assimilation, it is likely that the availability of transition metals has been a major control on the evolution of nitrogen assimilation pathways over Earth history (Zerkle et al., 2006; Glass et al., 2009). As an example, Fe and Mo are essential elemental components of the nitrogen-fixing enzyme, nitrogenase, and in enzymes responsible for the assimilation of inorganic nitrogen. Whereas Fe is scarce in the well-oxygenated modern oceans, it was abundant in anoxic Archean surface and deep oceans. Similar changes must have occurred in the abundance of Mo but with an opposite sense of direction, because Mo is more soluble in oxygenated than in reducing waters (Zerkle et al., 2006; Anbar, 2008; Scott et al., 2008). It was therefore hypothesized that photosynthesis-derived O_2 in late Archean oceans, particularly the "Great Oxidation Event," mobilized Mo and enabled the evolution of Mo-based N_2 fixation and the Mo-dependent enzymes for assimilation and denitrification by prokaryotes (Glass et al., 2009).

Summary

Nitrogen is one of the "biological elements" that constitutes organic compounds and its biogeochemical cycle is of utmost importance in the biosphere. The nitrogen cycle is mainly driven by prokaryotes and involves different nitrogen species, mainly N_{org} , NH_4^+ , N_2 , N_2O , NO_2^- , and NO_3^- . Nitrogen mostly occurs as dinitrogen (N_2) in the atmosphere and, in a dissolved form, in aquatic systems. Reductive conversion of inert N_2 into biologically accessible forms (organic compounds, NH_4) is an energy expensive process that requires specific enzymes (nitrogenase), and is only accomplished by a number of specialized organisms (diazotrophs). Further reactions used either for assimilatory purposes or in respiratory pathways involve anaerobic redox processes (anammox $NH_4^+ + NO_2^- \rightarrow N_2$), anaerobic/aerobic reductions (denitrification, $NO_3^- \rightarrow N_2$), and aerobic oxidations (nitrification; $NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) of the various nitrogen species. It is unknown whether and how the aerobic steps closing the modern nitrogen cycle were substituted early in Earth history, i.e., before the rise of oxygen. In the geological realm, nitrogen rarely occurs in a few highly soluble minerals such as saltpeter (KNO_3 , $NaNO_3$). In addition, it is a constituent element of clay minerals (ammonium), sedimentary organic matter, including kerogen, coal, natural gas, and crude oil, but its reservoir sizes and the modes of occurrence and formation are as yet not well constrained.

Bibliography

- Anbar, A. D., 2008. Elements and evolution. *Science*, **322**, 1481–1483.
- Bateman, E. J., and Baggs, E. M., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different

- water-filled pore space. *Biology and Fertility of Soils*, **41**, 379–388.
- Bashkin, V. N., 2002. *Modern Biogeochemistry*. Dordrecht: Kluwer Academic.
- Böhlke, J. K., Ericksen, G. E., and Revesz, K., 1997. Stable isotope evidence for an atmospheric origin of desert nitrate deposits in northern Chile and southern California, USA. *Chemical Geology*, **136**, 135–152.
- Brandes, J. A., Devol, A. H., and Deutsch, C., 2007. New developments in the marine nitrogen cycle. *Chemical Reviews*, **107**, 577–589.
- Cabello, P., Roldán, M. D., and Moreno-Vivián, C., 2004. Nitrate reduction and the nitrogen cycle in archaea. *Microbiology*, **150**, 3527–3546.
- Dalsgaard, T., Thamdrup, B., and Canfield, D. E., 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. *Research in Microbiology*, **156**, 457–464.
- Engström, P., Dalsgaard, T., Hulth, S., and Aller, R. C., 2005. Anaerobic ammonium oxidation by nitrite (anammox): Implications for N_2 production in coastal marine sediments. *Geochim. et Cosmochim. Acta*, **69**, 2057–2065.
- Ericksen, G. E., 1983. The Chilean nitrate deposits. *American Scientist*, **71**, 366–374.
- Gao, H., Schreiber, F., Collins, G., Jensen, M. M., Kostka, J. E., Lavik, G., de Beer, D., Zhou, H., and Kuypers, M. M. M., 2010. Aerobic denitrification in permeable Wadden Sea sediments. *The ISME Journal*, **4**, 417–426.
- Giovannoni, S., and Rappé, M., 2000. Evolution, diversity and molecular ecology of marine prokaryotes. In Kirchman, D. L. (ed.), *Microbial Ecology of the Oceans*. New York: Wiley, pp. 47–84.
- Glass, J. B., Wolfe-Simon, F., and Anbar, A., 2009. Coevolution of metal availability and nitrogen assimilation in cyanobacteria and algae. *Geobiology*, **7**, 100–123.
- Gruber, N., and Galloway, J. N., 2008. An Earth-system perspective of the global nitrogen cycle. *Nature*, **451**, 293–296.
- Hulth, S., Aller, R. C., Canfield, D. E., Dalsgaard, T., Engstrom, P., Gilbert, F., Sundback, K., and Thamdrup, B., 2005. Nitrogen removal in marine environments: recent findings and future research challenges. *Marine Chemistry*, **94**, 125–145.
- Intergovernmental Panel on Climate Change (IPCC), Fourth Assessment Report 2007 (AR4); Working Group I Report "The physical science basis", Ch. 2.
- Kuypers, M. M. M., Sliekers, A. O., Lavik, G., Schmid, M., Jørgensen, B. B., Kuenen, J. G., Sinninghe Damsté, J. S., Strous, M., and Jetten, M. S. M., 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*, **422**, 608–611.
- Kuypers, M. M. M., Lavik, G., Woebken, D., Schmid, M., Fuchs, B. M., Amann, R., Jørgensen, B. B., and Jetten, M. S. M., 2005. Massive nitrogen loss from the Benguela upwelling system through anaerobic ammonium oxidation. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 6478–6483.
- Krooss, B. M., Jurisch, A., and Plessen, B., 2006. Investigation of the fate of nitrogen in Palaeozoic shales of the Central European Basin. *Journal of Geochemical Exploration*, **89**, 191–194.
- Libes, S. M., 1992. *An Introduction to Marine Biogeochemistry*. New York: John Wiley & Sons, 734 pp.
- Madigan, M. T., and Martinko, J. M., 2006. *Brock Biology of Microorganisms*, 11th edn. Englewood Cliffs NJ: Prentice Hall.
- Mamo, M., Taylor, R. W., and Shuford, J. W., 1993. Ammonium fixation by soil and pure clay minerals. *Communications in Soil Science and Plant Analysis*, **24**, 1115–1126.
- Meiklejohn, M., 2006. The nitrifying bacteria: a review. *European Journal of Soil Science*, **4**, 59–68.

- Michalski, G., Böhlke, J. K., and Thiemens, M., 2004. Long term atmospheric deposition as the source of nitrate and other salts in the Atacama Desert, Chile: new evidence from mass-independent oxygen isotopic compositions. *Geochimica et Cosmochimica Acta*, **68**, 4023–4038.
- Mingram, B., Hoth, P., Lüders, V., and Harlov, D., 2005. The significance of fixed ammonium in Palaeozoic sediments for the generation of nitrogen-rich natural gases in the North German Basin. *International Journal of Earth Science*, **94**, 1010–1022.
- Muehe, E. M., Gerhardt, S., Schink, B., and Kappler, A., 2009. Ecophysiology and the energetic benefit of mixotrophic Fe(II) oxidation by various strains of nitrate-reducing bacteria. *FEMS (Federation of European Microbiological Societies) Microbiology – Ecology*, **70**, 335–343.
- Oyarzun, J., and Oyarzun, R., 2007. Massive volcanism in the Altiplano-Puna volcanic plateau and formation of the huge atacama desert nitrate deposits: a case for thermal and electric fixation of atmospheric nitrogen. *International Geology Review*, **49**, 962–968.
- Purvaja, R., Ramesh, R., Ray, A. K., and Rixen, T., 2008. Nitrogen cycling: a review of the processes, transformations and fluxes in coastal ecosystems. *Current Science*, **94**, 1419–1438.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J., Ettwig, K. F., Rijpstra, W. I., Schouten, S., Damsté, J. S., Op den Camp, H. J., Jetten, M. S., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 878–879.
- Reeburgh, W. S., 1997. Figures summarizing the global cycles of biogeochemically important elements. *Bulletin of the Ecological Society of America*, **78**, 260–267.
- Rosenfeld, J. K., 1979. Ammonium adsorption in nearshore anoxic sediments. *Limnology and Oceanography*, **24**, 356–364.
- Saito, M. A., Sigman, D. M., and Morel, F. M. M., 2003. The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean-Proterozoic boundary? *Inorganica Chimica Acta*, **356**, 308–318.
- Schmid, M., Maas, B., Dapena, A., van de Pas-Schoonen, K., van de Vossenberg, J., Kartal, B., van Niftrik, L., Schmidt, I., Cirpus, I., Kuenen, J. G., Wagner, M., Damsté, J. S. S., Kuypers, M., Revsbech, N. P., Mendez, R., Jetten, M. S. M., and Strous, M., 2005. Biomarkers for in situ detection of anaerobic ammonium-oxidizing (anammox) bacteria. *Applied and Environmental Microbiology*, **71**, 1677–1684.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X., and Anbar, A. D., 2008. Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature*, **452**, 456–459.
- Schott, J., Griffin, B. M., and Schink, B., 2010. Anaerobic phototrophic nitrite oxidation by *Thiocapsa* sp. strain KS1 and *Rhodospseudomonas* sp. strain LQ17. *Microbiology*, **156**, 2428–2437.
- Stal, L. J., and Zehr, J. P., 2008. Cyanobacterial nitrogen fixation in the ocean: diversity, regulation, and ecology. In Herrero, A., and Flores, E. (eds.), *The Cyanobacteria: Molecular Biology, Genomics and Evolution*. Norwich: Horizon Scientific Press, pp. 423–471.
- Ward, B. B., 2003. Significance of anaerobic ammonium oxidation in the ocean. *Trends in Microbiology*, **11**, 408–410.
- Weeks, M. E., 1934. Daniel Rutherford and the discovery of nitrogen. *Journal of Chemical Education*, **11**, 101–107.
- Zerkle, A. L., House, C. H., Cox, R. P., and Canfield, D. E., 2006. Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle. *Geobiology*, **4**, 285–297.

Cross-references

[Archaea](#)
[Bacteria](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Nitrogen Fixation](#)
[Thiotrophic Bacteria](#)

NITROGEN FIXATION

Nitrogen fixation is the conversion of diatomic nitrogen (N₂) into ammonia (NH₃), which is then used for biosynthesis of organic compounds. See entry “[Nitrogen](#)” for further reading.

O

ORES, MICROBIAL PRECIPITATION AND OXIDATION

Beda A. Hofmann
Natural History Museum Bern, Bern, Switzerland

Synonyms

Gossan; Ore deposits; Oxidation zone; Supergene enrichment

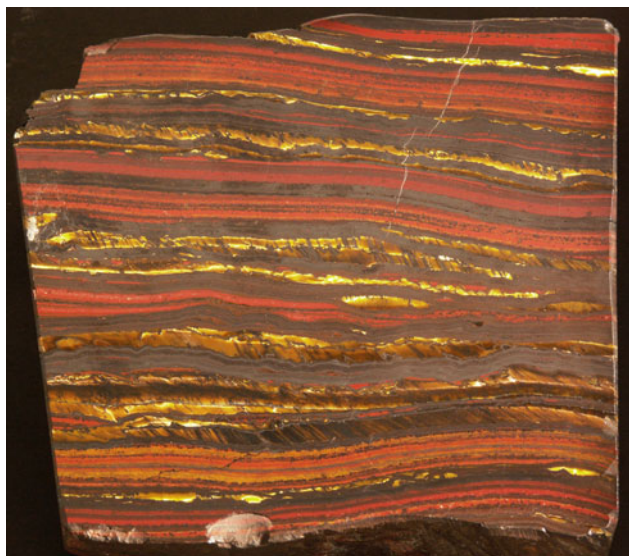
Definition

Ore deposits are natural enrichments of chemical elements of economic interest. While all natural elements are present in certain background concentrations in rocks and minerals, they are typically not economically extractable at these levels. Geological processes may lead to enrichments of elements in such a way that orebodies are formed from which large quantities of elements may be extracted at a much lower cost. The formation of orebodies generally occurs in three steps: (1) element extraction from a large volume of rock or melt; (2) transport of elements; (3) deposition of elements in a volume of rock much smaller than the extracted volume. These steps may occur in magmatic melts, hydrothermal systems, diagenetic environments, and at the Earth's surface. Melts, solutions, gases, and solid phases (minerals) may be involved in these processes. In many cases, ore forming processes occur in magmatic and hydrothermal systems well beyond the temperature limit of microbial growth. However, important ore forming systems are also known within the temperature realm of microbial life (maximum 121°C). These are mainly confined to sedimentary and diagenetic environments and the low-temperature end of hydrothermal systems. A microbial involvement in the different

steps of ore formation may be due to direct interaction, i.e., ore precipitation as a result of microbial activity. On the other hand, indirect influences of life may include the conditioning of the environment (e.g., oxidizing atmosphere), allowing transport of certain elements in a soluble form, and so influencing ore forming processes in systems where microbes cannot live. During the weathering of ore deposits under near-surface conditions, microbes may also play an important role. In general, the direct involvement of microbes in ore formation is one of catalyzing redox reactions such as the oxidation of Fe and Mn and the reduction of S, Cr, and U. The role of low-temperature redox fronts as sites of ore formation and availability of energy for microbes have been reviewed by Hofmann (1999).

The possible role of microbes in ore precipitation has long been recognized (Macqueen and Coope, 1985; Southam and Saunders, 2005). Precipitation of ore minerals as a direct result of microbial activity can be inferred from the fact that many microbes can harvest energy from redox reactions, leading to immobilization of elements. Such reactions include the reduction of U(VI), Cr(VI), and Au(I) (Kashefi and Lovley, 2000; Kashefi et al., 2001; Labrenz et al., 2000; Moreau et al., 2004), the reduction of As and Se (Stolz and Oremland, 1999), and possibly also the indirect reduction of oxidized elements by H₂S (Mohagheghi, 1985).

Arsenic is an important element in ore deposits. Reduced arsenic (As-I) leads to the precipitation of Ni, Co, Fe, and other elements as arsenides. Native As, As(0), also is relatively common. Native As of distinct low-temperature origin occurs in a stratigraphically well-defined horizon over approximately 300 km² in the Middle Triassic of southern Germany/N Switzerland (Hofmann, 1989a; Hofmann and Gehlen, 1993). While the microbial reduction of As(V) to As(III) is well known



Ores, Microbial Precipitation and Oxidation, Figure 1 Banded iron formation. Bands of black hematite, red chert, and yellow (altered) riebeckite, a metamorphic mineral. Mt. Brockman, Hamersley Range, Western Australia. Width of sample is 9 cm. (Photograph by Peter Vollenweider.)

(Oremland et al., 2005), an involvement of microbes in the reduction of As to lower valence states is also known (Bentley and Chasteen, 2002) and may possibly play a role in the precipitation of arsenides and native As.

Oxidation reactions leading to ore mineral precipitation are particularly significant in the case of Fe (Konhauser, 1998; Konhauser et al., 2002; Widdel et al., 1993) and Mn (Tebo et al., 1997).

There are still other possible mechanisms of microbial involvement in ore precipitation such as the microbial destruction of complexants, e.g., of thiosulfate in the case of gold and possibly silver (Lengke and Southam, 2005). Similar processes may apply for cyanate.

Ore deposit types for which microbial involvement in ore deposition has been postulated

Banded iron formations (BIFs) are mainly of Proterozoic age and are temporally related to the oxygenation of the atmosphere and the sea (Figure 1). BIFs are also known from the Archean to the Phanerozoic. The involvement of photosynthesizing microbes in the origin of BIF is controversial. Konhauser et al. (2002) demonstrated the feasibility of microbial precipitation. Anoxygenic photosynthesis (Widdel et al., 1993; Kappler et al., 2005) has been proposed as a major possible process. In a discussion, Klein (2005) concludes that there is no proof of direct microbial iron oxidation in the formation of BIFs. The various genetic models are discussed by Beukes (2004). Iron isotope studies also do not provide a signal distinctive from that of nonbiological fractionation

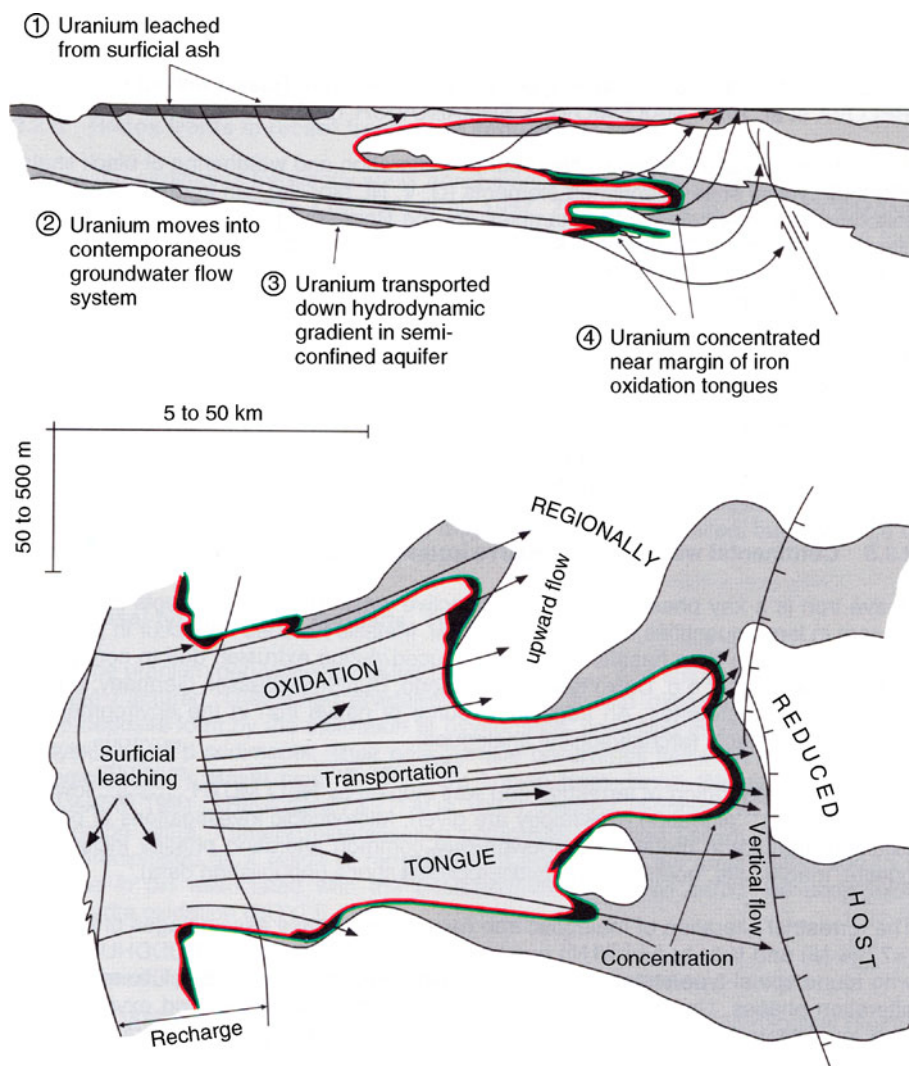
(Anbar, 2004). Even though there is no direct proof of microbial precipitation, it is very likely that the oxidant involved in the transformation of mobile Fe(II) to immobile Fe(III) was of biogenic origin.

Other iron deposits. Phanerozoic sedimentary iron deposits commonly display oolitic or pisolitic textures and are of syngenetic–diagenetic origin. Dahanayake and Krumbein (1986) proposed a biogenic involvement in ore precipitation based on the presence of filaments in ooids.

Sedimentary manganese deposits including ferromanganese nodules. The coincidence of environmental oxidation in the Proterozoic with the precipitation of the Kalahari manganese deposits, the world's largest manganese orefield, is taken as evidence that deposit formation was induced by biological oxygen production (Kirschvink et al., 2000; Schaefer et al., 2001).

Sandstone-hosted uranium deposits: Major sandstone-hosted uranium deposits most commonly occur in Mesozoic and Cenozoic rocks in the western USA, but also in Australia, southern France, and the former Soviet Union. A summary of uranium deposit types is found in (Dahlkamp, 1993). The sandstone-hosted deposits are often formed where detrital terrestrial organic matter produced local reducing environments. Sandstone-hosted uranium deposits also occur in Proterozoic rocks of the Francevillian Basin of Gabon, where the reducing traps are related to hydrocarbon-bearing faults. Phanerozoic deposits exist in tabular and roll-type (Figure 2) geometries. Closely related are redox-boundary controlled uranium deposits in altered phonolitic rocks at Pocos de Caldas, Brazil (Figure 3, see Waber et al., 1992). Both for roll-type deposits (Goldhaber et al., 1978; Reynolds et al., 1982; Warren, 1972) and tabular U–V deposits (Goldhaber et al., 1990; Meunier et al., 1987; Mohagheghi et al., 1985; Wanty et al., 1990) biogenic versus abiogenic reduction processes have been discussed. Considering that these deposits formed in near-surface environments at close to ambient temperatures that they do contain sulfide minerals indicating low-T sulfate reduction, and that they represented redox fronts providing a lot of chemical energy, the absence of a microbial involvement would be surprising. However, no clear biosignatures have been described. Light sulfur isotopes from these deposits have been interpreted biologically (Jensen, 1958; Rackley, 1972), but also nonbiologically (Warren, 1971). The possible involvement of intermediate-valence sulfur species (e.g., thiosulfate), through biological (Bak and Cypionka, 1987) or nonbiological pathways, seem possible.

Unconformity-related uranium deposits: For this major type of uranium deposits (mainly occurring in the Proterozoic of Canada and Australia) a microbial involvement appears generally unlikely due to high formation temperatures of 100–250°C (Suzuki and Banfield, 1999). An involvement of microbial activity has been postulated by Donnelly and Ferguson (1980). There is an interesting paragenetic similarity (predominance of U, Co, Ni, Bi,



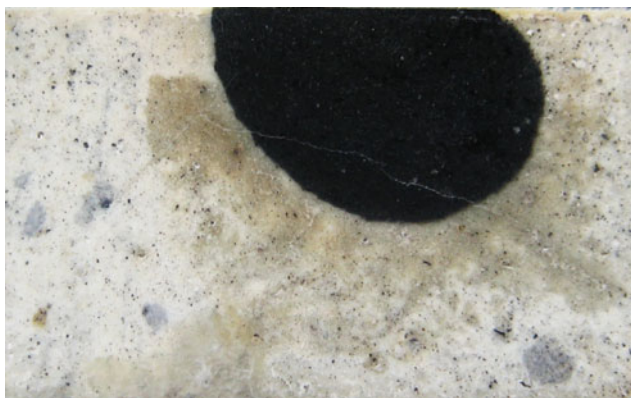
Ores, Microbial Precipitation and Oxidation, Figure 2 Roll-front type uranium deposit, South Texas, formed by infiltration of oxidized waters into permeable reduced sandstones. Red denotes oxidized sandstone, black denotes the zone of uranium mineralization, and green is the unaltered reduced rock. (From Hofmann 1999, based on Dahlkamp, 1993.)

Ag) with reduction spheroids, for which a microbial involvement appears likely.

Deep-sea hydrothermal vents: Sulfide-rich ore deposits originating from the venting of metal-rich hydrothermal fluids in the deep sea are dominated by Fe–Cu-sulfides (with Au, Pb–Zn-rich in back-arc and sedimentary settings). Based on sulfur isotope data, a part of the sulfide sulfur is of microbial origin (Fallick et al., 2001; Taylor, 2004). In case of Irish-type carbonate-hosted massive sulfide deposits, similar evidence has been provided by Wilkinson et al. (2005).

Mississippi-Valley-Type (MVT) and related Pb–Zn deposits. MVT deposits are formed not only from basinal brines, mainly in carbonate rocks, but also in sandstones at the edge of sedimentary basins. Ore deposition is due to cooling, wall-rock reactions, fluid mixing, or

a combination thereof (Burstein et al., 1993; Leach et al., 1996). The possible role of microbial sulfate reduction in the precipitation of ores is controversial. Reports on fossilized microbes (Kucha et al., 2005) and sulfur isotopic variations have been interpreted as proof of biogenic involvement, e.g., in salt caprock-associated (Saunders and Swann, 1994) and alpine type deposits (Schroll, 1996). Features of thermochemical sulfate reduction (TSR) as compared with biogenic sulfate reduction (BSR) are discussed by (Machel, 2001), concluding that most MVT deposits are due to TSR, while BSR produces iron-dominated sulfide accumulations. The range of MVT deposits is very large and ore textures range from coarsely crystalline to submicroscopic. This variability, also including sulfur isotopes, and the near-surface origin of a part of the deposits, may indicate that both TSR and



Ores, Microbial Precipitation and Oxidation,

Figure 3 Uraninite-rich nodule in altered phonolite from the reduced side of the redox front at Poços de Caldas, Minas Gerais, Brazil. A microbial origin of this local uranium enrichment appears possible, based on its isolated occurrence, low-T origin, and geometry. Field of view is 17 mm.

BSR are involved in MVT genesis. Clear criteria for a differentiation have yet to be found.

Low-temperature hydrothermal ore deposits. Low-temperature (epithermal) ore deposits are formed in a temperature range that may overlap with possible microbial growth. Fluctuating systems may provide conditions at least temporarily suitable for microbes. Reports of microbial activity in such occurrences are rather scarce. Hofmann (1989b) reported filamentous forms from the Krunkelbach uranium deposit in (Germany, Figures 4 and 5) and similar features were observed in other Schwarzwald occurrences (Reitner, 2004). Based on morphological similarities, a microbiological origin of features described as “stalactites” from Creede, Colorado (Campbell and Barton, 1996; Hofmann, 1989b; Hofmann et al., 1998; Reitner, 2004), may be ascribed to be due to mineralized microbial fabrics (Hofmann et al., 2008).

Reduction spheroids. Small-scale reduction phenomenon in red beds with accumulations of numerous elements (U, V, Co, Ni, As). Due to geochemical similarities with sandstone-hosted U-V deposits and certain low-T hydrothermal veins, these features of probable microbial origin may present an important genetic link see *Reduction Spheroids*.

Black shales. Sediments deposited under oxygen-poor to oxygen-free conditions, with low sedimentation rates, are typically enriched in redox-sensitive elements such as U, V, and Mo (Lehmann et al., 2007; Leventhal, 1998). Trace element-rich black shales are a result of the activity of sulfate-reducing bacteria in the water column and/or at the water-sediment interface. Typically, black shales are of subeconomic grade, e.g., the U-enriched Cambrian Alum Shale of Scandinavia. Under conditions of extremely low sedimentation rates, economic ore grades may be reached as in case of the early Cambrian of China (Lehmann et al., 2007). Secondary processes such as deep diagenetic redox fronts (Kupferschiefer of Germany and Poland) or supergene enrichment, e.g., at Ronneburg and



Ores, Microbial Precipitation and Oxidation, Figure 4 Banded quartz mineralized with pyrite and uraninite, encrusting silicified granite. Krunkelbach uranium deposit, Schwarzwald, Germany. Low-temperature hydrothermal precipitation with possible involvement of microorganisms. Note the change in mineralization in banded quartz at bend. Sample width 3 cm.



Ores, Microbial Precipitation and Oxidation, Figure 5 Filamentous forms preserved as uraninite in low-T hydrothermal quartz from the Krunkelbach uranium deposit, Schwarzwald, Germany (compare Figure 4). Field of view 350 μm . Automontage image.

Marsberg, Germany (Stribny and Puchelt, 1991), may enrich black shale protores to economic grades.

Weathering and supergene enrichment of ore deposits

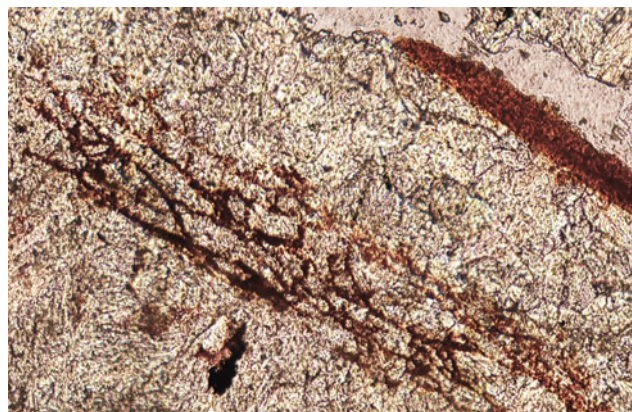
Long after the formation of orebodies, interactions with their environment may take part; most commonly observed are weathering and supergene enrichment. In certain cases the processes of weathering lead to a secondary, near-surface enrichment of the ore (supergene enrichment) along a redox front between unaltered



Ores, Microbial Precipitation and Oxidation,
Figure 6 Specimen of duftite $\text{PbCu}(\text{AsO}_4)\text{OH}$ on calcite from the second oxidation zone of the Tsumeb mine, Namibia (depth range 600–1,000 m below surface). This oxidation zone mineral shows a peculiar filament-like form indicating the incrustation of microbial filaments. Maximum field of view is 10 cm. (Photograph by Peter Vollenweider.)

and oxidized ores. Supergene enrichment may be critical for economic extraction. An involvement of microorganisms in the processes of ore oxidation and supergene enrichment has been reported in many cases from terrestrial (Sillitoe et al., 1996) and marine settings (Glynn et al., 2006). Microbial sulfide oxidation is well studied, mainly with reference to biomining and acid rock drainage.

Probably the most significant microbial process in the oxidation zone is the oxidation of sulfide (Nordstrom and Southam, 1997) and ferrous minerals. Subsurface filamentous fabrics SFF likely of microbial origin are very common in oxidation zone mineral assemblages (Figures 6 and 7). Such morphological evidence of microbial activity in the oxidation zone of ore deposits is



Ores, Microbial Precipitation and Oxidation,
Figure 7 Microphotograph of a thin section across filamentous form of duftite from Tsumeb, Namibia (compare Figure 6). The interior of duftite strand shows filamentous forms preserved as iron hydroxides, probably mineralized microbial filaments. Image width 600 μm .

corroborated by stable isotopic data (Rainbow et al., 2006; Melchiorre and Williams, 2001; Melchiorre et al., 2001).

In the zone of supergene enrichment, oxidized fluids from near-surface parts of the ore interact with reduced, unaltered ore (commonly sulfides). A microbial involvement in such processes of supergene enrichment has been demonstrated in the Morenci copper mine, Arizona (Enders et al., 2006), and in the case of ore-grade supergene sphalerite from Nevada (Bawden et al., 2003). Based on nannobacteria-like forms, Sillitoe et al. (1996) assumed a microbial origin of cementative copper ore at Chuquicamata, Chile.

Conclusions

It is a fact that microorganisms do perform transformations (mainly redox changes) for a number of key elements in ore deposits. In many cases such microbial transactions are advocated to explain the genesis of orebodies, and in other cases they are excluded. A critical review of the existing literature indicates, however, that proofs of microbial involvements often are weak, while the probability of microbial involvement, based on knowledge in microbial ecology, is highly likely. Clearly, the range of arguments to prove or disprove biological interactions in ore deposit formation needs to be extended.

Bibliography

- Anbar, A. D., 2004. Iron stable isotopes: beyond biosignatures. *Earth and Planetary Science Letters*, **217**, 223–236.
- Bak, F., and Cypionka, H., 1987. A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. *Nature*, **326**, 891–892.
- Bawden, T. M. et al., 2003. Extreme ^{34}S depletions in ZnS at the Mike gold deposit, Carlin Trend, Nevada: evidence for bacteriogenic supergene sphalerite. *Geology*, **31**(10), 913–916.

- Bentley, R., and Chasteen, T. G., 2002. Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiology and Molecular Biology Reviews*, **66**(2), 250–271.
- Beukes, N. J., 2004. Early options in photosynthesis. *Nature*, **431**, 522–523.
- Burstein, I. B., Shelton, K. L., Gregg, J. M., and Hagni, R. D., 1993. Complex, multiple ore-fluids in the world-class Southeast Missouri Pb-Zn-Cu deposits: sulfur isotope evidence. In Hagni, R. D. (ed.), *Geology and Geochemistry of Mississippi Valley-Type Ore Deposits*. Rolla: University of Missouri, pp. 1–15.
- Campbell, W. R., and Barton, P. B., 1996. Occurrence and significance of stalactites within the epithermal deposits at Creede, Colorado. *The Canadian Mineralogist*, **34**, 905–930.
- Dahanayake, K., and Krumbein, W. E., 1986. Microbial structures in oolitic iron formations. *Mineralium Deposita*, **21**, 85–94.
- Dahlkamp, F. J., 1993. *Uranium Ore Deposits*. Berlin: Springer, p. 459.
- Donnelly, T. H., and Ferguson, J., 1980. A stable isotope study of three deposits in the Alligator Rivers uranium field. In Ferguson, J., and Goleby, A. B. (eds.), *Uranium in the Pine Creek Geosyncline*. Vienna: International Atomic Energy Agency, pp. 387–406.
- Enders, M. S., Knickerbocker, C., Titley, S. R., and Southam, G., 2006. The role of bacteria on the supergene environments of the Morenci porphyry copper deposit, Greenlee County, Arizona. *Economic Geology*, **101**, 59–70.
- Fallick, A. E., Ashton, J. H., Boyce, A. J., Ellam, R. M., and Russell, M. J., 2001. Bacteria were responsible for the magnitude of the world-class hydrothermal base metal sulphide orebody at Navan, Ireland. *Economic Geology*, **96**, 885–890.
- Glynn, S. et al., 2006. The role of prokaryotes in supergene alteration of submarine hydrothermal sulfides. *Earth and Planetary Science Letters*, **244**, 170–185.
- Goldhaber, M. B. et al., 1990. Genesis of the tabular-type Vanadium-Uranium Deposits of the Henry Basin, Utah. Part II. Mechanisms of ore and gangue mineral formation at the interface between brine and meteoric water. *Economic Geology*, **85**, 215–269.
- Goldhaber, M. B., Reynolds, R. L., and Rye, R. O., 1978. Origin of a South Texas roll-type uranium deposit: II. sulfide petrology and sulfur isotope studies. *Economic Geology*, **73**, 1690–1705.
- Hofmann, B., 1989a. Erzminerale in palaeozoischen, mesozoischen und tertiären Sedimenten der Nordschweiz und Südwestdeutschlands. *Schweizerische Mineralogische und Petrographische Mitteilungen*, **69**, 345–357.
- Hofmann, B., 1989b. *Genese, Alteration und rezentes Fließ-System der Uranlagerstätte Krunkelbach (Menzenschwand, Südschwarzwald)*. Baden, Switzerland: Nagra, pp. 88–30.
- Hofmann, B., and v. Gehlen, K., 1993. Formation of stratiform sulfide mineralizations in the Lower Muschelkalk (Middle Triassic) of Southwestern Germany and Northern Switzerland: constraints from sulfur isotope data. *Schweizerische Mineralogische und Petrographische Mitteilungen*, **73**, 365–374.
- Hofmann, B. A., 1999. Geochemistry of Natural Redox Fronts – A Review. NTB 99–05, Wettingen, Switzerland: Nagra.
- Hofmann, B. A., Helfer, M., Diamond, I., Villa, I., and Sharp, Z. D., 1998. Late-Alpine epithermal breccia mineralization at Grimsel, Central Swiss Alps. *Terra Nostra*, **98**(1), 17.
- Hofmann, B. A., Farmer, J. D., von Blanckenburg, F., and Fallick, A. E., 2008. Subsurface filamentous fabrics: An evaluation of possible modes of origins based on morphological and geochemical criteria, with implications for exoplanetology. *Astrobiology*, **8**(1), 87–117.
- Jensen, M. L., 1958. Sulfur isotopes and the origin of sandstone-type uranium deposits. *Economic Geology*, **53**, 598–616.
- Kappler, A., Pasquero, C., and Konhauser, K. O., 2005. Deposition of banded iron formations by anoxygenic phototrophic Fe(II)-oxidizing bacteria. *Geology*, **33**(11), 865–868.
- Kashefi, K., and Lovley, D. R., 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100°C by *Pyrobaculum islandicum*. *Applied and Environmental Microbiology*, **66**(3), 1050–1056.
- Kashefi, K., Tor, J. M., Nevin, K. P., and Lovley, D. R., 2001. Reductive precipitation of gold by dissimilatory Fe(III)-reducing bacteria and archaea. *Applied and Environmental Microbiology*, **67**(7), 3275–3279.
- Kirschvink, J. L. et al., 2000. Paleoproterozoic snowball Earth: Extreme climatic and geochemical global change and its biological consequences. *Proceedings of the National Academy of Sciences*, **97**(4), 1400–1405.
- Klein, C., 2005. Some Precambrian banded iron-formations (BIFs) around the world: their age, geologic setting, mineralogy, metamorphism, geochemistry, and origin. *American Mineralogist*, **90**, 1473–1499.
- Konhauser, K. O., 1998. Diversity of bacterial iron mineralization. *Earth-Science Reviews*, **43**, 91–121.
- Konhauser, K. O. et al., 2002. Could bacteria have formed the Precambrian banded iron formations? *Geology*, **30**, 1079–1082.
- Kucha, H., Schroll, E., and Stumpf, E. F., 2005. Fossil sulphate-reducing bacteria in the Bleiberg lead-zinc deposit, Austria. *Mineralium Deposita*, **40**, 123–126.
- Labrenz, M. et al., 2000. Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. *Science*, **290**, 1744–1747.
- Leach, D. L., Viets, J. G., and Gent, C. A., 1996. Sulfur isotope geochemistry of ore and gangue minerals from the Silesia-Cracow Mississippi Valley-type ore district, Poland. *Prace Panstwowego Instytutu Geologicznego*, **154**, 123–137.
- Lehmann, B. et al., 2007. Highly metalliferous carbonaceous shale and Early Cambrian seawater. *Geology*, **35**(5), 403–406.
- Lengke, M. F., and Southam, G., 2005. The effect of thiosulfate-oxidizing bacteria on the stability of the gold-thiosulfate complex. *Geochimica et Cosmochimica Acta*, **69**(15), 3759–3772.
- Leventhal, J. S., 1998. Metal-rich black shales: formation, economic geology and environmental considerations. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Stuttgart: Näglerle u. Obermiller.
- Machel, H. G., 2001. Bacterial and thermochemical sulfate reduction in diagenetic settings – old and new insights. *Sedimentary Geology*, **140**, 143–175.
- Macqueen, R. W., and Coope, J. A., 1985. Role of organisms and organic matter in ore deposition. *Canadian Journal of Earth Sciences*, **22**, 1890–1892.
- Melchiorre, E. B., and Williams, P. A., 2001. Stable isotopic characterization of the thermal profile and subsurface biological activity during oxidation of the Great Australia Deposit, Cloncurry, Queensland, Australia. *Economic Geology*, **96**, 1685–1693.
- Melchiorre, E. B., Williams, P. A., and Bevins, R. E., 2001. A low temperature oxygen isotope thermometer for cerussite, with applications at Broken Hill, New South Wales, Australia. *Geochimica et Cosmochimica Acta*, **65**(15), 2527–2533.
- Meunier, J. D., Landais, P., Monthieux, M., and Pagel, M., 1987. Oxidation – reduction processes in the genesis of the uranium – vanadium tabular deposits of the Cottonwood Wash mining area (Utah, USA): evidence from petrological study and organic matter analysis. *Bulletin of Mineralogy*, **110**, 145–156.
- Mohagheghi, A., 1985. *The Role of Aqueous Sulfide and Sulfate-Reducing Bacteria in the Kinetics and Mechanisms of the Reduction of Uranyl Ion*. PhD thesis, Colorado School of Mines, Colorado.

- Mohagheghi, A., Updegraff, D. M., and Goldhaber, M. B., 1985. The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores. *Geomicrobiology Journal*, **4**(2), 153–173.
- Moreau, J. W., Webb, R. I., and Banfield, J. F., 2004. Ultrastructure, aggregation state, and crystal growth of biogenic nanocrystalline sphalerite and wurtzite. *American Mineralogist*, **89**, 950–960.
- Nordstrom, D. K., and Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation. In Nealson, K. H. (ed.), *Geomicrobiology. Reviews in Mineralogy*. Washington: Mineralogical Society of America, pp. 361–390.
- Oremland, R. S. et al., 2005. A microbial arsenic cycle in a salt-saturated extreme environment. *Science*, **308**, 1305–1308.
- Rackley, R. L., 1972. Environment of Wyoming tertiary uranium deposits. *American Association of Petroleum Geologists Bulletin*, **56**, 755–774.
- Rainbow, A., Kyser, T. K., and Clark, A. H., 2006. Isotopic evidence for microbial activity during supergene oxidation of a high-sulfidation epithermal Au-Ag deposit. *Geology*, **34**(4), 269–272.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie. 74. Jahrestagung der Paläontologischen Gesellschaft, Göttingen, 02. bis 08. Oktober 2004. Kurzfassungen der Vorträge und Poster*. Universitätsdruckerei Göttingen, Göttingen.
- Reynolds, R. L., Goldhaber, M. B., and Carpenter, D. J., 1982. Biogenic and nonbiogenic ore-forming processes in the South Texas uranium district: Evidence from the Panna Maria deposit. *Economic Geology*, **77**, 541–556.
- Saunders, J. A., and Swann, C. T., 1994. Mineralogy and geochemistry of a cap-rock Zn-Pb-Sr-Ba occurrence at the Hazlehurst salt dome, Mississippi. *Economic Geology*, **89**(2), 381–390.
- Schaefer, M. O., Gutzmer, J., and Beukes, N. J., 2001. Late proterozoic Mn-rich oncoids: earliest evidence for microbially mediated Mn precipitation. *Geology*, **29**(9), 835–838.
- Schroll, E., 1996. The Triassic carbonate-hosted Pb-Zn mineralization in the Alps (Europe): the genetic position of Bleiberg Type deposits. In Sangster, D. F. (ed.), *Carbonate-Hosted Lead-Zinc Deposits*. Michigan: Society of Economic Geologists, Special Publication 4.
- Sillitoe, R. H., Folk, R. L., and Saric, N., 1996. Bacteria as mediators of copper sulfide enrichment during weathering. *Science*, **272**, 1153–1155.
- Southam, G., and Saunders, J. A., 2005. The Geomicrobiology of ore deposits. *Economic Geology*, **100**(6), 1067–1084.
- Stolz, J. S., and Oremland, R. S., 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiology Reviews*, **23**(5), 615–627.
- Strubny, B., and Puchelt, H., 1991. Geochemical and metallogenic aspects of organic carbon-rich pelitic sediments in Germany. In Pagel, M., and Leroy, J. L. (eds.), *Source, Transport and Deposition of Metals*. Rotterdam: Balkema, pp. 593–598.
- Suzuki, Y., and Banfield, J. F., 1999. Geomicrobiology of uranium. In Burns, P. C., and Finch, R. (eds.), *Uranium: Mineralogy, Geochemistry and the Environment*. Washington: Mineralogical Society of America, pp. 393–432.
- Taylor, B. E., 2004. Biogenic and thermogenic sulfate reduction in the Sullivan Pb-Zn-Ag deposit, British Columbia (Canada): Evidence from micro-isotopic analysis of carbonate and sulfide in bedded ores. *Chemical Geology*, **204**, 215–236.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: Insights into manganese(II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between microbes and minerals. Reviews in Mineralogy*, No. 35. Washington: Mineralogical Society of America.
- Waber, N., Schorscher, H. D., and Peters, T., 1992. Hydrothermal and supergene uranium mineralization at the Osamu Utsumi mine, Poços de Caldas, Minas Gerais, Brazil. *Journal of Geochemical Exploration*, **45**, 53–112.
- Wanty, R. B., Goldhaber, M. B., and Northrop, H. R., 1990. Geochemistry of vanadium in an epigenetic, sandstone-hosted vanadium-uranium deposit, Henry Basin, Utah. *Economic Geology*, **85**, 270–284.
- Warren, C. G., 1971. A method for discriminating between biogenic and chemical origins of the ore-stage pyrite in a roll-type uranium deposit. *Economic Geology*, **66**, 919–928.
- Warren, C. G., 1972. Sulfur isotopes as a clue to the genetic geochemistry of a roll-type uranium deposit. *Economic Geology*, **67**, 759–767.
- Widdel, F. et al., 1993. Ferrous iron oxidation by anoxygenic phototropic bacteria. *Nature*, **362**, 834–836.
- Wilkinson, J. J., Eyre, S. L., and Boyce, A. J., 2005. Ore-forming processes in Irish-type carbonate-hosted Zn-Pb deposits: evidence from mineralogy, chemistry, and isotopic composition of sulfides at the Lisheen mine. *Economic Geology*, **100**(1), 63–86.

Cross-references

[Acid Rock Drainage](#)
[Banded Iron Formations](#)
[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Biosignatures in Rocks](#)
[Black Shales](#)
[Carbon \(Organic, Degradation\)](#)
[Extreme Environments](#)
[Hydrogen](#)
[Hydrothermal Environments, Fossil](#)
[Nan\(n\)obacteria](#)
[Origin of Life](#)
[Radioactivity \(Natural\)](#)
[Reduction Spheroids](#)
[Subsurface Filamentous Fabrics](#)
[Sulfate-Reducing Bacteria](#)
[Sulfide Mineral Oxidation](#)

ORGANIC CARBON

Carbon that occurs in materials of ultimately biological origin that can be oxidized. Organic matter is material that contains organic carbon. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#)” for further reading.

ORGANOMINERALIZATION

Christian Défarge
 Université d'Orléans and Université François-Rabelais de Tours, Orléans, France

Synonyms

Organomineral formation

- Mohagheghi, A., Updegraff, D. M., and Goldhaber, M. B., 1985. The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores. *Geomicrobiology Journal*, **4**(2), 153–173.
- Moreau, J. W., Webb, R. I., and Banfield, J. F., 2004. Ultrastructure, aggregation state, and crystal growth of biogenic nanocrystalline sphalerite and wurtzite. *American Mineralogist*, **89**, 950–960.
- Nordstrom, D. K., and Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation. In Nealson, K. H. (ed.), *Geomicrobiology. Reviews in Mineralogy*. Washington: Mineralogical Society of America, pp. 361–390.
- Oremland, R. S. et al., 2005. A microbial arsenic cycle in a salt-saturated extreme environment. *Science*, **308**, 1305–1308.
- Rackley, R. L., 1972. Environment of Wyoming tertiary uranium deposits. *American Association of Petroleum Geologists Bulletin*, **56**, 755–774.
- Rainbow, A., Kyser, T. K., and Clark, A. H., 2006. Isotopic evidence for microbial activity during supergene oxidation of a high-sulfidation epithermal Au-Ag deposit. *Geology*, **34**(4), 269–272.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie. 74. Jahrestagung der Paläontologischen Gesellschaft, Göttingen, 02. bis 08. Oktober 2004. Kurzfassungen der Vorträge und Poster*. Universitätsdruckerei Göttingen, Göttingen.
- Reynolds, R. L., Goldhaber, M. B., and Carpenter, D. J., 1982. Biogenic and nonbiogenic ore-forming processes in the South Texas uranium district: Evidence from the Panna Maria deposit. *Economic Geology*, **77**, 541–556.
- Saunders, J. A., and Swann, C. T., 1994. Mineralogy and geochemistry of a cap-rock Zn-Pb-Sr-Ba occurrence at the Hazlehurst salt dome, Mississippi. *Economic Geology*, **89**(2), 381–390.
- Schaefer, M. O., Gutzmer, J., and Beukes, N. J., 2001. Late proterozoic Mn-rich oncoids: earliest evidence for microbially mediated Mn precipitation. *Geology*, **29**(9), 835–838.
- Schroll, E., 1996. The Triassic carbonate-hosted Pb-Zn mineralization in the Alps (Europe): the genetic position of Bleiberg Type deposits. In Sangster, D. F. (ed.), *Carbonate-Hosted Lead-Zinc Deposits*. Michigan: Society of Economic Geologists, Special Publication 4.
- Sillitoe, R. H., Folk, R. L., and Saric, N., 1996. Bacteria as mediators of copper sulfide enrichment during weathering. *Science*, **272**, 1153–1155.
- Southam, G., and Saunders, J. A., 2005. The Geomicrobiology of ore deposits. *Economic Geology*, **100**(6), 1067–1084.
- Stolz, J. S., and Oremland, R. S., 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiology Reviews*, **23**(5), 615–627.
- Strubny, B., and Puchelt, H., 1991. Geochemical and metallogenic aspects of organic carbon-rich pelitic sediments in Germany. In Pagel, M., and Leroy, J. L. (eds.), *Source, Transport and Deposition of Metals*. Rotterdam: Balkema, pp. 593–598.
- Suzuki, Y., and Banfield, J. F., 1999. Geomicrobiology of uranium. In Burns, P. C., and Finch, R. (eds.), *Uranium: Mineralogy, Geochemistry and the Environment*. Washington: Mineralogical Society of America, pp. 393–432.
- Taylor, B. E., 2004. Biogenic and thermogenic sulfate reduction in the Sullivan Pb-Zn-Ag deposit, British Columbia (Canada): Evidence from micro-isotopic analysis of carbonate and sulfide in bedded ores. *Chemical Geology*, **204**, 215–236.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: Insights into manganese(II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between microbes and minerals. Reviews in Mineralogy*, No. 35. Washington: Mineralogical Society of America.
- Waber, N., Schorscher, H. D., and Peters, T., 1992. Hydrothermal and supergene uranium mineralization at the Osamu Utsumi mine, Poços de Caldas, Minas Gerais, Brazil. *Journal of Geochemical Exploration*, **45**, 53–112.
- Wanty, R. B., Goldhaber, M. B., and Northrop, H. R., 1990. Geochemistry of vanadium in an epigenetic, sandstone-hosted vanadium-uranium deposit, Henry Basin, Utah. *Economic Geology*, **85**, 270–284.
- Warren, C. G., 1971. A method for discriminating between biogenic and chemical origins of the ore-stage pyrite in a roll-type uranium deposit. *Economic Geology*, **66**, 919–928.
- Warren, C. G., 1972. Sulfur isotopes as a clue to the genetic geochemistry of a roll-type uranium deposit. *Economic Geology*, **67**, 759–767.
- Widdel, F. et al., 1993. Ferrous iron oxidation by anoxygenic phototropic bacteria. *Nature*, **362**, 834–836.
- Wilkinson, J. J., Eyre, S. L., and Boyce, A. J., 2005. Ore-forming processes in Irish-type carbonate-hosted Zn-Pb deposits: evidence from mineralogy, chemistry, and isotopic composition of sulfides at the Lisheen mine. *Economic Geology*, **100**(1), 63–86.

Cross-references

[Acid Rock Drainage](#)
[Banded Iron Formations](#)
[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Biosignatures in Rocks](#)
[Black Shales](#)
[Carbon \(Organic, Degradation\)](#)
[Extreme Environments](#)
[Hydrogen](#)
[Hydrothermal Environments, Fossil](#)
[Nan\(n\)obacteria](#)
[Origin of Life](#)
[Radioactivity \(Natural\)](#)
[Reduction Spheroids](#)
[Subsurface Filamentous Fabrics](#)
[Sulfate-Reducing Bacteria](#)
[Sulfide Mineral Oxidation](#)

ORGANIC CARBON

Carbon that occurs in materials of ultimately biological origin that can be oxidized. Organic matter is material that contains organic carbon. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#)” for further reading.

ORGANOMINERALIZATION

Christian Défarge
 Université d'Orléans and Université François-Rabelais de Tours, Orléans, France

Synonyms

Organomineral formation

Definition

Organomineralization is a process of mineral formation mediated by organic matter (OM), independent of the living organisms which the OM derives from. The organic compounds may be excretion products or detached parts of living organisms, or relics and by-products of dead organisms that have been released into waters or incorporated into soils, sediments, or rocks. Abiotic OM may also induce the formation of minerals in nature or in the laboratory.

Organominerals are the mineralic products of organomineralization. They should be distinguished from minerals precipitated through inorganic processes, among which some may contain OM entombed or complexed during crystal growth, from organic minerals such as graphite and from biominerals produced by living organisms.

Genesis of the organomineralization concept

The term *organomineralization* was introduced in 1993 by Trichet and Défarge (1995) at the 7th International Symposium on Biomineralization. It aimed to denote the processes of mineral formation that are similar to biomineral formation by living organisms, because induced by organic templates, compartments and compounds (*Biomineralization*, qv), but distinct because occurring in waters, soils, sediments or rocks, independently of the living organisms which the mineralizing OM derives from. The substantive *organomineral* was also introduced at the same symposium to refer to the mineral products of organomineralization, to be distinguished from the biominerals produced by living organisms (Défarge and Trichet, 1995). These definitions were supported by illustrations of organomineral formation processes including (Défarge and Trichet, 1995; Trichet and Défarge, 1995) diagenetic calcification of gastropod fecal pellets; post-mortem encrustation of cyanobacterial filaments, fish organs, and soft tissues of trilobites by calcium carbonate (CC), calcium salts of fatty acids, or pyrite; formation of CC bodies and phosphate pellets under the influence of sedimentary OM; intervention of soil organic compounds in the crystallization of aluminum and iron oxides and hydroxides; precipitation of metal oxides and sulfides following the demixion of organometallic complexes subjected to geothermal heating; and laboratory formation of CC ooids in the presence of OM rich in acidic groups.

The first hypotheses on the role of sedimentary OM in the precipitation of minerals in natural environments, believed to be analogous to that played by organic templates in biomineralization, were published in the 1960s in papers presented on the formation of calcareous microbial deposits, oolites, and coprolites (Trichet, 1967, 1968; Mitterer, 1968; for later references see Morse et al., 2007). These hypotheses were founded on the similarities in composition of the organic fraction of these carbonate bodies with that of biomineral matrices, which are both rich in acidic amino acids, and on earlier work evidencing that

dissolved organic compounds can favor CC precipitation from waters (see references in Mitterer, 1968; Trichet, 1968; or Trichet and Défarge, 1995).

A decisive step in this research and in the genesis of the concept of organomineralization was made, thanks to studies on carbonate formation in the modern lacustrine *microbialites* (qv) of Pacific atolls (Défarge and Trichet, 1995): The mineral fraction of these sediments was shown to be principally composed of magnesian calcite micrite clusters that had precipitated within the alveoli, on the wall surfaces of an organic honeycomb-like network formed by the reorganization of *extracellular polymeric substances* (EPS, qv) secreted by *cyanobacteria* (qv). These organic templates of precipitation thus had the same origin as those of CC biomineralization in cyanobacteria, which is associated with the cell sheath (Merz-Preiß, 2000). In addition, it was demonstrated that this OM fixed divalent metallic cations at acidic sites, whose affinities with calcium ions explain why they act as nucleation sites for CC biomineralization (Addadi and Weiner, 1992; Gilbert et al., 2005). When they can be individually observed, the magnesian calcite crystals were shown to grow with their *c*-axis perpendicular to the organic template (e.g., Défarge et al., 1996, Figure 5c), as is frequently observed in epitaxial CC biomineralization (Addadi and Weiner, 1992), e.g., on algal cell surfaces (Weiner and Dove, 2003). This process of micrite precipitation thus appeared analogous to carbonate biomineralization, although distinct because of being mediated by nonliving, sedimentary OM. These micrite organominerals were accompanied by tubular ones, resulting from the post-mortem encrustation of cyanobacterial filaments, which thus controlled the final mineral shape, and by biomineralized cyanobacterial sheaths (Défarge and Trichet, 1995). These carbonate microbialites thus revealed a continuum of mineralizing organic–mineral interactions in nature, from biomineralization through organomineralization processes (Défarge and Trichet, 1995; Défarge et al., 1996).

Organomineralization versus biomineralization

The existence of such continua among biomineralization processes *sensu stricto* had already been established: Lowenstam and Weiner (1989) distinguished “biologically controlled mineralization”, characterized by cellular controls on crystal nucleation and growth, delineated spaces of mineral formation, and specific habits of the biominerals produced, and “biologically induced mineralization”, which designates biotic processes that are not specifically designed for mineralization but result in mineral being formed. However, in these latter cases too, organisms exercised some control on mineral formation (Lowenstam and Weiner, 1989; Weiner and Dove, 2003).

Another continuum bridges biologically induced mineralization occurring through crystal nucleation on organic secretions outside of organisms (see Weiner and Dove, 2003, Figure 4), and organomineralization on sedimentary organic substrates derived from EPS described by

Défarge and Trichet (1995). The distinction between the two processes, however, is justified, as nucleating organic substrates may act remote in space and time from the organisms which they derive from, as in the examples documented by Trichet and Défarge (1995) for carbonate sediments or for metal minerals formed through geothermal heating of organometallic complexes.

In all cases of organomineralization, biotic processes (such as photosynthesis or sulfate reduction in the case of CC; Dupraz and Visscher, 2005) may also participate in solution supersaturation leading to crystal formation on the nonliving organic templates. However, the global process cannot be simply described as “biologically induced” when these nonliving substrates appear to play a necessary role in crystallization, in particular crystal heterogeneous nucleation, or cessation or confinement of crystal growth. In introducing the concept of organomineralization, Trichet and Défarge (1995) aimed to point out that organic matrices and compounds inherited from living organisms may retain mineralizing properties after they have been released into waters and incorporated into soils, sediments, and rocks.

Evolution of the organomineralization domain

The same year in which Trichet and Défarge (1995) introduced the term *organomineralization* at the 7th International Symposium on Biomineralization (1993), Reitner (1993) and then Reitner et al. (1995) at the same conference, hypothesized that organic matrix-mediated processes were responsible for carbonate automicrite and peloid formation in modern microbialites and sponge tissues from reef caves. These processes appeared analogous to those postulated for the precipitation of CC organominerals in Polynesian microbialites (Défarge and Trichet, 1995; Trichet and Défarge, 1995), and have therefore been considered organomineralization processes (Neuweiler et al., 1999; Reitner, 2004). Thereafter, a number of papers have documented CC organomineralization in natural environments or in the laboratory (see review in Défarge et al., 2009). New illustrations included for example, automicrite formation in Lower Cretaceous carbonate *mud mounds* (qv; Neuweiler et al., 1999) or calcification of the housing tubes of modern and fossil polychaete worms (Fischer et al., 2000). Riding (2000) and Schlager (2003) identified organomineralization as one of the main processes of carbonate precipitation in *microbial mats* (qv) and *biofilms* (qv), or more widely in benthic marine sediments. Many *in vitro* studies continued to demonstrate the control exerted by biological compounds on the crystallization of various mineral species (Davies et al., 2003). Thanks to experiments that induced the precipitation of carbonate bodies in the presence of OM extracted from the Murchison CM2 meteorite, Reitner (2004) pointed out that abiotic OM may also induce organomineral formation.

Recently, Dupraz and Visscher (2005) proposed distinguishing carbonate organomineralization mediated

by crystal nucleation on recomposed EPS, as in the former examples documented by Défarge and Trichet (1995) in Polynesian microbialites, from carbonate precipitation following bacterial degradation of EPS: Bacterial degradation liberates EPS-bound ions such as Ca^{2+} , Mg^{2+} , or HCO_3^- , thus leading to outside solution supersaturation for Ca and Mg carbonates. The latter process is not considered by Dupraz and Visscher (2005) to be organomineralization *sensu* Trichet and Défarge (1995). However, sedimentary EPS may also concentrate ions independent of their living producers (Trichet and Défarge, 1995; Westall et al., 2000). In the case of carbonate precipitation induced by degradation of that sedimentary OM, since ion binding by EPS is a prerequisite for mineralization, this process should be considered a subcategory of organomineralization. The distinction between organomineralization induced by the liberation in solution of ions previously concentrated by sedimentary EPS and organomineralization involving a nucleating role of EPS corresponds to the distinction originally introduced by Trichet and Défarge (1995) between “organically induced” and “organically supported” organomineralization.

Geobiological consequences of organomineralization

Impact on present-day and past geological processes

Although difficult to evaluate, because it is frequently hard to distinguish from the direct role of biotic activity, and neglected by most geobiologists, the impact of organomineralization on sediment and soil formation is likely to be far from negligible. The works cited above (reviewed by Défarge et al., 2009) have already documented occurrences of organomineralization processes in the principal environments at the surface of the Earth, from deep cold water to shallow warm marine, through hyposaline to alkaline lakes, and terrestrial soils. They are involved in the formation of sedimentary calcareous buildups such as microbialites, mud mounds, and reefs (qv). Organomineral products include micrites, ooids, peloids, lithified cyanobacterial sheaths, worm housing tubes, animal tissues, and fecal pellets. The rapid authigenic phosphatization of tissues after death, leading to the preservation of soft-bodied fossils, likely involves matrix-mediated organomineralization processes (see Briggs, 2003, p. 292). The principal mineral species forming biominerals (Weiner and Dove, 2003) are also represented by organominerals: Ca carbonates and phosphates, and metal sulfides, oxides, and hydroxides; the silicification of EPS that occurs in biofilms independent of those of the microorganisms (Westall et al., 2000) may also be regarded as an organomineralization.

Défarge et al. (1996) showed that organomineralization processes are probably commonly involved in microbialite calcification; this was then confirmed in numerous cases (Défarge et al., 2009). The process of carbonate–fluorapatite precipitation mediated by dead

bacterial cells, proposed by Sanchez-Navas and Martin-Algarra (2001), in phosphate *stromatolites* (qv) that are representative of microbial structures frequently encountered in pelagic sediments from Mesozoic Alpine-Mediterranean paleomargins, may also be considered an organomineralization. The involvement of mineralizing OM remains the most plausible hypothesis for explaining the formation of magnesian calcite radial ooids composing numerous shallow marine carbonate sands (Morse et al., 2007). Neuweiler et al. (1999) provided evidence for the involvement of micrite organomineralization in the building of Lower Cretaceous mud mounds that are significant examples of calcareous buildups, possibly originating in the Neoproterozoic and common during the Phanerozoic. As EPS are essential components of microbial biofilms, which are widespread in nature and persistent throughout the history of life on the Earth (Westall et al., 2000), EPS-mediated organomineralization has probably been active at least since the appearance of living organisms, and may represent a geologically early process of mineral production coexisting with, or even preexisting, biomineralization.

Implications for astrobiological interpretations

Organominerals formed through the mediation of biologically derived OM may serve as *biosignatures* (qv) in the search for evidence of life in the geological rock record or on extraterrestrial bodies devoid of fossils or bioconstructions. Westall et al. (2000) stressed that mineralized EPS could be used as markers of the presence of bacteria in terrestrial or extraterrestrial materials. However, these biotic EPS need to be distinguished from potential prebiotic EPS that might have the same abilities for fossilization (Westall et al., 2000). Laboratory experiments by Reitner (2004) have shown that OM, likely abiotic, extracted from the Murchinson CM2 meteorite, was able to precipitate CC organominerals similar to those known from terrestrial microbial sediments. More generally, organic molecules were proved to be able to precipitate, in sterile conditions, Ca carbonate or phosphate mineral products resembling biologically induced minerals, in particular those interpreted as fossilized “*nan(n)obacteria*” (qv; Kirkland et al., 1999; Reitner, 2004; see other references in Défarge et al., 2009). Astrobiological studies for the search and the origin of life should keep in mind this spectrum of potential prebiotic, abiotic, or biologically derived organominerals in nature.

Potential engineering applications

Biomineralization processes have already inspired the design of new organic matrix-mediated materials by synthetic analogues of the matrices of natural biominerals (Davies et al., 2003). Such manufactured materials are artificial organominerals, although they can also be regarded, from a global geobiological perspective, as biominerals, because they are formed under direct biological, i.e., human, control. Similarly, organomineralization

sensu stricto might, in the future, inspire further engineering processes based on the precipitation of minerals or the extraction of ions from solutions in sterile conditions, applied, for example, to water and wastewater treatment, soil remediation and improvement, protection and restoration of building stones, etc.

Summary and perspectives

The direct role of life in the functioning and the evolution of our Planet is beginning to be fully recognized; witness is this encyclopedia. The geobiological impact of OM, however, which is in part included in the indirect role of biotic activity but may also be due to prebiotic or abiotic processes, is far from being equally well acknowledged and studied. Since the mineralizing role of OM has now been proved in various environments and geological periods, it should be searched for and identified in other case studies, in particular in carbonate formation that took place before the rise of calcareous organisms. While organomineralization research has mainly been devoted until now to EPS calcification, studies on organomineralization processes leading to the formation of other common biomineral species, such as phosphates, metal sulfides and oxihydroxides, or silica, should be continued, and the case of other species such as sulfates might be considered. The role of other types of sedimentary and soil OM also needs to be investigated. The possible involvement of abiotic OM in organomineral formation should be taken into account in astrobiological studies for the search of life on the primitive Earth and on extraterrestrial bodies. In addition to materials applications based on biomineralization processes, other studies might be devoted, in the future, to organomineralization-inspired engineering.

Bibliography

- Addadi, L., and Weiner, S., 1992. Control and design principles in biological mineralization. *Angewandte Chemie International Edition in English*, **31**, 153–169.
- Briggs, D. E. G., 2003. The role of decay and mineralization in the preservation of soft-bodied fossils. *Annual Review of Earth and Planetary Sciences*, **31**, 275–301.
- Davies, S. A., Dujardin, E., and Mann, S., 2003. Biomolecular inorganic materials chemistry. *Current Opinion in Solid State and Materials Science*, **7**, 273–281.
- Défarge, C., and Trichet, J., 1995. From biominerals to ‘organominerals’: The example of the modern lacustrine calcareous stromatolites from Polynesian atolls. In Allemand, D., and Cuif, J. P. (eds.), *Proceedings 7th International Symposium on Biomineralization. Bulletin de l’Institut Océanographique de Monaco*, n° spéc. 14, Vol. 2, 265–271.
- Défarge, C., Trichet, J., Jaunet, A. M., Robert, M., Tribble, J., and Sansone, F. J., 1996. Texture of microbial sediments revealed by cryo-scanning electron microscopy. *Journal of Sedimentary Research*, **66**, 935–947.
- Défarge, C., Gautret, P., Reitner, J., and Trichet, J., 2009. Defining organominerals: comment on ‘Defining biominerals and organominerals: Direct and indirect indicators of life’ by Perry et al. (2007, *Sedimentary Geology*, 201, 157–179). *Sedimentary Geology*, **213**, 152–155.

- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Fischer, R., Pernet, B., and Reitner, J., 2000. Organomineralization of cirratulid annelid tubes – Fossil and Recent examples. *Facies*, **42**, 35–50.
- Gilbert, P. U. P. A., Abrecht, M., and Frazer, B. H., 2005. The organic-mineral interface in biominerals. *Reviews in Mineralogy and Geochemistry*, **59**, 157–185.
- Kirkland, B. L., Lynch, F. L., Rahnis, M. A., Folk, R. L., Molineux, I. J., and McLean, R. J. C., 1999. Alternative origins for nanobacteria-like objects in calcite. *Geology*, **27**, 347–350.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. Oxford: Oxford University Press.
- Merz-Preiß, M., 2000. Calcification in cyanobacteria. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 50–56.
- Mitterer, R. M., 1968. Amino acid composition of organic matrix in calcareous oolites. *Science*, **162**, 1498–1499.
- Morse, J. W., Arvidson, R. S., and Lüttge, A., 2007. Calcium carbonate formation and dissolution. *Chemical Reviews*, **107**, 342–381.
- Neuweiler, F., Gautret, P., Thiel, V., Lange, R., Michaelis, W., and Reitner, J., 1999. Petrology of Lower Cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40.
- Reitner, J., 2004. Organomineralization: a clue to the understanding of meteorite-related “bacteria-shaped” carbonate particles. In Seckbach, J. (ed.), *Origins. Genesis, Evolution and Diversity of Life*. Dordrecht: Kluwer, pp. 195–212.
- Reitner, J., Gautret, P., Marin, F., and Neuweiler, F., 1995. Automicrites in a modern marine microbialite. Formation model via organic matrices (Lizard Island, Great Barrier Reef, Australia). In Allemand, D., and Cuif, J. P. (eds.), *Proceedings 7th International Symposium on Biomineralization. Bulletin de l'Institut Océanographique de Monaco*, n° spéc. 14, Vol. 2, 237–263.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47** (Suppl. 1), 179–214.
- Sanchez-Navas, A., and Martin-Algarra, A., 2001. Genesis of apatite in phosphate stromatolites. *European Journal of Mineralogy*, **13**, 361–376.
- Schlager, W., 2003. Benthic carbonate factories of the Phanerozoic. *International Journal of Earth Sciences*, **92**, 445–464.
- Trichet, J., 1967. Essai d'explication du dépôt d'aragonite sur des substrats organiques. *Comptes Rendus de l'Académie des Sciences de Paris, Série D*, **265**, 1464–1467.
- Trichet, J., 1968. Etude de la composition de la fraction organique des oolites. Comparaison avec celle des membranes des bactéries et des cyanophycées. *Comptes Rendus de l'Académie des Sciences de Paris, Série D*, **267**, 1492–1494.
- Trichet, J., and Défarge, C., 1995. Non-biologically supported organomineralization. In Allemand, D., and Cuif, J. P. (eds.), *Proceedings 7th International Symposium on Biomineralization. Bulletin de l'Institut Océanographique de Monaco*, n° spéc. 14, Vol. 2, 203–236.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–29.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., McKay, D., Gibson, E., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24511–24527.

Cross-references

[Biofilms](#)
[Biosignatures in Rocks](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomineralization](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Mud Mounds](#)
[Nan\(n\)obacteria](#)
[Reefs](#)
[Stromatolites](#)

ORIGIN OF LIFE

Michael J. Russell
 California Institute of Technology, Pasadena, CA, USA

Definition

Life exploits free energy potentials to produce effluent at much reduced value. Put another way, it takes a low entropy feed, transforming it to high entropy waste. At the root or origin of the evolutionary tree as well as at the base of the food chain, life essentially draws down carbon dioxide by reducing it with hydrogen from a variety of sources, aided by a diversity of other aqueous solutes. Thus, life is a coded and organized entropy generator that can search and evolve to find further appropriate potential energies and materials.

Approaches to the origin of life

Origin of life theory has yet to reach a stage where it can take its place as the introductory chapter in textbooks in the life sciences. Why is this? Many researchers have concentrated on what life *is* rather than what life *does*, and have cast around for organic molecules that might have constituted the “building blocks” for the first cells – nucleic and amino acids, carbohydrates, and lipids. Thus comets (Ehrenfreund et al., 2006; see Chapter [Asteroid and Comet Impacts](#)), carbonaceous meteorites (Deamer, 1985), cosmic dust particles (Maurette, 1998), the products of lightning in a reduced atmosphere (Miller, 1953; Miller and Urey, 1959), and Fischer–Tropsch reactions within the Earth’s crust (Holm and Charlou, 2001) have all been appealed to in what may be called organogenic or heterogenic theories of origin. But research taking this tack has had little experimental success. Nor does it suggest an evolutionary link between such “prebiotic chemistry” and biochemistry. However, if instead of approaching life in terms of its constituents and where they might come from, but rather ask “what does life do,” it can be apprehended rather as a processor of energy and a generator of entropy, so inviting consideration of a thermodynamic, geochemical, and evolutionary context for the emergence of life. This approach has led to the autogenic theories for life’s origin, theories that view as

- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Fischer, R., Pernet, B., and Reitner, J., 2000. Organomineralization of cirratulid annelid tubes – Fossil and Recent examples. *Facies*, **42**, 35–50.
- Gilbert, P. U. P. A., Abrecht, M., and Frazer, B. H., 2005. The organic-mineral interface in biominerals. *Reviews in Mineralogy and Geochemistry*, **59**, 157–185.
- Kirkland, B. L., Lynch, F. L., Rahnis, M. A., Folk, R. L., Molineux, I. J., and McLean, R. J. C., 1999. Alternative origins for nanobacteria-like objects in calcite. *Geology*, **27**, 347–350.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. Oxford: Oxford University Press.
- Merz-Preiß, M., 2000. Calcification in cyanobacteria. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 50–56.
- Mitterer, R. M., 1968. Amino acid composition of organic matrix in calcareous oolites. *Science*, **162**, 1498–1499.
- Morse, J. W., Arvidson, R. S., and Lüttge, A., 2007. Calcium carbonate formation and dissolution. *Chemical Reviews*, **107**, 342–381.
- Neuweiler, F., Gautret, P., Thiel, V., Lange, R., Michaelis, W., and Reitner, J., 1999. Petrology of Lower Cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40.
- Reitner, J., 2004. Organomineralization: a clue to the understanding of meteorite-related “bacteria-shaped” carbonate particles. In Seckbach, J. (ed.), *Origins. Genesis, Evolution and Diversity of Life*. Dordrecht: Kluwer, pp. 195–212.
- Reitner, J., Gautret, P., Marin, F., and Neuweiler, F., 1995. Automicrites in a modern marine microbialite. Formation model via organic matrices (Lizard Island, Great Barrier Reef, Australia). In Allemand, D., and Cuif, J. P. (eds.), *Proceedings 7th International Symposium on Biomineralization. Bulletin de l'Institut Océanographique de Monaco*, n° spéc. 14, Vol. 2, 237–263.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47** (Suppl. 1), 179–214.
- Sanchez-Navas, A., and Martin-Algarra, A., 2001. Genesis of apatite in phosphate stromatolites. *European Journal of Mineralogy*, **13**, 361–376.
- Schlager, W., 2003. Benthic carbonate factories of the Phanerozoic. *International Journal of Earth Sciences*, **92**, 445–464.
- Trichet, J., 1967. Essai d'explication du dépôt d'aragonite sur des substrats organiques. *Comptes Rendus de l'Académie des Sciences de Paris, Série D*, **265**, 1464–1467.
- Trichet, J., 1968. Etude de la composition de la fraction organique des oolites. Comparaison avec celle des membranes des bactéries et des cyanophycées. *Comptes Rendus de l'Académie des Sciences de Paris, Série D*, **267**, 1492–1494.
- Trichet, J., and Défarge, C., 1995. Non-biologically supported organomineralization. In Allemand, D., and Cuif, J. P. (eds.), *Proceedings 7th International Symposium on Biomineralization. Bulletin de l'Institut Océanographique de Monaco*, n° spéc. 14, Vol. 2, 203–236.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–29.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., McKay, D., Gibson, E., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24511–24527.

Cross-references

[Biofilms](#)
[Biosignatures in Rocks](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomineralization](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Mud Mounds](#)
[Nan\(n\)obacteria](#)
[Reefs](#)
[Stromatolites](#)

ORIGIN OF LIFE

Michael J. Russell
 California Institute of Technology, Pasadena, CA, USA

Definition

Life exploits free energy potentials to produce effluent at much reduced value. Put another way, it takes a low entropy feed, transforming it to high entropy waste. At the root or origin of the evolutionary tree as well as at the base of the food chain, life essentially draws down carbon dioxide by reducing it with hydrogen from a variety of sources, aided by a diversity of other aqueous solutes. Thus, life is a coded and organized entropy generator that can search and evolve to find further appropriate potential energies and materials.

Approaches to the origin of life

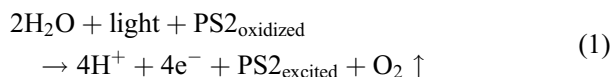
Origin of life theory has yet to reach a stage where it can take its place as the introductory chapter in textbooks in the life sciences. Why is this? Many researchers have concentrated on what life *is* rather than what life *does*, and have cast around for organic molecules that might have constituted the “building blocks” for the first cells – nucleic and amino acids, carbohydrates, and lipids. Thus comets (Ehrenfreund et al., 2006; see Chapter [Asteroid and Comet Impacts](#)), carbonaceous meteorites (Deamer, 1985), cosmic dust particles (Maurette, 1998), the products of lightning in a reduced atmosphere (Miller, 1953; Miller and Urey, 1959), and Fischer–Tropsch reactions within the Earth’s crust (Holm and Charlou, 2001) have all been appealed to in what may be called organogenic or heterogenic theories of origin. But research taking this tack has had little experimental success. Nor does it suggest an evolutionary link between such “prebiotic chemistry” and biochemistry. However, if instead of approaching life in terms of its constituents and where they might come from, but rather ask “what does life do,” it can be apprehended rather as a processor of energy and a generator of entropy, so inviting consideration of a thermodynamic, geochemical, and evolutionary context for the emergence of life. This approach has led to the autogenic theories for life’s origin, theories that view as

paramount consideration of the conditions upon the early Earth that drove life into being that see the emergence of life as a geological issue.

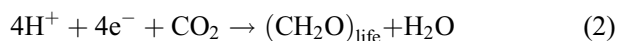
Therefore, the method applied in this entry is broadly evolutionary, autogenic, and mechanistic. We consider the onset of life as a natural and inevitable outcome of the well-established far-from-equilibrium conditions obtaining on our planet more than 4 billion years ago, especially the potential between hydrothermal hydrogen and atmospheric carbon dioxide. However, more reduced molecules bearing one carbon atom such as formate (CHOO^-), and methyl thiolate (CH_3S^-) may be required to get life going initially. Moreover, electrochemical energy is also seen to be needed in the form of the protonmotive force. Indeed, the first microbial vehicles were “hybrids,” driven into being by chemical potential and steep pH, redox, and temperature gradients across their membranes. So, first looking at what life does, we then investigate how life, as an energy and materials processor, may have begun. Only after metabolism’s engine is ticking over could genetic regulation and then replication emerge.

Life now

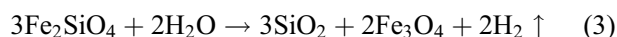
At root life takes molecules that are out of equilibrium at or near the surface of the Earth, reacts them together (i.e., processes or metabolizes them) within cells that can grow, reproduce, and evolve. None of this can happen without cell mortality and the excretion and disposal of relatively disordered waste products. In human metabolism the disequilibrium is between hydrogen released from foodstuffs (by dehydrogenases to nicotinamide dinucleotide (NAD to NADH)) and atmospheric oxygen (Williams and Ramsden, 2007). The waste products are carbon dioxide and water from energy production and the excreted disordered (higher entropy) organic molecules of lower energy rejected or degenerated during biosynthesis. But we live off others. At the base of the food chain are organisms that directly exploit the disequilibrium between hydrogen released from water in some manner, and from the carbon dioxide gleaned from the atmosphere. The obvious examples of the food-chain’s base are the plants and the cyanobacteria (see Chapter *Cyanobacteria*). They split hydrogen from water into four protons and four electrons during oxygenic photosynthesis using, amongst other mechanisms, a complex enzyme known as photosystem 2 (PS2) (Blankenship, 2002); oxygen is released as waste:



The protons and electrons are then “fed” to the carbon dioxide, which with further enzymatic assistance, is thereby reduced to cellular carbon and some waste water:



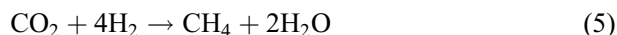
However, there is a still more basic metabolism whereby prokaryotes use the hydrogen emanating naturally from hot springs in a geochemical, rather than a photosynthetic, reaction (Spear et al., 2005; see Chapter *Hydrogen*). The hydrogen is released during hydrothermal convection as the water oxidizes the ferrous iron in minerals such as fayalite olivine (Fe_2SiO_4) in the Earth’s crust:



This hydrogen can reduce carbon dioxide in certain prokaryotes through the involvement of nicotinamide dinucleotide (NAD), chemiosmosis, iron-nickel hydrogenases, and iron-sulfur proteins (Daniel and Danson, 1995; Volbeda and Fontecilla-Camps, 2005a; Williams and Ramsden, 2007; McGlynn et al., 2009; see Chapters *Hydrothermal Environments*, *Marine* and *Nickel*, *Biology*). In such cases the carbon wastes are either acetic acid as in the homoacetogens (and see Chapter *Acetogens*):



or methane as in the methanogens (see Chapter *Methane*, *Origin*):

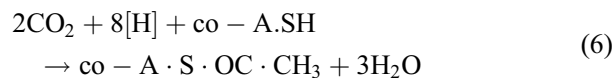


Based on the uniformitarian principle (that the present is the key to the past) we expect today’s food chain to recapitulate the evolutionary tree of metabolic pathways, thereby giving us a strong indication of what the earliest biochemistry was like. From this knowledge we might enquire whether there is a match between such a biochemical expectation and the very slow aqueous geochemical reactions arising from the initial conditions on our planet four or more billion years ago – reactions that could have been quickened through the emergence of life. A positive response to the question has led to a hydrothermal theory of the origin of life that at first sight seems to lie strictly in the autogenic camp – a theory that expects that the first metabolism was based on acetogenesis and methanogenesis (see Chapters *Acetogens* and *Methane*, *Origin*).

Autogenic theory

The class of theory that invokes the simplest of components such as carbon dioxide and ammonia to explain the emergence of life was first termed “autogenic” by Haeckel (1870, p. 302; 1892, p. 414). Mereschowsky (1910, p. 360), Harvey (1924), and Corliss et al. (1981) thought the same way. Because *appropriate* organic molecules were not to be had on the early Earth, an origin of life based on minerals and their component clusters was generally assumed (Leduc, 1911; Goldschmidt, 1952; Hall et al., 1971). A modern autogenic version of the theory considers the early Earth as having surface geochemistries that fed into the overall reactions as identified in Equations 2, 4, or 5 (Shock 1992; Russell and Arndt, 2005; Russell and Hall, 2006). Of these, what is the most

likely first reaction and thereby the most plausible pathway to life? Obviously, if hydrogen were to have been provided freely to emergent life, the putative first organism is hardly likely to have managed (or bothered) to develop such a complicated system of accessing it from water near the turbulent ocean's surface in the face of destructive ultraviolet radiation. That leaves reaction 4 as the likely energizer of life and source substrate for its structures. Reaction 5 could only supply energy. However, the generation of the intermediate radicals formyl ($-\text{HCO}$), methenyl ($-\text{CH}^+$), methylene ($-\text{CH}_2$), and methyl ($-\text{CH}_3$) in both reactions 4 and 5 are kinetically (Shock, 1990) and, in the early steps, thermodynamically (Maden, 2000) challenged, requiring both catalysis and energy for their resolution, (Figure 1). Yet these intermediates are themselves points of departure for biosynthesis (Maden, 2000). The kinetic barrier is higher for methane synthesis because the addition of a carbon-bearing radical ($-\text{COX}$) to a methyl ($-\text{CH}_3$) group to produce acetate ($\text{H}_3\text{C}-\text{COO}^-$) faces less of an obstacle than does the addition of a hydride (H^-) to make methane (CH_4) (Maden, 2000). Thus a pathway to acetate was probably the first to develop (Fuchs, 1989; Crabtree, 1997). Exploiting such a metabolism is the task of the acetogenic bacteria via what is known as the acetyl coenzyme-A pathway (Russell and Martin, 2004; see Chapter *Acetogens*):

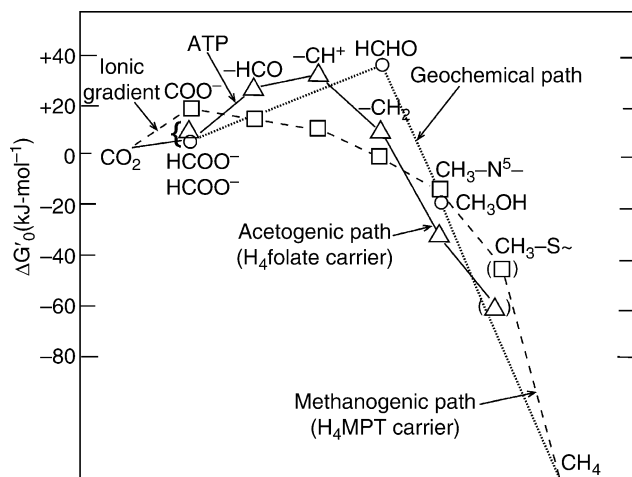


However, the path to methane production has much in common with the acetogenic pathway and could not have been far behind in evolutionary terms (Martin and Russell, 2007). Nevertheless, the microbes that excrete methane are so different from the acetogens that they have been assigned to a different domain of life, viz., the archaea (Woese et al., 1990, see Chapter *Archaea*). In this entry we will limit ourselves to an explication of the emergence of autotrophic acetogenesis and methanogenesis as biochemistry's first essay into processing chemical and electrochemical energy and generating cellular carbon. Given these qualifications, is there a likely context for where these reactions might have been promoted on the early Earth? To answer this we need to take a look at the conditions on our planet around 4 billion years ago.

Conditions at ~4 Ga

Presently taking up ~70% of the planet's surface, the Earth 4 billion years ago was probably completely covered with water. Radiogenic, gravitational, and tidal heat rendered any continental massifs differentiated by then so hot as to be too pliable and plastic to break the surface of the Hadean Ocean (Russell and Arndt, 2005). Subject to gales and hurricanes and given to high tides on a planet where the day lasted a few hours, this ocean was in a state of constant turmoil. There was certainly no place near the UV-saturated chaotic surface where organic molecules had the peace to organize into anything more complex than small ephemeral slicks. And the atmosphere was dominated by CO_2 , mainly from a myriad of highly active volcanoes derived from the Earth's mantle that had lost its native iron through rapid gravitation to the Earth's core (Wood et al., 1990; Delano, 2001; Boyet and Carlson, 2005; Wood and Halliday, 2005). Also exhaled from volcanoes, pyrophosphate rained into the ocean (Yamagata et al., 1991; Hagan et al., 2007). Any reduced carbon molecules were photo-oxidized to more CO_2 . The carbonic ocean was acidic (pH 5–6) (Macleod et al., 1994; Lowell and Keller, 2003; Halevy et al., 2007). Acidic too were (and are) the 400°C springs exhaling at ocean spreading centers and above mantle plumes (see Chapter *Hydrothermal Environments, Marine*). Because the Fe: H_2S ratio was $\gg 1$ in these very hot fluids emanating into the reservoir of that acidic ocean (Kump and Seyfried, 2005; Konhauser et al., 2009). These metals (e.g., Fe, Mn, Zn, Ni, Co, V, Mo, and W) are just the ones that contribute to metalloenzymes, particularly important to the autotrophic prokaryotes (Cody, 2004; Volbeda and Fontecilla-Camps 2006; McGlynn et al., 2009; see Chapter *Metalloenzymes*).

In contrast to the acidic hot springs, a large number of alkaline springs would have emanated at some distance from the oceanic spreading centers, similar in many ways to those found today at Lost City, 15 km from the



Origin of Life, Figure 1 Free-energy profiles of acetogenic and methanogenic reduction pathways (the reverse eastern branch of the acetyl coenzyme-A pathway, Ragsdale, 1997), compared to the geochemical pathway (cf. Seewald et al., 2006). Note how adenosine triphosphate (ATP) or an ionic gradient are required to drive early stages of CO_2 reduction in the acetogens and methanogens respectively. Without such a boost of energy the geochemical reduction of CO_2 to formaldehyde is minor and the abiotic production of acetate or methane is extremely slow. Life emerged as a processor to quicken the reactions which are exergonic overall. (Redrawn and recast from Maden, 2000.)

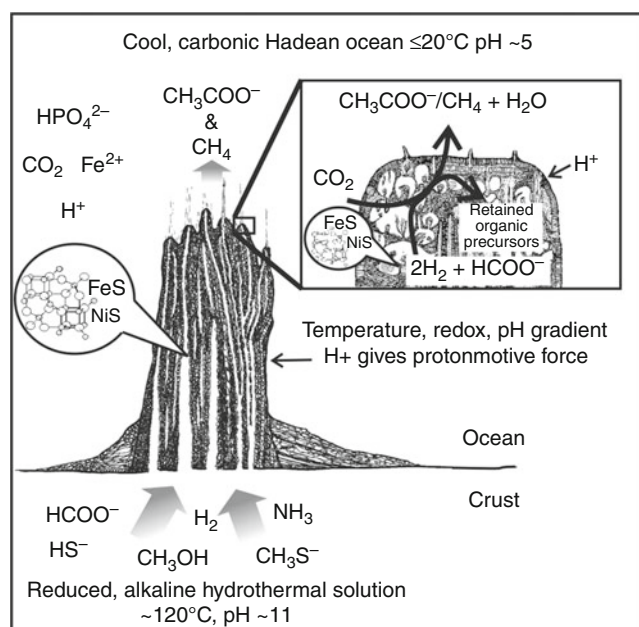
Mid-Atlantic Ridge (Kelley et al., 2001). The Lost City fluids exhale at moderate temperatures ($\leq 90^\circ\text{C}$), are alkaline ($\text{pH} \leq 11$) and contain up to 15 mmol/kg of dissolved hydrogen (Kelley et al., 2001; Martin et al., 2008; and see Macleod et al., 1994). They have exhaled without a break for at least 35,000 years (Früh-Green et al., 2003). Apart from hydrogen, other reduced entities likely delivered by moderate temperature alkaline springs on the early Earth were formate (HCOO^-), ammonia (NH_3), hydrosulfide (HS^-), methanol (CH_3OH), methane thiol (CH_3S^-), and methane (CH_4) (Schulte and Shock, 1995; Russell and Hall, 1997; Shock et al., 1998; McCollom and Seewald, 2003). Metals of potential biochemical interest carried in these moderate temperature fluids are molybdenum and tungsten. These solutes from alkaline springs – when juxtaposed with the carbon dioxide, transition metals and protons in the Hadean Ocean – provide all the basic nutrient and appropriate energy requirements for chemolithotrophic life as outlined below (Figure 2).

The theory of the onset of life at an alkaline hydrothermal vent

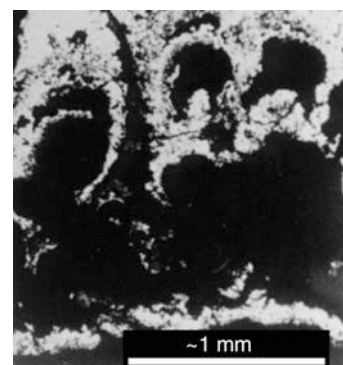
The hydrogenations of atmospheric carbon dioxide on the early Earth to either acetate or methane (Equations 4 and 5), as well as of the organic by-products of these reactions (and their intermediates) that comprise living cells, are faced with both thermodynamic and kinetic barriers (Figure 1). This is the theoretical challenge to an evolutionary theory of the emergence of life. To conquer kinetic barriers, there must be common naturally occurring

minerals that not only have the appropriate catalytic propensities but also are likely to be easily sequestered by organic molecules that they might evolve to protoenzymes. To override the thermodynamic barriers at the early stages of reduction (Figure 1), extra electrochemical energy is required to drive reactions 4 and 5 toward completion; energy supplied through chemiosmosis and the protonmotive force as alluded to below (Mitchell, 1967, 1979; Kell, 1988).

A plausible context where the necessary thermodynamic/electrochemical drive and natural catalysts comes into play is at a long-lasting hydrothermal vent. Here such alkaline solutions feed springs of constant temperature ($\leq 100^\circ\text{C}$) and $\text{pH} (\leq 11)$ that exhale at a distance from ocean-floor spreading centers into a somewhat acidic ancient ocean (Shock, 1992; Russell et al., 1994) (Figure 2). As this alkaline solution titrates with the metal-bearing acidulous ocean, porous and semi-permeable hydrothermal mounds form through precipitation of the metals as sulfides, carbonates, clays, and oxyhydroxides (green and white rust) on the ocean floor. Evidence for the porous nature of mound structure derives from modern examples (Marteinsson et al., 2001; Kelley et al., 2005) as well as from 350 million year old fossil representatives at mineral deposits in Ireland. These latter structures formed from similar titrations to those defined above, though where the pH of the two solutions was inverted (Figure 3) (Russell and Hall, 1997). Like these



Origin of Life, Figure 2 A submarine mound with the properties of a natural self-restoring flow reactor and fractionation column growing on the Hadean ocean floor above an alkaline spring. (After Russell and Martin, 2004.)

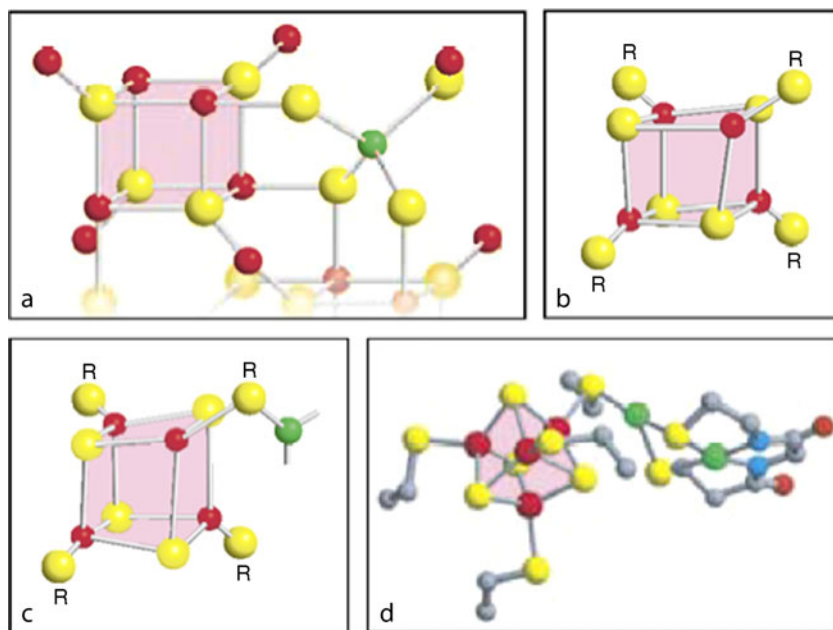


Origin of Life, Figure 3 Polished cross section of iron sulfide botryoids (bubbles or compartments) from the Tynagh mineral deposit in Ireland. These structures inspired the idea that the first compartments involved in the emergence of life were of comparable structure. It should be noted that the sulfide comprising what is now pyrite (FeS_2) in these 350 million year old submarine deposits was derived through bacterial sulfate reduction that took place in somewhat alkaline and saline seawater while the ferrous iron was contributed by exhaling acidic hydrothermal solutions. On the early Earth the sulfide would have been carried in the alkaline solution in some springs as abiotic bisulfide (HS^-), whereas the ferrous iron would have been contributed from the acidulous Hadean ocean. On mixing of the two solutions, nickel bearing mackinawite ($\text{Fe}(\text{Ni})\text{S}$) and greigite (Fe_3NiS_8) would have precipitated to contribute to inorganic barriers or membranes at their interface. (From Russell et al., 1994.)

morphologies, the growing surface of Hadean mounds probably comprised bubbles and compartments made of iron sulfide (FeS) and hydroxide gels with subordinate nickel, wherever the alkaline solutions were particularly sulfidic. Microcavities within initially colloidal iron monosulfide and hydroxide precipitates acted as the original chemically and electrochemically driven catalytic culture chambers for early metabolism and embryonic life (Russell et al., 1994). Thus the hydrothermal mound takes on the attributes of a continually renewed catalytic flow reactor and fractionation column, fed by hydrogen from within, and protons from without (Russell and Hall, 1997; Stone and Goldstein, 2004; Martin and Russell, 2007). Here, fresh catalytic iron sulfide and iron hydroxide nanocrystalline surfaces are continually offered as catalytic reaction sites between hydrogen (separated into electrons and protons) plus formate in the hydrothermal solution, and the carbon dioxide dissolved in the ancient ocean. The sulfides are mainly mackinawite (FeS) and greigite (Fe₃S₄) (see Chapter *Iron Sulfide Formation*) – sulfides which accommodate nickel, an effective and common catalyst. The atomic lattices of these sulfides have affinities with those of the active centers of enzymes involved in the acetyl coenzyme-A pathway mentioned above (Figure 4) (see Chapter *Metalloenzymes*).

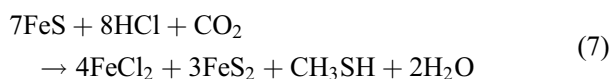
Mackinawite (FeS) comprises rhomboids of FeSMS (where M = Fe, Ni or Co and S = sulfur) whereas in the

rather more oxidized greigite [NiFe₅S₈] these rhombs associate to form Fe₄S₄ cuboids with a distal iron or nickel atom ligated through two sulfur atoms (Figure 4a). Further oxidation (sulfidation) to pyrite (FeS₂) is prevented by formaldehyde (HCHO) (Rickard et al., 2001), significant because a formyl (–CHO) group is an early intermediate produced along the acetyl coenzyme-A pathway (Figure 1) (see Chapter *Acetogens*). The mackinawite rhomboids have a similar topology to the active centers of Fe–Fe and Fe–Ni hydrogenases involved in acetogenesis and methanogenesis (McGlynn et al., 2009). The greigite structure (Figure 4a), by contrast, is topologically similar to that of the [4Fe–4S]^{0/+} centers of the ferredoxins (proteins that act as electron transfer agents) (Figure 4b); to the active center of carbon monoxide dehydrogenase (CODH) (e.g., [Fe₄S₄]S–Ni or [Fe₃NiS₄]Fe) (Figure 4c) where CO₂ is reduced to CO; and to the [Fe₄S₄]cys–Ni–cys₂–Ni] center of acetyl coenzyme-A synthase (ACS) where the acetyl group (CH₃CO[–]) is assembled (Figure 4d) (Vaughan and Ridout, 1971; Rickard and Luther, 2007; Dobbek et al., 2001; Drennan et al., 2001; Volbeda and Fontecilla-Camps, 2005a, b). (An animation of the mechanism by which an Fe–Ni hydrogenase, with the involvement of two 4Fe–4S centers and a 3Fe–4S center, splits hydrogen into 2 protons and 2 electrons, is beautifully illustrated at <http://www.kcl.ac.uk/ip/richardcammack/H2/animation/movie1.html>).



Origin of Life, Figure 4 The molecular structure of the mineral greigite (a) is very similar to that of the thiocubane unit (b) of the ferredoxin protein, as well as to the cuboidal complex (c) in the active site of the enzyme acetyl-CoA synthase/carbon monoxide dehydrogenase (shown in schematic form). The X-ray crystal structure (d) for the so-called A cluster of the latter confirms this similarity. Atoms are colored as follows: iron, red; sulfur, yellow; nickel, green; carbon, gray; nitrogen, blue. R signifies links through sulfur to the remainder of the protein. Part (d) is modified from Darnault et al. (2003) (and see Fontecilla-Camps and Ragsdale, 1999). (From Russell 2006, with permission.)

Is there experimental evidence to favor catalytic involvement of such iron-nickel sulfides or of nickel in the synthesis of acetate as we might expect from Equation 4 and in the conditions outlined above? No. The stumbling block appears to be the reduction of carbon dioxide to carbon monoxide in the analogy with a reversed “eastern branch” of the acetyl coenzyme-A pathway (see Ragsdale, 1997) (Figure 1). However, though yields are low, Heinen and Lauwers (1996) have reduced carbon dioxide to methyl sulfide (CH_3S^-) (in a short-cut through a reversed “western branch”) irreversibly from hydrogen sulfide and carbon dioxide in acidic conditions in the presence of ferrous sulfide where pyrite was apparently also produced at the liquid–vapor boundary:



Though a much simpler molecule, methylsulfide ($\text{CH}_3 \cdot \text{SH}$) is comparable to the thiol acetyl-coenzyme-A ($\text{Co-A} \cdot \text{SH}$) that expedites the leaving of activated acetate from ACS. In theory methane thiol activities would rise a 1000-fold when generated from H_2 and CO (rather than CO_2) in the crust or the hydrothermal mound (Schulte and Rogers, 2004).

The production of pyrite in the Heinen–Lauwers experiment is an expectation of the “Pyrite-Pulled Theory” of Wächtershäuser (1988a,b) and we now explore some experiments based on his hypothesis. Wächtershäuser, and his collaborator Claudia Huber, did synthesize the energy-rich methyl thioacetate ($\text{CH}_3 \cdot \text{COSCH}_3$) from the methane thiol (as produced by Heinen and Lauwers, 1996). However, apart from the thiol, they also used carbon monoxide gas rather than the much more stable dioxide (Huber and Wächtershäuser, 1997):



In a further departure from the biochemical pathway it should be noted that carbon monoxide is not stable in alkaline solution. However, a single carbonyl radical ($=\text{C}=\text{O}$) could perhaps be produced locally at each nickel site in greigite, dissociated from hydrothermal formate in the mound in a reaction driven to the right by the high CO_2 and H_2 partial pressures obtaining at an alkaline vent:

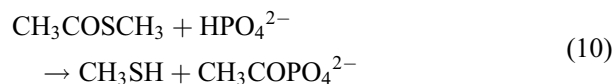


However, the Huber–Wächtershäuser reaction (8) is comparable to the addition of a carbonyl group to the methyl sulfide facilitated by acetyl coenzyme-A synthase (ACS) (Figure 4d) (Crabtree, 1997; Schink, 1997; Amend and Shock, 2001). The synthesis works best when catalyzed by NiS in acidic or alkaline conditions or with nickel sulfate in alkaline conditions – a result to be expected from knowledge of the biochemical reaction, the hydrothermal context, and also the role of nickel in the active site of ACS (Figure 4d) (Ragsdale, 2004). As we shall see, this energetic thioester ($\text{CH}_3 \cdot \text{COSCH}_3$) could be variously

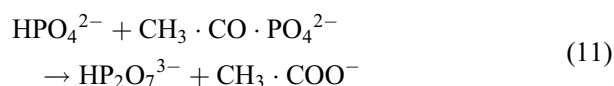
carboxylated, condensed, aminated, or used to make pyrophosphate. In the latter case, acetate is the waste product as it is of the acetogens. We turn now to energy requirements for further biosynthetic reactions that are also thermodynamically uphill. Where might this energy have come from at the emergence of life?

Pyrophosphate

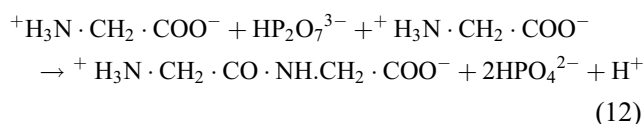
Life requires measures whereby energy can be stored. Now it employs various pyrophosphates for the job, notably adenosine triphosphate (ATP) produced either from substrate reactions (Gottschalk, 1985) or via the protonmotive force (Mitchell, 1967, 1979). ATP is too complex a molecule to be considered a geochemical product. It must be a product of early biological evolution. Thus we need to consider a precursor. One possibility is inorganic pyrophosphate (Westheimer, 1987; Baltscheffsky et al., 1999). And, seeing that acetate is one likely early product of emergent biochemistry, the first organic phosphate may have been acetyl phosphate ($\text{CH}_3 \cdot \text{CO} \cdot \text{PO}_4^{2-}$), perhaps produced by substrate phosphorylation of the acetyl thioester (from Equation 8) (de Duve, 1991):



Reacting this acetyl phosphate product with inorganic phosphate (HPO_4^{2-}) in the presence of Fe^{II} minerals, de Zwart et al. (2004) have generated pyrophosphate with a yield of 25% at $\sim 40^\circ\text{C}$,



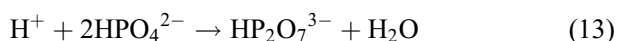
comparable to the way ATP is produced by the phosphorylation of adenosine diphosphate (ADP) in the acetyl coenzyme-A pathway. FeS was found to strongly retard hydrolysis of, and thus preserve, the pyrophosphate (de Zwart et al., 2004). Either the acetyl phosphate or the resulting pyrophosphate (PPi) (Equation 10) could have provided a proportion of the energy to drive hydrogenations at the formate-to-formyl step along the acetyl coenzyme-A pathway (for the acetogens in Figure 1) (Romero et al., 1991; Baltscheffsky et al., 1999). It might also have polymerized amino acids. For example, an overall condensation of glycine ($^+\text{H}_3\text{N} \cdot \text{CH}_2 \cdot \text{COO}^-$) with pyrophosphate on a mineral surface could theoretically produce diglycine:



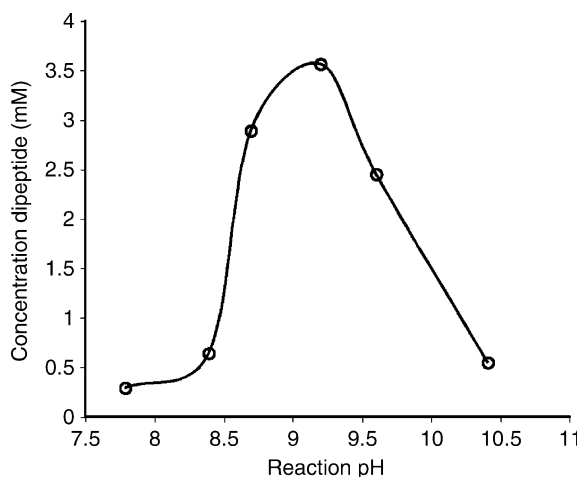
However, this amount of pyrophosphate is a small proportion of the energy required for biosynthesis (Amend and Shock, 2001; Martin and Russell, 2007). The extra energy

could only be supplied by protons, via the protonmotive force as detailed next.

At the origin of life the iron sulfide/hydroxide precipitates do more than merely provide catalytic surfaces. They also act as compartment walls, tending to trap organic products that become involved in further reactions, while permitting the transfer of anions across the membrane driven by proton flow (Russell et al., 1994). This natural protonmotive force, a consequence of the pH gradient operating across these inorganic membranes, is also the energy with the potential to drive pyrophosphate synthesis and thereby further hydrogenations and condensations (Mitchell, 1979). If the fluid inside the compartments has a pH of ~10 and the ocean has a pH of ~5, then the “protonic” potential approximates 300 mV; enough geochemical energy to have driven metabolism on early Earth (Russell et al., 2003). That the pH-dependent boundary between the mono- and di-phosphate fields intersects the (primarily) Eh-dependent iron sulfide fields demonstrates how “proticity” (a proton current) could have driven the condensation of inorganic phosphate (Pi) to pyrophosphate (PPi) (Figure 5). So in theory the proton gradient could have been simply coupled through the membrane to this dehydration or condensation of monophosphate on nanocrystalline surfaces within the inorganic membrane (Russell and Hall, 2006):



In biochemical energetics the process is known as “oxidative phosphorylation,” a reference to the fact that protons in living systems are initially driven to the outside of the membrane (to maintain charge balance with outflowing electrons to an acceptor) before returning to recharge the



Origin of Life, Figure 5 Dependence of the formation of phenylalanine dipeptide on pH (and see Huber and Wächtershäuser, 1998, 2003) for comparable results. (From Leman et al., 2004, supporting on-line material www.sciencemag.org/cgi/content/full/1101400/DC1, with permission.)

phosphate (Mitchell, 1967). The protonmotive force is the power behind metabolism and is therefore indispensable to life. So the fact that this ambient force is a feature of the redox and acid-to-alkaline interface separated by a semiconducting inorganic membrane suggests that the emergence of life was driven by similar energetic gradients as obtain in modern living systems. Nevertheless, the “ATPase” responsible for the conversion in today’s organisms is a complex rotating turbo-motor driven by a flow of protons from the membrane’s exterior (Elston et al., 1998). We assume that phosphate adhering to the mineral surfaces comprising the inorganic membrane was sequestered by short chains of amino acids (peptides) in a prototype of the phosphate-binding loop (the so-called P-loop) to form a pyrophosphatase as explained in a later section (Milner-White and Russell, 2005, 2008). This eventually evolved to the relatively simple, static H^+ -pyrophosphatase (Baltscheffsky et al., 1999; Hirono et al., 2007). That the amino acids and the subsequent heterochiral peptides required for such sequestering can be generated in an alkaline hydrothermal environment at moderate temperatures has been demonstrated by Wächtershäuser and his collaborators (Huber and Wächtershäuser, 1998; Huber et al., 2003).

Amino acid and peptide synthesis

Huber and Wächtershäuser (2003) have successfully aminated α -keto acids (like the pyruvate synthesized hydrothermally by Cody et al., 2000), but only in alkaline conditions. Amino acid yields are highest around pH 9, close to the pK_a of ammonium at 9.25 (Huber and Wächtershäuser, 2003). In these experiments $\text{Fe}(\text{OH})_2$ (“white rust”; a constituent, along with FeS , of the precipitate membranes) was shown to be just as effective a catalyst as iron monosulfide.

Similarly, alkaline (pH ~ 9) conditions are required in the condensation of amino acids to short peptides, though in these experiments an iron-nickel sulfide slurry was used as catalyst and carbon monoxide employed as condensing agent (Huber and Wächtershäuser, 1998, 2003). Of significance to the debate on the origin of chirality, lepto-tyrosine was found to racemize on condensation to give alternating L and D residues (Huber and Wächtershäuser, 1998, 2003). Leman et al. (2004) also condensed amino acids at similar alkaline pH, though using carbonyl sulfide in place of CO (Figure 5). The formation of peptides heralded a new stage in complexity at the emergence of life as we shall see. However, before this stage we must consider how organic molecules produced in these kinds of reactions might have been concentrated in the inorganic compartments comprising the mound.

Organic concentration in inorganic compartments

At the origin of life the iron-nickel precipitates did more than merely provide catalytic surfaces. They also acted as compartment walls, tending to trap organic products that became involved in further reactions. Yet they likely

permitted the transfer of anions across the membrane driven by the flow of protons from the acidulous Hadean Ocean through to the alkaline interior (Russell et al., 1994). And the protons had the potential to drive the condensation of phosphate to pyrophosphate; an example of ambient chemiosmosis. Evidence that such compartment walls could have existed in such an environment is afforded by what appear to be hollow but contiguous iron sulfide bubbles found at the 350 million year old Tynagh metal sulfide deposit in Ireland (Russell and Hall, 1997) (Figure 3). This ephemeral trapping of the rising hydrothermal solution, and its interfacing with the carbonic ocean across the inorganic membrane, also placed it at the mercy of physical as well as chemical gradients in an environment that could cause crowding of product (cf. Ellis, 2001).

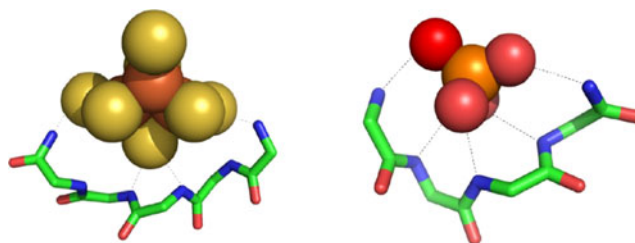
Concentration through thermal diffusion

Quantities of the organic intermediates and further products of geochemical acetogenesis and methanogenesis, such as amino acids and short peptides, will tend to be trapped in solution within the inorganic compartments comprising the hydrothermal mound. Noting that the hydrothermal mound could act as an affinity column, some of the organic anions will be concentrated on the compartment wall. However, Braun and Libchaber (2004) demonstrate *in vitro* how thermophoresis (thermal diffusion) accumulates charged polymers, with the potential to drive them to high concentrations in the cooler, stagnant portions of individual reaction microchambers. Such a process would be most effective on the outer margins of a hydrothermal mound where the temperature gradient was high and ranged between 50 and 100°C; just the kind of gradient to be expected in the envisaged mounds growing at off-ridge hydrothermal springs and seepages. Such “crowding” of newly generated macromolecules would decrease the activity of water and thence inhibit hydrolysis and thus further enhance the assembly of oligomeric structures and their subsequent folding into functional peptides. It would also quicken the rates of reaction along metabolic pathways; a prelude to an organic takeover from mineral catalysis and compartmentation.

An organic takeover

Protoenzymes and coenzymes

The consanguinity between the first structural and catalytic sulfide precipitates (Figure 4) and pyrophosphate with early enzymes (Russell and Hall, 1997; Beinert et al., 1997; Baymann et al., 2003; McGlynn et al., 2009) suggests that these structures were sequestered by the short heterochiral peptides similar to those produced in the Huber–Wächtershäuser experiments. Eck and Dayhoff (1966), pointing to the likely ubiquity and catalytic propensity of FeS on the early Earth, made the suggestion that the ferredoxins had the longest pedigree of all enzymes. Even before genetic control similar structures



Origin of Life, Figure 6 Ball and stick pictures showing early nests of short heterochiral peptides. The inorganic moieties are ligated through hydrogen bonds to the nitrogen atoms in the main chain of the polypeptides: *Left*, $[\text{Fe}_3\text{S}_4](\text{RS})_4^{3-}/4^-$ bound to a heterochiral peptide nest (cf. the ferredoxin in Figure 4b); *Right*, phosphate bound to a peptide nest (cf. the P- or phosphate loop). [carbon, green; oxygen, red; iron, rust; nitrogen blue; phosphorus, orange; sulfur, yellow]. (From Milner-White and Russell 2008, with permission.)

may have formed spontaneously. For example, Bonomi et al. (1985) demonstrate the synthesis in water of iron sulfide clusters ligated to organic sulfides (e.g., thioethanol) to produce $[\text{Fe}_4\text{S}_4](\text{RS})_4^{2-}$ anions in aqueous solution. Mildly hydrophobic short peptides are attracted to these anionic complexes by virtue of the δ^+ charge on the amino group nitrogen atoms along the backbone of the peptide chain ($\text{N}^{\delta+} \text{H} \cdot \text{CH}_2 \cdot \text{CO}^{\delta-} \cdot \text{N}^{\delta+} \text{H} \cdot \text{CH}_2 \cdot \text{CO}^{\delta-}$). These bend and bow to satisfy the negatively charged clusters (Figure 6). Cosseted in such “peptide nests,” the active centers (“eggs”) would be partially protected from dissolution, nucleation, and crystallization. Also, given the high surface-to-volume ratio of the clusters, they would remain highly active, yet spaced at distances appropriate for electron tunneling that allowed multi-electron bond-forming and breaking catalysis at high rates (Milner-White and Russell, 2005, 2008; Moser et al., 2006; Hengeveld and Fedonkin, 2007).

While these protoferredoxins were involved in electron transfer, phosphate anions were required for the storage of energy used in biosynthesis. These anions could also be sequestered by the backbones of short peptides. Indeed, a similar structure – the P-loop – is still a protein-motif of the phosphatases in prokaryotes to this day, the conformation now permitted by the one or more of the non-chiral amino acid glycine residues featured in the structure (Milner-White and Russell, 2008) (Figure 6). The “ready-made” aspect of these catalytically active molecular clusters is at one with the view that there was an evolutionary continuum between hydrothermal chemistry on the early Earth and emergent biochemistry (Russell and Hall, 1997; Martin and Russell, 2007). However, the short peptides may have had another important role as we see below.

Amyloid as the first organic membrane

Although FeS-bounded “cells” are plausible candidates for the hatcheries of life, they are flimsy, leaky, and poor

insulators. In the absence of lipids, which appear to be a late microbiological invention (Koga et al., 1998), peptidic membranes and walls to protocells offer several advantages to a metabolizing system before the advent of a genetic control. For example, sticky amyloid peptide would have made protective insulating layers and thus may have constituted the first organic membranes and cell walls (Zhang et al., 1993; Chernoff, 2004; Carny and Gazit, 2005; Liu et al., 2008). Some amyloid strands are like the nests described above but are flatter (Milner-White and Russell, 2008). Once concentrated by thermophoresis to the exclusion of inorganic anions, peptides will bond with each other. The δ^- of the carbonyl oxygens of one peptide ($\cdot\text{N}^{\delta+}\text{H}\cdot\text{CH}_2\cdot\text{CO}^{\delta-}\cdot\text{N}^{\delta+}\text{H}\cdot\text{CH}_2\cdot\text{CO}^{\delta-}\cdot$) will interact electrostatically with the δ^+ of the nitrogen atoms along the backbone of the next. Thus sheets of amyloid are formed that rapidly aggregate to blocks. Composites of this type of amyloid might coat the inside of the iron sulfide compartments, and eventually take over from it entirely. However, the amyloid is not limited to merely acting as an inert and impermeable membrane. In cases where the amyloid blocks are juxtaposed such that their charges are the same, then the natural repulsion between, for example the δ^- of the carbonyl groups will generate a natural channel that could allow transmission of particular ions, e.g., H^+ and K^+ (Milner-White and Russell, 2008).

Because this type of amyloid is composed of alpha-sheet, it could truss clusters such as the $[\text{Fe}_4\text{S}_4][\text{RS}]_4^{2-}$ anionic centers within its structure as stabilizers and electron transfer agents (Figure 6). It could also sequester the phosphates and pyrophosphates $[\text{HP}_2\text{O}_7^{3-}]$ required for early biosynthesis. Moreover, as glycine comprised much of the first peptide (Hennet et al., 2001), tetraglycines could sequester single atoms of Ni, Co and other transition elements as reduction- and group-transfer enzymes, as do the tetrapyrroles in acetogenesis and methanogenesis (Eschenmoser, 1988; Thauer, 1998; Milner-White and Russell, 2008).

The emergence of the code

We have seen how the mound plausibly acts as the organic-molecule factory, i.e., as a compartmentalized flow reactor. Acetate, amino acids, and short peptides are synthesized in it. And natural iron-nickel sulfide catalysts, and phosphate condensing agents, are sequestered by short peptides to produce protoenzymes and coenzymes. The inorganic, compartmentalizing membrane may then be superseded by a peptidic/amyloid membrane. The hydrothermal feed of hydrogen, formate, ammonia, and sulfide continues without interruption and meets the carbon dioxide, ferrous iron and phosphate in the acidic Hadean Ocean. Unlimited energy in the form of hydrothermal hydrogen and of protons and electron acceptors in the ocean guarantees continual synthesis of

organic molecules. What we do not have is replication – a reliable regulator, translation, or memory – we do not have genes.

Yet at first no special place in this decentralized “unintentional” world should be given to RNA beyond it being metabolically and homeostatically useful and thereby a surviving molecule (Hengeveld and Fedonkin, 2007). Prior to the involvement of nucleic acids in RNA and DNA, the nucleosides had metabolic roles, for example as pyrophosphate-bearing molecules like ATP and GTP (White, 1976). In time, and as polymers, they took over as information molecules, helping to regulate metabolic interactions, replicating and coding for propagation. But how were the ribose bases first assembled?

Although an unstable entity, once formed RNA would be less mutable when secured upon a mineral surface, especially in the presence of highly reduced fluids. Nevertheless, the synthesis of nucleic acids, composed of a phosphorylated ribose sugar attached to one of four possible bases, is a problem more daunting than that of the amino and carboxylic acids. As a start we note that pyrophosphate, introduced through volcanoes to the early oceans, remained in solution in the relatively acidic ocean, although some would have been precipitated as vivianite ($\text{Fe}_2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$) and as a condensed pyrophosphate on mixing with moderate temperature alkaline fluids at the hydrothermal mound (Rouse et al., 1988; Yamagata et al., 1991; Russell and Hall, 1997; de Zwart et al., 2004; Hagan et al., 2007). An explanation of how RNA bases were first synthesized in the mound still escapes us. One possibility is that they were constructed upon mineral surfaces in a sequence not very different from the way they are generated in the cell, for example with the simple entities; aspartate, glutamine, glycine, formyl phosphate, ammonia, and carbon dioxide (Martin and Russell, 2007). The synthesis of ribose phosphate, the particular pentose sugar attached to the bases, is also difficult to understand but may have been produced from phosphoenolpyruvate, itself a product of the acetyl coenzyme-A pathway (Martin and Russell, 2007). Anyway, apart from the easily synthesized adenine, only small concentrations of the rest were needed because, as we shall see, RNA polymers were the “moulds” that may have produced a myriad of peptide “casts.”

Emergence of the RNA world

In living cells amino acids are sequenced and polymerized in a process centered on the ribosome, composed essentially of RNA that ratchets along messenger RNA (Ban et al., 2000). Such a complex process must have evolved from a simpler system. Just what this was is the most challenging problem facing origin of life research. What follows is a speculative attempt to point to research directions. Neglecting their side chains, amino acids comprise a negatively charged carboxyl group ($-\text{COO}^-$) and a positively charged amino group ($-\text{NH}_3^+$). Amino acids

are polymerized by the loss of the constituents of water, OH^- from the carboxyl of one amino acid and of H^+ from the next. Though experiments show that condensation may be driven by carbon monoxide, carbonyl sulfide, or by drying (Ferris et al., 1996; Huber and Wächtershäuser, 1998; Huber et al., 2003; Leman et al., 2004), pyrophosphate phosphorylation is a possibility more in line with an alkaline hydrothermal origin though in conditions where water activity was low (see Baltscheffsky et al., 1999; Baaske et al., 2007) (Equation 18). While this may have happened in the first membranes, a polymerase would have been more efficient.

Woese (1967) suggested that genetic information was first transferred to protein sequences directly by selection through a somewhat indiscriminate “codon-amino acid pairing.” This relied upon the affinity between the shape and charge of the codon (a triplet of three nucleic acids) with the shape and charge of the amino acid and especially of its side chain (Woese, 1967, pp. 174–175). Thus, what is known as the peptidyl transferase reaction of an RNA molecule might have evolved via direct translation on a protoribosome. Although somewhat indiscriminate, triplets of side chains of RNA facilitating polymerization tended to favor particular amino acid side chains to the polymerizing amino acid sequence. Mellersh (1993) emphasized that RNA triplets offered a cleft-like (tridentate) conformation to attract amino acids in this way when adhering to a solid phase such as clay. The rows of RNA triplets then gripped and juxtaposed amino acid monomers in such a way as to offer the carboxyl group of one amino acid to the amino group of the next for bonding (Mellersh, 1993). At the same time the affinity between the clefts of RNA and the side chains of the amino acids would happen to effect a crude selection by codon-amino acid pairing as envisaged by Woese (1967). For example, clefts in which uracil was the central base would tend to attract only amino acids with hydrophobic side chains such as that of methionine ($\text{NH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{SH} \cdot \text{CH}_3 \cdot \text{COOH}$), while those in which adenine was central would show affinity for the hydrophilic charged, or polar, amino acids.

Concentrated by thermal diffusion, amino and nucleic acids occupied the cooler zones within the mineral and/or amyloid compartments (Braun and Libchaber, 2004). Where water activity was low, RNAs on the surface of nanocrystals may themselves have replicated by Watson–Crick hydrogen bonding whereby A (adenine) bonded to U (uracil) and G (guanine) bonded to C (cytosine) (Béland and Allen, 1994). The “protoribosome” would then have operated as a replicase, via the replication of triplets (the “triplicase” of Poole et al., 1999). The first cycle of replication provided an antisense codon, so that a GCC triplet (the codon for alanine and the most likely first triplet) produced the antisense codon “read” in the opposite direction as GGC (glycine) (Trifonov, 2000). Therefore, as touched on above, if the initial triplet coded for a hydrophilic amino acid (with adenine occupying the central position), then its opposite coded for a

hydrophobic residue (with uracil as the central base) (Béland and Allen, 1994; Konecny et al., 1995).

These triplets, or point (single base) mutations therefrom, tended to code for just those amino acids synthesized by Hennet et al. (1992) in their hydrothermal experiments, i.e., glycine (GGC), alanine (GCC), aspartate (GAC), and serine (UCC). More astonishing is the fact that these amino acids constitute the common sequence in the ancient ferredoxins (Eck and Dayhoff, 1966; Trifonov et al., 2001). This system of direct coding was relatively robust in that mutations not involving the central RNA monomer attracted amino acids with similar side chains and thereby similar properties.

However, during the organic takeover the protoribosome required another surface in place of a mineral such as a peptide sequence rich in positively charged side chains. Such a peptide would have attracted the phosphates of RNA that they might polymerize and still offer the triplet clefts. With amino groups on their side chains, lysine, arginine, and ornithine are equally useful in such a peptide. Mellersh and Wilkinson (2000) demonstrated that poly-adenosine, which includes the clefts AAA expected to have affinity for lysine, does stereoselectively bind L-lysine from dilute aqueous solution of L-amino acids. Moreover, about half the amount of L-arginine and L-ornithine also was found to bind with poly-adenosine. As adenosine was likely the most common of the nucleic acids, and lysine and probably ornithine can be made abiotically, then we have the makings of a feedback cycle that involves the transfer of information.

In this scenario the chance stereochemistry of the short RNA polymer determines the decision of whether it catalyzed the polymerization of D- or L-amino acids into peptides (Mellersh, 1993). To achieve a low energy state, as with mineral growth we might expect RNA to tend to lengthen while preserving either left or right chiralities, i.e., a favorable packing arrangement (Joyce et al., 1984). Were a monomer with the opposite stereochemistry to be added to a growing chain, growth would be thwarted (Sanders, 2003). That filter would have been sufficient to tip the holochirality scale since, despite the presence of a racemic mixture of amino acids in the microcavities only amino acids of the same α -carbon configuration (similar stereochemistry) would preferentially have ended up in peptides, to yield a population of distinctly handed peptides. The rest are eluted to the Hadean Ocean. Some of the retained peptides are eventually fed back in a hypercyclic manner to favor the syntheses of their “stereochemically appropriate” polymerizing template.

Eventually the more robust but less reactive DNA (deoxyribonucleic acid) molecules took over from RNA and thence survived. Braun and Libchaber (2004) demonstrate that secondary convection and thermophoresis, driven by temperature gradients within microcavities in a hydrothermal mound, could concentrate, elongate, and drive the replication of DNA. It remains to be seen if RNA can be elongated and replicated by the same process.

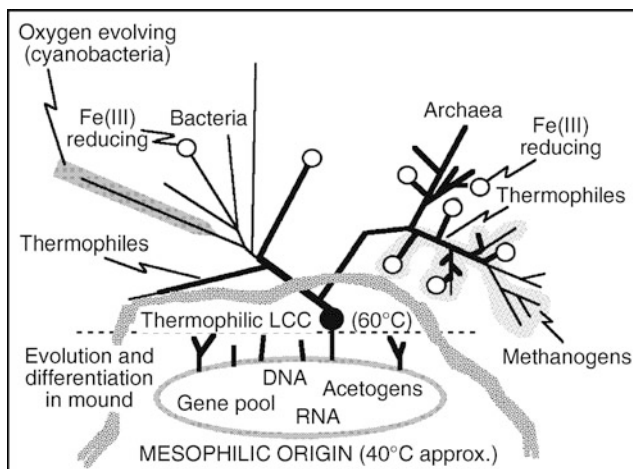
Of course, all this begs the question as to how RNA itself was first generated.

Replication by a natural PCR (polymerase chain reaction) in the mound

According to Koonin and Martin (2005) replication of genetic information and protein coding was probably expedited by populations of virus-like RNA molecules operating within the system of contiguous iron sulfide compartments. Braun and Libchaber (2004) demonstrated in vitro how laminar thermal convection can drive DNA replication and how thermophoresis (thermal diffusion) accumulates the charged polymers (Baaske et al., 2007; Koonin, 2007). These authors point out that such systematic evolution of ligands by exponential amplification is likely to have progressed naturally in porous hydrothermal mounds where the temperature gradient was high and ranged between 50°C and 100°C. This is just the kind of gradient to be expected in off-ridge hydrothermal springs and seepages like Lost City, an alkaline spring that has been operating at least for the last 35,000 years (Früh-Green et al., 2003). While convective temperature cycling encourages the polymerase chain reaction, thermophoresis concentrates DNA and other polymers in cool stagnant zones of the reaction chamber or pore space which act as a thermogravitational microcolumn (Baaske et al., 2007). These first “genes” would stabilize selection. Such “crowding” of newly generated macromolecules would further enhance the assembly of oligomeric structures and their subsequent folding into functional proteins and speed up the rates of reaction along metabolic pathways (Ellis, 2001; Spitzer and Poole, 2009).

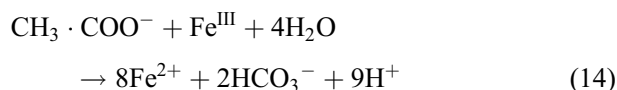
Early evolution and the common ancestral community

So far we have seen how geochemical processes may have evolved into biochemical processes. We now enter an evolutionary realm with an empirical base. The universal ancestor of life probably comprised a community of single-celled organisms still housed within its hydrothermal hatchery that possessed all of the attributes common to all bacteria and archaea: the genetic code, the ribosome; DNA; a supporting core and intermediate metabolism needed to supply the constituents of its reproduction; replication; compartmentation from the environment; redox chemistry; and the use of a proton gradient. This last common community (the LCC of Woese, 1998; Macalady and Banfield, 2003) existed in the hydrothermal mound at the dawn of the biochemical revolution where metal sulfide catalysts had been replaced by metalloenzymes, where genes and proteins were complexifying and diversifying into a myriad of functions, and where new pathways and cofactors were being invented to augment and substitute their mineral and RNA precursors (Eck and Dayhoff, 1966; Hall et al., 1971; Martin and Russell, 2003) (Figure 7).



Origin of Life, Figure 7 Evolutionary tree (after Woese et al., 1990; Stetter, 1996; Martin and Russell, 2003). The last common community (the LCC) occupied the mound within which life emerged. It is argued that the first acetogens are the root organisms of the *Bacterial domain* and that the first methanogens evolved into the *Archaeal domain* (the methanogens are found only amongst the archaea). Escape from the vent of these first microbes down into the contiguous ocean floor was only possible when genetically encoded lipid synthesis and cell wall synthesis had been achieved, and when autogenous formyl pterin synthesis as well as *ab initio* ion-pumping mechanisms had been developed for bioenergetic reasons relating to energy conservation efficiency, but in independent lineages of energetically sustainable and genetically replicating ensembles within the network of FeS-bearing compartments (Koonin and Martin, 2005; Russell and Hall, 2006; Martin and Russell, 2007). Here anaerobic microbial communities could be both autotrophic and heterotrophic, the autotrophs utilizing H_2 and formate as substrate while the first heterotrophs could use nitric oxide, elemental sulfur and ferric iron (Fe^{III}) as electron acceptors (Vargas et al., 1998; Philippot et al., 2007; Ducluzeau et al., 2009; see Chapters *Geobacter* and *Shewanella*).

Evolution in the mound extended beyond mere optimization of the acetate and methane reactions (Martin and Russell, 2003). A next step was adaptation that exploited the reduced carbon and energy to be found in waste products and dead cells:



Ferric iron (Fe^{3+}), as an electron acceptor, provided a means of such respiration (oxidative metabolism) (Liu et al., 1997; Vargas et al., 1998), as did elemental sulfur (Philippot et al., 2007) and nitric oxide and its close derivatives (Ducluzeau et al., 2009).

The last common ancestral community occupied the very hatchery in which life first emerged. The acetogenic proto-bacteria and the methanogenic proto-archaea emerged at moderate temperatures (40–50°C) (Brochier and Philippot, 2002) (Figures 1 and 7). A period of high

ambient temperature, caused either by a meteorite impact or a carbon dioxide greenhouse (Kasting and Ackerman, 1986; Kasting and Brown, 1998; Nisbet and Sleep, 2001) could explain why the last common community, residing in the ocean crust contiguous with the mound where life originated, may have been thermophilic, perhaps living at 50–60°C (Gaucher et al., 2003; Schwartzman and Lineweaver, 2004; see Chapter *Basalt (Glass, Endoliths)*).

Conclusions

The onset of life is assumed here to be an inevitable evolutionary outcome of the far-from-equilibrium conditions obtaining on a planet such as ours. These geophysical and geochemical states of the Earth 4 billion years ago are well-established. Life emerged as the ever-renewing catalyst that resolved the potential between carbon dioxide in the atmosphere/ocean and hydrogen in hydrothermal solution. Its hatchery may have been in iron-sulfide-bearing pores within a submarine mound precipitated above a hydrogen-rich alkaline spring. A natural protonmotive force, a consequence of a proton gradient across the inorganic walls of the pores spaces from the acid exterior to the alkaline interior, provided much of the energy for biosynthesis. The autotrophic acetyl coenzyme-A pathway was the first metabolic process to develop within this natural hydrothermal reactor. One variant of the autotrophic pathway produced acetate as waste, the other produced methane. This differentiation between the first microbial processors may have had a profound evolutionary consequence. The protoacetogens perhaps were the forerunners of the bacterial domain, while the protomethanogens evolved into the archaeal domain.

Acknowledgments

I thank Allan Hall and Isik Kanik for help and support. This entry was written at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration for Astrobiology: Exobiology and Evolutionary Biology and supported by NASA's Astrobiology Institute (Icy Worlds).

Bibliography

- Amend, J. P., and Shock, E. L., 2001. Energetics of overall metabolic reactions of thermophilic and hyperthermophilic Archaea and Bacteria. *FEMS Microbiological Reviews*, **25**, 175–243.
- Baaske, P., Weinert, F., Duhr, S., Lemke, K., Russell, M. J., and Braun, D., 2007. Extreme accumulation of nucleotides in simulated hydrothermal pore systems. *Proceedings of the National Academy of Science, USA*, **104**, 9346–9351.
- Baltscheffsky, M., Schultz, A., and Baltscheffsky, H., 1999. H⁺ - PPases: a tightly membrane-bound family. *FEBS Letters*, **457**, 527–533.
- Ban, N., Nissen, P., Hansen, J., Moore, P. B., and Steitz, T. A., 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science*, **289**, 905–920.
- Baymann, F., Lebrun, E., Brugna, M., Schoepp-Cothenet, B., Giudici-Orticoni, M. T., and Nitschke, W., 2003. The redox protein construction kit: pre-last universal common ancestor evolution of energy-conserving enzymes. *Philosophical Transactions of the Royal Society of London*, **358B**, 267–274.
- Beinert, H., Holm, R. H., and Münck, E., 1997. Iron-sulfur clusters: nature's modular, multipurpose structures. *Science*, **277**, 653–659.
- Béland, P., and Allen, T. F. H., 1994. The origin and evolution of the genetic code. *Journal of Theoretical Biology*, **170**, 359–365.
- Blankenship, R. E., 2002. *Molecular Mechanisms of Photosynthesis*. Oxford: Blackwell Science.
- Bonomi, F., Werth, M. T., and Kurtz, D. M., 1985. Assembly of Fe_nS_n (SR)²⁻ (n = 2, 4) in aqueous media from iron salts, thiols and sulfur, sulfide, thiosulfide plus rhodanase. *Inorganic Chemistry*, **24**, 4331–4335.
- Boyet, M., and Carlson, R. W., 2005. ¹⁴²Nd evidence for early (>4.53 Ga) global differentiation of the silicate Earth. *Science*, **309**, 576–581.
- Braun, D., and Libchaber, A., 2004. Thermal force approach to molecular evolution. *Physical Biology*, **1**, 1–8 (doi: 10.1088/1478-3967/1/1/P01).
- Brochier, C., and Philippe, H., 2002. A non-hyperthermophilic ancestor for bacteria. *Nature*, **417**, 244.
- Campbell, I. H., Griffiths, R. W., and Hill, R. I., 1989. Melting in an Archaean mantle plume: heads it's basalts, tails it's komatiites. *Nature*, **339**, 697–699.
- Carny, O., and Gazit, E., 2005. A model for the role of short self-assembled peptides in the very early stages of the origin of life. *FASEB Journal*, **19**, 1051–1055.
- Chernoff, Y. O., 2004. Amyloidogenic domains, prions and structural inheritance: rudiments of early life or recent acquisition? *Current Opinion in Chemical Biology*, **8**, 665–671.
- Cody, G. D., 2004. Transition metal sulfides and the origin of metabolism. *Annual Review of Earth and Planetary Science*, **32**, 569–599.
- Cody, G. D., Boctor, N. Z., Filley, T. R., Hazen, R. M., Scott, J. H., Sharma, A., and Yoder, H. S., 2000. Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science*, **289**, 1337–1340.
- Corliss, J. B., Baross, J. A., and Hoffman, S. E., 1981. An hypothesis concerning the relationship between submarine hot springs and the origin of life on Earth: In *Proceedings 26th International Geological Congress, Geology of Oceans Symposium*, Paris. July 7–17, 1980, *Oceanologica Acta*, N° SP, pp. 59–69.
- Crabtree, R. H., 1997. Where smokers rule. *Science*, **276**, 222.
- Daniel, R. M., and Danson, M. J., 1995. Did primitive microorganisms use nonheme iron proteins in place of NAD(P)? *Journal of Molecular Evolution*, **40**, 559–563.
- Darnault, C., Volbeda, A., Kim, E. J., Legrand, P., Vernède, X., Lindahl, P. A., and Fontecilla-Camps, J. C., 2003. Ni-Zn-[Fe₄-S₄] and Ni-Ni-[Fe₄-S₄] clusters in closed and open subunits of acetyl-CoA synthase/carbon monoxide dehydrogenase. *Nature Structural Biology*, **10**, 271–279.
- Deamer, D. W., 1985. Boundary structures are formed by organic components of the Murchison carbonaceous chondrite. *Nature*, **317**, 792–794.
- de Duve, C., 1991. *Blueprint for a Cell: The Nature and Origin of Life*. Burlington, NC: Neil Patterson Publishers.
- Delano, J. W., 2001. Redox history of the Earth's interior since 3900 Ma: implications for prebiotic molecules. *Origins of Life and Evolution of the Biosphere*, **31**, 311–341.
- de Zwart, I. I., Meade, S. J., and Pratt, A. J., 2004. Biomimetic phosphoryl transfer catalysed by iron(II)-mineral precipitates. *Geochimica et Cosmochimica Acta*, **68**, 4093–4098.
- Dobbeck, H., Svetlitchnyi, V., Gremer, L., Huber, R., and Meyer, O., 2001. Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. *Science*, **293**, 1281–1285.

- Drennan, C. L., Heo, J., Sintchak, M. D., Schreiter, E., and Ludden, P. W., 2001. Life on carbon monoxide: x-ray structure of *Rhodospirillum rubrum* Ni-Fe-S carbon monoxide dehydrogenase. *Proceedings of the National Academy of Science, USA*, **98**, 11973–11978.
- Ducluzeau, A.-L., van Lis, R., Duval, S., Schoepp-Cothenet, B., Russell, M. J., and Nitschke, W., 2009. Was nitric oxide the first deep electron sink? *Trends in Biochemical Science*, **34**, 9–15.
- Eck, R. V., and Dayhoff, M. O., 1966. Evolution of the structure of ferredoxin based on living relics of primitive amino acid sequences. *Science*, **152**, 363–366.
- Ehrenfreund, P., Rasmussen, S., Cleaves, J., and Chen, L., 2006. Experimentally tracing the key steps in the origin of life: the aromatic world. *Astrobiology*, **6**, 490–520.
- Einsle, O., Tezcan, F. A., Andrade, S. L. A., Schmid, B., Yoshida, M., Howard, J. B., and Rees, D. C., 2002. Nitrogenase MoFe-protein at 1.16 Å resolution: a central ligand in the FeMo-cofactor. *Science*, **297**, 1696–1700.
- Ellis, R. J., 2001. Macromolecular crowding: obvious but underappreciated. *Trends in Biochemical Science*, **26**, 597–604.
- Elston, T., Wang, H., and Oster, G., 1998. Energy transduction in ATP synthase. *Nature*, **391**, 510–513.
- Eschenmoser, A., 1988. Vitamin B12: experiments concerning the origin of its molecular structure. *Angewandte Chemie (International edition in English)*, **27**, 5–39.
- Ferris, J. P., Hill, A. R., Liu, R., and Orgel, L. E., 1996. Synthesis of long prebiotic oligomers on mineral surfaces. *Nature*, **381**, 59–61.
- Fontecilla-Camps, J. C., and Ragsdale, S. W., 1999. Nickel-iron-sulfur active sites: Hydrogenase and CO dehydrogenase. *Advances in Inorganic Chemistry*, **47**, 283–333.
- Früh-Green, G. L., Kelley, D. D., Bernasconi, S. M., et al., 2003. 30,000 years of hydrothermal activity at the Lost City vent field. *Science*, **301**, 495–498.
- Fuchs, G., 1989. Alternative pathways of autotrophic CO₂ fixation. In Schlegel, H. G., and Bowien, B. (eds.), *Autotrophic Bacteria*. Madison, WI: Science Tech Publishers, pp. 365–382.
- Gaucher, E. A., Thomson, J. M., Burgan, M., and Benner, S. A., 2003. Inferring the palaeoenvironment of ancient bacteria on the basis of resurrected proteins. *Nature*, **425**, 285–288.
- Goldschmidt, V. M., 1952. Geochemical aspects of the origin of complex organic molecules on Earth, as precursors to organic life. *New Biology*, **12**, 97–105.
- Gottschalk, G., 1985. *Bacterial Metabolism*. New York: Springer-Verlag.
- Haeckel, E., 1870. *Natürliche schöpfungsgeschichte: Gemeinverständliche wissenschaftliche vorträge über die entwickelungslehre im allgemeinen und diejenige von Darwin, Goethe und Lamarck im besonderen, über die anwendung derselben auf den ursprung des menschen und andere damit zusammenhängende grundfragen der naturwissenschaft*. Berlin: Georg Reimer.
- Haeckel, E., 1892. *The History of Creation: or the Development of the Earth and its Inhabitants by the Action of Natural Causes, a Popular Exposition of Evolution in General, and that of Darwin, Goethe and Lamark in Particular*, volume 1, Lankester, E. R. (trans), 4th edn. London, Kegan Paul, Trench, Trübner & Co.
- Hagan, W. J., Parker, A., Steuerwald, A., and Hathaway, M., 2007. Phosphate solubility and the cyanate-mediated synthesis of pyrophosphate. *Origins of Life and Evolution of the Biosphere*, **37**, 113–122.
- Halevy, I., Zuber, M. T., and Schrag, D. P., 2007. A sulfur dioxide climate feedback on early Mars. *Science*, **318**, 1903–1907.
- Hall, D. O., Cammack, R., and Rao, K. K., 1971. Role for ferredoxins in the origin of life and biological evolution. *Nature*, **233**, 136–138.
- Harvey, R. B., 1924. Enzymes of thermal algae. *Science*, **50**, 481–482.
- Heinen, W., and Lauwers, A. M., 1996. Organic sulfur compounds resulting from the interaction of iron sulfide, hydrogen sulfide and carbon dioxide in an anaerobic aqueous environment. *Origins of Life and Evolution of the Biosphere*, **26**, 131–150.
- Hengeveld, R., 2007. Two approaches to the study of the origin of life. *Acta Biotheoretica*, **55**, 97–131.
- Hengeveld, R., and Fedonkin, M. A., 2007. Bootstrapping the energy flow at the beginning of life. *Acta Biotheoretica*, **55**, 181–226.
- Hennet, R. J.-C., Holm, N. G., and Engel, M. H., 1992. Abiotic synthesis of amino acids under hydrothermal conditions and the origin of life: a perpetual phenomenon? *Naturwissenschaften*, **79**, 361–365.
- Hirono, M., Nakanishi, Y., and Maeshima, M., 2007. Essential amino acid residues in the central transmembrane domains and loops for energy coupling of *Streptomyces coelicolor* A3(2) H⁺-pyrophosphatase. *Biochimica et Biophysica Acta*, **1767**, 930–939.
- Holm, N. G., and Charlou, J. L., 2001. Initial indications of abiotic formation of hydrocarbons in the Rainbow ultramafic hydrothermal system, Mid-Atlantic Ridge. *Earth and Planetary Science Letters*, **191**, 1–8.
- Huber, C., and Wächtershäuser, G., 1997. Activated acetic acid by carbon fixation on (Fe,Ni)S under primordial conditions. *Science*, **276**, 245–247.
- Huber, C., and Wächtershäuser, G., 1998. Peptides by activation of amino acids on (Fe,Ni)S surfaces: implications for the origin of life. *Science*, **281**, 670–672.
- Huber, C., and Wächtershäuser, G., 2003. Primordial reductive amination revisited. *Tetrahedron Letters*, **44**, 1695–1697.
- Huber, C., Eisenreich, W., Hecht, S., and Wächtershäuser, G., 2003. A possible primordial peptide cycle. *Science*, **301**, 938–940.
- Joyce, G. F., Visser, G. M., van Boeckel, C. A. A., van Boom, J. H., Orgel, L. E., and Westrenen, J., 1984. Chiral selection in poly (C)-directed synthesis of oligo(G). *Nature*, **310**, 602–604.
- Kasting, J. F., and Ackerman, T. P., 1986. Climatic consequences of very high carbon dioxide levels in the earth's early atmosphere. *Science*, **234**, 1383–1385.
- Kasting, J. F., and Brown, L. L., 1998. The early atmosphere as a source of biogenic compounds. In Brack, A. (ed.), *The Molecular Origins of Life*. Cambridge: Cambridge University Press, pp. 35–56.
- Kell, D. B., 1988. Protonmotive energy-transducing mechanisms: some physical principles and experimental approaches. In Anthony, C. (ed.), *Bacterial Energy Transduction*. London, Academic, pp. 429–490.
- Kelley, D. S., Karson, J. A., Blackman, D. K., et al., 2001. An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature*, **412**, 145–149.
- Kelley, D. S., Karson, J. A., Früh-Green, G. L., et al., 2005. A serpentinite-hosted ecosystem: the lost city hydrothermal field. *Science*, **307**, 1428–1434.
- Koga, Y., Kyuragi, T., Nishihara, M., and Sone, N., 1998. Did archaeal and bacterial cells arise independently from noncellular precursors? A hypothesis stating that the advent of membrane phospholipid with enantiomeric glycerophosphate backbones caused the separation of the two lines of descent. *Journal of Molecular Evolution*, **46**, 54–63.
- Konecny, J., Schöniger, M., and Hofacker, G. L., 1995. Complementary coding conforms to the primeval comma-less code. *Journal of Theoretical Biology*, **173**, 263–270.
- Konhauser, K. O., Pecoits, E., Lalonde, S. V., Papineau, D., Nisbet, E. G., Barley, M. E., Arndt, N. T., Zahnle, K., and Kamber, B. S., 2009. Oceanic nickel depletion and a methanogen famine before the Great Oxidation Event. *Nature*, **458**, 750–753.

- Koonin, E. V., 2007. An RNA-making reactor for the origin of life. *Proceedings of the National Academy of Science, USA*, **104**, 9105–9106.
- Koonin, E. V., and Martin, W., 2005. On the origin of genomes and cells within inorganic compartments. *Trends in Genetics*, **21**, 647–654.
- Kump, L. R., and Seyfried, W. E., 2005. Hydrothermal Fe fluxes during the Precambrian: effect of low oceanic sulfate concentrations and low hydrostatic pressure on the composition of black smokers. *Earth and Planetary Science Letters*, **235**, 654–662.
- Leduc, S., 1911. *The Mechanism of Life*. London, Rebman Ltd.
- Leman, L., Orgel, L., and Ghadiri, M. R., 2004. Carbonyl sulfide-mediated prebiotic formation of peptides. *Science*, **306**, 283–286.
- Liu, S. V., Zhou, J., Zhang, C., Cole, D. R., Gajdarziska-Josifovska, M., and Phelps, T. J., 1997. Thermophilic Fe(III)-reducing bacteria from the deep subsurface: the evolutionary implications. *Science*, **277**, 1106–1109.
- Liu, P., Ni, R., Mehta, A. K., Childers, W. S., Lakdawala, A., Pingali, S. V., Thiyagarajan, P., and Lynn, D. G., 2008. Nucleobase-directed amyloid nanotube assembly. *Journal of the American Chemical Society*, **130**, 16867–16869.
- Lowell, R. P., and Keller, S. M., 2003. High-temperature seafloor hydrothermal circulation over geologic time and Archean banded iron formations. *Geophysical Research Letters*, **30**, 1391, doi:10.1029/2002GL016536.
- Macalady, J., and Banfield, J. F., 2003. Molecular geomicrobiology: genes and geochemical cycling. *Earth and Planetary Science Letters*, **209**, 1–17.
- MacLeod, G., McKeown, C., Hall, A. J., and Russell, M. J., 1994. Hydrothermal and oceanic pH conditions of possible relevance to the origin of life. *Origins of Life and Evolution of the Biosphere*, **24**, 19–41.
- Maden, B. E. H., 2000. Tetrahydrofolate and tetrahydromethanopterin compared: functionally distinct carriers in C1 metabolism. *Biochemical Journal*, **350**, 609–629.
- Marteinsson, V. T., Kristjánsson, J. K., Kristmannsdóttir, H., et al., 2001. Discovery of giant submarine smectite cones on the seafloor in Eyjafjörður, Northern Iceland, and a novel thermal microbial habitat. *Applied and Environmental Microbiology*, **67**, 827–833.
- Martin, W., and Russell, M. J., 2003. On the origin of cells: an hypothesis for the evolutionary transitions from abiotic geochemistry to chemoautotrophic prokaryotes, and from prokaryotes to nucleated cells. *Philosophical Transactions of the Royal Society of London*, **358B**, 27–85.
- Martin, W., and Russell, M. J., 2007. On the origin of biochemistry at an alkaline hydrothermal vent. *Philosophical Transactions of the Royal Society of London (Series B)*, **362**, 1887–1925.
- Martin, W., Baross, J., Kelley, D., and Russell, M. J., 2008. Hydrothermal vents and the origin of life: nature reviews. *Microbiology*, **6**, 805–814.
- Maurette, M., 1998. Carbonaceous micrometeorites and the origin of life. *Origins of Life and Evolution of the Biosphere*, **28**, 385–412.
- McCollom, T., and Seewald, J. S., 2003. Experimental constraints on the hydrothermal reactivity of organic acids and acid anions: I. Formic acid and formate. *Geochimica et Cosmochimica Acta*, **67**, 3625–3644.
- McGlynn, S. H., Mulder, D. W., Shepard, E. M., Broderick, J. B., and Peters, J. W., 2009. Hydrogenase cluster biosynthesis: organometallic chemistry nature's way. *Dalton Transactions*, doi: 10.1039/b821432h.
- Mellersh, A. R., 1993. A model for the prebiotic synthesis of peptides which throws light on the origin of the genetic code and the observed chirality of life. *Origins of Life and Evolution of the Biosphere*, **23**, 261–274.
- Mellersh, A. R., and Wilkinson, A.-S., 2000. RNA bound to a solid phase can select an amino acid and facilitate subsequent amide bond formation. *Origins of Life and Evolution of the Biosphere*, **30**, 3–7.
- Mereschkowsky, C., 1910. Theorie der zwei Plasmaarten als Grundlage der Symbiogenese, einer neuen Lehre von der Entstehung der Organismen. *Biol. Centralbl.*, **30**, 278–288, 289–303, 321–347, and 353–367.
- Miller, S. L., 1953. A production of amino acids under possible primitive Earth conditions. *Science*, **117**, 528–529.
- Miller, S. L., and Urey, H. C., 1959. Organic compound synthesis on the primitive Earth. *Science*, **130**, 245–251.
- Milner-White, E. J., and Russell, M. J., 2005. Nests as sites for phosphates and iron-sulfur thiolates in the first membranes. 3 to 6 residue anion-binding motifs. *Origins of Life and Evolution of the Biosphere*, **35**, 19–27.
- Milner-White, E. J., and Russell, M. J., 2008. Predicting the conformations of peptides and proteins in early evolution. *Biology Direct*, **3**, 3 doi:10.1186/1745-6150-3-3.
- Mitchell, P., 1967. Proton-translocation phosphorylation in mitochondria, chloroplasts and bacteria: natural fuel cells and solar cells. *FASEB*, **26**, 1370–1379.
- Mitchell, P., 1979. Keilin's respiratory chain concept and its chemiosmotic consequences. *Science*, **206**, 1148–1159.
- Moser, C. C., Page, C. C., and Dutton, P. L., 2006. Darwin at the molecular scale: selection and variance in electron tunnelling proteins including cytochrome *c* oxidase. *Philosophical Transactions of the Royal Society of London (Series B)*, **361**, 1295–1305.
- Nisbet, E. G., and Sleep, N. H., 2001. The habitat and nature of early life. *Nature*, **409**, 1083–1091.
- Philippot, P., Van Zuilen, M., Lepot, K., Thomazo, C., Farquhar, J., and Van Kranendonk, M. J., 2007. Early Archaeal microorganisms preferred elemental sulfur, not sulfate. *Science*, **317**, 1534–1537.
- Poole, A. M., Jeffares, D. C., and Penny, D., 1999. Early evolution: prokaryotes, the new kids on the block. *BioEssays*, **21**, 880–889.
- Ragsdale, S. W., 1997. The eastern and western branches of the Wood/Ljungdahl pathway: how the east and west were won. *BioFactors*, **6**, 3–11.
- Ragsdale, S. W., 2004. Life with carbon monoxide. *Critical Reviews in Biochemistry and Molecular Biology*, **39**, 165–195.
- Rickard, D., Butler, I. B., and Olroyd, A., 2001. A novel iron sulphide switch and its implications for earth and planetary science. *Earth and Planetary Science Letters*, **189**, 85–91.
- Rickard, D., and Luther, G. W., 2007. Chemistry of iron sulfides. *Chemical Reviews*, **107**, 514–562.
- Romero, I., Gómez-Priego, A., and Celis, H., 1991. A membrane-bound pyrophosphatase from respiratory membranes of *Rhodospirillum rubrum*. *Journal of General Microbiology*, **137**, 2611–2616.
- Rouse, R. C., Peacor, D. R., and Freed, R. L., 1988. Pyrophosphate groups in the structure of canaphite, $\text{Ca}_2\text{Na}_2\text{PO}_7 \cdot 4\text{H}_2\text{O}$: the first occurrence of a condensed phosphate mineral. *American Mineralogist*, **73**, 168–171.
- Russell, M. J., 2006. First life. *American Scientist*, **94**, 32–39.
- Russell, M. J., and Arndt, N. T., 2005. Geodynamic and metabolic cycles in the Hadean. *Biogeosciences*, **2**, 97–111.
- Russell, M. J., and Hall, A. J., 1997. The emergence of life from iron monosulphide bubbles at a submarine hydrothermal redox and pH front. *Journal of the Geological Society of London*, **154**, 377–402.
- Russell, M. J., and Hall, A. J., 2006. The onset and early evolution of life. In Kesler, S. E., and Ohmoto, H., (eds.), *Evolution of Early Earth's Atmosphere, Hydrosphere, and Biosphere—Constraints from Ore Deposits*. Geological Society of America, Memoir 198, pp. 1–32.

- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Science*, **29**, 358–363.
- Russell, M. J., Daniel, R. M., Hall, A. J., and Sherrington, J., 1994. A hydrothermally precipitated catalytic iron sulphide membrane as a first step toward life. *Journal of Molecular Evolution*, **39**, 231–243.
- Russell, M. J., Hall, A. J., and Mellersh, A. R., 2003. On the dissipation of thermal and chemical energies on the early Earth: The onsets of hydrothermal convection, chemiosmosis, genetically regulated metabolism and oxygenic photosynthesis. In Ikan, R., (ed.), *Natural and Laboratory-Simulated Thermal Geochemical Processes*. Dordrecht: Kluwer Academic Publishers pp. 325–388.
- Sanders, P. G. H., 2003. A toy model for the generation of homochirality during polymerization. *Origins of Life and Evolution of the Biosphere*, **33**, 575–587.
- Schink, B., 1997. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiology and Molecular Biology Reviews*, **61**, 262–280.
- Schulte, M. D., and Rogers, K. L., 2004. Thiols in hydrothermal solution: standard partial molar properties and their role in the organic geochemistry of hydrothermal environments. *Geochimica et Cosmochimica Acta*, **68**, 1087–1097.
- Schulte, M. D., and Shock, E. L., 1995. Thermodynamics of Strecker synthesis in hydrothermal systems. *Origins of Life and Evolution of the Biosphere*, **25**, 161–173.
- Schwartzman, D., and Lineweaver, C. H., 2004. The hyperthermophilic origin of life revisited. *Biochemical Society Transactions*, **32**, 168–171.
- Seewald, J. S., Zolotov, M. Y., and McCollom, T., 2006. Experimental investigation of single carbon compounds under hydrothermal conditions. *Geochimica et Cosmochimica Acta*, **70**, 446–460.
- Seyfried, W. M., and Bischoff, J. L., 1981. Experimental seawater-basalt interaction at 300°C, 500 bars: chemical exchange, secondary mineral formation, and implications for transport of heavy metals. *Geochimica et Cosmochimica Acta*, **45**, 135–147.
- Shock, E. L., 1990. Geochemical constraints on the origin of organic compounds in hydrothermal systems. *Origins of Life and Evolution of the Biosphere*, **20**, 331–367.
- Shock, E. L., 1992. Chemical environments of submarine hydrothermal systems. *Origins of Life and Evolution of the Biosphere*, **22**, 67–107.
- Shock, E. L., McCollom, T., and Schulte, M. D., 1998. The emergence of metabolism from within hydrothermal systems. In Wiegel, J., and Adams, M. W. W., (eds.), *Thermophiles: The Keys to Molecular Evolution and the Origin of Life*. Washington: Taylor and Francis, pp. 59–76.
- Spear, J. R., Walker, J. J., McCollom, T. M., and Pace, N. R., 2005. Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proceedings of the National Academy of Science, USA*, **102**, 2555–2560.
- Spitzer, J., and Poole, B., 2009. The Role of biomacromolecular crowding, ionic strength and physicochemical gradients in the complexities of life's emergence. *Microbiology and Molecular Biology Reviews*, **73**, 371–388.
- Stetter, K. O., 1996. Hyperthermophilic prokaryotes. *FEMS Microbiology Reviews*, **18**, 149–158.
- Stone, D. A., and Goldstein, R. E., 2004. Tubular precipitation and redox gradients on a bubbling template. *Proceedings of the National Academy of Science, USA*, **101**, 11537–11541.
- Svetlitchnyi, V., Dobbeck, H., Meyer-Klaucke, W., Meins, T., Thiele, B., Römer, P., Huber, R., and Meyer, O., 2004. A functional Ni-Ni-[4Fe4S] cluster in the monomeric acetyl-CoA synthase from *Carboxydotherrmus hydrogenoformans*. *Proceedings of the National Academy of Science, USA*, **101**, 446–451.
- Thauer, R. K., 1998. Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiology*, **144**, 2377–2406.
- Trifonov, E. N., 2000. Consensus temporal order of amino acids and evolution of the triplet code. *Gene*, **261**, 139–151.
- Trifonov, E. N., Kirzhner, A., Kirzhner, V. M., and Berezovsky, I. N., 2001. Distinct stages of protein evolution as suggested by protein sequence analysis. *Journal of Molecular Evolution*, **53**, 394–401.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L., and Lovley, D. R., 1998. Microbial evidence for Fe(III) reduction on early Earth. *Nature*, **395**, 65–67.
- Vaughan, D. J., and Ridout, M. S., 1971. Mössbauer studies of some sulfide minerals. *Journal of Inorganic Nuclear Chemistry*, **33**, 741–747.
- Volbeda, A., and Fontecilla-Camps, J. C., 2005a. Structure-function relationships of nickel-iron sites in hydrogenase and a comparison with the active sites of other nickel-iron enzymes. *Coordination Chemistry Reviews*, **249**, 1609–1619.
- Volbeda, A., and Fontecilla-Camps, J. C., 2005b. Structural bases for the catalytic mechanism of Ni-containing carbon monoxide dehydrogenases. *Dalton Transactions*, **2005**, 3443–3450.
- Volbeda, A., and Fontecilla-Camps, J. C., 2006. Catalytic nickel-iron-sulfur clusters: from minerals to enzymes. In Simmonneaux, G. (ed.), *Topics in Organometallic Chemistry*. Berlin: Springer, Vol. 17, pp. 57–82.
- Wächtershäuser, G., 1988a. Before enzymes and templates: theory of surface metabolism. *Microbiological Reviews*, **52**, 452–484.
- Wächtershäuser, G., 1988b. Pyrite formation, the first energy source for life: a hypothesis. *Systematic Applied Microbiology*, **10**, 207–210.
- Wächtershäuser, G., 2006. From volcanic origins of chemoautotrophic life to Bacteria, Archaea and Eukarya. *Philosophical Transactions of the Royal Society of London (Series B)*, **361**, 1787–1808.
- Westheimer, F. H., 1987. Why nature chose phosphates. *Science*, **235**, 1173–1178.
- White, H. B., 1976. Coenzymes as fossils of an earlier metabolic state. *Journal of Molecular Evolution*, **7**, 101–104.
- Wilde, S. A., Valley, J. W., Peck, W. H., and Graham, C. M., 2001. Evidence from detrital zircons for the existence of continental crust and oceans on the Earth 4.4 Gyr ago. *Nature*, **409**, 175–178.
- Williams, A. C., and Ramsden, D. B., 2007. Hydrogen symbioses in evolution and disease. *Quarterly Journal Medicine*, doi:10.1093/qjmed/hcm045.
- Woese, C. R., 1967. *The Genetic Code: The Molecular Basis for Genetic Expression*. New York: Harper & Row, Publishers.
- Woese, C. R., 1998. The universal ancestor. *Proceedings of the National Academy of Science, USA*, **95**, 6854–6859.
- Woese, C. R., Kandler, O., and Wheelis, M. L., 1990. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Science, USA*, **87**, 4576–4579.
- Wood, B. J., Bryndzia, L. T., and Johnson, K. E., 1990. Mantle oxidation state and its relation to tectonic environment and fluid speciation. *Science*, **248**, 337–345.
- Wood, B. J., and Halliday, A. N., 2005. Cooling of the Earth and core formation after the giant impact. *Nature*, **437**, 1345–1348.
- Yamagata, Y., Wanatabe, H., Saitoh, M., and Namba, T., 1991. Volcanic production of polyphosphates and its relevance to prebiotic evolution. *Nature*, **352**, 516–519.
- Zhang, S., Holmes, T., Lockshin, C., and Rich, A., 1993. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proceedings of the National Academy of Science, USA*, **90**, 3334–3338.

Cross-references

[Acetogens](#)
[Archaea](#)
[Asteroid and Comet Impacts](#)
[Basalt \(Glass, Endoliths\)](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Geobacter](#)
[Hydrogen](#)
[Hydrothermal Environments, Marine](#)
[Iron Sulfide Formation](#)
[Metalloenzymes](#)
[Methane, Origin](#)
[Nickel, Biology](#)
[Shewanella](#)

ORIGINS OF THE METAZOA

Daniel J. Jackson
 University of Göttingen, Göttingen, Germany

Synonyms

Animals

Definition

Metazoans – heterotrophic, multicellular organisms that possess eukaryotic cells.

Heterotrophs – organisms that must ingest organic carbon to support growth, unlike plants that are autotrophic and can use light to photosynthesize organic compounds.

Evolutionary concepts

A major event in the time line of Earth's history was the evolution of multicellular animal life, known scientifically as the Metazoa (Knoll and Carroll, 1999; Knoll et al., 2006; Budd, 2008; Love et al., 2009; Xiao and Laflamme, 2009). Metazoans possess eukaryotic cells which contain a distinct membrane bound nucleus, nuclear material (DNA) packaged into multiple linear chromosomes, membrane bound organelles, and modes of cellular division distinct from that of Bacteria and Archaea (Cooper and Hausman, 2009). Metazoans are primarily set apart from plants (also multicellular eukaryotes) in being heterotrophic, and in lacking a rigid cell wall (Campbell et al., 2008).

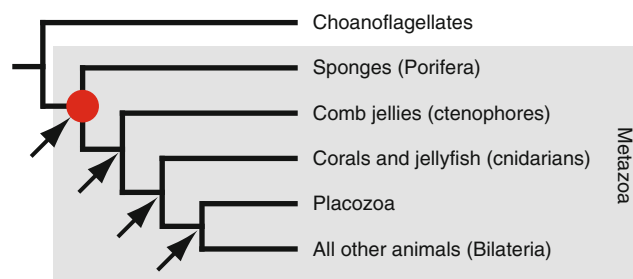
While all other forms of life (Bacteria, Archaea, and plants) display extensive genetic and metabolic diversity (Venter et al., 2004), the metazoans through 600+ million years of evolution (Peterson et al., 2008) display the greatest variety of locomotory habits, behaviors, and morphologies. Indeed, a fundamental feature of the Metazoa is the morphological diversity displayed by the 35+ (Collins and Valentine, 2001) major groups of animals that constitute the Kingdom Animalia (Carroll, 2001).

Metazoan morphological diversity is essentially generated by two means. Firstly through an ability of

a collection of cells to accurately communicate with one another during the process of development, and secondly for members of that collection of cells to adopt fates with distinct functions. This is most clearly conceptualized when considering the process of metazoan development. Typically, unicellular gametes (sperm and egg cells) fuse to form a zygote (a fertilized egg), which then divides to produce a multicelled embryo and eventually a reproductively mature adult (Gilbert, 2006). During this process, undifferentiated embryonic cells become progressively specified to a particular cell fate (e.g., muscle, neuron, skin, gonad, and eye). Without cell fate specification, tissues (e.g., muscle), organs (e.g., hearts), and organ systems (e.g., digestive system) and the variety of functions that these collections of cells fulfill would not develop (Arendt, 2008).

Evolutionary changes to the metazoan developmental program are ultimately responsible for generating diversity in the adult phenotype. This realization has fuelled the growth of the field of evolutionary-developmental biology (Carroll, 2005). Comparisons of the genes that regulate different metazoan developmental programs now holds the exciting promise of explaining on a molecular level how metazoan morphological diversity is generated (Martindale et al., 2003; Matus et al., 2006). Additionally, recent developments in high-throughput DNA sequencing technologies have seen the completion of a variety of metazoan genomes (King et al., 2008; Putnam et al., 2008; Chapman et al., 2010). These large-scale datasets allow exhaustive comparisons to be made between disparate animal phyla, and deepen our understanding of the genetic innovations that accompanied the diversification of metazoan life.

Related to these efforts are attempts to understand how metazoan life first arose. This requires that we accurately understand how metazoan and pre-metazoan groups are evolutionarily related, and calls on the field of



Origins of the Metazoa, Figure 1 A phylogenetic tree represents a hypothesis regarding the evolutionary relationships of a given set of organisms. Speciation events (indicated by an arrow) generate new lineages of organisms that in turn speciate to generate new "sublineages" thereby increasing diversity. This simplified phylogenetic tree summarizes a recently proposed hypothesis (Pick et al., 2010) that suggests the last common ancestor of the Metazoa (indicated by the red circle) was a sponge-like animal that was descended from a choanoflagellate-like organism.

P

PARASITISM

Parasitism is a form of symbiosis, in which one organism benefits but the other is harmed. See entry “*Symbiosis*” for further reading.

PEDOGENIC CARBONATES

Eric P. Verrecchia
Université de Lausanne, Lausanne, Switzerland

Synonyms

Secondary terrestrial carbonates; Soil carbonates; Vadose zone carbonates

Definition

Definition and mineralogy

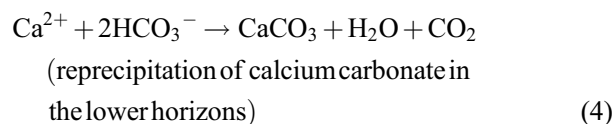
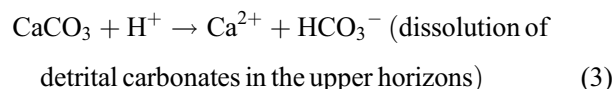
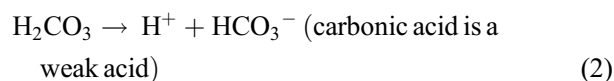
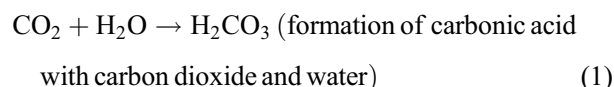
Pedogenic carbonates are authigenic (or secondary) carbonate deposits precipitated in soils (Lal et al., 2000). When found in the soil but directly inherited from parent materials, carbonates are defined as detrital or lithogenic (Kraimer et al., 2005). Pedogenic carbonates are characterized by a wide diversity of shapes, from secondary diffuse micritic (micron-size) crystals inside the soil matrix to coalescent hardened layers, sometimes capping whole landscapes. They are mainly composed of calcium carbonate as calcite and rarely as aragonite (Schaetzl and Anderson, 2005), of magnesian calcite, dolomite (Whipkey et al., 2002), and occasionally of iron-rich carbonate such as ferroan calcite, siderite, and ankerite (Table 1). Iron-rich carbonates are generally found in organic-rich soils and soils under the influence of

water-table fluctuations with active oxidation–reduction processes.

Origin

Carbonate physicochemical equilibria

Various processes are involved in pedogenic carbonate formation. The first significant process is related to the leaching of carbonate through the soil profile. In the upper horizons, inherited calcium carbonate is dissolved by carbon dioxide enriched waters and displaced in solution toward deeper horizons where it precipitates. This precipitation is supposed to occur if the concentration in Ca^{2+} increases, the pCO_2 decreases, or if soil solutions concentrate due to water loss (evaporation). In other words, all these processes are driven by the following general equations when lithogenic (detrital) carbonates are involved:

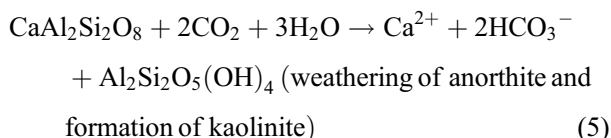


A second process is related to the transformation, in surficial environments, of primary minerals which can also lead to the precipitation of pedogenic carbonates. For

Pedogenic Carbonates, Table 1 Mineralogy and occurrence of pedogenic carbonates

Mineral	Formula	Occurrence of pedogenic carbonate
Calcite	CaCO ₃	All latitudes
Aragonite	CaCO ₃	Detected in some temperate carbonate soils
Magnesian calcite	[Ca _{0.90–0.95} , Mg _{0.05–0.10}]CO ₃	Detected in carbonate soils from evaporitic and tropical environments
Dolomite	(Ca, Mg)(CO ₃) ₂	Detected in carbonate soils from evaporitic or paralic environments
Ferroan calcite	[Ca(Fe ^{II})]CO ₃	In carbonate soils with an active iron cycle
Siderite	[Fe ^{II} (Ca, Mg)]CO ₃	In carbonate soils with active oxidation–reduction processes and organic matter
Ankerite	[Ca, Fe ^{II} (Mg, Mn)](CO ₃) ₂	In carbonate soils with active oxidation–reduction processes and organic matter

example, weathering of alkaline feldspar (plagioclase) by water enriched in carbon dioxide leads to the solubilization of calcium ions as well as hydrogen carbonate (i.e., bicarbonate) ions according to the equation:



The presence of calcium and hydrogen carbonate ions can lead to pedogenic carbonates, according to [equation \(4\)](#) if, for example, appropriate pH conditions are reached. Such pedogenic carbonates include carbonate cements, calcitic coatings associated with pores (mostly related to soil desiccation and/or root suction), and matrix impregnation by carbonate crystals of various sizes, from sparite (>20 µm) to micrite (<2 µm), during displacement of equilibria in soil solution (Deutz et al., 2001).

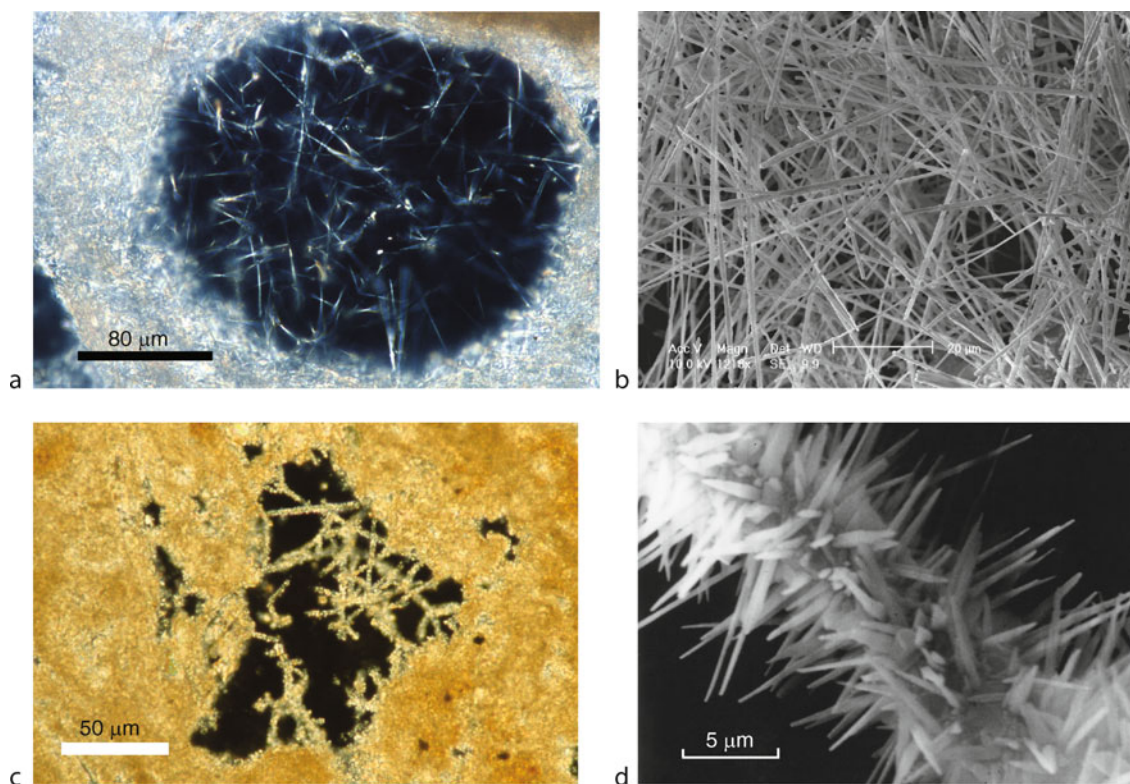
Although often presented as the engine of main pedogenic carbonate accumulations, these physicochemical equilibria remain fairly theoretical and do not incorporate all the complexities in the field. Processes in nature cannot be reduced to a simple set of equilibrium equations. First, kinetics of the carbonate system depend on many factors such as CO₂ diffusion as well as relationships between soil pores and water or the presence of specific ions such as Mg²⁺ or phosphorous, making the system much more complex than it seems. Second, the role of life is barely taken into account in such equations. Indeed, pedogenic carbonates are probably much more related to biomineralization processes (directly or indirectly) rather than simple displacement of physicochemical equilibria. To illustrate this point, just consider the distribution of the pCO₂ in a soil. The deeper the horizon, the higher the pCO₂. So why are pedogenic carbonates precipitating in the solum where the conditions seem to be the least favorable, that is, in the deeper horizons? The explanation can be found when the contribution of life is explored.

The role of microorganisms

Bacteria undoubtedly play an important role in the formation of pedogenic carbonates. It has been demonstrated that precipitation of CaCO₃ could be the reaction of heterotrophic bacterial guilds to an increase in soil

organic matter. Another example is given by the oxalate–carbonate pathway (Cailleau et al., 2005). In soils enriched in oxalate by plants and/or fungi, oxalotrophic bacteria can use oxalic acid and/or calcium oxalate as an electron donor and a carbon source. The result is a change in soil pH as well as the production of hydrogen carbonate ions. Calcium carbonate can precipitate when pH is high enough and the carbonate species and calcium are in appropriate concentrations, even in acidic environments such as tropical soils. Therefore, bacterial activity not only contributes to pedogenic carbonates but also changes chemical soil properties. In soils where nitrate and sulfate minerals are available, heterotrophic reducing bacteria can use these substrates as electron donors to produce CaCO₃ (as long as calcium is present). When iron is present with calcium sulfate, bacterial activity can lead to the concomitant precipitation of calcium carbonate and iron sulfide. This process assumes some soil redox conditions. Another example is given by methanogenic bacteria which can transform soluble organic matter from the soil solution percolating in deep horizons. This transformation occurs in the phreatic zone, under soils enriched in organic matter or peats, that is, in a distinctly anoxic environment. Secondary calcium carbonate appears as coatings in cracks of the parent rock. In conclusion, bacteria influence the kinetics and the reactions involved in pedogenic carbonates, even if their direct role in crystal nucleation is still difficult to prove beyond doubt. In any case, bacteria influence the composition of the soil solution and the biogeochemical cycles of major elements.

Fungi are also important factors in the formation of pedogenic carbonates. Their growth is related to the development of mycelia which can form a network in the soil. In their need to absorb Ca²⁺ for their metabolism, filamentous fungi developed the ability to concentrate these ions in their thallus as calcium oxalate or calcium carbonate salts, inside or outside their membrane. In addition, fungi excrete large quantities of organic acids, especially oxalic acid. Two common fungal features in pedogenic carbonates can be observed: calcified filaments and hyphae associated with needle fiber calcite (called NFC; [Figure 1a](#) and [b](#)). Calcified filaments are fungi covered with oxalate crystals ([Figure 1c](#) and [d](#)). The way in which fungi can form such accumulations of calcium oxalate around their cell walls is still unclear, although the excretion of oxalic acid must play a role in the formation of calcium oxalate



Pedogenic Carbonates, Figure 1 Microbial pedogenic carbonates. (a) needle fiber calcite inside a root channel, carbonate soil on chalk, Galilee, Israel. Cross-polarized light. Bar: 80 μm . (b) scanning electron microscope (SEM) view of needle fiber calcite released from a fungal sheath. Carbonate soil, Jura Mountains, Switzerland. (c) calcified filaments inside a carbonate soil pore, Galilee, Israel. Plane-polarized light. Bar: 50 μm . (d) SEM view of a calcified filament composed of polyhydrate calcium oxalate, carbonate soil, Galilee, Israel. Bar: 5 μm .

as well as in the dissolution of carbonate parent rocks. It is likely that part of the dissolution products diffuse inside the pores of the matrix and reprecipitate as secondary calcium carbonate a bit further away, contributing to soil matrix induration. Regarding the oxalates, they constitute a carbon and an electron source for oxalotrophic bacteria. As stipulated above, this oxalotrophic pathway also contributes to secondary calcium carbonate accumulations in soils. The origin of needle fiber calcite is also controversial (Verrecchia and Verrecchia, 1994; Cailleau et al., 2009b), often interpreted as a purely physicochemical product related to plant suction or other specific soil conditions. In fact, NFC seems to be mainly related to fungal activity, as it is always associated with fungal filaments (rhizomorphs). NFC crystallographic properties show that the crystals are complex, often polygenic, and undergo early diagenetic evolution, such as epitactic growth, inside the soil pores. Finally, some authors have observed nanorods associated with fungal filaments. These features are often considered either calcitic nanocrystals, a former stage of NFC, or mineralized bacteria. Recent research seems to demonstrate that these nanorods are decayed cell wall fibrous material from fungal rhizomorphs (Bindschedler et al., 2010). This material acts as a template for calcium carbonate

precipitation, leading to a mineralized mesh covering soil aggregates (Cailleau et al., 2009a). In conclusion, microorganisms play a prominent role in pedogenic carbonates by changing the biogeochemical conditions in microenvironments, by their direct influence on the precipitation of calcium carbonate, as well as their potential role to serve as a template for CaCO_3 crystal nucleation.

The role of the rhizosphere

The impact of the rhizosphere in the formation of pedogenic carbonates is related to two main processes: dissolution of carbonate parent material, leading to the redistribution of calcium carbonate in the soil matrix, and indirect and direct precipitation of carbonate due to root activity (Becze-Deak et al., 1997). The dissolution of carbonate parent rock by roots increases the soil porosity as well as the saturation index of the soil solution in carbonates. Although dissolution seems to be a destructive process, it is associated with a redistribution of soil carbonates leading to soil induration. Indeed, when organic acids are excreted by roots, dissolution of lithogenic carbonate occurs, increasing the concentration of dissolved carbonate inside the soil solution. On the other hand, when conditions are dry, roots need water: they pump

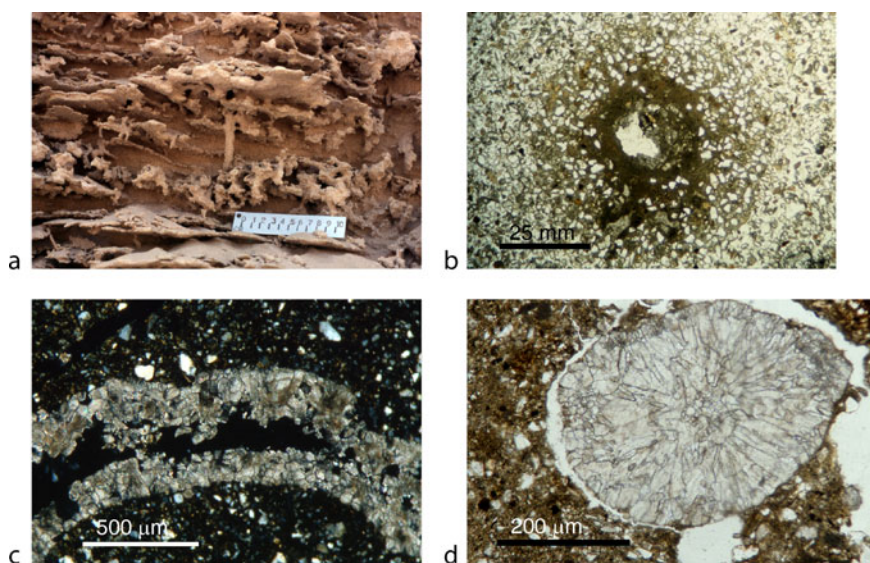
water from the soil solution, consequently increasing the concentration of calcium and carbonate ions in the solution around the root, leading to periporal precipitation of secondary calcium carbonate. Simple soil desiccation through the root channel may produce the same results. Such a cementation process, i.e., impregnation by secondary calcium carbonate of the soil matrix around root traces, is at the origin of rhizoliths (pedogenic carbonates having the shape of roots, Figure 2a and b). Not only do roots excrete large quantities of organic acids, protons, and even hydrogen carbonates, but they also absorb potassium, nitrogen, water, and calcium. Some plants have the ability to accumulate CaCO_3 in their vacuole in order to detoxify a possible excess of Ca^{2+} . These cellular biomineralizations are called calcified root cells (Jaillard et al., 1991; Figure 2c) and they are pedogenic carbonates directly related to the interaction of plant roots with soil solution. Finally, regarding pedogenic carbonate nodules found in present-day soils as well as paleosols, they probably result from the complex interactions between microorganisms, plant roots, and the composition of soil phases (soil solution and gas).

Animals may also play an important role in pedogenic carbonate formation inside the rhizosphere. For example, termites both mine carbonate from deep parent rock (bioturbation) and biomineralize the calcite in their body (Liu et al., 2007). Earthworms contribute to calcium carbonate accumulation in soils (Canti and Pearce, 2003). They excrete calcitic biospheroids in their casts (Figure 2d). The biospheroid formation occurs in the

calciferous cells of the epithelium that secrete amorphous calcium carbonate, crystallizing in the lumen of the esophageal pouch. These spheruliths of calcite are usually up to 2 mm in diameter with an average weight of 1.5 mg. They indicate the presence of earthworms. When, through diagenesis, obvious traces of earthworms are missing in buried paleosols, the presence of biospheroids provides good arguments for relative stability of the soil-sedimentary environment, i.e., no rapid burial and no drastic prolonged water stress.

Calcretes

The term “calcrete” should only be used for complex terrestrial carbonate accumulations, often hardened and showing specific features such as nodular, lamellar, massive, and laminar horizons. However, calcretes are characterized microscopically by many features similar and/or related to pedogenic carbonates (root traces, calcified root cells, needle fiber calcite, matrix nodulization, calcitic coatings), which explains why the term has been inappropriately used as an equivalent of pedogenic carbonates in many studies (Nash and McLaren, 2007). The origin of vadose calcretes is still controversial. Soil scientists consider calcretes as soils: calcium carbonate is leached from the upper horizon toward the lower horizon, until CaCO_3 infills all the pores leading to a plugged horizon. Sedimentologists interpret calcrete as complex (polygenic) sedimentary formations where pedogenesis, biogenic processes, and sedimentation episodes interact. In this case,



Pedogenic Carbonates, Figure 2 Pedogenic carbonates and the rhizosphere: (a) vertical root traces inside a paleosol developed on eolianites, Israeli Mediterranean coast. Scale in centimeters. (b) cross section of a root. From *center* to *periphery*: hole left by the decayed root, accumulation of dense pedogenic micrite, transition to secondary accumulation of pedogenic micrite diffusing toward the sandy matrix, carbonate-depleted zone, and sandy host rock. Plane-polarized light. Bar: 25 mm. (c) longitudinal section of a calcified root. The cells form a hollow tube. Loessic soil, Bk2b horizon, Wallertheim, Germany. Cross-polarized light. Bar: 500 μm . (d) calcitic biospheroid formed by earthworms. Carbonate alluvial soil, Rhone River terrasse, Holocene, France. Plane polarized light. Bar: 200 μm .

calcretes record a long history of landscape evolution whereas, in the soil model, calcretes are only related to a single process of dissolution and reprecipitation in a relatively stable environment. For example, for soil scientists, mature calcretes are characterized by a laminar horizon resulting from cementation inside a carbonate-rich perched water table, in which the soil solution is unable to percolate further down because it is stopped by the plugged horizon. This horizon is therefore posterior to the upper soft soil. Sedimentologists consider this laminar horizon as a surficial deposit (in contact with the atmosphere) related to cyanobacterial activity and sedimentary processes (weak runoff, eolian dust incorporation). The upper soft soil, where present, is therefore posterior to the laminar crust.

Pedogenic carbonates as a carbon sink?

The question of a potential role of pedogenic carbonates as a carbon sink is often asked (Lal et al., 2000; Landi et al., 2003; Cailleau et al., 2004). Researchers consider that the residence time of carbon in pedogenic carbonates is 3–4 orders of magnitude higher than that of soil organic matter. In order to answer this question, the origin of calcium (Ca) has to be considered (Van der Hoven and Quade, 2002). There are two possible origins for the Ca in pedogenic carbonates. Ca can be inherited from weathering of preexisting CaCO_3 (e.g., dust or parent rock limestone). Recrystallization of pedogenic CaCO_3 allows atmospheric CO_2 catchment but, in contrast, fossil CO_2 has been released during weathering, leading to a zero net balance for the carbon cycle. Thus, this process has to be considered only a molecular CO_2 substitution from fossil limestone to pedogenic carbonates. A second possible origin of Ca is when released from the weathering of noncarbonate minerals (see e.g., Equation 5) and precipitated as CaCO_3 . In this case, the catchment of atmospheric CO_2 by the released Ca into authigenic pedogenic carbonates acts as a carbon sink.

Summary

Pedogenic carbonates are authigenic deposits found in soils. Their origin is related to biological activity involving bacteria, fungi, roots, and soil animals, as well as complex kinetics between minerals and soil solution. Consequently, their biogeochemical signature as a proxy should be used with caution to reconstruct paleoenvironments (Quast et al., 2006). On the other hand, they can constitute a carbon sink if the cations originate from noncarbonate substratum.

Bibliography

- Becze-Deak, J., Langohr, R., and Verrecchia, E. P., 1997. Small scale secondary CaCO_3 accumulations in selected sections of the European loess belt. Morphological forms and potential for paleoenvironmental reconstruction. *Geoderma*, **76**, 221–252.
- Bindschedler, S., Millière, L., Cailleau, G., Job, D., and Verrecchia, E. P., 2010. Calcitic nanofibres in soils and caves: a putative

fungus contribution to carbonatogenesis. In Pedley, H. M., and Rogerson, M. (eds.), *Tufas and Speleothems: Unravelling the Microbial and Physical Controls*. London: Geological Society, Special Publications, 336.

- Canti, M. G., and Pearce, T. G., 2003. Morphology and dynamics of calcium carbonate granules produced by different earthworm species. *Pedobiologia*, **47**, 511–521.
- Cailleau, G., Braissant, O., Dupraz, C., Aragno, M., and Verrecchia, E. P., 2005. Biologically induced accumulations of CaCO_3 in orthox soils of Biga, Ivory Coast. *Catena*, **59**, 1–17.
- Cailleau, G., Braissant, O., and Verrecchia, E. P., 2004. Biomineralization in plants as a long term carbon sink. *Naturwissenschaften*, **91**, 191–194.
- Cailleau, G., Dadras, M., Abolhassani-Dadras, S., Braissant, O., and Verrecchia, E. P., 2009a. Evidence for an organic origin of pedogenic nanofibres. *Journal of Crystal Growth*, **311**, 2490–2495.
- Cailleau, G., Verrecchia, E. P., Braissant, O., and Emmanuel, L., 2009b. The biogenic origin of needle fibre calcite. *Sedimentology*, **56**, 1858–1875.
- Deutz, P., Montañez, I. P., Monger, H. C., and Morrison, J., 2001. Morphology and isotope heterogeneity of Late Quaternary pedogenic carbonates: implications for paleosol carbonates as paleoenvironmental proxies. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **166**, 293–317.
- Jaillard, B., Guyon, A., and Maurin, A. F., 1991. Structure and composition of calcified roots, and their identification in calcareous soils. *Geoderma*, **50**, 197–210.
- Kraimer, R. A., Monger, H. C., and Steiner, R. L., 2005. Mineralogical distinctions of carbonates in desert soils. *Soil Science Society of America Journal*, **69**, 1773–1781.
- Lal, R., Kimble, J. M., Eswaran, H., and Stewart, B. A., 2000. *Global Climate Change and Pedogenic Carbonates*. Boca Raton: Lewis Publishers.
- Landi, A., Mermut, A. R., and Anderson, D. W., 2003. Origin and rate of pedogenic carbonate accumulation in Saskatchewan soils, Canada. *Geoderma*, **117**, 143–156.
- Liu, X., Monger, H. C., and Whitford, W. G., 2007. Calcium carbonate in termite galleries – biomineralization or upward transport? *Biogeochemistry*, **82**, 241–250.
- Nash, D. J., and McLaren, S. J., 2007. *Geochemical Sediments and Landscapes*. Oxford: Blackwell Publishing.
- Quast, A., Hoefs, J., and Paul, J., 2006. Pedogenic carbonates as a proxy for palaeo- CO_2 in the Palaeozoic atmosphere. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **242**, 110–125.
- Schaetzl, R., and Anderson, S., 2005. *Soils – Genesis and Geomorphology*. Cambridge: Cambridge University Press.
- Van der Hoven, S. J., and Quade, J., 2002. Tracing spatial and temporal variations in the sources of calcium in pedogenic carbonates in a semiarid environment. *Geoderma*, **108**, 259–276.
- Verrecchia, E. P., and Verrecchia, K. E., 1994. Needle-fibre calcite: a critical review and a proposed classification. *Journal of Sedimentary Research*, **A64**, 650–664.
- Whipkey, C. E., Capo, R. C., Hsieh, J. C. C., and Chadwick, O. A., 2002. Development of magnesian carbonates in Quaternary soils on the island of Hawaii. *Journal of Sedimentary Research*, **72**, 158–165.

Cross-references

Calcite Precipitation, Microbially Induced
Carbon Cycle
Carbonates
Fungi and Lichens
Microbial Degradation
Soils

PERMAFROST MICROBIOLOGY

David A. Gilichinsky, Elizaveta M. Rivkina
Russian Academy of Sciences, Pushchino, Russia

Synonyms

Microbiology of perennially frozen ground

Definition

Permafrost is the ground (soil or rock) beneath the surface of the earth that remains at or below 0°C for at least two consecutive years. Permafrost is defined on the basis of temperature. It is not necessarily frozen; moisture in the ice form may or may not be present (van Everdingen, 1998). For microbiology is important the usage of permafrost that requires also the presence the moisture in the ice form. In other cases the following terms are used: (a) overcooled, because the freezing point of the included water may be depressed several degrees below 0°C; (b) frosty, if the moisture content is close to zero (Basic foundation of geocryology, 1959).

Introduction: Why permafrost?

Because the occurrence of a viable Cenozoic generation of microorganisms within the permafrost is intriguing as a window into microbial life as it was before the impact of humans (Tiedje et al., 1994) and as a storage of the ancient genetic pool – the preexisting life, which hypothetically has long since vanished on the Earth surface.

Because the answer for fundamental question “how long the life might be preserved,” could be found within permafrost – natural observatory of viable systems over geological time, where paleoorganisms realize unknown possibilities and unique mechanisms of physiological and biochemical adaptation that allow them to maintain viability for very long cold periods.

Because permafrost represents a wide range of possible habitats and inhabitants on the planets without obvious surface ice such as in Mars, and if life existed during the early stages of development on this Earth-like planet, then, similar to the Earth, remnants of primitive forms may be found within frozen material.

Because permafrost thawing due to anthropogenic or natural processes exposes ancient life to modern ecosystems and ancient biota became easily involved anew in present-day ecological processes.

Only these problems demonstrate the significance and perspectives of permafrost microbiology.

Cryobiosphere

The terrestrial Cryosphere consists of two parts: Glaciosphere (snow and ice) and Cryolithosphere, the frozen part of the Lithosphere, containing long-term and seasonally cryogenic formations with ancient and recent viable microorganisms, respectively. The comparison of

number of the cells survived over geological time at temperature above 0°C in halite (Lipman, 1928; Dombrowski, 1963; Norton et al., 1993; Denner et al., 1994; Vreeland et al., 2000; Stan-Lotter et al., 2003) and amber (Galippe, 1920; Cano and Borucki, 1995; Lambert et al., 1998; Greenblatt et al., 1999) and at temperature below 0°C in Cryosphere, shows that the latter is the most spread and rich terrestrial depository of ancient viable microorganisms. Cryosphere that is found between the upper atmospheric layers (Wainwright et al., 2003), including overcooled cloud droplets (Sattler et al., 2001) and the bottom of Antarctic Ice Sheet, represents a significant part of Biosphere, the Cryobiosphere, where life is confined in ice and permafrost.

Icy world

Biota of Greenland Ice Sheet, 120,000-years old, and Antarctic Ice Sheet, ~400 Kyr have been widely studied to the depth more than 3 km (Abyzov, 1993; Kapitsa et al., 1996; Priscu et al., 1998; Karl et al., 1999; Petit et al., 1999; Skidmore et al., 2000; Deming, 2002; Miteva et al., 2004; Miteva and Brenchley, 2005). The age of the oldest glacial ice as well as immured bacteria are still under discussion: >500,000 years at Guliya ice cap on Tibetan Plateau (Thompson et al., 1997; Christner et al., 2003), ~2 million years at the bottom of Vostok ice core (Salamatin et al., 2004) or even ~8.1 million years (Sudgen et al., 1996; Bidle et al., 2007) in Beacon Valley, Antarctica. Microbial population and traces of life in ice sheets are interpreted to be the Earth's most representative models of extraterrestrial icy habitats (like Jupiter's Ice-covered moon Europa, or Enceladus – Icy moon in Saturn system).

The initial number of mostly air-borne microorganisms isolated from snow and seasonally ice covers is not great (10^2 cells per ml) and of the same order as viable cells within the ancient ice sheets core's. Such fact could be interpreted as absence of reduction of microbial population once immured in ice for thousand years and could be explained, for example, by the near zero background radiation in the ice. The ultra small ($<\mu^3$) cells dominated in its population (Miteva and Brenchley, 2005) and the number of viable cells in ice cores increases sharply in the presence of dust particles (Abyzov, 1993). Unlikely they occupying the narrow liquid veins (Price et al., 2004) only, because data from Vostok cores showed that the most abundant are the upper layers in spite of extremely low temperatures (-50°C). Distribution of cells along the hole indicates that the populous of viable cells in Antarctic Ice Sheet decrease with the increasing age of embedding ice.

Greenland ice cores also indicate a good preservation of relatively young, 2–4 Kyr, genomic DNA (Willerslev et al., 1999), as well as bacterial and plant viruses varied in age 0.5–100,000 years (Castello et al., 2005). Therefore, freezing prompts viral endurance. Unfortunately, this relates to human viruses too – in Arctic, RNA of influenza

A is preserved in high concentrations in lake's seasonally ice (Shoham, 2005; Zhang et al., 2006).

The last review of icy Biosphere made by Priscu and Christner (2004) and the recent level of the problem are shown in the book *Life in Ancient Ice* Castello and Rogers (2005).

Cryolithosphere

However, the most inhabited part of Cryosphere, represented by Cryolithosphere or permafrost, underlie about a quarter of the Earth's land surface. Permafrost is the integral part of Arctic (the northern reaches of North America and Eurasia) and ice-free regions of Antarctica and Greenland. The so-called, Alpine permafrost is wide spread in the high mountains of all continents: Europe, western China, and both Americas, as well as offshore permafrost occupied the polar shelf surrounding Arctic and, probably, Antarctica.

This considerable frozen mass, up to several hundreds of meters deep, where microorganisms are adsorbed on organic or mineral particles, harbors a numerous (up to dozen millions cells per 1 g of dry soil – gdw) of various ecological and morphological viable microbial groups survived under permafrost conditions since the time of its formation. They have been isolated from frozen cores with permanently constant ground temperatures (–1)–(–2)°C near the south border of permafrost in Siberia and down to lowest in Arctic: –17°C, on 80°N, Ellesmere Island – the most northern latitude (Steven et al., 2007) and in Antarctica: –27°C on 78°S, Dry Valleys – the most southern latitude (Gilichinsky et al., 2007a). Viable microorganisms were found down to 400-m depth in Mackenzie Delta (Gilichinsky, 2002) and up to 4700-m elevation in Qinghai-Tibet Plateau (Zhang et al., 2007). The age of the isolates corresponds to the longevity of the permanently frozen state of the sediments and dates back from few thousand to 2–3 million years in northeastern Siberia and to 5–8 million years and probably older in Antarctica (Gilichinsky et al., 2007a). This great mass of living matter is peculiar to permafrost only, and taken into account the thickness of Cryolithosphere (50–1000 m), it is easy to conclude that permafrost contain a total biomass of cold adapted microorganisms many times higher than that of the modern soil cover.

In contrast to the ice sheets, where the overwhelming majority of discovered microorganisms came from the atmosphere, microorganisms isolated from permafrost are similar to those of surface and aquatic cold ecosystems and habitats. The total cell number counted by epifluorescence microscopy reached 10^7 – 10^8 cells/gdw in Arctic (Siberian and Canadian) permafrost (Vorobyova et al., 1997; Steven et al., 2007) and 10^5 – 10^6 cells/gdw in Antarctic permafrost (Gilichinsky et al., 2007a). This indicates the initial low level of microbial population in Antarctic Dry Valley environment in comparison with Arctic. Antarctic permafrost also is several orders of magnitude lower in the number of viable cells, and has less

microbial diversity, than Arctic one. The fraction of the total bacterial population that could be recovered from frozen samples (the number of cells that grow on nutrient media) varied 10^3 – 10^7 cells/gdw, ranging 0.01–1.5% of the total amount counted by epifluorescence microscopy (Vishnivetskaya et al., 2000) and this conclusion is supported by experimental data from different cores and regions. Note that cold adapted organisms of the microbial community within the permafrost has the optimal growth at room temperature, i.e., according to the growth temperature classification of Morita (1975, 1997), are not psychrophilic, or restricted to permanently cold habitats (Gounot, 1986), but are predominantly psychrotrophic.

Permafrost is not only bacterial (aerobic and anaerobic, spore-forming and spore less, Gram-positive and Gram-negative) archival depository, but also contain viable cyanobacteria and green alga (Vishnivetskaya, 2009), yeast (Dmitriev et al., 1997; Faizutdinova et al., 2005), actino- and micromycetes (Kochkina et al., 2001), as well as their metabolic-end products: biologically active macromolecules – free intra- and extracellular enzymes (invertase, catalase, protease and amylase) (Vorobyova et al., 1996), pigments (chlorophyll *a*, *b*, and pheophytin) (Erokhina et al., 2004), and biogenic gases, such as methane (Rivkina et al., 2007) and even biological objects of higher level: mosses (Gilichinsky et al., 2001), seeds (Porsild et al., 1967; Yashina et al., 2002), and protozoa (amoeba, infusorium and flagellates) (Shatilovich et al., 2005).

This is why permafrost could be considered as a unique subsurface complex, where the long-term impact of constant physicochemical regimes, including subzero temperatures, should be regarded not as the extreme and limiting but rather as a stabilizing factor. The frozen environment is characterized by solid and liquid water phases. The solid phase (ice) makes up 93–98% of total water volume and serves as a cryoconservant, a reagent favored for conservation of biological objects at temperatures below 0°C. At subzero temperatures the rates of biochemical reactions and biological processes become extremely low, and ensure preservation of biological system. Concomitant with the solid phase, the remaining few percent of the water is in an unfrozen state and occurs most commonly as thin films enveloping soil particles and, occasionally, as brine pockets. In contrast to ice, the unfrozen water serves as a cryoprotector and plays the leading role in the preservation of microorganisms. These films, by coating the organic and mineral particles, protect the viable cells sorbed onto their surface from mechanical destruction by growing ice crystals and make possible the mass transfer of microbial metabolic end-products within the permafrost, preventing the cell's biochemical death. This is why the unfrozen water serves as a main ecological niche for microorganisms (Gilichinsky et al., 1995).

The quantity of the unfrozen water and the thickness of its films are independent of the total ice content, but decrease with subzero temperature decreasing (Nersesova and Tsytoich, 1966; Anderson, 1967). Given these

characteristics of Arctic permafrost at temperatures between -3 to -12°C , the amount of unfrozen water can be estimated as 3–8% by weight, which depends upon the ion content and texture. In the coarse Antarctic sands, because of the low temperatures, the unfrozen water values are so small that the instrumental methods fail to record them. Unfrozen water in Antarctic permafrost must, therefore, only be firmly bound “liquid” water with binding molecules, which indicates a “biologically dry” permafrost environment.

Life below freezing point

The subzero temperatures themselves are not the limiting factor, and microorganisms are able to grow at these temperatures if the media is not frozen, at least as low as -10°C for one year in the presence of glycerol as cryoprotector (Gilichinsky et al., 1993). This correlates with the lower temperature limit for microorganisms growth (Russell and Hamamoto, 1998), but the conditions of experiments are too far from the permafrost environment in situ. Such conditions (temperatures down to -10°C and up to 200 g/L salt concentrations) are present within the lenses of cryopegs – overcooled water brines in permafrost, where metabolic activity without cell division was shown using resazurin reduction as indicator (Bakermans et al., 2003) and uptake of D- $[^{14}\text{C}]$ glucose into the bacteria biomass (Gilichinsky et al., 2003, 2005). Hypersaline waters are defined as having salt concentrations greater than that of sea water (3.5% w/v) while the salinity of cryopeg lenses reaches 15–20%.

The unfrozen water films, high in salts, represent the same microcryopegs, and most investigators indicate that at least part of permafrost community (20%, according to Steven et al., 2006) growth at temperatures between -2 and -10°C (Shcherbakova et al., 2004, 2005; Ponder et al., 2006; Rodrigues et al., 2006; Bakermans et al., 2006), while 95% of the isolated cells of aerobic bacteria did not grow at temperatures above 30°C (Gilichinsky et al., 1995).

Biotic survival in the late Cenozoic frozen and overcooled high-salt aquatic environments and Permian-Triassic salt deposits (Stan-Lotter et al., 2002, etc.) indicates unknown bacterial adaptations. It remains to be determined whether the salt tolerance of these cells may also be associated with cold tolerance on a geological scale. Experiments showed that under subzero temperatures halophilic bacteria survive better than non-halophilic and remain viable at -80°C in the presence of 25% NaCl and extreme halophiles require NaCl concentrations above 15.6% (w/v) for growth (Rothschild and Mancinelli, 2001; Mancinelli et al., 2004).

The permafrost, mostly psychro-halo-tolerant microbial community, has been described as “a community of survivors” (Friedmann, 1994), with the starvation-survival lifestyle as the normal physiological state (Morita, 2000). We can conclude that this community represents the only the selected members of buried surface or

aquatic ecosystems adapted to permafrost conditions and have efficient repair mechanisms that allow for these and not, for example, other tundra organisms to continue to live in permafrost (Ponder et al., 2006). This is why from one hand, permafrost bacteria have been previously isolated from Arctic tundra soils (Bab’eva and Sizova, 1983), and from the another one – many types of bacteria present in the Arctic soils, were not isolated from permafrost samples (Shi et al., 1997).

The preservation of a viable microbial community in permafrost over geological times at subzero temperatures raises the significant possibility of metabolic reactions for these conditions. The following facts provide indirect evidence that ancient microorganisms can be active in the permafrost nutritional-temperature regime and allows speculation concerning the possibility of biogeochemical processes in situ: the ability of immobilized enzymes in permafrost to become instantly activated (Vorobyova et al., 1996); the presence of usually metastable ferrous sulfides (Siegert, 1987) and nitrites (Jansen and Bock, 1994); the ability of cells to grow on media after being exposed to ground radiation during up to millions of years. This ability implies their capacity to repair DNA damage even in the frozen environment, i.e., at the stable rate of damage accumulation a comparable rate of repair must also exist.

However, the question about the metabolic state of microorganisms, microbiological and biogeochemical processes, and life forms within permafrost still remains open to debate. For recent microbial communities measurements show that Antarctic lichens may be active at a temperature of -17°C (Kappen et al., 1996) and evidence was obtained of low rates of bacterial DNA and protein synthesis at subzero temperatures (-12 to -17°C), which indicates that the organisms divided in snow at the South Pole (Carpenter et al., 2000). For ancient microorganisms to the moment we can refer only to results of few experiments, suggesting that permafrost microorganisms are not, as previously thought, in a frozen resting state. The incorporation of ^{14}C -labeled acetate into lipids shows that in permafrost 2–3 million years old, at temperatures down to -20°C , anabolic metabolism is possible, resulting in the formation of bacterial lipids with the minimum 160 days doubling time (Rivkina et al., 2000). Also, using the ^{14}C -labeled substrates (H^{14}CO_3 and $^{14}\text{CH}_3\text{CO}_2$), hydrogenotrophic and acetoclastic methanogenesis was measured in the late Pliocene to Holocene permafrost samples at temperatures down to -16.5°C (minimal permafrost temperature in Arctic) and even to -28°C (permafrost temperature in Antarctica), indicating the energetic metabolism – the ability of microorganisms to carry out redox reactions after thousands to millions years of existence in permafrost (Rivkina et al., 2004, 2005, 2007; Gilichinsky et al., 2007).

From the biological point of view, taking into account the natural radiation background of 1–2 mGy/year, the dose from radio nuclides diffused through the permafrost is far from sufficient for complete sterilization even the

oldest microbial complexes, i.e., it is not fatal to viable cells, but it is highly enough to cause selection effect and to destroy the DNA of ancient cells. The calculated data correlate with the number of viable cells in permafrost of different age and with experimental results. The cell viability and growth on media implies a high capacity for DNA repair and we can conclude that DNA repair occurs in the frozen environment, i.e., at the stable rate of damage accumulation, while a comparable or lower rate of reparation also exists (Gilichinsky et al., 2007b). The same suggestion made Johnson et al. (2007). Using a combination of molecular biology techniques and direct measurement of CO₂ production from permanently frozen samples they find strong evidence that long-term survival is closely tied to cellular metabolic activity and DNA repair as a mechanism in sustaining bacteria viability.

The DNA preservation and reparation in permafrost is a huge area of important problems. Microbial communities began now characterized by newly emerging culture-independent molecular techniques – PCR amplification of diverse environmental 16S rRNA genes as well as the diversity of fungi – by 18S rRNA genes. Long-term persistence of bacterial, archaeal, and fungal DNA were found in relatively young, late Pleistocene-Holocene, Canadian (Steven et al., 2007) and Tibetan (Zhang et al., 2006) permafrost and within late Pliocene–Pleistocene Siberian (Shi et al., 1997; Lydolph et al., 2005; Vishnivetskaya et al., 2006) and Antarctic (Spirina et al., 2003; Gilichinsky et al., 2007a). These studies shows that DNA, usually degraded rapidly in most environments, survived within permafrost over geological time and the question is how long the DNA could be preserved. Series papers (Willerslev et al., 2003, 2004; Hansen et al., 2006; Johnson et al., 2007) on the basis of observed rates of DNA degradation indicate a limit of ca. 400–600,000 years beyond which PCR amplifications are prevented. This conclusion is still under discussion because based on the same few samples only and has not the statistical support and even its authors agree that studied organisms maintain a metabolic activity to repair DNA damages. Also because in another studies the viable microorganisms were isolated and DNA was extracted from permafrost samples up to 3–5-million-years old.

Conclusion

Microbiological and molecular studies of paleobiological objects in permafrost expand our knowledge about spatio-temporal limits of Biosphere and form the basis for new knowledge in various scientific multidisciplinary (geo/bio/cryo) fields: quaternary geology, geocryology, bacterial paleontology, geo- cryo-, paleo-, and exobiology. Also, the advances in biotechnology evidently indicate that in the visible future the genetic recourses will be even more important than geological one, and the permafrost microorganisms, as well as their metabolic-end products might be of potential value for industrial application, for

example, on the base of abilities to operate at subzero temperatures.

Acknowledgments

This research was supported by Russian Fund for Basic Research (grants 07-05-00953 and 08-05-00268).

Bibliography

- Abyzov, S., 1993. The microorganisms in the Antarctic ice. In Friedmann, E. I. (ed.), *Antarctic Microbiology*, New York: Wiley, pp. 265–297.
- Anderson, D. M., 1967. Ice nucleation and substrate-ice interface. *Nature*, **216**, 563–566.
- Bab'eva, I. P., and Sizova, E. T., 1983. Micromycetes in the soil of Arctic tundra ecosystem. *Pochvovedenie*, **10**, 98–101 (in Russian).
- Bakermans, C., Tsapin, A. I., Souza-Egipsy, V., Gilichinsky, D. A., and Neelson, K. H., 2003. Reproduction and metabolism at –10°C of bacteria isolated from Siberian permafrost. *Environmental Microbiology*, **5**(4), 321–326.
- Bakermans, C., Ayala-del-Rio, H. L., Ponder, M., Vishnivetskaya, T., Gilichinsky, D., Thomashow, M. F., and Tiedje, J. M., 2006. *Psychrobacter cryohalolentis* sp. nov. and *Psychrobacter arcticus* sp. nov., isolated from Siberian permafrost. *International Journal of Systematic and Evolutionary Microbiology*, **56**, 1285–1291.
- Basic foundation of geocryology, 1959. Shvetsov, P. F., and Dostovalov, B. N. (eds.), *The main conceptions and definitions*. Moscow: Russian Academy of Sciences, pp. 7–10.
- Bidle, K. D., Lee, S., Marchant, D. R., and Falkowski, P. G., 2007. Fossil genes and microbes in the oldest ice on Earth. *PNAS*, **104**(33), 13455–13460.
- Cano, R., and Borucki, M., 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. *Science*, **268**, 1060–1064.
- Carpenter, E. J., Lin, S. J., and Capone, D. G., 2000. Bacterial activity in South Pole snow. *Applied and Environmental Microbiology*, **66**(10), 4514–4517.
- Castello, J. D., and Rogers, S. O. (eds.), 2005, *Life in Ancient Ice*. Princeton: Princeton University Press.
- Castello, J. D., Rogers, S. O., Smith, J. E., Starmer, W. T., and Zhao, Y., 2005. Plant and bacterial viruses in the Greenland ice sheet. In Castello, J. D., and Rogers, S. O. (eds.), *Life in Ancient Ice*. Princeton: Princeton University Press, pp. 196–207.
- Christner, B. C., Mosley-Thompson, E., Thompson, L. G., and Reeve, J. N., 2003. Bacterial recovery from ancient glacial ice. *Environmental Microbiology*, **5**, 433–436.
- Deming, J. W., 2002. Psychrophiles and polar regions. *Current Opinion in Microbiology*, **5**, 301–309.
- Dmitriev, V., Gilichinsky, D., Faizutdinova, R., Shershunov, I., Golubev, V., and Duda, V., 1997. Detection of viable yeast in 3-million-year-old permafrost soils of Siberia. *Microbiology*, **66**, 546–550.
- Dombrowski, H., 1963. Bacteria from paleozoic salt deposits. *Annals of the New York Academy of Sciences*, **108**, 453–460.
- Erokhina, L., Shatilovich, A., Kaminskaya, O., and Gilichinsky, D., 2004. Spectral properties of ancient green algae from Antarctic Dry Valley permafrost. *Microbiology*, **73**(4), 485–487.
- Faizutdinova, R., Suzina, N., Duda, V., Petrovskaya, L., and Gilichinsky, D., 2005. Yeasts isolated from ancient permafrost. In Castello, J. D., and Rogers, S. O. (eds.), *Life in Ancient Ice*. Princeton: Princeton University Press, pp. 118–126.

- Friedmann, E. I., 1994. Permafrost as microbial habitat. In Gilichinsky, D. A. (ed.), *Viable Microorganisms in Permafrost*. Pushchino: Russian Academy of Sciences, pp. 21–26.
- Galippe, V., 1920. Recherches sur la resistance de *Microzymas* a l'action du temps et sur leur survivance dans l'amber. *Paris, Comptes-Rendus de l'Académie des Sciences*, **170**, 856–858.
- Gilichinsky, D., 2002. Permafrost as a microbial habitat. In Bitton, G. (ed.), *Encyclopedia of Environmental Microbiology*. Wiley, New York, pp. 932–956.
- Gilichinsky, D. A., Soina, V. S., and Petrova, M. A., 1993. Cryoprotective properties of water in the Earth cryolithosphere and its role in exobiology. *Origins of Life and Evolution of the Biosphere*, **23**(1), 65–75.
- Gilichinsky, D. A., Wagener, S., and Vishnivetskaya, T. A., 1995. Permafrost microbiology. *Permafrost and Periglacial Processes*, **6**, 281–291.
- Gilichinsky, D., Shatilovich, A., Vishnivetskaya, T., Spirina, E., Faizutdinova, R., Rivkina, E., Gubin, S., Erokhina, L., Vorobyova, E., and Soina, V., 2001. The terrestrial permafrost model for Astrobiology. In Giovannelli, F. (ed.), *The Bridge between Big Bang and Biology. How long the life might be preserved?* International Workshop, Stromboli, Italy, September 13–17, 1999. Consiglio Nazionale delle Ricerche of Italy, Rome, pp. 362–369.
- Gilichinsky, D., Rivkina, E., Shcherbakova, V., Laurinavichus, K., and Tiedje, J., 2003. Supercooled water brines within permafrost – an unknown ecological niche for microorganisms: a model for astrobiology. *Astrobiology*, **3**(2), 331–341.
- Gilichinsky, D., Rivkina, E., Bakermans, C., Shcherbakova, V., Petrovskaya, L., Ozerskaya, S., Ivanushkina, N., Kochkina, G., Laurinavichus, K., Pecheritsina, S., Fattakhova, R., and Tiedje, J., 2005. Biodiversity of cryopegs in permafrost. *FEMS Microbiology Ecology*, **53**(1), 117–128.
- Gilichinsky, D., Wilson, G., Friedmann, E. I., McKay, C., Sletten, R., Rivkina, E., Vishnivetskaya, T., Erokhina, L., Ivanushkina, N., Kochkina, G., Shcherbakova, V., Soina, V., Spirina, E., Vorobyova, E., Fyodorov-Davydov, D., Hallet, B., Ozerskaya, S., Sorokovikov, V., Laurinavichus, K., Shatilovich, A., Chanton, J., Ostroumov, V., and Tiedje, J., 2007a. Microbial populations in Antarctic permafrost: biodiversity, state, age, and implication for astrobiology. *Astrobiology*, **7**(2), 275–311.
- Gilichinsky, D., Vishnivetskaya, T., Petrova, M., Spirina, E., Mamykin, V., and Rivkina, E., 2007b. Bacteria in permafrost. In Margesin, R., Schinner, F., Marx, J.-C., and Gerday, C. (eds.), *Psychrophiles: from Biodiversity to Biotechnology*. Berlin: Springer, Chapter 6, pp. 83–102.
- Gounot, A., 1986. Psychrofilic and psychrotrophic microorganisms. *Experientia*, **42**, 1192–1197.
- Greenblatt, C. L., Davis, A., Clement, B. G., Kitts, C. L., Cox, T., and Cano, R. J., 1999. Diversity of microorganisms isolated from amber. *Microbial Ecology*, **38**(1), 58–68.
- Hansen, A. J., Mitchell, D. L., Wiuf, C., Paniker, L., Brand, T. B., Binladen, J., Gilichinsky, D. A., Rønn, R., and Willerslev, E., 2006. Crosslinks rather than strand breaks determine access to ancient DNA sequences from frozen sediments. *Genetics*, **173**, 1175–1179.
- Jansen, H., and Bock, E., 1994. Profiles of ammonium, nitrite and nitrate in the permafrost soils. In Gilichinsky, D. (ed.), *Viable Microorganisms in Permafrost*. Pushchino: Russian Academy of Sciences, pp. 27–36.
- Johnson, S., Hebsgaard, M., Christensen, T., Mastepanov, M., Nielsen, R., Munch, K., Brand, T., Gilbert, M., Zuber, M., Bunce, M., Rønn, R., Gilichinsky, D., and Froese, D., Willerslev, E., 2007. Ancient bacteria show evidence of DNA repair. *PNAS*, **104**(36), 14401–14405.
- Kapitsa, A. P., Ridley, J. K., Robin, G. D., Siegert, M. J., and Zotikov, I. A., 1996. A large deep freshwater lake beneath the ice of central East Antarctica. *Nature*, **381**, 684–686.
- Kappen, L., Schroeter, B., Scheidegger, C., Sommerkorn, M., and Hestmark, G., 1996. Cold resistance and metabolic activity of lichens below 0°C. *Advances in Space Research*, **18**(12), 119–128.
- Karl, D., Bird, D., Bjorkman, K., Houlihan, T., Shackelford, R., and Tupas, L., 1999. Microorganisms in the accreted ice of Lake Vostok, Antarctica. *Science*, **286**(5447), 2144–2146.
- Kochkina, G., Ivanushkina, N., Karasev, S., Gavrish, E., Gurina, L., Evtushenko, L., Spirina, E., Vorobyova, E., Gilichinsky, D., and Ozerskaya, S., 2001. Micromycetes of Arctic and Antarctic under the long-term cryoconservation. *Microbiology*, **70**(3), 356–364.
- Lambert, L. H., Cox, T., Mitchell, K., Rossello-Mora, R. A., Del Cueto, C., Dodge, D. E., Orkand, P., and Cano, R. J., 1998. *Staphylococcus succinus* sp. nov., isolated from Dominican amber. *International Journal of Systematic Bacteriology*, **48** (Pt 2), 511–518.
- Lipman, C., 1928. The discovery of living microorganisms in ancient sediments. *Science*, **68**, 272–273.
- Lydolph, M., Jacobsen, J., Arctander, P., Thomas, M., Gilbert, P., Gilichinsky, D., Hansen, A., Willerslev, E., and Lange, L., 2005. Beringian paleoecology inferred from permafrost-preserved fungal DNA. *Applied and Environmental Microbiology*, **71**(2), 1012–1017.
- Mancinelli, R. L., Fahlen, T. F., Landheim, R., and Klovstad, M. R., 2004. Brines and evaporites: analogs for Martian life. *Advances in Space Research*, **33**, 1244–1246.
- Miteva, V. I., and Brenchley, J. E., 2005. Detection and isolation of ultrasmall microorganisms from a 120,000-year-old Greenland glacier ice core. *Applied and Environmental Microbiology*, **71**, 7806–7818.
- Miteva, V. I., Sheridan, P. P., and Brenchley, J. E., 2004. Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. *Applied and Environmental Microbiology*, **70**(1), 202–213.
- Morita, R., 1975. Psychrophilic bacteria. *Bacteriological Reviews*, **39**, 144–167.
- Morita, R., 1997. *Bacteria in Oligotrophic Environments*, New York: Chapman & Hall, 529 p.
- Morita, R., 2000. Low-temperature environments. In Lederberg, J. (ed.), *Encyclopedia of Microbiology*, New York: Academic, Vol. 3 L–P, pp. 93–98.
- Nersesova, Z., and Tsyrovich, N., 1966. *Unfrozen Water in Frozen Soils*. Washington, DC: First Permafrost International Conference, National Academy of Sciences, National Research Council, pp. 230–234.
- Norton, C., McGenity, T., and Grant, W., 1993. Archaeal halophiles (halobacteria) from two British salt mines. *Journal of General Microbiology*, **139**, 1077–1081.
- Petit, J., Jouzel, J., Raynaud, D., Barkov, N., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., Delaygue, G., Delmotte, M., Kotlyakov, V., Legrand, M., Lipenkov, V., Lorius, C., Pépin, L., Ritz, C., Saltzman, E., and Stievenard, M., 1999. Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica. *Nature*, **399**, 429–436.
- Ponder, M. A., Gilmour, S. J., Bergholz, P. W., Mindock, C. A., Hollingsworth, R., Thomashow, M. F., and Tiedje, J. M., 2006. Characterization of potential stress responses in ancient Siberian permafrost psychrotrophic bacteria. *FEMS Microbiology Ecology*, **53**, 103–115.
- Porsild, A., Harington, C., and Milligan, G., 1967. Grown seeds of Pleistocene age. *Science*, **158**, 113–114.
- Priscu, J. C., and Christner, B. C., 2004. Earth's icy biosphere. In Bull, A. T. (ed.), *Microbial Diversity and Bioprospecting*. Washington: ASM Press, pp. 130–145.

- Priscu, J. C., Fritsen, C. H., Adams, E. E., Giovannoni, S. J., Paerl, H. W., McKay, C. P., Doran, P. T., Gordon, D. A., Lanol, B. D., and Pinckney, J. L., 1998. Perennial Antarctic lake ice: an oasis for life in a polar desert. *Science*, **280**(5372), 2095–2098.
- Rivkina, E., Friedmann, E. I., McKay, C. P., and Gilichinsky, D., 2000. Metabolic activity of permafrost bacteria below the freezing point. *Applied and Environmental Microbiology*, **66**(8), 3230–3233.
- Rivkina, E., Laurinavichius, K., McGrath, J., Tiedje, J., Shcherbakova, V., and Gilichinsky, D., 2004. Microbial life in permafrost. *Advances in Space Research*, **33**(8), 1215–1221.
- Rivkina, E., Laurinavichius, K., and Gilichinsky, D., 2005. Microbial life below freezing point within permafrost. In Castello, J. D., and Rogers, S. O. (eds.), *Life in Ancient Ice*. Princeton: Princeton University Press, pp. 106–118.
- Rivkina, E., Shcherbakova, V., Laurinavichius, K., Petrovskaya, L., Krivushin, K., Kraev, G., Pecheritsina, S., and Gilichinsky, D., 2007. Biogeochemistry of methane and methanogenic archaea in Permafrost. *FEMS Microbiology Ecology*, **61**, 1–15.
- Rodrigues, D. F., Goris, J., Vishnivetskaya, T., Gilichinsky, D., Thomashow, M. F., and Tiedje, J. M., 2006. Characterization of *Exiguobacterium* isolates from the Siberian permafrost. Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles*, **10**, 285–294.
- Russell, N., and Hamamoto, T., 1998. Psychrophiles. In Horikoshi, K., and Grant, W. (eds.), *Extremophiles. Microbial Life in Extreme Environments*. New York: Wiley, pp. 25–46.
- Salamatin, N., Tsyganova, E. A., Lipenkov, V. Ya., and Petit, J. R., 2004. Vostok (Antarctica) ice-core time scale from datings of different origins. *Annals of Glaciology*, **39**, 283–292.
- Sattler, B., Puxbaum, H., and Psenner, R., 2001. Bacterial growth in supercooled cloud droplets. *Geophysics Research Letter*, **28**, 239–242.
- Shatilovich, A., Shmakova, L., Gudkov, A., Gubin, S., and Gilichinsky, D., 2005. Viable Protozoa from permafrost and buried soil. *Doklady Biological Sciences*, **401**(5), 136–138.
- Shcherbakova, V., Rivkina, E., Laurinavichius, K., Pecheritsina, S., and Gilichinsky, D., 2004. Physiological characteristics of bacteria isolated from water brines within permafrost. *International Journal of Astrobiology*, **3**(1), 37–43.
- Shcherbakova, V. A., Chuvilskaya, N. A., Rivkina, E. M., Pecheritsyna, S. A., Laurinavichius, K. S., Suzina, N. E., Osipov, G. A., Lysenko, A. M., Gilichinsky, D. A., and Akimenko, V. K., 2005. Novel psychrophilic anaerobic spore-forming bacterium from the overcooled water brine in permafrost: description *Clostridium algoriphilum* sp. nov. *Extremophiles*, **9**, 239–246.
- Shi, T., Reeves, R., Gilichinsky, D., and Friedmann E. I., 1997. Characterization of viable bacteria from Siberian permafrost by 16S rDNA sequencing. *Microbial Ecology*, **33**, 169–179.
- Shoham, D., 2005. Viral pathogens of humans likely to be preserved in natural ice. In Castello, J. D., and Rogers, S. O. (eds.), *Life in Ancient Ice*. Princeton: Princeton University Press, pp. 208–226.
- Siebert, C., 1987. Greigite and mackinawite in the Quaternary permafrost sediments of Central Yakutiya, *Mineral J*, **9**, 75–80 (in Russian).
- Skidmore, M. L., Foght, J. M., and Sharp, M. J., 2000. Microbial life beneath a high Arctic glacier. *Applied and Environmental Microbiology*, **66**, 3214–3220.
- Spirina, E., Cole, J., Chai, B., Gilichinsky, D., and Tiedje, J., 2003. High throughput approach to study ancient microbial phylogenetic diversity in permafrost as a terrestrial model of Mars. *Astrobiology*, **2**(4), 542–543.
- Stan-Lotter, H., Pfaffenhuemer, M., Legat, A., Busse, H. J., Radax, C., and Gruber, C., 2002. *Halococcus dombrowskii* sp. nov., an archaeal isolate from a Permian alpine salt deposit. *International Journal of Systematic and Evolutionary Microbiology*, **52**(Pt 5), 1807–1814.
- Steven, B., L'evenill'e, R., Pollard, W. H., and Whyte, L. G., 2006. Microbial ecology and biodiversity in permafrost. *Extremophiles*, **10**, 259–267.
- Steven, B., Briggs, G., McKay, C. P., Pollard, W. H., Greer, Ch. W., and Whyte, L. G., 2007. Characterization of the microbial diversity in a permafrost sample from the Canadian high Arctic using culture-dependent and culture-independent methods. *FEMS Microbiology Ecology*, **59**, 513–523.
- Sudgen, D. E., Marchant, D. R., Potter, N., Souchez, R. A., Denton, G. H., Swisher, C. C., and Tison, J. L., 1996. Preservation of Miocene glacier ice in East Antarctica. *Nature*, **376**, 412–414.
- Thompson, L., Yao, T., Davis, E., Henderson, K., Mosley-Thompson, E., Lin, P.-N., Beer, J., Synal, H.-A., Cole-Dai, J., and Boizan, J., 1997. Tropical climate instability: the last glacial cycle from a qinghai-tibetan ice core. *Science*, **276**, 1821–1825.
- Tiedje, J. M., Smith, G. B., Simkins, S., Holben, W. E., Finney, C., and Gilichinsky, D. A., 1994. Recovery of DNA, denitrifiers and patterns of antibiotic sensitivity in microorganisms from ancient permafrost soils of Eastern Siberia. In Gilichinsky, D. (ed.), *Viable Microorganisms in Permafrost*. Pushchino: Russian Academy of Sciences, pp. 83–98.
- van Everdingen, R. (ed.), 2005. *Multi-language Glossary of Permafrost and Related Ground-ice Terms, 1998 Revised*, Boulder, CO: National Snow and Ice Data Center/World Data Center for Glaciology.
- Vishnivetskaya, T., Kathariou, S., McGrath, J., Gilichinsky, D., and Tiedje, J., 2000. Low-temperature recovery strategies for the isolation of bacteria from ancient permafrost sediments. *Extremophiles*, **3**, 165–173.
- Vishnivetskaya, T., Petrova, M., Urbance, J., Ponder, M., Moyer, C., Gilichinsky, D., and Tiedje, J., 2006. Bacterial community in ancient Siberian permafrost as characterized by culture and culture-independent methods. *Astrobiology*, **6**(3), 400–414.
- Vishnivetskaya, T. A., 2009. Viable Cyanobacteria and Green Algae from the Permafrost Darkness. In Margesin, R. (ed.), *Permafrost Soil (Soil Biology series)*, Springer-Verlag. Chapter 6, pp. 73–84.
- Vorobyova, E., Soina, V., and Mulukin, A., 1996. Microorganisms and enzyme activity in permafrost after removal of long-term cold stress. *Advances in Space Research*, **18**, 103–108.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Mamukelashvili, A., Zalinova, N., Gilichinsky, D., Rivkina, E., and Vishnivetskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Vreeland, R., Rosenzweig, W., and Powers, D., 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*, **407**, 897–900.
- Wainwright, M., Wickramasinghe, N. C., Narlikar, J. V., and Rajaratnam, P., 2003. Microorganisms cultured from stratospheric air samples obtained at 41 km. *FEMS Microbiology Letter*, **218**(1), 161–165.
- Willerslev, E., Hansen, A., Christensen, B., Steffensen, J., and Arstander, P., 1999. Diversity of Holocene life forms in fossil glacier ice. *PNAS*, **96**, 8017–8021.
- Willerslev, E., Hansen, A., Binladen, J., Brand, T., Thomas, M., Gilbert, P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D., and Cooper, A., 2003. Diverse plant and animal genetic records from holocene and pleistocene sediments. *Science*, **300**, 791–795.
- Willerslev, E., Hansen, A., Ronn, R., Brand, T., Barnes, I., Wiuf, C., Gilichinsky, D., Mitchell, D., and Cooper, A., 2004. Long-term persistence of bacterial DNA. *Current Biology*, **14**(1), R9–R10.
- Yashina, S., Gubin, S., Shabaeva, E., Egorova, E., and Maximovich S., 2002. Viability of late pleistocene higher-plant seeds from permafrost as determined in vitro culturing. *Doklady Biological Sciences*, **383**(5), 151–154.

- Zhang, G., Shoham, D., Gilichinsky, D., Davydov, S., Castello, J. D., and Rogers, S. O., 2006. Evidence for influenza A virus RNA in Siberian lake ice. *Virology Journal*, **80**(24), 12229–12235.
- Zhang, G., Max, X., Niu, F., Dong M., Feng, H., An, L., and Cheng, G., 2007. Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai–Tibet Plateau permafrost region. *Extremophiles*, **11**, 415–424.

Cross-references

Algae (Eukaryotic)
 Archaea
 Astrobiology
 Bacteria
 Chemolithotrophy
 Cyanobacteria
 Extreme Environments
 Methanogens
 Microbial Communities, Structure, and Function
 Microbial Ecology of Submarine Caves
 Protozoa (Heterotroph, Eukaryotic)
 Snowball Earth
 Soils

PHOSPHORUS, PHOSPHORITES

Karl B. Föllmi
 University of Lausanne, Lausanne, Switzerland

Definition

Phosphorus is a chemical element (P) with atomic number 15, which occurs mainly in the form of phosphate (PO_4^{3-}). P is essential for life, but may negatively impact existing ecosystems when present in excess. The P cycle is tightly coupled with the carbon cycle through photosynthetic and biogeochemical weathering processes.

Introduction

Phosphorus (P) occurs most widely in the form of phosphate (PO_4^{3-}) and plays – as an essential and often limiting nutrient – an important role in the overall functioning of the biosphere and its environment. It is in this capacity that this element is heavily recycled within the biosphere, and the most important source is its release from preexisting stocks within the biosphere. Its primordial source, however, is related to the biochemical weathering of P-containing rocks and the consequent transfer into the biosphere (Lerman et al., 1975; Froelich et al., 1982; Compton et al., 2000; Ruttenberg, 2005).

P exerts important interactions with the carbon cycle through the interfaces of photosynthesis and biogeochemical weathering. In the first case, P may drive the transformation of atmospheric CO_2 into organic C in those ecosystems where P is limiting, and in the second case, the amount of atmospheric CO_2 determines the amount of P liberated from its host rock and transferred to the biosphere (Mackenzie et al., 1993).

The study of the present-day P cycle on a worldwide scale together with the reconstruction of temporal changes in the P cycle during the Earth's history is essential in the reconstruction of global environmental change at present and in the past (Cook and McElhinny, 1979; Bendor, 1980; Arthur and Jenkyns, 1981; Filippelli and Delaney, 1994; Föllmi, 1995, 1996; Trappe, 1998; Slomp and Van Cappellen, 2007).

The global phosphorus cycle

The principal release mechanism of P to the environment consists in the biogeochemical weathering of P-containing crystalline, volcanic, and sedimentary rocks (Froelich et al., 1982; Föllmi et al., 2009). Volcanic and hydrothermal activity provides only minor amounts of P, if at all – except eventually for basaltic volcanism and the formation of P-enriched deposits such as carbonatite and anorthosite (Baturin, 1982; Notholt et al., 1989; Feely et al., 1990). Once transferred into the biosphere, P predominantly resides in soils, where it becomes part of the organic fraction and may also be included into secondary mineral phases such as iron oxyhydroxides (Walker and Syers, 1976; Frossard et al., 1989). Approximately 3–6% of the total terrestrial P stock is incorporated in vegetation, with organic P:C ratios of 1:500–1:800 (Delwiche and Likens, 1977; Berner et al., 1993).

P is transported to oceans via rivers and wind, where the eolian fraction corresponds to 7–15% of the total preanthropogenic P freight (Graham and Duce, 1979; Compton et al., 2000). Rivers transport P as dissolved inorganic P (DIP), dissolved organic P (DOP) and in particulate forms (PIP, POP). PIP transport occurs in the form of detrital P and P absorbed onto iron oxyhydroxides and clay minerals (Meybeck, 1982, 1993; Compton et al., 2000; Seitzinger et al., 2005; Harrison et al., 2005). Recent estimates show that presently approximately 85% of the total P freight to the ocean is transported in particulate form (Beusen et al., 2005).

Once in the ocean, P enters a recycling loop between organic and inorganic forms, and only approximately 1% of the total amount of P used in primary productivity is permanently stored in the sedimentary reservoir per time unit. The P:C ratio in oceanic primary producers is significantly lower than that for terrestrial plants and amounts to 1:106–117 (Redfield, 1958; Anderson et al., 2001). The present-day residence time of oceanic P is estimated as between 10,000 and 17,000 years (Ruttenberg, 1993, 2005; Colman and Holland, 2000).

Recent attempts to quantify P reservoirs and flux rates on a global scale have been published in Mackenzie et al. (1993), Compton et al. (2000), Harrison et al. (2005), Beusen et al. (2005), and Ruttenberg (2005).

Phosphorus, microbes and life

The degree to which P may limit primary productivity in certain ecosystems is judged differently in the literature (Falkowski et al., 1992; Tyrrell, 1999). A vast array of

- Zhang, G., Shoham, D., Gilichinsky, D., Davydov, S., Castello, J. D., and Rogers, S. O., 2006. Evidence for influenza A virus RNA in Siberian lake ice. *Virology Journal*, **80**(24), 12229–12235.
- Zhang, G., Max, X., Niu, F., Dong M., Feng, H., An, L., and Cheng, G., 2007. Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai–Tibet Plateau permafrost region. *Extremophiles*, **11**, 415–424.

Cross-references

Algae (Eukaryotic)
 Archaea
 Astrobiology
 Bacteria
 Chemolithotrophy
 Cyanobacteria
 Extreme Environments
 Methanogens
 Microbial Communities, Structure, and Function
 Microbial Ecology of Submarine Caves
 Protozoa (Heterotroph, Eukaryotic)
 Snowball Earth
 Soils

PHOSPHORUS, PHOSPHORITES

Karl B. Föllmi
 University of Lausanne, Lausanne, Switzerland

Definition

Phosphorus is a chemical element (P) with atomic number 15, which occurs mainly in the form of phosphate (PO_4^{3-}). P is essential for life, but may negatively impact existing ecosystems when present in excess. The P cycle is tightly coupled with the carbon cycle through photosynthetic and biogeochemical weathering processes.

Introduction

Phosphorus (P) occurs most widely in the form of phosphate (PO_4^{3-}) and plays – as an essential and often limiting nutrient – an important role in the overall functioning of the biosphere and its environment. It is in this capacity that this element is heavily recycled within the biosphere, and the most important source is its release from preexisting stocks within the biosphere. Its primordial source, however, is related to the biochemical weathering of P-containing rocks and the consequent transfer into the biosphere (Lerman et al., 1975; Froelich et al., 1982; Compton et al., 2000; Ruttenger, 2005).

P exerts important interactions with the carbon cycle through the interfaces of photosynthesis and biogeochemical weathering. In the first case, P may drive the transformation of atmospheric CO_2 into organic C in those ecosystems where P is limiting, and in the second case, the amount of atmospheric CO_2 determines the amount of P liberated from its host rock and transferred to the biosphere (Mackenzie et al., 1993).

The study of the present-day P cycle on a worldwide scale together with the reconstruction of temporal changes in the P cycle during the Earth's history is essential in the reconstruction of global environmental change at present and in the past (Cook and McElhinny, 1979; Bendor, 1980; Arthur and Jenkyns, 1981; Filippelli and Delaney, 1994; Föllmi, 1995, 1996; Trappe, 1998; Slomp and Van Cappellen, 2007).

The global phosphorus cycle

The principal release mechanism of P to the environment consists in the biogeochemical weathering of P-containing crystalline, volcanic, and sedimentary rocks (Froelich et al., 1982; Föllmi et al., 2009). Volcanic and hydrothermal activity provides only minor amounts of P, if at all – except eventually for basaltic volcanism and the formation of P-enriched deposits such as carbonatite and anorthosite (Baturin, 1982; Notholt et al., 1989; Feely et al., 1990). Once transferred into the biosphere, P predominantly resides in soils, where it becomes part of the organic fraction and may also be included into secondary mineral phases such as iron oxyhydroxides (Walker and Syers, 1976; Frossard et al., 1989). Approximately 3–6% of the total terrestrial P stock is incorporated in vegetation, with organic P:C ratios of 1:500–1:800 (Delwiche and Likens, 1977; Berner et al., 1993).

P is transported to oceans via rivers and wind, where the eolian fraction corresponds to 7–15% of the total preanthropogenic P freight (Graham and Duce, 1979; Compton et al., 2000). Rivers transport P as dissolved inorganic P (DIP), dissolved organic P (DOP) and in particulate forms (PIP, POP). PIP transport occurs in the form of detrital P and P absorbed onto iron oxyhydroxides and clay minerals (Meybeck, 1982, 1993; Compton et al., 2000; Seitzinger et al., 2005; Harrison et al., 2005). Recent estimates show that presently approximately 85% of the total P freight to the ocean is transported in particulate form (Beusen et al., 2005).

Once in the ocean, P enters a recycling loop between organic and inorganic forms, and only approximately 1% of the total amount of P used in primary productivity is permanently stored in the sedimentary reservoir per time unit. The P:C ratio in oceanic primary producers is significantly lower than that for terrestrial plants and amounts to 1:106–117 (Redfield, 1958; Anderson et al., 2001). The present-day residence time of oceanic P is estimated as between 10,000 and 17,000 years (Ruttenger, 1993, 2005; Colman and Holland, 2000).

Recent attempts to quantify P reservoirs and flux rates on a global scale have been published in Mackenzie et al. (1993), Compton et al. (2000), Harrison et al. (2005), Beusen et al. (2005), and Ruttenger (2005).

Phosphorus, microbes and life

The degree to which P may limit primary productivity in certain ecosystems is judged differently in the literature (Falkowski et al., 1992; Tyrrell, 1999). A vast array of

regional studies in which limiting capacities have been demonstrated exists. This appears especially the case in terrestrial, lacustrine, and near-shore oceanic environments, whereas open-ocean environments are often limited by nitrate or micronutrients such as iron (especially in high nutrient – low chlorophyll areas; Martin, 1991). Recently, a synergistic effect on primary productivity across most ecosystems has been proposed in case additions of nitrate and P are combined (Elser et al., 2007).

The above mentioned C:P ratios in primary producers predict that under P-limiting conditions, the addition of a single bioavailable P atom will lead to the photosynthetic transformation of more than 100 CO₂ molecules in oceans and 500–800 CO₂ molecules on the continent into organic carbon. This underlines the importance of P in environmental change.

Principal players in the transfer and transformation processes of P from solid, biounavailable forms into bioavailable forms and vice versa are microbes. They are involved in the solubilization and liberation of P from different substrata such as P-containing rocks and organic matter (Ehrlich, 1981; Krajewski et al., 1994), and they also play an active role in the remobilization of P in anoxic sedimentary environments and its reflux to primary producers (Gächter et al., 1988; Ingall et al., 1993; Van Cappellen and Ingall, 1994). Microbes also act in the reverse process – in the transfer of P into a solid mineral phase (in soils and sediments; Lucas and Prévôt, 1985; Soudry and Lewy, 1988; Krajewski et al., 1994; Schulz and Schulz, 2005).

Phosphogenesis and phosphorites

The precipitation of phosphate minerals in sediments is termed “phosphogenesis.” The principal mineral containing P in such environments is francolite ($\text{Ca}_{10-a-b}\text{Na}_a\text{Mg}_b(\text{PO}_4)_{6-x}(\text{CO}_3)_{x-y-z}(\text{CO}_3\text{F})_y(\text{SO}_4)_z\text{F}_2$; Jarvis et al., 1994). Phosphogenesis is a process which occurs in the first few top centimeters of marine and lacustrine sediments. Favorable to this process are the sufficient supply of reactive P and overall low sediment accumulation rates. The supply of P may be from internal sources such as the decomposition of trapped organic matter or the desorption of P from iron and manganese compounds, or external sources such as the supply from bottom waters across microbial mats at the sediment–water interface (Krajewski et al., 1994). The concentration of thus formed phosphates into phosphorites, i.e., P-enriched deposits which are sedimentary and generally of marine origin, is largely a mechanical affair, related to bottom-water currents and gravity-flow-related redeposition (Glenn et al., 1994a; Föllmi, 1996).

Presently, phosphorites form in a limited number of marine localities, which are almost all related to oceanic sites where the upwelling of deeper, P-enriched waters occurs (offshore Peru and Chile, Baja California, Namibia, SW India; Veeh et al., 1973; Burnett, 1977; Baturin, 1982; Jahnke et al., 1983; Garrison and Kastner,

1990; Glenn et al., 1994b). Recent phosphorite formation has also been reported from E Australia and Pacific atolls and guyots (O’Brien et al., 1990). Phosphogenesis and the formation of minor amounts of authigenic francolite are, however, phenomena in marine sedimentary environments which are more widespread than previously thought (Ruttenberg 1993, 2005; Delaney, 1998).

Phosphorus during the Earth’s history

Periods of formation of exploitable phosphorite deposits are unevenly spread throughout the Earth’s history. Important deposits are known from the following time windows (Cook and McElhinny, 1979): Precambrian–Cambrian boundary (China, Australia); Ordovician (Baltic countries); Permian (Wyoming, Idaho); Jurassic (Siberia, Mexico); late Cretaceous–Eocene (N Africa and Middle East countries); and Miocene (Florida). The unequal occurrence of P-enriched deposits in time suggests important fluctuations in the global P cycle, which are confirmed by other approximations of temporal behavior of the P cycle, such as a detailed analysis of P contents and variations therein in sedimentary cores retrieved during the Deep Sea Drilling Project and Ocean Drilling Program (Cook, 1984; Filippelli and Delaney, 1994; Föllmi, 1995). These temporal changes have been related to changes in global geochemical weathering output, which again is dependent on climate conditions and the creation of relief through orogenetic processes. Global change in the output of reactive, bioavailable P engendered important changes in the environment and life during the Earth’s history, such as shifts from oligotrophic to eutrophic ecosystems, accompanied by the widespread disappearance of reef and carbonate-platform systems and the enrollment of oceanic anoxic episodes (Hallock and Schlager, 1986; Hallock, 1987; Föllmi et al., 1994, 2006; Mort et al., 2007).

Phosphorus, eutrophication and anthropogenic impact

Recent estimates suggest that approximately 65% of the total DIP river flux to the ocean is anthropogenically derived (Harrison et al., 2005). The increased load of reactive P has a considerable impact on the environment in that the so-called dead zones without free oxygen develop and expand, and that wider zones with diminished oxygen contents are created, both in lakes as well as in marginal and open marine environments (Joos et al., 2003; Mee, 2006). Furthermore, shifts occur in the composition of ecosystems which may negatively affect oligotrophic ecosystems such as coral reefs (Hallock, 1987; Roberts et al., 2002). Some possible consequences of excessive P availability to the environment are (Föllmi, 1996):

- Development of dysaerobic to anaerobic mid-water and/or bottom-water conditions
- Severe damage to marine oligotrophic ecosystems such as coral reefs

- Limitation of marine and lacustrine benthic communities in dysaerobic bottom-water conditions
- Shorter feeding networks and simplified ecosystems
- Change to opportunistic phytoplankton species
- Increase in the intensity and frequency of “red tides,” i.e., toxic blooms of dinoflagellates and planktonic algae
- Toxic blooms of benthic cyanobacteria
- Higher rates of primary productivity
- Increased burial rates of lacustrine and marine organic matter

Summary

The strong interferences between the element P, life, and the environment render this element of particular interest. From the certainly biased view of a geologist, important recent contributions and potential pathways of future research relate to the following topics:

- The extend of the capacity of P as a limiting nutrient
- Precise quantifications of reservoir and flux rates and of residence times
- Possible interferences between present-day anthropogenic change in the P cycle and the environment
- Coupling between the P cycle and other cycles of biophile elements and molecules
- Weathering of P-containing rocks and the release of P to the biosphere
- Pathways and conditions leading to P mineralization and accumulation in sedimentary environments
- Temporal changes in the global P cycle during the Earth's history and their impact on the paleoenvironment.

Bibliography

- Anderson, L. D., Delaney, M. L., and Faul, K. L., 2001. Carbon to phosphorus ratios in sediments: implications for nutrient cycling. *Global Biogeochemical Cycles*, **15**, 65–79.
- Arthur, M. A., and Jenkyns, H. C., 1981. Phosphorites and paleoceanography. *Oceanologica Acta*, (Special Volume), 83–96.
- Baturin, G. N., 1982. Phosphorites on the sea floor. In *Developments in Sedimentology*. Amsterdam: Elsevier, Vol. 33, p. 343.
- Bentor, Y. K., 1980. Phosphorites – the unsolved problems. In Bentor, Y. K. (ed.), *Marine Phosphorites – Geochemistry, Occurrence, Genesis*. Tulsa: Society of Economic Paleontologists and Mineralogists. Special publication, 29, pp. 3–18.
- Berner, R. A., Rittenberg, K. C., Ingall, E. D., and Rao, J. L., 1993. The nature of phosphorus burial in modern marine sediments. In Wollast, R., Mackenzie, F. T., and Chou, L. (eds.), *Interactions of C, N, P and S Biochemical Cycles and Global Change*. Berlin: Springer, NATO ASI Series, 14, pp. 365–378.
- Beusen, A. H. W., Dekkers, A. L. M., Bouwman, A. F., Ludwig, W., and Harrison, J., 2005. Estimation of global river transport of sediments and associated particulate C, N, and P. *Global Biogeochemical Cycles*, **19**, GB4S05.
- Burnett, W. C., 1977. Geochemistry and origin of phosphorite deposits from off Peru and Chile. *Bulletin of the Geological Society of America*, **88**, 813–823.
- Colman, A. S., and Holland, H. D., 2000. The global diagenetic flux of phosphorus from marine sediments to the oceans: redox sensitivity and the control of atmospheric oxygen levels. In Glenn, C. R., Prévôt-Lucas, L., and Lucas, J. (eds.), *Marine Authigenesis: From Global to Microbial*. Tulsa: Society of Sedimentary Geology, SEPM special volume, 66, pp. 53–75.
- Compton, J., Mallinson, D., Glenn, C. R., Filippelli, G., Föllmi, K. B., Shields, G., and Zanin, Y., 2000. Variations in the global phosphorus cycle. In Glenn, C. R., Lucas, J., and Prévôt-Lucas, L. (eds.), *Marine Authigenesis: From Global to Microbial*. Tulsa: Society of Economic Paleontologists and Mineralogists. Special publication, 66, pp. 21–34.
- Cook, P. J., 1984. Spatial and temporal controls on the formation of phosphate deposits – a review. In Nriagu, J. O., and Moore, P. B. (eds.), *Phosphate Minerals*. Heidelberg: Springer, pp. 242–274.
- Cook, P. J., and McElhinny, M. W., 1979. A re-evaluation of the spatial and temporal distribution of sedimentary phosphate deposits in the light of plate tectonics. *Economic Geology*, **74**, 315–330.
- Delaney, M. L., 1998. Phosphorus accumulation in marine sediments and the oceanic phosphorus cycle. *Global Biogeochemical Cycles*, **12**, 563–572.
- Delwiche, C. C., and Likens, G. E., 1977. Biological response to fossil fuel combustion products. In Stumm, W. (ed.), *Global Chemical Cycles and Their Alterations by Man*. Berlin: Dahlem Konferenzen, pp. 73–88.
- Ehrlich, H. L., 1981. *Geomicrobiology*. New York: Marcel Dekker, p. 393.
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, **10**, 1–8.
- Falkowski, P. G., Greene, R. M., and Geider, R. J., 1992. Physiological limitations on phytoplankton productivity in the ocean. *Oceanography*, **5**, 84–91.
- Feely, R. A., Massoth, G. J., Baker, E. T., Cowen, J. P., Lamb, M. F., and Kroglund, K. A., 1990. The effect of hydrothermal processes on midwater phosphorus distributions in the northeast Pacific. *Earth and Planetary Science Letters*, **96**, 305–318.
- Filippelli, G. M., and Delaney, M. L., 1994. The oceanic phosphorus cycle and continental weathering during the Neogene. *Paleoceanography*, **9**, 643–652.
- Föllmi, K. B., 1995. 160 m.y. record of marine sedimentary phosphorus burial: coupling of climate and continental weathering under greenhouse and icehouse conditions. *Geology*, **23**, 859–862.
- Föllmi, K. B., 1996. The phosphorus cycle, phosphogenesis, and marine phosphate-rich deposits. *Earth-Science Reviews*, **40**, 55–124.
- Föllmi, K. B., Weissert, H., Bisping, M., and Funk, H., 1994. Phosphogenesis, carbon-isotope stratigraphy, and carbonate-platform evolution along the lower Cretaceous northern Tethyan margin. *Geological Society of America Bulletin*, **106**, 729–746.
- Föllmi, K. B., Godet, A., Bodin, S., and Linder, P., 2006. Interactions between environmental change and shallow-water carbonate build-up along the northern Tethyan margin and their impact on the early Cretaceous carbon-isotope record. *Paleoceanography*, **21**, PA4211, doi:10.1029/2006PA001313.
- Föllmi, K. B., Hosein, R., Arn, K., and Steinmann, P., 2009. Weathering and the mobility of phosphorus in the catchments and forefields of the Rhône and Oberaar glaciers, central Switzerland: implications for the global phosphorus cycle on glacial-interglacial timescales. *Geochimica et Cosmochimica Acta*, **73**, 2252–2282.

- Froelich, P. N., Bender, M. L., Luedtke, N. A., Heath, G. R., and De Vries, T., 1982. The marine phosphorus cycle. *American Journal of Science*, **282**, 474–511.
- Frossard, E., Stewart, J. W. B., and Arnaud, R. J. S., 1989. Distribution and mobility of phosphorus in grassland and forest soils of Saskatchewan. *Canadian Journal of Soil Science*, **69**, 401–416.
- Gächter, R., Meyer, J. S., and Mares, A., 1988. Contribution of bacteria to release and fixation of phosphorus in lake sediments. *Limnology and Oceanography*, **33**, 1542–1558.
- Garrison, R. E., and Kastner, M., 1990. Phosphatic sediments and rocks recovered from the Peru margin during Leg 112 of the ocean drilling program. In Suess, E., von Huene, R. et al. (eds.), *Proceedings ODP, Scientific Results*. Washington, Vol. 112, pp. 111–134.
- Glenn, C. R., Föllmi, K. B., Riggs, S. R., Baturin, G. N., Grimm, K. A., Trappe, J., Abed, A. M., Galli-Olivier, C., Garrison, R. E., Ilyin, A., Jehl, C., Rohrlach, V., Sadaqah, R., Schidlowski, M., Sheldon, R. E., and Siegmund, H., 1994a. Phosphorus and phosphorites: sedimentology and environments of formation. In Föllmi, K. B. (ed.), *Concepts and Controversies in Phosphogenesis*. *Eclogae geologicae Helvetiae*, **87**, pp. 747–788.
- Glenn, C. R., Arthur, M. A., Resig, J. M., Burnett, W. C., Dean, W. E., and Jahnke, R. A., 1994b. Are modern and ancient phosphorites really so different? In Iijima, A., Abed A. M., and Garrison R. E. (eds.), *Siliceous, Phosphatic and Glauconitic Sediments of the Tertiary and Mesozoic*. *Proceedings of the 29th International Geological Congress, Part C*. Utrecht: VSP, pp. 159–188.
- Graham, W. F., and Duce, R. A., 1979. Atmospheric pathways of the phosphorus cycle. *Geochimica et Cosmochimica Acta*, **43**, 1195–1208.
- Hallock, P., 1987. Fluctuations in the trophic resource continuum: a factor in global diversity cycles? *Paleoceanography*, **2**, 457–471.
- Hallock, P., and Schlager, W., 1986. Nutrient excess and the demise of coral reefs and carbonate platforms. *Palaios*, **1**, 389–398.
- Harrison, J. A., Seitzinger, S. P., Bouwman, A. F., Caraco, N. F., Beusen, A. H. W., and Vörösmarty, C. J., 2005. Dissolved inorganic phosphorus export to the coastal zone: results from a spatially explicit, global model. *Global Biogeochemical Cycles*, **19**, GB4S03.
- Ingall, E. D., Bustin, R. M., and Van Cappellen, P., 1993. Influences of water column anoxia on the burial and preservation of carbon and phosphorus in marine shales. *Geochimica et Cosmochimica Acta*, **57**, 303–316.
- Jahnke, R. A., Emerson, S. R., Roe, K. K., and Burnett, W. C., 1983. The present day formation of apatite in Mexican continental margin sediments. *Geochimica et Cosmochimica Acta*, **47**, 259–266.
- Jarvis, I., Burnett, W. C., Nathan, Y., Almbaydin, F., Attia, K. M., Castro, L. N., Flicoteaux, R., Hilmy, M. E., Husain, V., Qutawna, A. A., Serjani, A., and Zanin, Y. N., 1994. Phosphorite geochemistry: state-of-the-art and environmental concerns. In Föllmi, K. B. (ed.), *Concepts and Controversies in Phosphogenesis*. *Eclogae geologicae Helvetiae*, Vol. 87, pp. 643–700.
- Joos, F., Plattner, G. K., Stocker, T. F., Körtzinger, A., and Wallace, D. W. R., 2003. Trends in marine dissolved oxygen: implications for ocean circulation changes and the carbon budget. *Eos, Transactions of the American Geophysical Union*, **81**, 197–201.
- Krajewski, K. P., Van Cappellen, P., Trichet, J., Kuhn, O., Lucas, J., Martín-Algarra, A., Prévôt, L., Tewari, V. C., Gaspar, L., Knight, R. I., and Lamboy, M., 1994. Biological processes and apatite formation in sedimentary environments. In Föllmi, K. B. (ed.), *Concepts and Controversies in Phosphogenesis*. *Eclogae geologicae Helvetiae*, Vol. 87, pp. 701–745.
- Lerman, A., Mackenzie, F. T., and Garrels, R. M., 1975. Modeling of geochemical cycles: phosphorus as an example. *Memoir of the Geological Society of America*, **142**, 205–218.
- Lucas, J., and Prévôt, L., 1985. The synthesis of apatite by bacterial activity: mechanism. *Sciences Géologiques, Mémoire*, **77**, 83–92.
- Mackenzie, F. T., Ver, L. M., Sabine, C., and Lane, M., 1993. C, N, P, S Global biochemical cycles and modeling of global change. In Wollast, R., Mackenzie, F. T., and Chou, L. (eds.), *Interactions of C, N, P and S Biochemical Cycles and Global Change*. Berlin: Springer. NATO ASI Series, **14**, pp. 1–61.
- Martin, J. H., 1991. Iron, Liebig's law, and the greenhouse. *Oceanography*, **4**, 52–55.
- Mee, L., 2006. Reviving dead zones. *Scientific American*, **295/5**, 54–61.
- Meybeck, M., 1982. Carbon, nitrogen, and phosphorus transport by world rivers. *American Journal of Science*, **282**, 401–450.
- Meybeck, M., 1993. C, N, P and S in rivers: from sources to global inputs. In Wollast, R., Mackenzie, F. T., and Chou, L. (eds.), *Interactions of C, N, S and P Biochemical Cycles and Global Change*. Berlin: Springer. NATO ASI Series, **14**, pp. 163–193.
- Mort, H., Adatte, T., Föllmi, K. B., Keller, G., Steinmann, P., Matera, V., Berner, Z., and Stüben, D., 2007. Phosphorus and the roles of productivity and nutrient recycling during oceanic anoxic event 2. *Geology*, **35**, 483–486.
- Notholt, A. J. G., Sheldon, R. P., and Davidson, D. F., 1989. *Phosphate Deposits of the World: Vol. 2. Phosphate Rock Resources*. Cambridge: Cambridge University Press, p. 566.
- O'Brien, G. W., Milnes, A. R., Veeh, H. H., Heggie, D. T., Riggs, S. R., Cullen, D. J., Marshall, J. F., and Cook, P. J., 1990. Sedimentation dynamics and redox iron-cycling: controlling factors for the apatite-glaucinite association on the east Australian continental margin. In Notholt, A. J. G., and Jarvis, I. (eds.), *Phosphorite Research and Development*. London: Geological Society, Geological Society of London. Special publication, **52**, pp. 61–86.
- Redfield, A. C., 1958. The biological control of chemical factors in the environment. *American Scientist*, **46**, 205–221.
- Roberts, C. M., McClean, C. J., Veron, J. E. N., Hawkins, J. P., Allen, G. R., McAllister, D. E., Mittermeier, C. G., Schueler, F. W., Spalding, M., Wells, F., Wynne, C., and Werner, T. B., 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. *Science*, **295**, 1280–1284.
- Ruttenberg, K. C., 1993. Reassessment of the oceanic residence time of phosphorus. *Chemical Geology*, **107**, 405–409.
- Ruttenberg, K. C., 2005. The global phosphorus cycle. In Schlesinger, W. H. (ed.), *Biogeochemistry, Treatise on Geochemistry*. Amsterdam: Elsevier, Vol. 8, pp. 585–643.
- Schulz, H. N., and Schulz, H. D., 2005. Large sulfur bacteria and the formation of phosphorite. *Science*, **307**, 416–418.
- Seitzinger, S. P., Harrison, J. A., Dumont, E., Beusen, A. H. W., and Bouwman, A. F., 2005. Sources and delivery of carbon, nitrogen, and phosphorus to the coastal zone: an overview of global nutrient export from watersheds (NEWS): models and their application. *Global Biogeochemical Cycles*, **19**, GB4S01.
- Slomp, C. R., and Van Cappellen, P., 2007. The global marine phosphorus cycle: sensitivity to oceanic circulation. *Biogeosciences*, **4**, 155–171.
- Soudry, D., and Lewy, Z., 1988. Microbially influenced formation of phosphate nodules and megafossil moulds (Negev, Southern Israel). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **64**, 15–34.
- Trappe, J., 1998. Phanerozoic phosphorite depositional systems. In *Lecture Notes in Earth Sciences*. Berlin: Springer, Vol. 76, p. 316.
- Tyrrill, T., 1999. The relative influences of nitrogen and phosphorus on oceanic primary productivity. *Nature*, **400**, 525–531.

- Van Cappellen, P., and Ingall, E. D., 1994. Benthic phosphorus regeneration, net primary production, and ocean anoxia: a model of the coupled marine biogeochemical cycles of carbon and phosphorus. *Paleoceanography*, **9**, 677–692.
- Veeh, H. H., Burnett, W. C., and Soutar, A., 1973. Contemporary phosphorites on the continental margin of Peru. *Science*, **181**, 844–845.
- Walker, T. W., and Syers, J. K., 1976. The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–19.

Cross-references

Bacteria
Biogeochemical Cycles
Black Shales
Carbon Cycle
Microbial Biomining
Sediment Diagenesis – Biologically Controlled
Thiomargarita
Thiotrophic Bacteria

PHOTOSYNTHESIS

Kerstin Schmidt
Friedrich-Schiller-Universität Jena, Jena, Germany

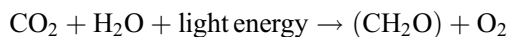
Definition

Process used by photoautotrophic organisms to combine carbon dioxide into carbohydrates utilizing the energy of sunlight.

Introduction

Photosynthesis is the most important biological process on Earth since it provides the reduced carbon and the molecular oxygen which are essential for almost all life on this planet. It is the basic metabolic reaction of organisms that contain chlorophyll where energy rich organic compounds are synthesized using the energy of sunlight. During the light-dependent reaction, the energy of the sunlight is converted into chemical energy with the help of light absorbing pigments, usually chlorophyll. This chemical energy is then used in a light-independent reaction to fix carbon dioxide and to produce carbohydrates.

The net equation of photosynthesis is:



Oxygenic photosynthesis which releases oxygen is found in green plants and cyanobacteria while anoxygenic photosynthesis is found in purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria.

Photosynthesis is almost as old as life itself. Oxygenic photosynthesis has evolved from anoxygenic photosynthesis, however there are different opinions on when this happened. Oxygenic photosynthesis was responsible for the Great Oxygenation Event about 2.300 million years ago when oxygen was released into the Earth's atmosphere, but it probably evolved at least several hundred

million years earlier (see Björn and Govindjee, 2009 for review).

Oxygenic photosynthesis

During the light-dependent reactions of oxygenic photosynthesis, light energy is absorbed by chlorophyll and converted to chemical energy in the form of ATP (adenosine triphosphate) and $\text{NADPH} + \text{H}^+$ (nicotinamide adenine dinucleotide triphosphate) which can then be used in the light-independent reactions to produce sugars from carbon dioxide. During the light reactions oxygen is released as a by-product (Figure 1a).

The net equation for the light reactions of the oxygenic photosynthesis is:



Plants and cyanobacteria, the organisms using oxygenic photosynthesis, contain two photosystems: photosystem I (PSI) and photosystem II (PSII). Each photosystem is surrounded by a light harvesting complex comprising a large number of pigments, mainly chlorophylls and carotenoids. The reaction center of PSII contains a pair of chlorophyll *a* molecules with an absorption maximum of 680 nm wavelength and is therefore called P680, while the chlorophyll *a* of PSI (P700) has an absorption maximum of 700 nm. In cyanobacteria, the photosystems are situated in the plasma membrane which is often folded to stacks called thylakoids. In plants, the thylakoid membrane can be found in the chloroplasts which are derived from cyanobacteria.

When sunlight strikes the light-harvesting complex of PSII, a photon excites an electron of a pigment molecule and the energy is passed on to the next pigment molecule until it reaches the reaction-center of PSII where an electron of the P680 pair of chlorophyll molecules is excited to a higher energy state. This electron is transferred to pheophytin, the primary electron acceptor, through charge separation. In the oxygen evolving complex, water is split into two electrons, two hydrogen ions and an oxygen atom. The electrons fill the gap in the P680 while the oxygen atom combines with another oxygen atom to form dioxygen. Meanwhile, the electron from the pheophytin is transported down an electron transport chain consisting of the electron carrier plastoquinone (PQ), a cytochrome complex (Cyt_{b_6}), and the protein plastocyanin (PC). A proton gradient, which is built up when electrons pass through the electron transport chain in thermodynamically favorable direction, is used by the enzyme ATP-synthase for ATP-synthesis via photophosphorylation, where ATP is generated by adding a phosphate group to ADP. In the reaction-center of PSI, an electron of P700 pair of chlorophyll is excited after light energy is transferred from the light-harvesting complex of this photosystem. This electron is passed on to the primary electron acceptor, a special chlorophyll molecule, while the P700 now

- Van Cappellen, P., and Ingall, E. D., 1994. Benthic phosphorus regeneration, net primary production, and ocean anoxia: a model of the coupled marine biogeochemical cycles of carbon and phosphorus. *Paleoceanography*, **9**, 677–692.
- Veeh, H. H., Burnett, W. C., and Soutar, A., 1973. Contemporary phosphorites on the continental margin of Peru. *Science*, **181**, 844–845.
- Walker, T. W., and Syers, J. K., 1976. The fate of phosphorus during pedogenesis. *Geoderma*, **15**, 1–19.

Cross-references

Bacteria
Biogeochemical Cycles
Black Shales
Carbon Cycle
Microbial Biomining
Sediment Diagenesis – Biologically Controlled
Thiomargarita
Thiotrophic Bacteria

PHOTOSYNTHESIS

Kerstin Schmidt
Friedrich-Schiller-Universität Jena, Jena, Germany

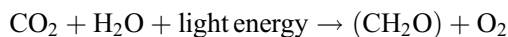
Definition

Process used by photoautotrophic organisms to combine carbon dioxide into carbohydrates utilizing the energy of sunlight.

Introduction

Photosynthesis is the most important biological process on Earth since it provides the reduced carbon and the molecular oxygen which are essential for almost all life on this planet. It is the basic metabolic reaction of organisms that contain chlorophyll where energy rich organic compounds are synthesized using the energy of sunlight. During the light-dependent reaction, the energy of the sunlight is converted into chemical energy with the help of light absorbing pigments, usually chlorophyll. This chemical energy is then used in a light-independent reaction to fix carbon dioxide and to produce carbohydrates.

The net equation of photosynthesis is:



Oxygenic photosynthesis which releases oxygen is found in green plants and cyanobacteria while anoxygenic photosynthesis is found in purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria.

Photosynthesis is almost as old as life itself. Oxygenic photosynthesis has evolved from anoxygenic photosynthesis, however there are different opinions on when this happened. Oxygenic photosynthesis was responsible for the Great Oxygenation Event about 2.300 million years ago when oxygen was released into the Earth's atmosphere, but it probably evolved at least several hundred

million years earlier (see Björn and Govindjee, 2009 for review).

Oxygenic photosynthesis

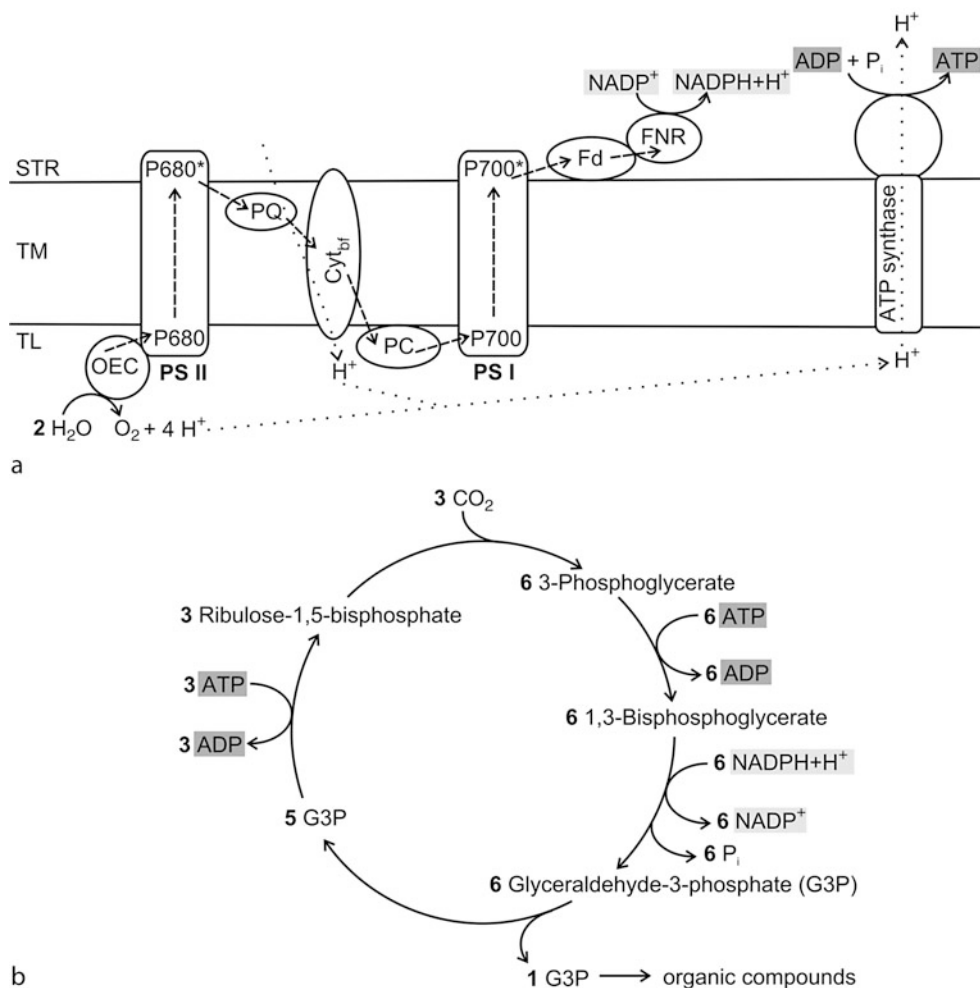
During the light-dependent reactions of oxygenic photosynthesis, light energy is absorbed by chlorophyll and converted to chemical energy in the form of ATP (adenosine triphosphate) and $\text{NADPH} + \text{H}^+$ (nicotinamide adenine dinucleotide triphosphate) which can then be used in the light-independent reactions to produce sugars from carbon dioxide. During the light reactions oxygen is released as a by-product (Figure 1a).

The net equation for the light reactions of the oxygenic photosynthesis is:



Plants and cyanobacteria, the organisms using oxygenic photosynthesis, contain two photosystems: photosystem I (PSI) and photosystem II (PSII). Each photosystem is surrounded by a light harvesting complex comprising a large number of pigments, mainly chlorophylls and carotenoids. The reaction center of PSII contains a pair of chlorophyll *a* molecules with an absorption maximum of 680 nm wavelength and is therefore called P680, while the chlorophyll *a* of PSI (P700) has an absorption maximum of 700 nm. In cyanobacteria, the photosystems are situated in the plasma membrane which is often folded to stacks called thylakoids. In plants, the thylakoid membrane can be found in the chloroplasts which are derived from cyanobacteria.

When sunlight strikes the light-harvesting complex of PSII, a photon excites an electron of a pigment molecule and the energy is passed on to the next pigment molecule until it reaches the reaction-center of PSII where an electron of the P680 pair of chlorophyll molecules is excited to a higher energy state. This electron is transferred to pheophytin, the primary electron acceptor, through charge separation. In the oxygen evolving complex, water is split into two electrons, two hydrogen ions and an oxygen atom. The electrons fill the gap in the P680 while the oxygen atom combines with another oxygen atom to form dioxygen. Meanwhile, the electron from the pheophytin is transported down an electron transport chain consisting of the electron carrier plastoquinone (PQ), a cytochrome complex (Cyt_{b_6}), and the protein plastocyanin (PC). A proton gradient, which is built up when electrons pass through the electron transport chain in thermodynamically favorable direction, is used by the enzyme ATP-synthase for ATP-synthesis via photophosphorylation, where ATP is generated by adding a phosphate group to ADP. In the reaction-center of PSI, an electron of P700 pair of chlorophyll is excited after light energy is transferred from the light-harvesting complex of this photosystem. This electron is passed on to the primary electron acceptor, a special chlorophyll molecule, while the P700 now

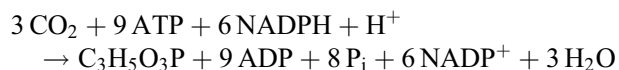


Photosynthesis, Figure 1 Oxygenic photosynthesis. (a) Simplified view of the thylakoid membrane of a chloroplast. The *dashed line* represents the electron flow through the photosystems PSII and PSI during the light reactions. *TM* thylakoid membrane, *STR* stroma, *TL* thylakoid lumen, *OEC* oxygen evolving complex, *PQ* Plastoquinone, *Cyt $_{bf}$* Cytochrome b_f , *PC* Plastocyanin, *Fd* Ferredoxin, *FNR* Ferredoxin-NADP $^+$ -oxidoreductase. (b) Calvin cycle. The regeneration steps from Glyceraldehyde-3-phosphate to Ribulose-1,5-bisphosphate are not shown.

accepts the electron that reaches the end of the electron transport chain of PSII. The excited electron of PSI is then transferred along a second electron transport chain and is then used to reduce NADP $^+$ to NADPH + H^+ . This electron transfer requires ferredoxin (Fd) and ferredoxin-NADP $^+$ -oxidoreductase (FNR; for more details on photosystems and light reactions, see, e.g., Whitmarsh and Govindjee, 1999; Lawlor, 2001; Renger, 2010).

The Calvin cycle uses the energy of ATP and the reducing power of NADPH + H^+ which are produced during the light reactions to convert carbon dioxide into carbohydrates. It is independent from sunlight and therefore often called dark reaction. The Calvin cycle takes place in the stroma of the chloroplasts of plants and in the cytoplasm of cyanobacteria.

The net equation for the light-independent reactions of the oxygenic photosynthesis is:



Carbon dioxide enters the Calvin cycle during carbon fixation, a process which is catalyzed by ribulose biphosphate carboxylase (rubisco). Each molecule of carbon dioxide is attached to a molecule of the five-carbon sugar ribulose-1,5-bisphosphate. The resulting unstable six-carbon sugar splits in two 3-phosphoglycerate molecules which then become 1,3-bisphosphoglycerate, receiving phosphate groups from ATP. With the help of NADPH + H^+ , each molecule of 1,3-bisphosphoglycerate

is then reduced to glyceraldehyde-3-phosphate which can be used to form glucose or other organic compounds. However, only one of six glyceraldehyde-3-phosphate molecules leaves the Calvin cycle while the rest is used for the regeneration of the carbon dioxide acceptor ribulose-1,5-bisphosphate which requires more ATP (Figure 1b; for more details on Calvin cycle, see, e.g., Lawlor, 2001; Heldt, 2003).

Anoxygenic photosynthesis

Anoxygenic photosynthesis, which can be found in purple bacteria, green sulfur bacteria, green non-sulfur bacteria, and heliobacteria, differs from oxygenic photosynthesis in that it requires only one photosystem and, since no splitting of water occurs, no oxygen is produced. The photosystem used by purple bacteria and green nonsulfur bacteria is similar to the PSII of plants and cyanobacteria, but contains bacteriochlorophyll instead of chlorophyll. The electron transport is cyclic since the electrons return to the reaction center after passing through a series of electron carriers. The product of the electron transport in the photosystem is ATP. Purple bacteria and green nonsulfur bacteria must oxidize inorganic or organic compounds such as H_2S , H_2 , succinate, or malate to provide the reduction potential necessary for carbon fixation. By using these compounds as electron donors, NAD^+ (nicotinamide adenine dinucleotide) is reduced to $\text{NADH} + \text{H}^+$ in a process called reverse electron transport. Purple bacteria use the Calvin cycle to fix carbon dioxide while green non-sulfur bacteria use a special process called 3-hydroxypropionate pathway.

Green sulfur bacteria and heliobacteria contain a photosystem that resembles the PSI of oxygenic photosynthetic organisms. It has a cyclic electron flow, but here NAD^+ can be reduced directly. Green sulfur bacteria use the reverse Krebs cycle to fix carbon dioxide, while heliobacteria are photoorganotrophs (for more details on anoxygenic photosynthesis, see, e.g., Whitmarsh and Govindjee, 1999; Madigan et al., 2009).

Summary

The light reactions of photosynthesis use the energy of sunlight to provide chemical energy and reducing power in the form of ATP and $\text{NADPH} + \text{H}^+$, respectively, which can then be used in the light-independent reactions to fix carbon dioxide and to convert it into carbohydrates. During the oxygenic photosynthesis of plants and cyanobacteria, water is split and dioxygen is released, whereas anoxygenic photosynthetic organisms such as purple bacteria, green sulfur bacteria, green nonsulfur bacteria, and heliobacteria do not produce oxygen.

Bibliography

Björn, L. O., and Govindjee, 2009. The evolution of photosynthesis and chloroplasts. *Current Science*, **96**, 1466–1474.
Heldt, H. W., 2003. *Pflanzenbiochemie*. Heidelberg: Spektrum Akademischer Verlag.

Lawlor, D. L., 2001. *Photosynthesis*. Oxford: BIOS Scientific Publishers.

Madigan, M. T., Martinko, J. M., Dunlap, P. V., and Clark, D. P., 2009. *Brock Biology of Microorganisms*. San Francisco: Pearson Benjamin Cummings.

Renger, G., 2010. The light reactions of photosynthesis. *Current Science*, **98**, 1305–1319.

Whitmarsh, J., and Govindjee, R., 1999. The photosynthetic process. In Singhal, G. S., Renger, G., Sopory, S. K., Irrgang, K.-D., and Govindjee, R. (eds.), *Concepts in Photobiology: Photosynthesis and Photomorphogenesis*. Boston: Kluwer, pp. 11–51.

Cross-references

Algae (Eukaryotic)
Bacteria
Banded Iron Formations
Carbon Cycle
Critical Intervals in Earth History
Cyanobacteria
Diatoms
Dinoflagellates
Endosymbiosis
Green Algae

PIEZOPHILIC BACTERIA

Jiasong Fang¹, Li Zhang²

¹Hawaii Pacific University, Kaneohe, HI, USA

²China University of Geosciences, Wuhan Hubei, China

Synonyms

Barophilic bacteria

Definition and introduction

Modern deep-sea microbiology was born in the twentieth century, thanks mainly to the efforts of Claude E. ZoBell, Frank H. Johnson, Richard Y. Morita, Holger W. Jannasch, A. Aristides Yayanos, Koki Horikoshi, and others. ZoBell and coworkers were the first group to study the effect of hydrostatic pressure on microbial activity in the deep sea (ZoBell and Johnson, 1949). They coined the term “barophile” to describe bacteria that have “a requirement for increased pressure for growth, or by increased growth at pressures higher than atmospheric pressure” (Jannasch and Taylor, 1984). Pelagic sediments taken from the Philippine Trench at more than 10,000 m (>100 MPa) contained 10^4 to 10^5 cells of viable bacteria g^{-1} wet weight of various physiological types (ZoBell and Morita, 1956) including nitrate reducers, sulfate reducers, and ammonifiers. Most of these organisms were obligate barophiles, unable to grow at atmospheric pressure (0.1 MPa).

Yayanos and his group were the first to successfully isolate a pressure-adapted bacterium (strain CNPT3) from amphipods collected from the deep sea at 5,700 m in the Pacific (Dietz and Yayanos, 1978). Strain MT41 was probably the first recognized obligate barophile isolated from a decomposing amphipod, *Hirondella gigas*, from a depth of 10,476 m of the Mariana Trench (Yayanos

- Aiello, I. W., and Fry, J. C., 2005. Deep sub-seafloor prokaryotes stimulated at interfaces over geologic time. *Nature*, **436**, 390–394.
- Tanaka, M., Ueno, A., Kawasaki, K., Yumoto, I., Ohgiya, S., Hoshino, T., Ishizaki, K., Okuyama, H., and Morita, N., 1999. Isolation of clustered genes that are notably homologous to the eicosapentaenoic acid biosynthesis gene cluster from the docosahexaenoic acid-producing bacterium *Vibrio marinus* strain MP-1. *Biotechnology Letters*, **21**, 939–945.
- Vezzi, A., et al. 2005. Life at depth: Photobacterium profundum genome sequence and expression analysis. *Science* **307**, 1459–1461.
- Wallis, J. G., Watts, J. L., and Browse, J., 2002. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends in Biological Science*, **27**, 467–473.
- Yayanos, A. A., 1986. Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. *Proceedings of the National Academy of Sciences of the USA*, **83**, 9542–9546.
- Yayanos, A. A., 1998. Empirical and theoretical aspects of life at high pressure in the deep sea. In Horikoshi, K., and Grant, W. D. (eds.), *Extremophiles, Microbial Life in Extreme Environments*. New York: Wiley, pp. 47–92.
- Yayanos, A. A., and DeLong, E. F., 1987. Deep-sea bacterial fitness to environmental temperatures and pressures. In Jannasch, H. W., Marquis, R. E., and Zimmerman, A. M. (eds.), *Current Perspectives in High Pressure Biology*. London: Academic Press, pp. 17–32.
- Yayanos, A. A., Dietz, A. S., and Van Boxtel, R., 1981. Obligately barophilic bacterium from the Mariana Trench. *Proceedings of the National Academy of Sciences of the USA*, **78**, 5212–5215.
- Yazawa, K. 1996. Production of eicosapentaenoic acid from marine bacteria. *Lipids*, **31**, S297–S300.
- ZoBell, C. E., and Johnson, F. H., 1949. The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. *Journal of Bacteriology*, **57**, 179–189.
- ZoBell, C. E., and Morita, R. Y., 1956. Barophilic bacteria in some deep sea sediments. *Journal of Bacteriology*, **73**, 563–568.

Cross-references

Bacteria
Biogeochemical Cycles
Biomarkers (Molecular Fossils)
Deep Biosphere of the Oceanic Deep Sea
Isotopes (Methods)
Terrestrial Deep Biosphere

PORE WATERS

Sabine Kasten
Alfred Wegener Institute for Polar and Marine Research,
Bremerhaven, Germany

Synonyms

Interstitial waters

Definition

The term *pore water* refers to the water contained in the interstices/pore space of aquatic sediments.

Introduction

Extracting pore waters and analyzing various dissolved constituents is a central approach in marine sediment

geochemistry, biogeochemistry, and geo(micro)biology. The ensuing pore-water concentration-depth profiles are used (a) to characterize the geochemical environment and redox zonation of aquatic sediments (Froelich et al., 1979; Berner, 1981; Postma and Jakobsen, 1996), (b) to infer and identify early diagenetic and biogeochemical processes by compounds which are either consumed or released by the particular reaction (Reeburgh, 1969; Froelich et al., 1979), (c) to calculate diffusive fluxes of dissolved constituents (Hensen et al., 2006; Zabel and Hensen, 2006), and (d) to model reaction and turnover rates of biogeochemical and diagenetic processes – including mineral dissolution and precipitation (Berner, 1980; Van Cappellen and Wang, 1995; Boudreau, 1996). Furthermore, pore-water profiles are applied to quantify the abundance of gas hydrates in marine deposits (Ussler and Paull, 2001; Bohrmann and Torres, 2006) and to study subsurface fluid flow (Saffer and Scretton, 2003).

Pore-water profiles evolve as a consequence of the complex interplay between successive microbial degradation of organic matter by various electron acceptors and a broad spectrum of secondary diagenetic reactions on the one hand and different transport processes (diffusion, advection, bioturbation, and bioirrigation) on the other hand. Due to the close coupling between geochemistry and microbiology, concentration-depth profiles of dissolved chemical species often point to “new” – i.e., to date unknown – biogeochemical processes and metabolic pathways and pave the way for the discovery of novel microorganisms or consortia of microbes which mediate the particular reactions. A well-known example is the process of anaerobic oxidation of methane by sulfate (AOM) which was already postulated to occur on the base of pore-water profiles in the late 1960s (Reeburgh, 1969). Only about 30 years later, new and advanced techniques in molecular biology ultimately were able to prove the existence of microbial consortia of archaea and sulfate-reducing bacteria mediating this process (Boetius et al., 2000; Orphan et al., 2001).

Extraction and analyses of pore waters

The classical ways of extracting pore waters are (a) by squeezers where pressure is applied either by an inert gas (e.g., nitrogen, argon) or with a hydraulic press, or (b) by centrifuging and subsequent decanting (Gieskes et al., 1991; Schlüter, 1990; De Lange et al., 1992; for a review see Schulz, 2006, and references therein). In recent years, a new technique for a rapid and more or less nondestructive collection of pore waters from marine sediments has been developed and established – the so-called rhizon sampling (Seeberg-Elverfeldt et al., 2005; Dickens et al., 2007). Rhizon samplers are thin tubes of hydrophilic porous polymer which have a minimum pore size of 0.1 µm. They are inserted into the sediment and are connected to either vacuum vials, spring-loaded syringes, or operated with peristaltic pumps which allow to

extract interstitial fluids without seriously altering the sediment structure.

Due to the often limited amount of pore water extractable – on average only a few milliliters – the analyses of individual dissolved constituents have to be performed on small sample volumes. Therefore, common methods of seawater analyses (Grasshoff et al., 1999) have been modified accordingly to allow the determination of the concentrations of nutrients and other dissolved chemical species from milliliter to submilliliter sample aliquots. A detailed overview of the techniques and instruments used in pore-water retrieval and analyses is available on <http://www.geochemie.uni-bremen.de/koelling/index.html>. For a concise introduction into the interpretation of pore-water profiles as well as the calculation of diffusive fluxes of dissolved pore-water constituents please refer to Schulz (2006).

Besides the extraction and analysis of pore water, chemical gradients at the sediment/water interface as well as in other environments characterized by a highly condensed geochemical zonation are generally determined by means of microsensors. Typical applications comprise the use of microelectrodes for oxygen, pH, and hydrogen sulfide as well as fiber-optic sensors – i.e., optodes and planar optodes – for oxygen to study and quantify transport processes as well as biogeochemical reactions in surface sediments (Archer et al., 1989; Jørgensen, 2001; Kühl and Revsbech, 2001; Wenzhöfer et al., 2001). The advantage of microelectrodes and optodes compared to the above-described pore-water sampling approaches is that they are sufficiently small to resolve steep chemical gradients and thus allow to accurately measure concentration differences and to calculate diffusive fluxes and model reaction rates. Determining chemical gradients at a high spatial resolution is of prime importance in geo(micro)biology in order to identify microenvironments and other small-scale geochemical heterogeneities as well as to determine steep concentration gradients as they typically exist in and across microbial mats and biofilms as well as at and around mineral and rock surfaces (De Beer and Kühl, 2001; Kühl, 2005; Krüger et al., 2008).

Biogeochemical processes and redox zonation

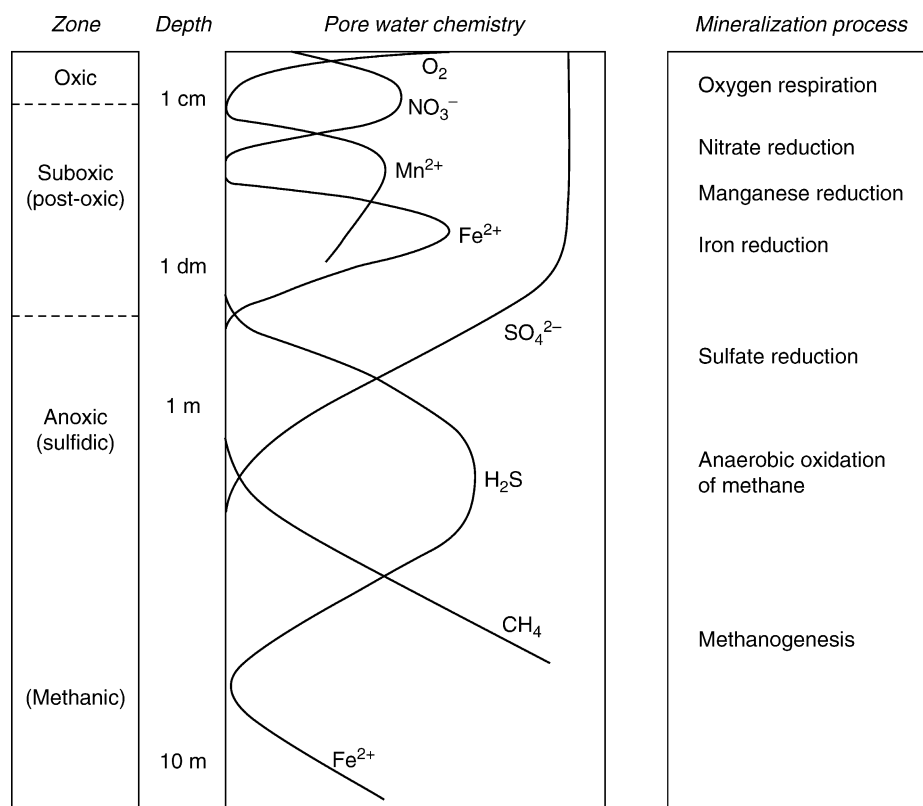
Particulate organic matter that is deposited on the seafloor is attacked by a broad range of organisms that all contribute to its degradation and gradual mineralization. At the sediment surface, macrofauna play a particularly important role by mechanically disintegrating the detritus and mixing and irrigating the upper sediment layer so that organic material becomes repeatedly exposed to oxygen. After the microbial fermentation/depolymerization of larger organic compounds into smaller organic molecules like, e.g., acetate, formate, and hydrogen microorganisms utilize a suite of external oxidants (electron acceptors) to mineralize these fermentation products (Froelich et al., 1979; Jørgensen, 2006). The different dissolved and solid

phase oxidants are used in the following order of decreasing energy yield: oxygen, nitrate, manganese (oxihydr)oxides, iron (oxihydr)oxides, and sulfate (Froelich et al., 1979). This sequence of oxidants used in the degradation of organic matter as well as the manifold secondary biotic and abiotic redox processes lead to the establishment of a typical redox or biogeochemical zonation (Froelich et al., 1979; Berner, 1981) which is shown schematically in Figure 1 together with the characteristic concentration-depth profiles of dissolved pore-water constituents. Besides marine sediments a similar segregation of electron-accepting processes in separate zones during the degradation of organic material is also found in lacustrine deposits as well as in groundwater environments.

It should be emphasized that the scheme depicted in Figure 1 is of course simplified and that in natural aquatic sediments the different redox processes in the degradation of organic matter can considerably overlap or the redox zones can even be reversed depending on the availability, abundance, and reactivity of the particular electron acceptors (Postma and Jakobsen, 1996). In any case, the evolving biogeochemical zonation – being itself significantly shaped by microbial metabolism (and activity of macrofauna at the sediment surface) – at the same time exerts a strong control on a broad spectrum of processes like (a) colonization by macro- and microorganisms, (b) different degrees of selective degradation and preservation of organic compounds (including paleoceanographic proxies) under oxic, anoxic or sulfidic conditions (Versteegh and Zonneveld, 2000), as well as (c) processes of mineral dissolution and precipitation (Torres et al., 1996; Peckmann et al., 2001; Kasten et al., 2003).

Mathematical modeling of pore-water profiles

Due to the fact that pore-water profiles are influenced by the sum of all reactions, process rates directly calculated from pore-water profiles may be highly inaccurate and always represent *net* rates. The development of numerical models that couple transport processes and (bio)geochemical reactions has therefore been a major advance that allows to more comprehensively interpret sediment pore-water profiles (Berner, 1980; Van Cappellen and Wang, 1995, 1996; Boudreau, 1996, 1997; Soetaert et al., 1996). Such early diagenetic reaction-transport models have been applied on various sediment depth scales, reaching from the simulation of processes close to the sediment surface like calcite dissolution and the cycling of iron and manganese (Van Cappellen and Wang, 1995, 1996; Adler et al., 2001; Pfeifer et al., 2002; Jourabchi et al., 2008) down to reactions occurring at and below the sulfate/methane transition zone in more deeply buried deposits (Riedinger et al., 2005; Wallmann et al., 2006). Reaction-transport models generally include a network of primary and secondary redox reactions as well as different transport processes and are fitted to the measured pore-water profiles. In this



Pore Waters, Figure 1 Schematic representation of the biogeochemical zonation in marine sediments. The names of the main zones were proposed by Froelich et al. (1979) and Berner (1981, in parenthesis). The depth scale is quasi-logarithmic; the exact depths, however, vary strongly and increase from the shelf to the deep sea. The pore-water chemistry shows relevant dissolved species. Peak heights and concentration scales are arbitrary. The chemical profiles reflect the depth sequence of the dominant mineralization processes through which organic matter is oxidized to CO_2 . (Modified from Froelich et al., 1979; Jørgensen and Kasten, 2006, with permission.)

way, reaction rates can be obtained which can, however, differ from *gross* reactions rates.

In the case of sulfate reduction, *gross* rates can be measured by experimental incubation of sediment samples after addition of radioactively labeled sulfate ($^{35}SO_4^{2-}$) [for reviews see Jørgensen (2006) and Jørgensen and Kasten (2006)]. While this experimental technique is optimal to determine process rates in shallow and highly dynamic surface sediments, a modeling approach is generally preferred in more deeply buried sediments (Jørgensen, 2006).

Conclusion

Pore-water profiles represent a powerful tool to determine the variations in redox and biogeochemical conditions over sediment depth which is a prerequisite for the identification and quantification of early diagenetic influences on the geological record and for an accurate interpretation of paleoceanographic proxies. Furthermore, chemical gradients in the pore water of aquatic sediments are used to infer and identify biogeochemical reactions and to model process rates. However, it should be emphasized that due to the fact that pore-water profiles result from the

interactive effects of production and consumption of multiple species during biogeochemical processes certain reaction products are not always detectable in pore water, although the particular reaction proceeds at high rates. For the same reason care has to be taken when process rates are derived from modeling of pore-water profiles. In general, numerical modeling has its strength in deep sediments while experimental measurements are more suitable to determine *gross* process rates in surface sediments.

Bibliography

- Adler, M., Hensen, C., Wenzhöfer, F., Pfeifer, K., and Schulz, H. D., 2001. Modeling of calcite dissolution by oxic respiration in supralysoclineal deep-sea sediments. *Marine Geology*, **177**, 167–189.
- Archer, D., Emerson, S., and Smith, C. R., 1989. Direct measurement of the diffusive sublayer at the deep sea floor using oxygen microelectrodes. *Nature*, **340**, 623–626.
- Berner, R. A., 1980. *Early Diagenesis: A Theoretical Approach*. Princeton: Princeton University Press, 241 pp.
- Berner, R. A., 1981. A new geochemical classification of sedimentary environments. *Journal of Sedimentary Petrology*, **51**, 359–365.

- Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R., Jørgensen, B. B., Witte, U., and Pfannkuche, O., 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature*, **407**, 623–626.
- Bohrmann, G., and Torres, M., 2006. Gas hydrates in marine sediments. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 481–512.
- Boudreau, B. P., 1996. A method-of-lines code for nutrient diagenesis in aquatic sediments. *Computers and Geosciences*, **22**, 479–496.
- Boudreau, B. P., 1997. *Diagenetic Models and Their Implementation: Modelling Transport and Reactions in Aquatic Sediments*. Berlin: Springer, 414 p.
- De Beer, D., and Kühl, M., 2001. Interfacial microbial mats and biofilms. In Boudreau, B. B., and Jørgensen, B. B. (eds.), *The Benthic Boundary Layer*. Oxford: Oxford University Press, pp. 374–394.
- De Lange, G. J., Cranston, R. E., Hydes, D. H., and Boust, D., 1992. Extraction of pore water from marine sediments: a review of possible artifacts with pertinent examples from the North Atlantic. *Marine Geology*, **109**, 53–76.
- Dickens, G., Kölling, M., Smith, D. C., Schnieders, L., and IODP Expedition 302 Scientists, 2007. Rhizon sampling of pore waters on scientific drilling expeditions: an example from the IODP Expedition 302, Arctic Coring Expedition (ACEX). *Scientific Drilling*, **4**, 22–25.
- Froelich, P. N., Klinkhammer, G. P., Bender, M. L., Luedtke, N. A., Heath, G. R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., and Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochimica et Cosmochimica Acta*, **43**, 1075–1090.
- Gieskes, J. M., Gamo, T., and Brumsack, H., 1991. Chemical methods for interstitial water analysis aboard *JOIDES Resolution*. ODP Tech. Note, 15.
- Grasshoff, K., Kremling, K., and Ehrhardt, M., 1999. *Methods of Seawater Analysis*, 2nd edn. Weinheim: Wiley-VCH, 600 p.
- Hensen, C., Zabel, M., and Schulz, H. N., 2006. Benthic cycling of oxygen, nitrogen and phosphorus. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 207–240.
- Jørgensen, B. B., 2001. Life in the diffusive boundary layer. In Boudreau, B. B., and Jørgensen, B. B. (eds.), *The Benthic Boundary Layer*. Oxford: Oxford University Press, pp. 348–373.
- Jørgensen, B. B., 2006. Bacteria and marine biogeochemistry. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*. Heidelberg: Springer, 2nd revised, updated and extended edition, pp. 169–206.
- Jørgensen, B. B., and Kasten, S., 2006. Sulfur cycling and methane oxidation. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 271–309.
- Jourabchi, P., Meile, C., Pasion, L. R., Van Cappellen, P., 2008. Quantitative interpretation of pore water O₂ and pH distributions in deep-sea sediments. *Geochimica et Cosmochimica Acta*, **72**, 1350–1364.
- Kasten, S., Zabel, M., Heuer, V., and Hensen, C., 2003. Processes and signals of nonsteady-state diagenesis in deep-sea sediments and their pore waters. In Wefer, G., Mulitza, S., and Ratmeyer, V. (eds.), *The South Atlantic in the Late Quaternary: Reconstruction of Material Budget and Current Systems*. Heidelberg: Springer, pp. 431–459.
- Krüger, M., Blumenberg, M., Kasten, S., Wieland, A., Känel, L., Klock, J.-H., Michaelis, W., and Seifert, R., 2008. A novel, multi-layered methanotrophic microbial mat system growing on the sediment of the Black Sea. *Environmental Microbiology*, **10**, 1934–1947.
- Kühl, M., 2005. Optical microsensors for analysis of microbial communities. *Methods in Enzymology*, **397**, 166–199.
- Kühl, M., and Revsbech, N. P., 2001. Biogeochemical microsensors for boundary layer studies. In Boudreau, B. B., and Jørgensen, B. B. (eds.), *The Benthic Boundary Layer*. Oxford: Oxford University Press, pp. 180–210.
- Orphan, V. J., House, C. H., Hinrichs, K.-U., McKeegan, K. D., and DeLong, E. F., 2001. Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. *Science*, **293**, 484–487.
- Peckmann, J., Reimer, A., Luth, U., Luth, C., Hansen, B. T., Heinicke, C., Hoefs, J., and Reitner, J., 2001. Methane-derived carbonates and authigenic pyrite from the northwestern Black Sea. *Marine Geology*, **177**, 129–150.
- Pfeifer, K., Hensen, C., Adler, M., Wenzhöfer, F., Weber, A., and Schulz, H. D., 2002. Modeling of subsurface calcite dissolution, including the respiration and reoxidation processes of marine sediments in the region of equatorial upwelling off Gabon. *Geochimica et Cosmochimica Acta*, **66**, 780–799.
- Postma, D., and Jakobsen, R., 1996. Redox zonation: equilibrium constraints on the Fe(III)/SO₄-reduction interface. *Geochimica et Cosmochimica Acta*, **60**, 3169–3175.
- Reeburgh, W. S., 1969. Observations of gases in Chesapeake Bay sediments. *Limnology and Oceanography*, **14**, 368–375.
- Riedinger, N., Pfeifer, K., Kasten, S., Garming, J. F. L., Vogt, C., and Hensen, C., 2005. Diagenetic alteration of magnetic signals by anaerobic oxidation of methane related to a change in sedimentation rate. *Geochimica et Cosmochimica Acta*, **69**, 4117–4126.
- Saffer, D. M., and Screatton, E. J., 2003. Fluid flow at the toe of convergent margins; interpretation of sharp pore-water geochemical gradients. *Earth and Planetary Science Letters*, **213**, 261–270.
- Schlüter, M., 1990. Zur Frühdiagenese von organischem Kohlenstoff und Opal in Sedimenten des südlichen und östlichen Weddellmeeres. *Berichte zur Polarforschung, Bremerhaven*, **73**, 156.
- Schulz, H. D., 2006. Quantification of early diagenesis: dissolved constituents in pore water and signals in the solid phase. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 73–124.
- Seeborg-Elverfeldt, J., Schlüter, M., Feseker, T., and Kölling, M., 2005. Rhizon sampling of pore waters near the sediment-water interface of aquatic systems. *Limnology and Oceanography: Methods*, **3**, 361–371.
- Soetaert, K., Herman, P. M. J., and Middelburg, J. J., 1996. A model of early diagenetic processes from the shelf to abyssal depths. *Geochimica et Cosmochimica Acta*, **60**, 1019–1040.
- Torres, M. E., Brumsack, H. J., Bohrmann, G., and Emeis, K. C., 1996. Barite fronts in continental margin sediments: a new look at barium remobilization in the zone of sulphate reduction and formation of heavy barites in diagenetic fronts. *Chemical Geology*, **127**, 125–139.
- Ussler, W. III, and Paull, C., 2001. Ion exclusion associated with marine gas hydrate deposits. In Paull, C., and Dillon, W. P. (eds.), *Natural Gas Hydrates – Occurrence, Distribution, and Detection*. Geophysical Monograph. Washington: AGU, Vol. 124, pp. 41–51.
- Van Cappellen, P., and Wang, Y., 1995. Metal cycling in surface sediments: modelling the interplay of transport and reaction. In Allen, H. E. (ed.), *Metal Contaminated Sediments*. Chelsea: Ann Arbor Press, pp. 21–62.
- Van Cappellen, P., and Wang, Y., 1996. Cycling of iron and manganese in surface sediments: a general theory for the coupled

- transport and reaction of carbon, oxygen, nitrogen, sulfur, iron and manganese. *American Journal of Science*, **296**, 197–243.
- Versteegh, G. J. M., and Zonneveld, K. A. F., 2002. Use of selective degradation to separate preservation from productivity. *Geology*, **30**, 615–618.
- Wallmann, K., Aloisi, G., Haeckel, M., Obzhairov, A., Pavlova, G., and Tishchenko, P., 2006. Kinetics of organic matter degradation, microbial methane generation, and gas hydrate formation in anoxic marine sediments. *Geochimica et Cosmochimica Acta*, **70**, 3905–3927.
- Wenzhöfer, F., Adler, M., Kohls, O., Hensen, C., Strotmann, B., Boehme, S., and Schulz, H. D., 2001. Calcite dissolution driven by benthic mineralization in the deep sea: in situ measurements of Ca^{2+} , pH, pCO_2 , and O_2 . *Geochimica et Cosmochimica Acta*, **65**, 2677–2690.
- Zabel, M., and Hensen, C., 2006. Quantification and regionalization of benthic reflux. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 429–456.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Carbon Cycle
 Carbon (Organic, Degradation)
 Microbial Degradation (of Organic Matter)
 Microsensors for Sediments, Microbial Mats, and Biofilms
 Sediment Diagenesis – Biologically Controlled
 Sulfate-Reducing Bacteria
 Sulfur Cycle

PROTOZOA (HETEROTROPH, EUKARYOTIC)

Jens Boenigk
 Austrian Academy of Sciences, Mondsee, Austria

Synonyms

Protist (pro parte); Protoctista (pro parte)

Definition

Protozoa (historical) Animals that can be reduced to the status of one cell.

Protozoa (modern) All eukaryotes after exclusion of the derived kingdoms such as Animalia, Fungi, Plantae, and Chromista.

Introduction

Protozoa are a loose grouping of organisms with similar (usually unicellular) organization and heterotrophic mode of nutrition. They are found in most soils, fresh water, and oceans. As components of the micro- and meiofauna, Protozoa are important consumers of bacteria and an important food source for microinvertebrates. While most are solitary individuals, various colonial forms exist. The taxonomic relationships of protozoans to one another and to other protists continue to be revised. Most are free-living, but some are commensalistic, mutualistic, or parasitic. Pathogenic Protozoa include species of

Plasmodium, the cause of human malaria, *Trypanosoma gambiense*, the cause of African trypanosomiasis, and *Entamoeba histolytica*, the cause of amebic dysentery. The smallest known protozoans are tiny blood parasites less than 2 microns long; the largest may be 16 mm long and visible to the naked eye. The cells show all typical features of a eukaryotic cell. Protozoan shapes vary, but all share such eukaryotic features as lipid–protein membranes and membrane-enclosed vacuoles and organelles. Ultrastructure as well as biochemical cell composition varies a lot due to the broad diversity of these organisms. They also show wide variation in modes of movement, nutrition, and reproduction. Reproduction is usually asexual, occurring mostly by cell division, or binary fission; some forms reproduce asexually by budding or by the formation of spores (reproductive cells that give rise to a new organism without fertilization). In several groups sexual reproduction also occurs.

The number of protozoan species is controversial. Only less than 10% of the species are described and the theoretical basis, that is, the species concept, is also controversial. The most widely used biological species concept is hardly applicable to Protozoa as reproductive isolation, which is the basis of the biological species concept, is rarely investigated for most taxa. In practice, protozoan species were largely defined based on morphological characteristics. However, the degree of molecular similarity between sexual conspecifics is increasingly used as a yardstick for asexual ones from comparable evolutionary lineages. For an increasing number of morphologically described species (morphospecies) molecular data demonstrate an insufficient taxon resolution. Consequently, the species number estimates are increasing. Current estimates of species numbers range from several hundreds of thousands to several million with around 70,000 described species. Further, environmental molecular surveys mostly based on the 18S rRNA gene demonstrate that only a minority of sequences is known and the species number estimates based on morphological analyses may underestimate the diversity by orders of magnitude.

Megasystematics and nomenclature

During the period of protistology, that is, from the first descriptions of microorganisms by van Leeuwenhoek in 1674 to date, the classification of Protozoa changed several times depending on the available methodology. Motility was the main or only criterion for the first observers to place these organisms within the animal kingdom; any signs of internal organization were also considered sufficient proof of the animal nature of these organisms. From the mid-nineteenth century onward, the mode of nutrition, that is, heterotrophy and specifically particulate food uptake versus autotrophy, became the dominating criterion in differentiating Protozoa (animals) from Protophyta (plants). The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. In consequence, alternative concepts arose, which shall be briefly introduced, starting with the historic context of the

E

EARLY EARTH

See entries “*Origin of Life*,” “*Salinity History of the Earth’s Ocean*,” and “*Critical Intervals in Earth History*.”

EARLY PRECAMBRIAN EUKARYOTES

Joachim Reitner
University of Göttingen, Göttingen, Germany

Definition

Eukaryotes are probably the result of endosymbiosis, and basically three types exist. The basic ones are the simplest eukaryotic cells with a nucleus only (*Giardia*) (Graczk, 2005). Secondly, protists have mitochondria, which have been proposed to have an alpha-proteobacterial origin (Sagan (Margulis), 1967). Finally, beside mitochondria, cells of eukaryotic algae contain chloroplasts, former single-celled cyanobacteria achieved via endosymbiosis (Mereschkowsky, 1905; Wallin, 1920; Sagan (Margulis), 1967). The geological record of protists begins long before the Ediacaran vendobionts. Possibly stem group protists were found in ca. 3 Ga-old rocks (Javaux et al., 2010). Within the Late Mesoproterozoic rocks, the divergence of major eukaryotic clades is observed. Beside the fossil record of early eukaryotes, related biomarkers (steranes) were found in up to 2.7 Ga-old rocks, which may support the early origin of eukaryotes before the Great Oxidation Event (Brocks et al., 1999; Brocks et al., 2003a, b). However, the syngeneity of the biomarkers used for the interpretation has recently been questioned (Rasmussen et al., 2008) and the first appearance of eukaryotic biomarkers is still disputed (Brocks

et al., 1999; George et al., 2008; Rasmussen et al., 2008; Waldbauer et al., 2009).

The oldest known single-celled eukaryotes are acritarchs, probably ancestors of dinoflagellates. Acritarchs played a significant role in Proterozoic oceans, and the first record comes from the 3.2 Ga-old Moodies Group of South Africa (Javaux et al., 2010). This finding is surprising, since eukaryotic metabolisms normally need oxygen, and the Great Oxidation Event took place about 700 My later, between 2.32 and 2.45 Ga. A similar discrepancy emerged from the first appearance of eukaryotic biomarkers in 2.7 Ga-old rocks (Brocks et al., 1999). Although often controversially debated, biomarker records may give excellent indications to the diversification of major phylogenetic clades. For instance, the distribution of 24-isopropylcholestane revealed a high abundance of (demo-)sponges in the Cryogenian (McCaffrey et al., 1994; Love et al., 2009), thus suggesting sufficient oxygen in shelf regions already 100 My prior to the Cambrian explosion. Furthermore, early Precambrian eukaryotic life is not only indicated by biomarker findings. In some diagenetically early silicified stromatolites, well-preserved eukaryotic cells have been described. The best material is known from the 1.88 Gy-old Gunflint Chert (Ontario, Canada) (Fralick et al., 2002; Awramik and Barghoorn, 1977). *Eosphaera tyleri* from these cherts is probably an eukaryotic cell (Kazmierczak, 1979). Beside the single-celled eukaryotes, few larger fossils which are most likely multicellular organisms are known, such as *Grypania spiralis* from the Mesoproterozoic Gaoyuzhuang Formation (China) and disk-shaped centimeter-sized organisms like *Chuaria* (Knoll et al., 2006).

In the Cryogenian and Ediacaran, the diversity of eukaryotes, including fungi and lichens, increased significantly. It is obvious that the increase of diversity and size

is related with the Snowball Earth scenarios and increase of oxygen in the atmosphere.

Bibliography

- Awramik, S. M., and Barghoorn, E. S., 1997. The Gunflint microbiota. *Precambrian Research*, **5**, 121–142.
- Brocks, J. J., Summons, R. E., Logan, G. A., and Buick, R., 2003a. Molecular fossils in Archean rocks II: composition, thermal maturity and integrity of hydrocarbons extracted from sedimentary rocks of the 2.78–2.45 billion years old Mount Bruce Supergroup, Pilbara Craton, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4289–4319.
- Brocks, J. J., Buick, R., Summons, R. E., and Logan, G. A., 2003b. A reconstruction of Archean biological diversity based on molecular fossils from the 2.78–2.45 billion year old Mount Bruce Supergroup, Hamersley Basin, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4321–4335.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of Eukaryotes. *Science*, **285**, 1033–1036.
- Fralick, P., Davis, D. W., and Kissin, S. A., 2002. The age of the Gunflint Formation, Ontario, Canada: single zircon U–Pb age determinations from reworked volcanic ash. *Canadian Journal of Earth Science*, **39**, 1085–1091.
- George, S. C., Volk, H., Dutkiewicz, A., Ridley, J., and Buick, R., 2008. Preservation of hydrocarbons and biomarkers in oil trapped inside fluid inclusions for >2 billion years. *Geochimica et Cosmochimica Acta*, **72**, 844–870.
- Graczyk, T. K., 2005. Is Giardia a living fossil? *Trends in Parasitology*, **21**, 104–107.
- Javaux, E., Marshall, C. P., and Bekker, A., 2010. Organic-walled microfossils in 3.2-billion-year-old shallow-marine siliciclastic deposits. *Nature*, **463**, 934–938.
- Kazmierczak, J., 1979. The eukaryotic nature of Eosphaera-like feriferous structures from the Precambrian Gunflint Iron Formation, Canada: a comparative study. *Precambrian Research*, **9**, 1–22.
- Knoll, A. H., Javaux, E. J., Hewitt, D. and Cohen, P., 2006. Eukaryotic organisms in Proterozoic oceans. Philosophical Transactions of the Royal Society of London B, **361**, 1023–1038.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–721.
- MaCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Palaeoenvironmental implications of novel C30 steranes in Precambrian to Cenozoic age petroleum and bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Mereschkowski, C., 1905. Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biologisches Centralblatt*, **25**, 593–604.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J., and Kilburn, M. R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, **455**, 1101–1104.
- Sagan, L. (Margulis, L.) 1967. On the origin of mitosing cells. *Journal of Theoretical Biology*, **14**(3), 255–274.
- Wallin, I. E., 1923. The Mitochondria Problem. *The American Naturalist*, **57**(650), 255–261.
- Waldbauer, J. R., Sherman, L. S., Sumner, D. Y., and Summons, R. E., 2009. Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis. *Precambrian Research*, **169**, 28–47.

Cross-references

Algae (Eukaryotic)
 Biomarkers (Molecular Fossils)
 Critical Intervals in Earth History
 Ediacara Biota
 Geomycology
 Lateral Gene Transfer
 Origin of Metazoa
 Protozoa (Heterotroph, Eukaryotic)
 Sponges (Porifera) and Sponge Microbes
 Symbiosis

ECOLOGICAL NICHE

Different from the habitat (see entry *Habitat*) (spatial), the niche defines the function of an organism. See entry “*Microbial Communities, Structure, and Function*” for further reading.

EDIACARAN BIOTA

Dmitriy Grazhdankin
 Russian Academy of Sciences, Novosibirsk, Russia

Definition

Ediacaran period: An interval in the history of earth after the Marinoan/Varanger glaciation of the Neoproterozoic Era (see Chapter *Snowball Earth*), but before the Cambrian radiations. This interval marks the introduction of complex macroscopic organisms, leading to a revolution in the structure and evolution of marine paleocommunities, including the establishment of multi-level trophic structures, coevolutionary predator–prey interactions and infauunal activity.

Ediacaran biota: A highly distinctive assemblage of Ediacaran-age macroscopic organisms preserved as casts and moulds in siliciclastic, volcanoclastic, and carbonate sediments.

Introduction

Ediacaran biota is a heterogeneous assemblage representing the earliest known communities of macroscopic organisms. Diverse Ediacaran fossil assemblages are coeval with the earliest phosphatized metazoan embryos (Xiao et al., 1998; but see Bailey et al., 2007; Xiao et al., 2007), the earliest undisputed traces of metazoan activities (Jensen, 2003), and the time of eumetazoan crown-group divergence estimated by some (but not all) molecular clocks (Peterson and Butterfield, 2005). Nonetheless, placing the Ediacaran morphologies into a metazoan phylogeny has been an enduring challenge to paleobiologists (Gehling, 1991; Fedonkin, 1992; Jenkins,

- transport and reaction of carbon, oxygen, nitrogen, sulfur, iron and manganese. *American Journal of Science*, **296**, 197–243.
- Versteegh, G. J. M., and Zonneveld, K. A. F., 2002. Use of selective degradation to separate preservation from productivity. *Geology*, **30**, 615–618.
- Wallmann, K., Aloisi, G., Haeckel, M., Obzhairov, A., Pavlova, G., and Tishchenko, P., 2006. Kinetics of organic matter degradation, microbial methane generation, and gas hydrate formation in anoxic marine sediments. *Geochimica et Cosmochimica Acta*, **70**, 3905–3927.
- Wenzhöfer, F., Adler, M., Kohls, O., Hensen, C., Strotmann, B., Boehme, S., and Schulz, H. D., 2001. Calcite dissolution driven by benthic mineralization in the deep sea: in situ measurements of Ca^{2+} , pH, pCO_2 , and O_2 . *Geochimica et Cosmochimica Acta*, **65**, 2677–2690.
- Zabel, M., and Hensen, C., 2006. Quantification and regionalization of benthic reflux. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*, 2nd revised, updated and extended edition. Heidelberg: Springer, pp. 429–456.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Carbon Cycle
 Carbon (Organic, Degradation)
 Microbial Degradation (of Organic Matter)
 Microsensors for Sediments, Microbial Mats, and Biofilms
 Sediment Diagenesis – Biologically Controlled
 Sulfate-Reducing Bacteria
 Sulfur Cycle

PROTOZOA (HETEROTROPH, EUKARYOTIC)

Jens Boenigk
 Austrian Academy of Sciences, Mondsee, Austria

Synonyms

Protist (pro parte); Protoctista (pro parte)

Definition

Protozoa (historical) Animals that can be reduced to the status of one cell.

Protozoa (modern) All eukaryotes after exclusion of the derived kingdoms such as Animalia, Fungi, Plantae, and Chromista.

Introduction

Protozoa are a loose grouping of organisms with similar (usually unicellular) organization and heterotrophic mode of nutrition. They are found in most soils, fresh water, and oceans. As components of the micro- and meiofauna, Protozoa are important consumers of bacteria and an important food source for microinvertebrates. While most are solitary individuals, various colonial forms exist. The taxonomic relationships of protozoans to one another and to other protists continue to be revised. Most are free-living, but some are commensalistic, mutualistic, or parasitic. Pathogenic Protozoa include species of

Plasmodium, the cause of human malaria, *Trypanosoma gambiense*, the cause of African trypanosomiasis, and *Entamoeba histolytica*, the cause of amebic dysentery. The smallest known protozoans are tiny blood parasites less than 2 microns long; the largest may be 16 mm long and visible to the naked eye. The cells show all typical features of a eukaryotic cell. Protozoan shapes vary, but all share such eukaryotic features as lipid–protein membranes and membrane-enclosed vacuoles and organelles. Ultrastructure as well as biochemical cell composition varies a lot due to the broad diversity of these organisms. They also show wide variation in modes of movement, nutrition, and reproduction. Reproduction is usually asexual, occurring mostly by cell division, or binary fission; some forms reproduce asexually by budding or by the formation of spores (reproductive cells that give rise to a new organism without fertilization). In several groups sexual reproduction also occurs.

The number of protozoan species is controversial. Only less than 10% of the species are described and the theoretical basis, that is, the species concept, is also controversial. The most widely used biological species concept is hardly applicable to Protozoa as reproductive isolation, which is the basis of the biological species concept, is rarely investigated for most taxa. In practice, protozoan species were largely defined based on morphological characteristics. However, the degree of molecular similarity between sexual conspecifics is increasingly used as a yardstick for asexual ones from comparable evolutionary lineages. For an increasing number of morphologically described species (morphospecies) molecular data demonstrate an insufficient taxon resolution. Consequently, the species number estimates are increasing. Current estimates of species numbers range from several hundreds of thousands to several million with around 70,000 described species. Further, environmental molecular surveys mostly based on the 18S rRNA gene demonstrate that only a minority of sequences is known and the species number estimates based on morphological analyses may underestimate the diversity by orders of magnitude.

Megasystematics and nomenclature

During the period of protistology, that is, from the first descriptions of microorganisms by van Leeuwenhoek in 1674 to date, the classification of Protozoa changed several times depending on the available methodology. Motility was the main or only criterion for the first observers to place these organisms within the animal kingdom; any signs of internal organization were also considered sufficient proof of the animal nature of these organisms. From the mid-nineteenth century onward, the mode of nutrition, that is, heterotrophy and specifically particulate food uptake versus autotrophy, became the dominating criterion in differentiating Protozoa (animals) from Protophyta (plants). The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. In consequence, alternative concepts arose, which shall be briefly introduced, starting with the historic context of the

definitions of the terms Protozoa, Protista, and Protoctista, which are often synonymously used despite the different conceptual background (Scarmadella, 1999).

Based on the German term “Urthiere” (von Oken, 1805) Georg August Goldfuß introduced the class **Protozoa** (first, or early animals) in 1820 for a class of organisms within Kingdom Animalia that consisted of Infusoria (called ciliates today), Lithozoa (corals), Phytozoa (e.g., Cryptomonas), and Medusinae. The term implies that these organisms are animals or animal-like and have been separated from the plant-like protophytes. After the formulation of the cell theory, Carl Theodor von Siebold defined in 1845 the Protozoa as animals that can be reduced to the status of a cell and subdivided his Phylum into the classes Infusoria (ciliates) and Rhizopoda (amoebae, foraminifera). In 1858, Richard Owen referred to this grouping as Kingdom Protozoa and defined the group by their lack of a certain number of characters, or “superadditions,” of true plants and animals. Since molecular systematics increasingly demonstrates a close relationship between phototrophic and phagotrophic microeukaryotes the term Protozoa was increasingly replaced by Protista (see below). Still, the term Protozoa is used by some scientists as a paraphyletic basal kingdom, that is, comprising the Amoebozoa, Choanoflagellates, Rhizaria, Excavata, and Alveolata (Cavalier-Smith, 2003).

Based on the problems to distinguish the Protozoa from the Protophyta, John Hogg proposed the term **Protoctista** in 1861 as a grouping of organisms having the common characters of both plants and animals. Hogg’s term “Protoctista” was expressive of organisms that came first in evolutionary time. What is significant in Hogg’s depiction of the Protoctista is that plants and animals share a common ancestry from the Protoctista. Protoctista are those microscopic and macroscopic eukaryotes that remain after exclusion of all animals developing from a blastula, all plants developing from embryonic stages, and all higher fungi without a flagellate stage in their life cycles (Margulis et al., 1990).

Agreeing with the erratic separation of Protozoa and Protophyta but disagreeing with Hogg’s view of Protoctista being the evolutionary first beings, Haeckel considered that these organisms evolved independent of the lineages of the animal and plant kingdoms. In 1866, he proposed the term **Protista** as a boundary kingdom intermediate between the animal and vegetable kingdoms containing organisms neither animals nor plants. According to the various definitions given by Haeckel himself, bacteria were Protista as well but Infusoria (ciliates) were placed in Kingdom Animalia as he (and other investigators) considered them multicellular animals. In the modern sense, the Protista embraces all eukaryotes that lack tissues, that is, a loose grouping of 30 or 40 disparate phyla with diverse combinations of trophic modes, mechanisms of motility, cell coverings, and life cycles.

In an ecological or functional context, Protozoa ($\approx$ phagotrophic protists) still is a meaningful term but due to the conceptual problems increasingly replaced by

the term protists or by applying narrower functional groups such as heterotrophic flagellates, amoebae, and ciliates. The major lineages of phagotrophic protists are (see Adl et al., 2005):

- Amoebozoa L  he, 1913, emend. Cavalier-Smith, 1998, that is, organisms with amoeboid locomotion generally with noneruptive morphologically variable pseudopodia (lobopodia). Flagellate stages may sometimes occur usually with a single flagellum. Note that several groups of amoeboid protists do not belong to the Amoebozoa.
- Choanomonada Kent, 1880 (choanoflagellates), that is, phagotrophic with collar of microvilli around a single flagellum. This group is traditionally divided into three groups based on the presence or absence of a cellulose theca or lorica of siliceous strips.
- Rhizaria Cavalier-Smith, 2002, that is, cells with fine pseudopodia (filopodia) varying as simple, branching, anastomosing, or supported by microtubules (axopodia). This group includes ecologically important benthic and soil protists such as the usually amoeboid and/or biflagellated Cercozoa and well as the palaeoecologically important Foraminifera and Radiolaria.
- Alveolata Cavalier-Smith, 1991, that is, protists with cortical alveolae. This group comprises the ciliates (Ciliophora Doflein 1901), the parasitic Apicomplexa including the malaria-parasite Plasmodium, and the Dinoflagellates.
- Several lineages within the Stramenopiles ($\rightarrow$ algae), which secondarily lost the plastid or reduced the plastid to a leucoplast. Important lineages are, for instance, the Bicosoecida and the chrysomonads (= chrysophytes). These lineages are closely related to major algal groups such as the (see entry *Diatoms*) (Bacillariophyceae) and the brown algae (Phaeophyceae).
- Cryptophyceae Pascher 1913 (= Cryptomonads), again a group with pigmented phototrophic members and colorless phagotrophic members.

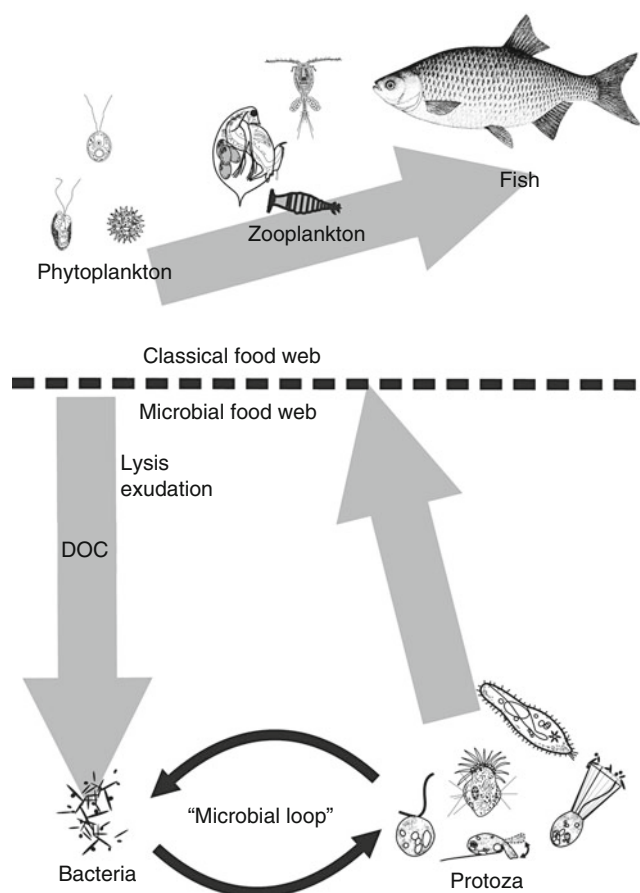
Ecology, microbial food web, and functional groups

In aquatic environments, protist abundances usually range between 10^2 mL^{-1} and 10^4 mL^{-1} . The dominant taxonomic groups among heterotrophic protist communities within different marine, brackish, and limnetic pelagic communities (heterokont taxa, dinoflagellates, choanoflagellates, katablepharids, and ciliates) and benthic communities (euglenids, bodonids, thaumatomonads, apusomonads, cercoconads, and ciliates) are relatively similar. Soil samples contain between 10^4 and 10^8 active protist individuals per gram soil and litter, with flagellates being the most dominant group followed by gymnamoebae, testate amoebae, and ciliates. Numbers decrease with increasing soil depth and are usually lower in unplanted soil. Bacterivorous protists are the most important among secondary saprotrophs; the main bacterivores belong to the bodonids and the cercoconads,

which comprise most soil flagellates, the colorless chrysomonad *Oikomonas*, the Vahlkampfiidae, the gymnamoebae, Testacealobosia, and dictyostelids (Amoebozoa), the filose testate amoebae, and the ciliates. Soil protists may be responsible for up to 70% of the soil animal respiration and for 14–66% and 20–40% of the mineralization of C and N, respectively.

In protozoan feeding, either phagotrophy or osmotrophy predominate in particular species. In addition, chlorophyll-bearing flagellates profit from photosynthesis. Some Protozoa, specifically small flagellata and some filter-feeding ciliates are among the most important consumers of bacteria whereas many of the larger Protozoa prefer algae and smaller Protozoa as food. Life observations on food uptake provided early insights in these functional differences. The more recent research foci on the plant–animal–fungi–protist distinction and the bacterial ingestion as basic in the serial endosymbiosis theory further extended this research direction. This line of thinking based on functional groups culminated in the 1980s in the formulation of the microbial loop concept with heterotrophic phagotrophic flagellates and ciliates as

consumers of bacteria (Figure 1). In general, phagotrophic protists ($\approx$ Protozoa) are a major component in any ecosystem and are responsible for the majority of eukaryotic respiration and production whereas their autotrophic counterparts are important primary producers. Protists are key mediators in the enhancement of nutrient flow by regulating decomposition rates and specific metabolic pathways to the benefit of plants and microorganisms in soil and aquatic habitats. Bacterial production is stimulated by protists excreting secondary metabolites, excess nitrogen, and phosphorous in the form of ammonium, inorganic phosphorous, or organic compounds. They channel carbon and nutrient flow from one of the largest standing stocks of living biomass, that is, bacteria (see entry *Bacteria*) and archaea (see entry *Archaea*), to higher trophic levels. Thus, with respect to global biogeochemical cycles (see entry *Biogeochemical Cycles*), phagotrophic protists are a major force that shape the movement and fate of microbial biomass in any ecosystem. Selective grazing has already been demonstrated with respect to prey size, cell wall chemistry, nutritional value, activity, and the ability to produce inhibitory compounds.

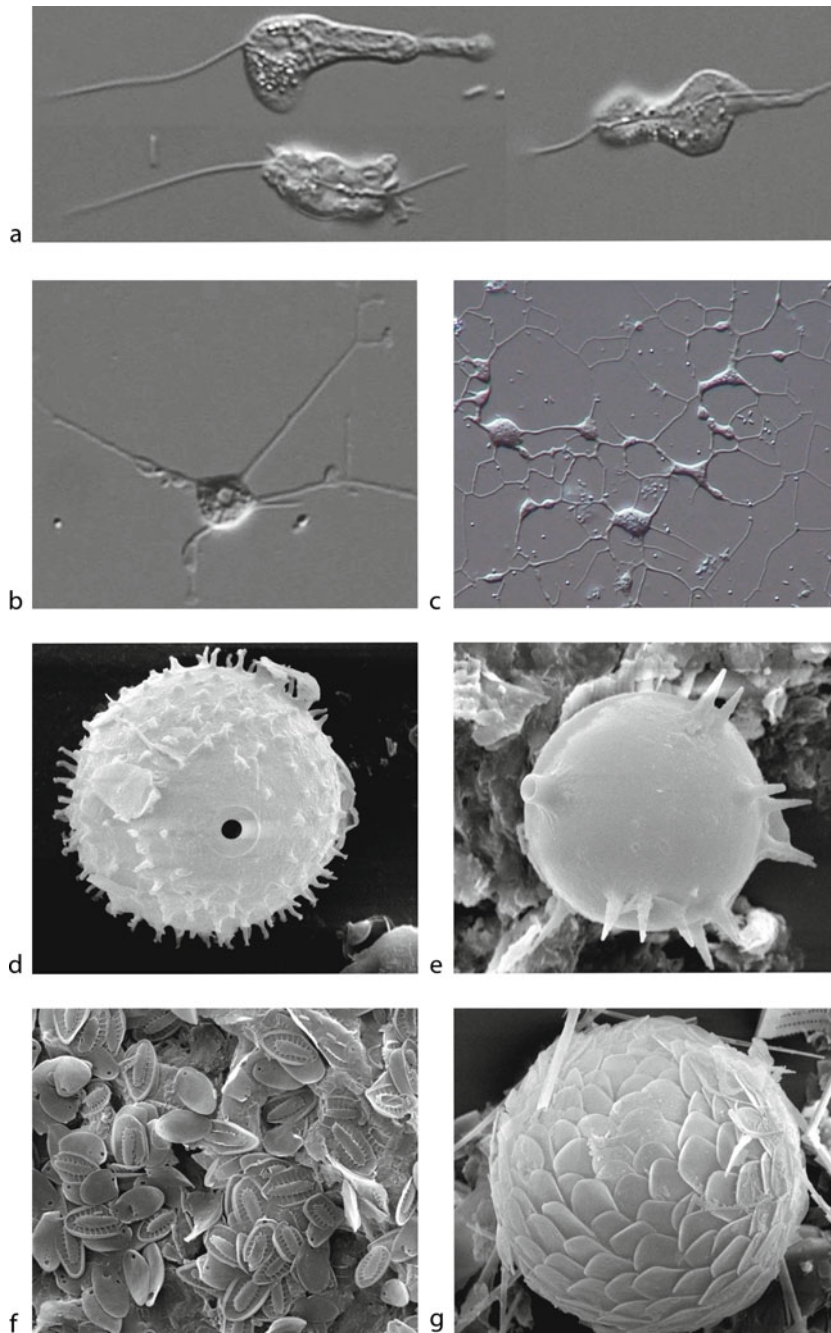


Protozoa (Heterotroph, Eukaryotic), Figure 1 The microbial food web. A substantial portion of the primary production is channeled through the microbial food web. Dissolved organic carbon is utilized by bacteria, which are grazed by protozoa.

Fossil record

Protozoa are important in reconstructing past ecological conditions as several protozoan taxa form microfossils. The fossil remains of Protozoa are naturally confined to

those classes or orders, which are shell-producing during life. They are most common in deposits of marine environments, but also occur in brackish water, fresh water, and terrestrial sedimentary deposits.



Protozoa (Heterotroph, Eukaryotic), Figure 2 (a–c) Cercozoa are abundant protozoa in soils and sediments. (a) *Cercomonas braziliensis*; (b) *Paracercomonas* sp. (c) *Filoreta marina*; (d–g) Unpigmented chrysomonad flagellates have been regarded as protozoa but are now placed within the Chromista. (d, e) Chrysomonad (=chrysophycean) resting stages (stomatocysts); (f) Surface scales of chrysophytes; (g) Resting stage covered in surface scales. Figures 2a, b and c are courtesy of David Bass, The Natural History Museum, London, UK.

Radiolaria (see entry [Radiolaria](#)) build mostly tests from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. They are important index fossils and a source of marine silica cements, chert (see entry [Cherts](#)), etc. Foraminiferans (see entry [Foraminifera](#)) have typically multichambered tests, although some are unilocular. Foraminiferans are important as biostratigraphic index fossils and as a reliable source of untransported carbonate shell material for stable-isotope analyses. Further, the Paleozoic Acritarchs (nonacid soluble organic structures, potentially cysts) and Chitinozoa (chitinous organic-walled flask-like chambers) potentially are also protozoan microfossils. Besides the fossils of heterotrophic taxa, specifically several Chromist taxa ([diatoms](#), golden algae) build important microfossils such as the diatoms (see entry [Diatoms](#)), the Coccolithophorids (see entry [Chroococcidiopsis](#)), and the golden algae ([Figure 2](#)).

Diversity and biogeography: “everything is everywhere”?

Two main hypotheses about the causes of nonrandom distribution of microorganisms continue to obtain conflicting experimental support: the idea that “everything is everywhere” is based on the assumption that the enormous dispersal capabilities of microorganisms allow them to spread into virtually any habitat. Absence of taxa is caused by unfavorable local conditions that prevent them from getting established everywhere. In a contrasting model, the rate of dispersal of microorganisms is not sufficiently high to overcome historical dispersal limitations and human influence. This allows for existence of endemic taxa many of which remain to be discovered. Adjusting the species concepts is a necessary precondition for drawing conclusions about derived theories such as flagellate biogeography and the “everything is everywhere” debate. The predominant view of a low to moderate number of nanoflagellate taxa, most of which seem to be globally distributed and ubiquitous, is increasingly replaced by the view of a tremendous number of taxa with a much more restricted distribution, both with respect to habitat type and geographic distance.

Summary

Protozoa are a loose grouping of organisms with usually unicellular organization and heterotrophic mode of nutrition. The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. Functional groups based on rough morphological characters comprise amoeboid, flagellated, and ciliated organisms. Protozoa are a major component in any ecosystem and are responsible for the majority of eukaryotic respiration and production whereas their autotrophic counterparts are important primary producers. Protists are key mediators in the enhancement of nutrient flow by regulating decomposition rates and specific metabolic pathways to the benefit of plants and microorganisms in

soil and aquatic habitats. They build important microfossils both in the marine and in freshwaters.

Bibliography

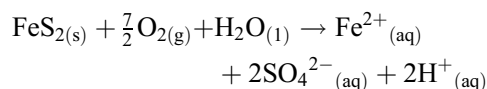
- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *The Journal of Eukaryotic Microbiology*, **52**(5), 399–451.
- Cavalier-Smith, T., 2003. Protist phylogeny and the high-level classification of Protozoa. *European Journal of Protistology*, **39**, 338–348.
- Hausmann, K., Hülsmann, N., and Radek, R., 2003. *Protistology*, 3rd edn. Schweizerbart'sche Verlagsbuchhandlung, 379 p.
- Margulis, L., Corliss, J. O., Melkonian, M., and Chapman, D. J., 1990. *Handbook of Protoctista*. Boston, MA: Jones & Barlett, 1024 p.
- Scarmadella, J. M., 1999. Not plants or animals: a brief history of the origin of Kingdoms Protozoa, Protista and Protoctista. *International Microbiology*, **2**, 207–216.
- von Oken, L., 1805. *Die Zeugung*. Bamberg, Würzburg: Göbhardt.

Cross-references

[Acritarchs](#)
[Algae \(Eukaryotic\)](#)
[Bacteria](#)
[Carbon Cycle](#)
[Coccolithophores](#)
[Diatoms](#)
[Foraminifera](#)
[Microbial Ecology of Submarine Caves](#)
[Radiolaria](#)
[Symbiosis](#)

PYRITE OXIDATION

The most common metal sulfide mineral is pyrite (FeS₂). When exposed to surface weathering, pyrite, as well as other metal sulfides, reacts with the oxygen in air in the presence of water, forming an acidic ferrous sulfate solution:



The resulting “[Acid Rock Drainage](#)” (see entry) is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. For details, see entry “[Sulfide Mineral Oxidation](#).” In aquatic settings, pyrite oxidation may result in the remobilization and subsequent enrichment of sedimentary sulfide iron. Please refer to entry “[Iron Sulfide Formation](#)” for further reading.

Radiolaria (see entry [Radiolaria](#)) build mostly tests from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. They are important index fossils and a source of marine silica cements, chert (see entry [Cherts](#)), etc. Foraminiferans (see entry [Foraminifera](#)) have typically multichambered tests, although some are unilocular. Foraminiferans are important as biostratigraphic index fossils and as a reliable source of untransported carbonate shell material for stable-isotope analyses. Further, the Paleozoic Acritarchs (nonacid soluble organic structures, potentially cysts) and Chitinozoa (chitinous organic-walled flask-like chambers) potentially are also protozoan microfossils. Besides the fossils of heterotrophic taxa, specifically several Chromist taxa ([diatoms](#), golden algae) build important microfossils such as the diatoms (see entry [Diatoms](#)), the Coccolithophorids (see entry [Chroococcidiopsis](#)), and the golden algae ([Figure 2](#)).

Diversity and biogeography: “everything is everywhere”?

Two main hypotheses about the causes of nonrandom distribution of microorganisms continue to obtain conflicting experimental support: the idea that “everything is everywhere” is based on the assumption that the enormous dispersal capabilities of microorganisms allow them to spread into virtually any habitat. Absence of taxa is caused by unfavorable local conditions that prevent them from getting established everywhere. In a contrasting model, the rate of dispersal of microorganisms is not sufficiently high to overcome historical dispersal limitations and human influence. This allows for existence of endemic taxa many of which remain to be discovered. Adjusting the species concepts is a necessary precondition for drawing conclusions about derived theories such as flagellate biogeography and the “everything is everywhere” debate. The predominant view of a low to moderate number of nanoflagellate taxa, most of which seem to be globally distributed and ubiquitous, is increasingly replaced by the view of a tremendous number of taxa with a much more restricted distribution, both with respect to habitat type and geographic distance.

Summary

Protozoa are a loose grouping of organisms with usually unicellular organization and heterotrophic mode of nutrition. The systematic position of the Protozoa and related organisms remained and still remains unclear for many lineages. Functional groups based on rough morphological characters comprise amoeboid, flagellated, and ciliated organisms. Protozoa are a major component in any ecosystem and are responsible for the majority of eukaryotic respiration and production whereas their autotrophic counterparts are important primary producers. Protists are key mediators in the enhancement of nutrient flow by regulating decomposition rates and specific metabolic pathways to the benefit of plants and microorganisms in

soil and aquatic habitats. They build important microfossils both in the marine and in freshwaters.

Bibliography

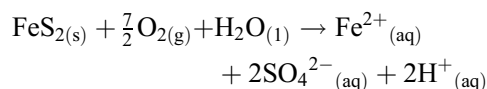
- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Andersen, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *The Journal of Eukaryotic Microbiology*, **52**(5), 399–451.
- Cavalier-Smith, T., 2003. Protist phylogeny and the high-level classification of Protozoa. *European Journal of Protistology*, **39**, 338–348.
- Hausmann, K., Hülsmann, N., and Radek, R., 2003. *Protistology*, 3rd edn. Schweizerbart'sche Verlagsbuchhandlung, 379 p.
- Margulis, L., Corliss, J. O., Melkonian, M., and Chapman, D. J., 1990. *Handbook of Protoctista*. Boston, MA: Jones & Barlett, 1024 p.
- Scarmadella, J. M., 1999. Not plants or animals: a brief history of the origin of Kingdoms Protozoa, Protista and Protoctista. *International Microbiology*, **2**, 207–216.
- von Oken, L., 1805. *Die Zeugung*. Bamberg, Würzburg: Göbhardt.

Cross-references

[Acritarchs](#)
[Algae \(Eukaryotic\)](#)
[Bacteria](#)
[Carbon Cycle](#)
[Coccolithophores](#)
[Diatoms](#)
[Foraminifera](#)
[Microbial Ecology of Submarine Caves](#)
[Radiolaria](#)
[Symbiosis](#)

PYRITE OXIDATION

The most common metal sulfide mineral is pyrite (FeS₂). When exposed to surface weathering, pyrite, as well as other metal sulfides, reacts with the oxygen in air in the presence of water, forming an acidic ferrous sulfate solution:



The resulting “[Acid Rock Drainage](#)” (see entry) is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. For details, see entry “[Sulfide Mineral Oxidation](#).” In aquatic settings, pyrite oxidation may result in the remobilization and subsequent enrichment of sedimentary sulfide iron. Please refer to entry “[Iron Sulfide Formation](#)” for further reading.

R

RADIOACTIVITY (NATURAL)

Beda A. Hofmann
Natural History Museum Bern, Bern, Switzerland

Synonyms

Natural radiation; Terrestrial radiation

Definition

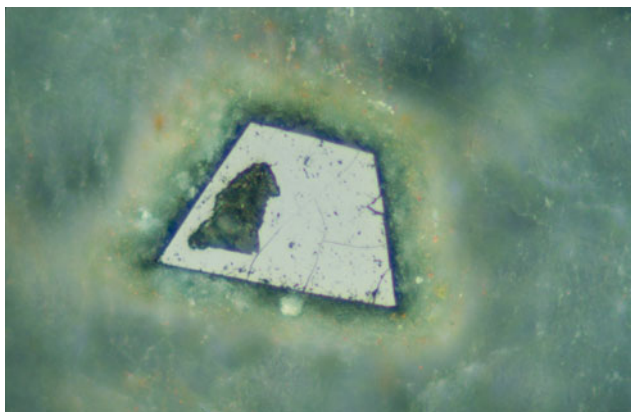
Natural radioactivity (NR) is predominantly due to the decay of ^{238}U , ^{235}U , ^{232}Th (and their chains of daughter elements), ^{87}Rb (27.8% of natural Rb), and ^{40}K (0.012% of natural K). Early in the Earth's history ^{244}Pu (half life 82.6 Ma) also was an important radioelement. These are all primordial isotopes formed prior to the origin of the solar system. The list of natural radioisotopes also includes primordial ^{147}Sm and ^{187}Re and short-lived cosmogenic isotopes such as ^{10}Be , ^{14}C , and ^{26}Al . Of all these elements, only U, Th (including daughter elements) Rb, and K represent significant sources of terrestrial natural radioactivity. U, Th, and daughters are emitters of alpha and beta particles and gamma rays; ^{87}Rb emits beta particles, ^{40}K is a beta and gamma emitter. Another source of NR are the cosmic rays, dominantly protons (and minor He nuclei) with a very wide range of energies. The higher energetic ones are of galactic origin, while the sun contributes on the lower energy side of the spectrum. Upon arrival in the atmosphere, a cascade of secondary particles is created (air shower) which ultimately may affect life on the surface. The Earth's surface is heavily shielded from cosmic rays by both its magnetic field and its atmosphere (Bauer and Lammer, 2004). The contribution of cosmic rays to the natural dose of radioactivity is strongly dependent on altitude and ranges from a minor fraction at sea level to dominant at high altitudes.

The radioactive elements U, Th, Rb, and K are strongly concentrated in the continental crust, and a large part of these elements are relatively evenly distributed with an average concentrations in crustal rocks of 2.7 ppm U, 9.6 ppm Th, 90 ppm Rb, and 2.1% K (2.5 ppm ^{40}K). The specific activities, including all daughter elements in secular equilibrium, are 179 Bq/kg for 1 ppm U, 41 Bq/kg for 1 ppm Th, 0.87 Bq/kg for 1 ppm Rb, and 302 Bq/kg for 1% K. Natural enrichment processes can lead to local concentrations much higher than the crustal average. High-grade ores may contain several percent U or Th and up to 52% K (sylvite). The most radioactive mineral is uraninite UO_2 with up to 88% uranium and a specific activity of $1.58 \cdot 10^8$ Bq/kg (Figures 1 and 2). Natural chemical or physical separations between U, Th, and their daughter elements can lead to U enrichments lacking daughters (until equilibrium is reached again) or enrichments of daughters unsupported by parent elements. Such disequilibria are of geologically short duration (few half lives) and do occur at sites of high element turnover, e.g., in submarine hot springs (Jolivet et al., 2003) and in the oxidation zone of uranium ores. Radioactive hot spots may form due to accumulation of short-lived daughter elements, e.g., ^{210}Po and ^{210}Pb in hydrothermal vents, and locally cause high doses to fauna (Cherry et al., 1992).

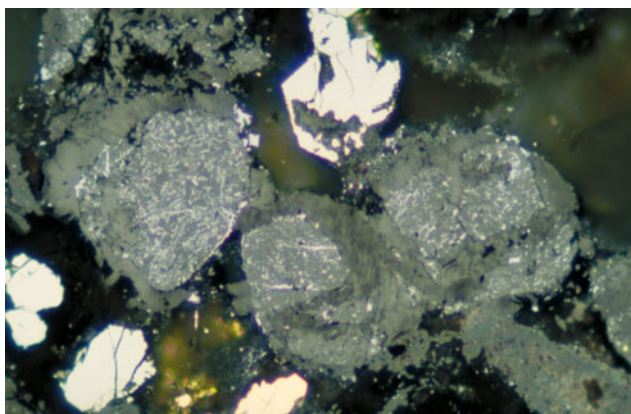
The separation of the short-lived ^{238}U daughter ^{222}Rn , a noble gas, and its migration in groundwater and soil gas is of particular importance for human radiation protection.

A special case of NR are the 1.97 Ga natural fission reactors of the Franceville basin, Gabun (Mathieu et al., 2001). During the activity of these natural reactors, they must have been the sites with the highest NR on Earth ever.

The two related actinide elements uranium and thorium show an important difference in chemical behaviour: Thorium only occurs in the tetravalent state Th(IV) and



Radioactivity (Natural), Figure 1 Uraninite as an accessory mineral in granite (Böttstein well, northern Switzerland). Around the crystal of the radioactive mineral a halo is visible corresponding to the range of alpha particles (approx. 20 μm) that destroyed (amorphized, metamictized) the crystal lattice of the enclosing K-feldspar within their range. Scattered red hematite particles are indicative of oxidizing conditions due to water radiolysis. The age of this uraninite is approximately 330 Ma. Reflected light, maximum diameter of crystal is 50 μm .



Radioactivity (Natural), Figure 2 Detrital uraninites from the Witwatersrand gold fields, South Africa (Blijvooruitzicht Mine). Uraninite is sprinkled with galena originating from radioactive decay of uranium, and mantled by kerogen-type organic matter (brownish without galena inclusions) due to the immobilization of mobile hydrocarbons as a result of irradiation. The age of these uraninites is in the order of 3000 Ma. Bright yellow mineral is pyrite. Reflected light, field of view is 220 μm .

is nearly immobile (very low solubility) under ambient conditions. Uranium occurs in two major states of oxidation: U(IV) is immobile as thorium, but U(VI), mainly occurring in the form of the uranyl ion UO_2^{2+} , is very soluble and the stable form in chemical equilibrium with the Earth's oxidizing modern atmosphere (Figure 3). Uranium accumulations due to reducing barriers and microbial redox interactions can therefore be expected in the case



Radioactivity (Natural), Figure 3 Precipitations of an amorphous uranium-(VI)-Al-silicate phase from water seeping out of fractures and a borehole on a granite tunnel wall. Krunkelbach uranium mine (240 m level), Menzenschwand, Germany, September 1987. This image demonstrates the high mobility of uranium in oxidized near-surface waters. Field of view 1 m.

of uranium, but not for thorium. Uranium thus plays a special role among natural radioactive materials because its minerals have the highest specific activity, and can be formed as a result of low-T redox processes.

There are a range of different interactions, certain and potential, between NR and life on Earth, and, potentially, on other celestial bodies. Such interactions may be direct (affecting organisms) or indirect (changes of the environment or of products of life). The most important interactions between life and NR are summarized here:

- (a) NR is a potentially detrimental factor for life, causing gene damage and, at high doses, cell death. The doses of NR are only relevant for gene damage with rare exceptions (Oklo reactors during their activity). For dormant microbial spores unable to perform repair processes, the situation may be drastically different and NR may pose a limiting factor for the long-term survival. NR in the form of cosmic rays is a limiting factor during the actual (manned space missions) and potential presence of life in space, e.g., dormant spores inside a meteoroid being transferred from one planetary body to another in a process called lithopanspermia (Mileikowski et al., 2000).
- (b) The low doses of NR on the Earth's surface are important for evolution in producing a range of mutations as a basis for natural selection.
- (c) Microbial life has found ways to adapt to high doses of NR by applying highly efficient DNA repair mechanisms. Such organisms include the bacteria *Deinococcus radiodurans* and *Thermococcus gammatolerans* (Jolivet et al., 2003). Apparently in *D. radiodurans* this mechanism, unlikely to evolve in environments of low NR, was developed as a

means to deal with desiccation (Mattimore and Battista, 1996).

- (d) NR may have played a role in the origin of life through production of reactive organic and inorganic radicals (Parnell, 2004). Natural heavy mineral enrichments (placers) containing radioactive minerals may have been such sites at a time when the specific activity of radioactive minerals was significantly higher than today due to the presence of ^{244}Pu and higher levels of ^{235}U .
- (e) NR can cause the immobilization of biogenic hydrocarbons during migration. Polymerization of hydrocarbons leads to coatings of immobile organics on radioactive minerals (Rasmussen, 2005; Rasmussen et al., 1993). Such coatings are tracers of hydrocarbon migration, and of life in general (Figure 2). Organic matter in ore deposits generally shows signs of radiation-induced maturation (Hofmann, 2004).
- (f) In the near-surface environment of low-shielded planetary bodies (e.g., Mars), destruction of biomolecules (e.g., amino acids) as a result of radiolytic destruction due to cosmic ray bombardment is potentially important over geological time scales (Kminek and Bada, 2006). This puts limits on the detectability of molecular biosignatures.
- (g) In certain deep subsurface environments, NR is a key source of chemical energy for microbial life. Through radiolysis of water, kinetically inert reductants (H_2) and oxidants (H_2O_2 as short-term product, sulfate as possible long-term product) may be formed. These and related compounds may then serve as an energy source for microbial growth (Hofmann, 1992; Jørgensen and D'Hondt, 2006; Lin et al., 2005; Lin et al., 2006; Savary and Pagel, 1997).
- (h) Generation of local anomalies of NR through direct or indirect microbial reduction of uranium. Both direct enzymatic reduction (Lovley et al., 1991, 1993) and indirect reduction due to microbial production of hydrogen sulphide (Mohagheghi et al., 1985) may be relevant. Microbial cells may become mineralized by uranium phases (Milodowski et al., 1990).
- (i) NR is the most important source of geothermal heat and thus a major driver of plate tectonics. This makes NR one of the key influences on geological processes, with which life on Earth is intimately interwoven.

Conclusions

Life on Earth would be different and maybe even nonexistent without natural radioactivity, and the environment would be a totally different one. Natural radioactivity plays only a minor role in the life of single individuals or generations of the bulk of microbial and higher life on Earth, but is a key factor in evolution. In specific extreme environments, like the deep subterranean biosphere, natural radioactivity may provide a crucial energy source.

Bibliography

- Bauer, S., and Lammer, H., 2004. *Planetary Aeronomy*. Berlin: Springer, 207 p.
- Cherry, R., Desbruyères, D., Heyraud, M., and Nolan, C., 1992. High levels of natural radioactivity in hydrothermal vent polychaetes: *Comptes rendus de l'Académie des sciences, Série III*, **315**, 21–26.
- Hofmann, B. A., 1992. Isolated reduction phenomena in red beds: a result of porewater radiolysis? In Maest, A. S. (ed.), *7th International Symposium on Water-Rock Interaction*: Park City: Balkema, pp. 503–506.
- Hofmann, B. A., 2004. Highly altered organic matter on Earth: biosignature relevance. In Derenne, L. S., Dutrey, A., Despois, D., Lazcano, A., and Robert, F. (eds.), *Astrobiology: Future Perspectives. Astrophysics and Space Science Library*, Amsterdam: Springer, Vol. 305, pp. 317–331.
- Jolivet, E., L'Haridon, S., Corre, E., Forterre, P., and Prieur, D., 2003. *Thermococcus gammatolerans* sp. nov., a hyperthermophilic archaeon from a deep-sea hydrothermal vent that resists ionizing radiation. *International Journal of Systematic and Evolutionary Microbiology*, **53**, 847–851.
- Jørgensen, B., and D'Hondt, S., 2006. A starving majority deep beneath the seafloor. *Science*, **314**, 932–934.
- Kminek, G., and Bada, J. L., 2006. The effect of ionizing radiation on the preservation of amino acids on Mars. *Earth and Planetary Science Letters*, **245**, 1–5.
- Lin, L.-H., Slater, G. F., Sherwood Lollar, B., Lacrampe-Couloume, G., and Onstott, T. C., 2005. The yield and isotopic composition of radiolytic H_2 , a potential energy source for the deep subsurface biosphere. *Geochimica et Cosmochimica Acta*, **69**, 893–903.
- Lin, L.-H., Wang, P.-L., Rumble, D., Lippmann-Pipke, J., Boice, E., Pratt, L. M., Sherwood Lollar, B., Brodie, E. L., Hazen, T. C., Andersen, G. L., DeSantis, T. Z., Moser, D. P., Kershaw, D., and Onstott, T. C., 2006. Long-term sustainability of a high-energy, low-diversity crustal biome. *Science*, **314**, 479–482.
- Lovley, D. R., Philips, E. J. P., Gorby, Y. A., and Landa, E. R., 1991. Microbial reduction of uranium. *Nature*, **350**, 413–416.
- Lovley, D. R., Roden, E. E., Philips, E. J. P., and Woodward, J. C., 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Marine Geology*, **113**, 41–53.
- Mathieu, R., Zetterström, L., Cuney, M., Gauthier-Lafaye, F., and Hidaka, H., 2001. Alteration of monazite and zircon and lead migration as geochemical tracers of fluid paleocirculations around the Oklo-Okélobondo and Bangombé natural nuclear reaction zones (Franceville basin, Gabon). *Chemical Geology*, **171**, 147–171.
- Mattimore, V., and Battista, J. R., 1996. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *Journal of Bacteriology*, **178**, 633–637.
- Mileikowski, C., Cucinotta, F., Wilson, J. W., Gladman, B., Horneck, G., Lindgren, L., Melosh, J., Rickman, H., Valtonen, M., and Zheng, J. Q., 2000. Natural transfer of viable microbes in space. 1. From Mars to Earth and Earth to Mars. *Icarus*, **145**, 391–427.
- Milodowski, A. E., West, J. M., Pearce, J. M., Hyslop, E. K., Basham, I. R., and Hooker, P. J., 1990. Uranium-mineralized microorganisms associated with uraniferous hydrocarbons in southwest Scotland. *Nature*, **347**, 465–467.
- Mohagheghi, A., Updegraff, D. M., and Goldhaber, M. B., 1985. The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores. *Geomicrobiology Journal*, **4**, 153–173.
- Parnell, J., 2004. Mineral radioactivity in sands as a mechanism for fixation of organic carbon in the early Earth. *Origins of Life and Evolution of the Biosphere*, **34**, 533–547.

- Rasmussen, B., 2005. Evidence for pervasive petroleum generation and migration in 3.2 and 2.63 Ga shales. *Geology*, **33**, 497–500.
- Rasmussen, B., Glover, J. E., and Foster, C. B., 1993. Polymerisation of hydrocarbons by radioactive minerals in sedimentary rocks: diagenetic and economic significance. In Landais, P. (ed.), *Bitumens in Ore Deposits*. Berlin: Springer, pp. 490–509.
- Savary, V., and Pagel, M., 1997. The effects of water radiolysis on local redox conditions in the Oklo, Gabon, natural fission reactors 10 and 16. *Geochimica Cosmochimica Acta*, **61**, 4479–4494.

Cross-references

Biosignatures in Rocks
Carbon (Organic, Degradation)
Extreme Environments
Hydrogen
Ores, Microbial Precipitation and Oxidation
Origin of Life

RADIOLARIA

Volker Thiel
University of Göttingen, Göttingen, Germany

Radiolaria are planktonic marine protozoa showing unicellular organization and heterotrophic mode of nutrition. They build tests varying in shape from simple scattered spicules to highly ornated geometric-shaped shells (Adl et al., 2005). Sizes usually range from hundredths to tenths of millimeters. The tests are made from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. The name “Radiolaria” derives from the marked radial skeletal spines that characterize many species. Radiolarians are known from the very beginning of the Paleozoic (early Cambrian of the Yangtze Platform; Braun et al., 2007). They are important index fossils and may significantly contribute to marine silica-rich sediments. Radiolarian cherts (radiolarites), a variety of chert (see entry *Cherts*) composed of radiolarian remains, indicate deep water deposition at depths below which siliceous sediments are stable, but carbonates are dissolved. See entry “*Protozoa (Heterotroph, Eukaryotic)*” for further reading.

Bibliography

- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Anderson, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology*, **52**, 399–451.
- Braun, A., Chen, J., Waloszek, D., Maas, A., 2007. First Early Cambrian Radiolaria. *Geological Society, London, Special Publications*, **286**, 143–149.

RAMAN MICROSCOPY (CONFOCAL)

Jan Toporski¹, Thomas Dieing¹, Christine Heim²
¹Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany
²University of Göttingen, Göttingen, Germany

Synonyms

Confocal Raman imaging (CRI)

Definition

Confocal Raman microscopy (CRM) is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area.

Introduction

Raman spectroscopy is well suited for studies in mineralogy and petrography, as it provides nondestructive mineral identification fast and with high specificity. In addition, Raman spectroscopy allows the characterization of complex organic materials, which makes it particularly useful in biogeoscience applications (Hild et al., 2008). This technique has long been applied in geosciences, for example, for the identification and characterization of minerals, or in the observation of mineral phase transitions in high and ultra-high pressure/temperature experiments. In most cases, measurements have been carried out in a micro-Raman set up, i.e., information was obtained from single or multiple points of interest on a sample. This way, little detail on the spatial distribution and association of components or mineral phases, or chemical variation could be observed, even though this information may contribute significantly to the understanding of a sample's complexity.

By means of CRM, such sample characteristics can be evaluated from large scale scans in the centimeter range to the finest detail with sub-micron resolution. Modern confocal Raman microscopes allow for such measurements with very high sensitivity and spatial as well as spectral resolution. CRM is a tool that not only provides complementary information to data obtained by e.g., electron microprobe (EMP), energy dispersive x-ray analysis (EDX), or secondary ion mass spectrometry (SIMS). In addition to the quantitative and semiquantitative elemental and/or isotopic data acquired by these techniques, CRM contributes the visualization of the distribution of molecular information over a defined sample area. Furthermore, considering that most geomaterials are transparent from the UV (NUV) to VIS and NIR to some degree, this information can be obtained three dimensionally due to the confocal set-up of the microscopes. The following discussion provides background information and examples that shall serve to highlight some key analytical features of this technique for applications in geosciences. A recent and comprehensive summary on the application of CRM in Geoscience can be found in Fries and Steele (2010).

- Rasmussen, B., 2005. Evidence for pervasive petroleum generation and migration in 3.2 and 2.63 Ga shales. *Geology*, **33**, 497–500.
- Rasmussen, B., Glover, J. E., and Foster, C. B., 1993. Polymerisation of hydrocarbons by radioactive minerals in sedimentary rocks: diagenetic and economic significance. In Landais, P. (ed.), *Bitumens in Ore Deposits*. Berlin: Springer, pp. 490–509.
- Savary, V., and Pagel, M., 1997. The effects of water radiolysis on local redox conditions in the Oklo, Gabon, natural fission reactors 10 and 16. *Geochimica Cosmochimica Acta*, **61**, 4479–4494.

Cross-references

Biosignatures in Rocks
Carbon (Organic, Degradation)
Extreme Environments
Hydrogen
Ores, Microbial Precipitation and Oxidation
Origin of Life

RADIOLARIA

Volker Thiel
University of Göttingen, Göttingen, Germany

Radiolaria are planktonic marine protozoa showing unicellular organization and heterotrophic mode of nutrition. They build tests varying in shape from simple scattered spicules to highly ornated geometric-shaped shells (Adl et al., 2005). Sizes usually range from hundredths to tenths of millimeters. The tests are made from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. The name “Radiolaria” derives from the marked radial skeletal spines that characterize many species. Radiolarians are known from the very beginning of the Paleozoic (early Cambrian of the Yangtze Platform; Braun et al., 2007). They are important index fossils and may significantly contribute to marine silica-rich sediments. Radiolarian cherts (radiolarites), a variety of chert (see entry *Cherts*) composed of radiolarian remains, indicate deep water deposition at depths below which siliceous sediments are stable, but carbonates are dissolved. See entry “*Protozoa (Heterotroph, Eukaryotic)*” for further reading.

Bibliography

- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Anderson, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology*, **52**, 399–451.
- Braun, A., Chen, J., Waloszek, D., Maas, A., 2007. First Early Cambrian Radiolaria. *Geological Society, London, Special Publications*, **286**, 143–149.

RAMAN MICROSCOPY (CONFOCAL)

Jan Toporski¹, Thomas Dieing¹, Christine Heim²
¹Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany
²University of Göttingen, Göttingen, Germany

Synonyms

Confocal Raman imaging (CRI)

Definition

Confocal Raman microscopy (CRM) is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area.

Introduction

Raman spectroscopy is well suited for studies in mineralogy and petrography, as it provides nondestructive mineral identification fast and with high specificity. In addition, Raman spectroscopy allows the characterization of complex organic materials, which makes it particularly useful in biogeoscience applications (Hild et al., 2008). This technique has long been applied in geosciences, for example, for the identification and characterization of minerals, or in the observation of mineral phase transitions in high and ultra-high pressure/temperature experiments. In most cases, measurements have been carried out in a micro-Raman set up, i.e., information was obtained from single or multiple points of interest on a sample. This way, little detail on the spatial distribution and association of components or mineral phases, or chemical variation could be observed, even though this information may contribute significantly to the understanding of a sample's complexity.

By means of CRM, such sample characteristics can be evaluated from large scale scans in the centimeter range to the finest detail with sub-micron resolution. Modern confocal Raman microscopes allow for such measurements with very high sensitivity and spatial as well as spectral resolution. CRM is a tool that not only provides complementary information to data obtained by e.g., electron microprobe (EMP), energy dispersive x-ray analysis (EDX), or secondary ion mass spectrometry (SIMS). In addition to the quantitative and semiquantitative elemental and/or isotopic data acquired by these techniques, CRM contributes the visualization of the distribution of molecular information over a defined sample area. Furthermore, considering that most geomaterials are transparent from the UV (NUV) to VIS and NIR to some degree, this information can be obtained three dimensionally due to the confocal set-up of the microscopes. The following discussion provides background information and examples that shall serve to highlight some key analytical features of this technique for applications in geosciences. A recent and comprehensive summary on the application of CRM in Geoscience can be found in Fries and Steele (2010).

- Rasmussen, B., 2005. Evidence for pervasive petroleum generation and migration in 3.2 and 2.63 Ga shales. *Geology*, **33**, 497–500.
- Rasmussen, B., Glover, J. E., and Foster, C. B., 1993. Polymerisation of hydrocarbons by radioactive minerals in sedimentary rocks: diagenetic and economic significance. In Landais, P. (ed.), *Bitumens in Ore Deposits*. Berlin: Springer, pp. 490–509.
- Savary, V., and Pagel, M., 1997. The effects of water radiolysis on local redox conditions in the Oklo, Gabon, natural fission reactors 10 and 16. *Geochimica Cosmochimica Acta*, **61**, 4479–4494.

Cross-references

Biosignatures in Rocks
Carbon (Organic, Degradation)
Extreme Environments
Hydrogen
Ores, Microbial Precipitation and Oxidation
Origin of Life

RADIOLARIA

Volker Thiel
University of Göttingen, Göttingen, Germany

Radiolaria are planktonic marine protozoa showing unicellular organization and heterotrophic mode of nutrition. They build tests varying in shape from simple scattered spicules to highly ornated geometric-shaped shells (Adl et al., 2005). Sizes usually range from hundredths to tenths of millimeters. The tests are made from opal (hydrated silica), some from silica and organic material, and some from strontium sulphate. The name “Radiolaria” derives from the marked radial skeletal spines that characterize many species. Radiolarians are known from the very beginning of the Paleozoic (early Cambrian of the Yangtze Platform; Braun et al., 2007). They are important index fossils and may significantly contribute to marine silica-rich sediments. Radiolarian cherts (radiolarites), a variety of chert (see entry *Cherts*) composed of radiolarian remains, indicate deep water deposition at depths below which siliceous sediments are stable, but carbonates are dissolved. See entry “*Protozoa (Heterotroph, Eukaryotic)*” for further reading.

Bibliography

- Adl, S. M., Simpson, A. G. B., Farmer, M. A., Anderson, R. A., Anderson, O. R., Barta, J. R., Bowser, S. S., Brugerolle, G., Fensome, R. A., Fredericq, S., James, T. Y., Karpov, S., Kugrens, P., Krug, J., Lane, C. E., Lewis, L. A., Lodge, J., Lynn, D. H., Mann, D. G., McCourt, R. M., Mendoza, L., Moestrup, Ø., Mozley-Standridge, S. E., Nerad, T. A., Shearer, C. A., Smirnov, A. V., Spiegel, F. W., and Taylor, M. F. J. R., 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology*, **52**, 399–451.
- Braun, A., Chen, J., Waloszek, D., Maas, A., 2007. First Early Cambrian Radiolaria. *Geological Society, London, Special Publications*, **286**, 143–149.

RAMAN MICROSCOPY (CONFOCAL)

Jan Toporski¹, Thomas Dieing¹, Christine Heim²
¹Wissenschaftliche Instrumente und Technologie GmbH, Ulm, Germany
²University of Göttingen, Göttingen, Germany

Synonyms

Confocal Raman imaging (CRI)

Definition

Confocal Raman microscopy (CRM) is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area.

Introduction

Raman spectroscopy is well suited for studies in mineralogy and petrography, as it provides nondestructive mineral identification fast and with high specificity. In addition, Raman spectroscopy allows the characterization of complex organic materials, which makes it particularly useful in biogeoscience applications (Hild et al., 2008). This technique has long been applied in geosciences, for example, for the identification and characterization of minerals, or in the observation of mineral phase transitions in high and ultra-high pressure/temperature experiments. In most cases, measurements have been carried out in a micro-Raman set up, i.e., information was obtained from single or multiple points of interest on a sample. This way, little detail on the spatial distribution and association of components or mineral phases, or chemical variation could be observed, even though this information may contribute significantly to the understanding of a sample's complexity.

By means of CRM, such sample characteristics can be evaluated from large scale scans in the centimeter range to the finest detail with sub-micron resolution. Modern confocal Raman microscopes allow for such measurements with very high sensitivity and spatial as well as spectral resolution. CRM is a tool that not only provides complementary information to data obtained by e.g., electron microprobe (EMP), energy dispersive x-ray analysis (EDX), or secondary ion mass spectrometry (SIMS). In addition to the quantitative and semiquantitative elemental and/or isotopic data acquired by these techniques, CRM contributes the visualization of the distribution of molecular information over a defined sample area. Furthermore, considering that most geomaterials are transparent from the UV (NUV) to VIS and NIR to some degree, this information can be obtained three dimensionally due to the confocal set-up of the microscopes. The following discussion provides background information and examples that shall serve to highlight some key analytical features of this technique for applications in geosciences. A recent and comprehensive summary on the application of CRM in Geoscience can be found in Fries and Steele (2010).

Principles of confocal Raman microscopy

CRM essentially merges two techniques into one. First, Raman spectroscopy, which allows nondestructive chemical analysis; secondly, Confocal microscopy which allows the user to examine samples with diffraction-limited resolution as well as to obtain three-dimensional information from the sample. The theory behind these two techniques will be explained in the following sections, followed by an illustration of how images with chemical sensitivity can be obtained using this combination of techniques.

Raman spectroscopy

When light of a certain wavelength interacts with a molecule, most photons are elastically scattered and therefore have the same energy as the incident photons. However, a very small fraction (approximately 1 in 10^6 – 10^7 photons) is inelastically scattered, which means that the energy of the scattered photon is different than the energy of the incident photon.

This is called the “Raman effect”, and it was discovered by Sir Chandrasekhara Raman in 1928 (Raman, 1928; Raman and Krishnan, 1928). Unlike today, he used a filtered beam of sunlight as an excitation source and his eye as a detector for the frequency shifted light. This was long before the development of the first laser by Maiman in 1960. Raman was awarded the Nobel Prize in 1930 for this discovery. The theory behind the Raman effect was derived five years earlier by Smekal (1923).

The tremendous importance of the Raman effect lies in the fact that the energy shift between the exciting and the Raman scattered photon is caused by the excitation (or annihilation) of a molecular vibration. This energy shift is thus characteristic and therefore a fingerprint for the type and coordination of the molecules involved in the scattering process.

Theory

The following section shall provide some basic descriptions and definitions relevant to Raman spectroscopy. Readers interested in a detailed theoretical background are referred to Ibach and Lüth (2003).

In quantum mechanics, the scattering process between a photon and a molecule is described as an excitation of a molecule to a virtual state lower in energy than a real electronic state and the (nearly immediate) de-excitation. The lifetime of the virtual state is extremely short and can be calculated by the Heisenberg uncertainty relation:

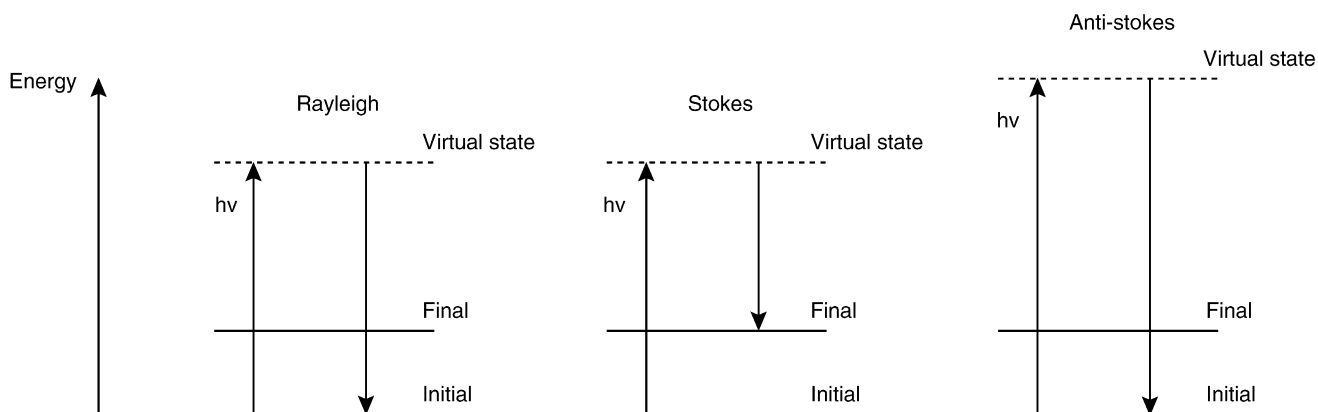
$$\Delta t \cdot \Delta E \geq \frac{\hbar}{2} \quad (1)$$

With typical photon energies of 1–2 eV, the lifetime of the excited state is only about 10^{-15} s. After this extremely short time, the molecule falls back either to the vibrational ground state or to an excited state (Figure 1). When the initial and final states are identical, the process is called Rayleigh scattering.

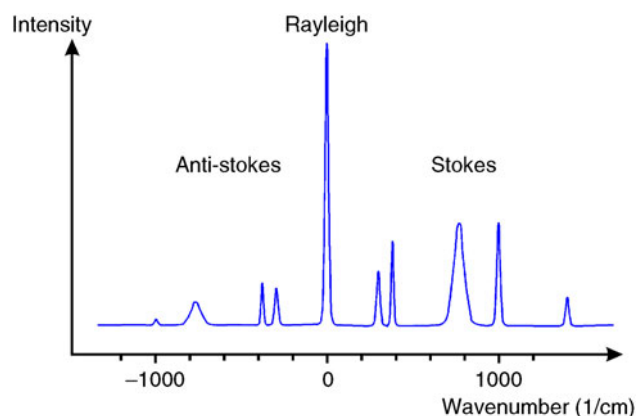
If the initial state is the ground and the final state a higher vibrational level, the process is called Stokes scattering, if the initial state is energetically higher than the final state, this is referred to as Anti-Stokes scattering. The difference in energy between the incident and the Raman scattered photon is equal to the energy of a vibration quantum of the scattering molecule. A plot of intensity of scattered light versus energy difference is called a Raman spectrum.

The position of a Raman line is usually given in relative wavenumbers ($1/\text{cm}$), which is the energy shift relative to the excitation line:

$$\bar{\nu} = \frac{1}{\lambda_{\text{incident}}} - \frac{1}{\lambda_{\text{scattered}}} \quad (2)$$



Raman Microscopy (Confocal), Figure 1 Energy level diagram for Raman scattering.



Raman Microscopy (Confocal), Figure 2 Typical Raman Spectrum.

$\lambda_{\text{incident}}$ and $\lambda_{\text{scattered}}$ are the wavelengths of the incident and Raman scattered photons, respectively.

As can be seen in Figure 2, a typical Raman spectrum is symmetric to the Rayleigh line and the Anti-Stokes lines are lower in intensity than the Stokes shifted lines.

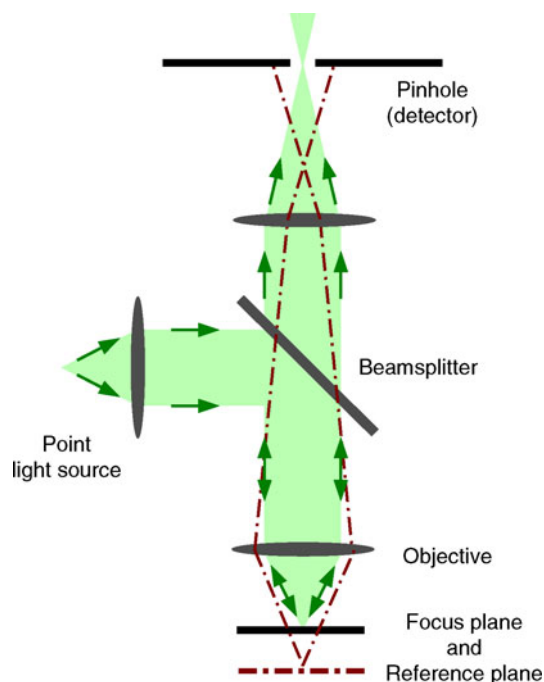
From classical scattering theory, one finds that the intensity I of scattered light is proportional to the 4th power of the excitation frequency

$$I \propto \nu^4 \quad (3)$$

Exciting a sample with blue light at 400 nm would therefore give a 16 times higher Raman signal than using red light at 800 nm. The problem of using blue (or UV) excitation light, however, is fluorescence. Many samples show fluorescence when they are excited with blue light and Raman emissions are extremely weak compared to fluorescence. If a sample shows significant fluorescence, obtaining a Raman spectrum is usually impossible because the fluorescence background covers the Raman signal. In the red (or even IR) region of the spectrum, fluorescence is usually not a problem, but the excitation intensity must be much higher ($I \propto \nu^4$). Another problem is that Silicon detectors cannot be used above 1100 nm (band gap energy of Si: 1.12 eV). Other IR detectors (such as InGaAs) show much more thermal and readout noise than silicon and photon counting detectors with low dark count rates are not available yet. In real experiments one must always find a compromise between detection efficiency and excitation power.

Confocal microscopy

Confocal microscopy allows the user to obtain three-dimensional information from the sample. It requires a point source (usually a laser), which is focused onto the sample. The reflected light (Rayleigh, Raman, fluorescence) is collected with the same objective and focused through a pinhole at the front of the detector (Figure 3). This ensures that only light from the image focal plane



Raman Microscopy (Confocal), Figure 3 Principal setup of a confocal microscope.

can reach the detector, which greatly increases image contrast and with the proper selection of pinhole size, slightly increases resolution (max. gain in resolution: factor $\sqrt{2}$). As can be seen from Figure 3, light originating from planes other than the focal plane will be out of focus at the pinhole. Therefore, its contribution to the detected signal is strongly reduced. Additionally, by changing the distance between the objective and the sample, the focal plane is moved within the sample thus allowing depth profiling or even 3D imaging (Wilson, 1990).

Pinhole size

The choice of the pinhole size is important because on one hand the signal should be as high as possible, while on the other hand the image should be as confocal as possible (highest depth resolution). To take full advantage of the lateral and depth resolution possible with confocal microscopy, the size of the pinhole should be adjusted and optimized. To obtain the highest lateral resolution, the pinhole size should be below $\nu_p = 0.5$. (The variable ν describes the position in optical coordinates and can be derived from $\nu = \frac{2\pi}{\lambda} \sqrt{x^2 + y^2} \sin \alpha$. Here λ is the excitation wavelength, x and y the sample coordinates in the focal plane and α half of the aperture angle. ν_p is the radius of the pinhole in optical coordinates when assuming a magnification of 1.) However, at this point the transmission through the pinhole is only 5% of the scattered intensity. In practice, the pinhole size can be up to $\nu_p = 4$ without significantly changing depth resolution and up to $\nu_p = 2$ without significantly changing lateral resolution. It can be shown that if $\nu_p > 4$, the

resolution of at least a conventional microscope remains. This is due to the fact that for a large detector the resolution is always determined by the diameter of the excitation laser spot. Only the depth resolution (and therefore contrast for a thick sample) is lost in this case. In most cases, a pinhole size of $v_p = 2.5$ is a good compromise since good depth resolution is maintained while $>75\%$ of the light still reaches the detector (see Figure 4).

For the experiment, the relation

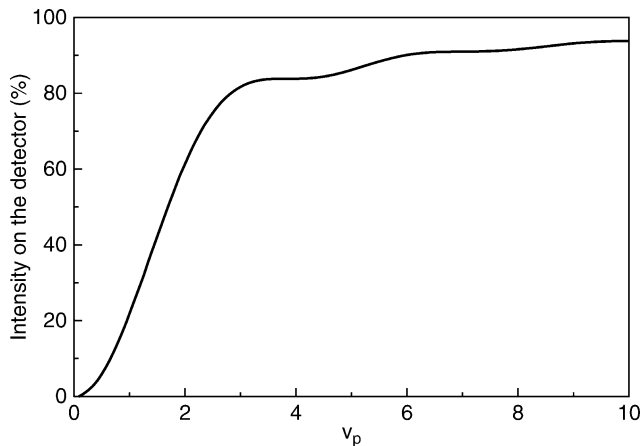
$$\frac{M}{NA} \geq \frac{\pi d_0}{v_p \lambda} \quad (4)$$

should be fulfilled, where M is the magnification, d_0 the diameter of the pinhole, and NA the numerical aperture of the objective. The left side of this equation is defined by the objective and the beam path and the right side by the wavelength, the pinhole size itself and v_p . If, for example, an objective with a magnification of $100\times$ and a numerical aperture of 0.9 is used at a wavelength of 532 nm the optimum pinhole size would be 50 μm for maximum depth resolution and 10 μm for maximum lateral resolution.

In actual experiments, one usually has to find a compromise between the highest resolution and collection efficiency. This is very important in CRM because Raman is an extremely weak effect. If a very small pinhole is used, the collection efficiency is strongly reduced (Figure 4).

This graphic shows the intensity on the detector as a function of pinhole size, normalized to the total intensity in the image plane. One can see that the collection efficiency is about 75% for maximum depth resolution ($v_p = 2.5$), but only 6% for maximum lateral resolution ($v_p = 0.5$).

Using the appropriate pinhole size, it is therefore always possible to obtain maximum depth resolution.



Raman Microscopy (Confocal), Figure 4 Collection efficiency as a function of the pinhole size normalized to the total power in the image plane.

Resolution

For sample scanning systems, the magnification printed on the objective used is of minor importance. The maximum scan range achievable by the sample scanner determines the maximum image size, independent of the magnification of the objective. The more important property of the objective is the numerical aperture, which together with the excitation wavelength determines the lateral resolution of the objective. The magnification is only important for the choice of the pinhole size.

The maximum resolution of a classical microscope is given by the Rayleigh criterion

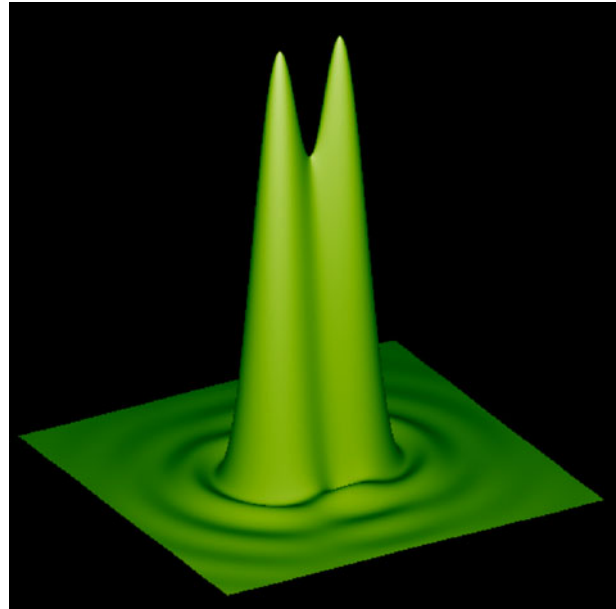
$$\Delta x = \frac{0.61 \lambda}{NA} \quad (5)$$

where Δx is the smallest distance between two point objects that will appear separated in the image plane, λ is the wavelength of the excitation light, and NA is the numerical aperture of the microscope objective. In this case, the image of two point objects will appear just separated (Figure 5).

Confocal Raman microscopy

Instrumentation considerations

When combining confocal microscopy and Raman spectroscopy the main challenge is the low signal intensity. As mentioned earlier, only one in about $10^6 - 10^7$ photons is frequency shifted by the Raman effect. Thus the number of photons reaching the detector is far less than is the case for confocal or fluorescence microscopy. The two obvious



Raman Microscopy (Confocal), Figure 5 The intensity distribution of two point sources which are separated by the Rayleigh criterion.

changes would be to increase the laser power and to increase the integration time. However, there are limitations to these possibilities as shown below:

- (a) *Laser power*: If a power of only 1 mW from a 532 nm laser is focused diffraction limited using a $100\times$ air objective with a NA of 0.9, the power density in the illuminated spot is approximately 10^6 W/cm². These levels are only possible because a single point is illuminated and the heat effectively dissipates in three dimensions. Nevertheless, the maximum laser power on the sample is therefore typically in the range of 1–20 mW.
- (b) *Integration time*: Increasing the integration time will increase the signal to noise ratio significantly. However, when CRM or mapping is applied, in which an image is produced by recording a spectrum at every image pixel, the integration time per spectrum needs to be kept to a minimum. An integration time of only 1 s per spectrum will, when recording an image of 128×128 pixels, result in a total acquisition time of about 4.5 h, which is an inconvenient time-span for routine application.

Therefore, a system for CRM must be capable of obtaining the Raman spectra with an acceptable signal-to-noise ratio in less than 50–100 ms. There are several parts of a confocal Raman microscope which should be optimized in order to allow such rapid data acquisition. These will be discussed in the following:

Laser power: As described above, the maximum laser power is limited and will heavily depend on the destruction and alteration threshold of the sample used.

Collection efficiency of the objective: Using objectives with high numerical apertures maximizes the collection efficiency since light from a larger solid angle is collected.

Throughput of the microscope: In order to enhance the throughput of the microscope, the components used within the beam path should be optimized and if possible minimized since every passive optical element introduces losses to the signal.

Efficiency of the grating: Highly efficient gratings with the correct blazing angle for the excitation wavelength should be used. As an example: a grating blazed at 500 nm will have an absolute efficiency of >60% up to 3000 rel.1/cm when using a 532 nm laser. Using the same grating with a 785 nm laser will cause the efficiency to drop to about 30% at 3000 rel.1/cm. The correct grating blazed at 750 nm, however, shows an efficiency >60% up to 3000 rel.1/cm when used with the 785 nm laser.

Efficiency of the spectrometer: There are a variety of spectrometers available on the market. While mirror-based spectrometers can generally be used over a wide spectral range, lens-based spectrometers often show a higher throughput in the spectral range they are designed for. Some lens-based spectrometers show a transmission at 532 nm of >60%. Spectrometers using a combination of lenses and prisms or mirrors generally have a significantly lower throughput. Commercially available mirror-based

Czerny-Turner spectrometers only have a transmission of about 30% at this wavelength.

Efficiency of the detector (CCD): Back-illuminated CCD cameras show a quantum efficiency (QE) of >90% over the entire spectral range of interest for 532 nm excitation. Deep depletion back-illuminated CCD cameras, on the other hand, show a QE of >90% for 785 nm excitation. As a comparison, front-illuminated CCD cameras do not exceed 55% quantum efficiency at any wavelength.

Additionally, the dark current of the cameras needs to be minimized, which is achieved through efficient Peltier cooling.

The readout noise is another limiting factor for small signals. As the analog to digital (A/D) converter of all cameras will add at least 5–10 electrons read-out noise to the signal, any signal below approximately 5 electrons will be lost in the noise. Additionally, the faster the A/D converter is operating, the higher the read-out noise will be. Electron-multiplying CCD (EM-CCD) cameras can be used to overcome this problem. With these cameras, the signal is amplified before the A/D conversion, allowing the detection of even single photons and reducing the necessary integration time down to milliseconds.

Principle of operation

Confocal Raman microscopes generally provide a variety of modes of operations. The most common are listed below:

Collection of Raman spectra at selected sample areas (single spectrum): Single Raman spectra can be collected at user-selectable sample areas with integration times ranging from ms to hours. The position of the collected spectrum can normally be fully controlled in 3D. A stable and precise positioning system must be included in the instrument to ensure that the point of interest will remain fixed under the excitation focus. This is very important when spectra with longer integration times for the best quality and signal to noise ratio are to be obtained from extremely small sample volumes. For example, using an oil immersion objective (NA 1.4) with a 532 nm laser and the proper pinhole size allows the sample volume to be as small as $\approx 230 \times 230 \times 550$ nm.

Collection of time series of Raman spectra at selected sample areas (time spectrum): With this mode, time series of Raman spectra can be obtained to analyze dynamic sample properties. Thousands of spectra can be obtained over time and analyzed with integration times ranging from ms to tens of seconds.

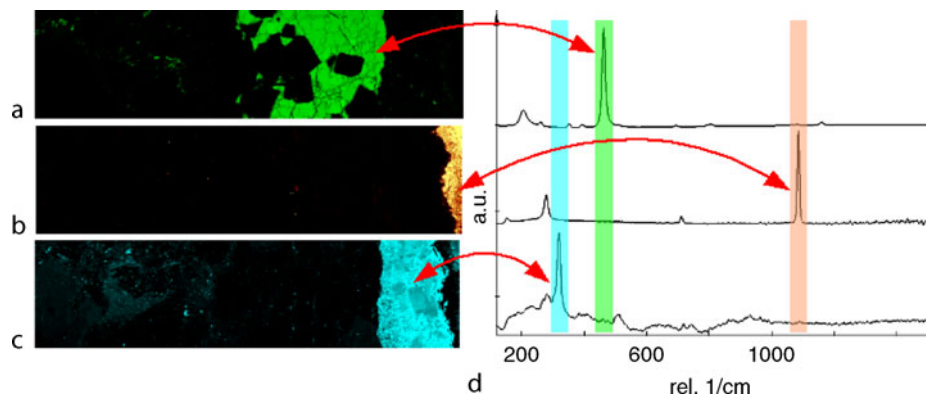
Raman spectral imaging: In the Raman spectral imaging mode, the sample is moved in X and Y and a full Raman spectrum is obtained at every pixel measured. From these data sets images of, e.g., the integrated intensity of various bands can be generated. This is illustrated in the following example.

A drill core section from the Äspö Hard Rock Laboratory, Sweden, was studied using the large area scan mode CRM, aiming to characterize secondary fracture fillings in

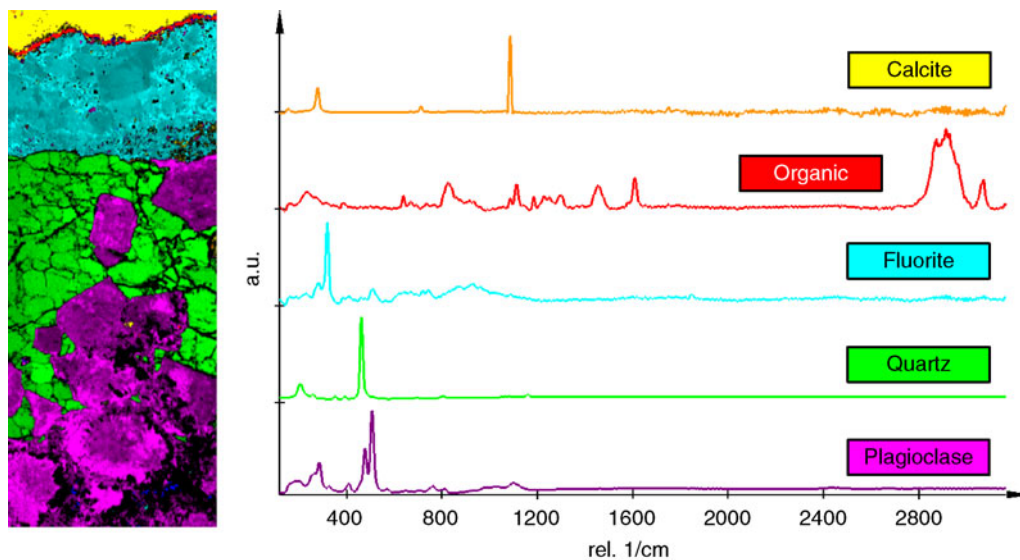
a 1.8–1.4 billion years old diorite. The rock sample was obtained from ~450 m below the surface (kindly provided by SKB, Swedish nuclear fuel and waste management). This sample was examined with a 532 nm laser and a 50× air objective (NA 0.55). The scan range was 8000 μm in X and 2000 μm in Y with 800×200 points resolution. Each spectrum was integrated for 36 ms. Figure 6 shows the characteristic spectra of calcite, fluorite and quartz found in the sample. Integrating over, for example, the area marked in green in Figure 6d results in a single value for each of the 160,000 spectra. These spectra can be displayed as a color-coded image as shown in Figure 6a for quartz. Here, brighter values indicate a higher integrated intensity of the quartz peak and the distribution of quartz can thus be obtained from this image.

Other spectra of the same scan show the characteristic features of calcite or fluorite (Figure 6d). Using these, the calcite (Figure 6b) and fluorite (Figure 6c) images can be generated by using additional integral filters for the marked regions. Other features of the spectra such as the width of peaks or their position can also be evaluated by applying the corresponding filters.

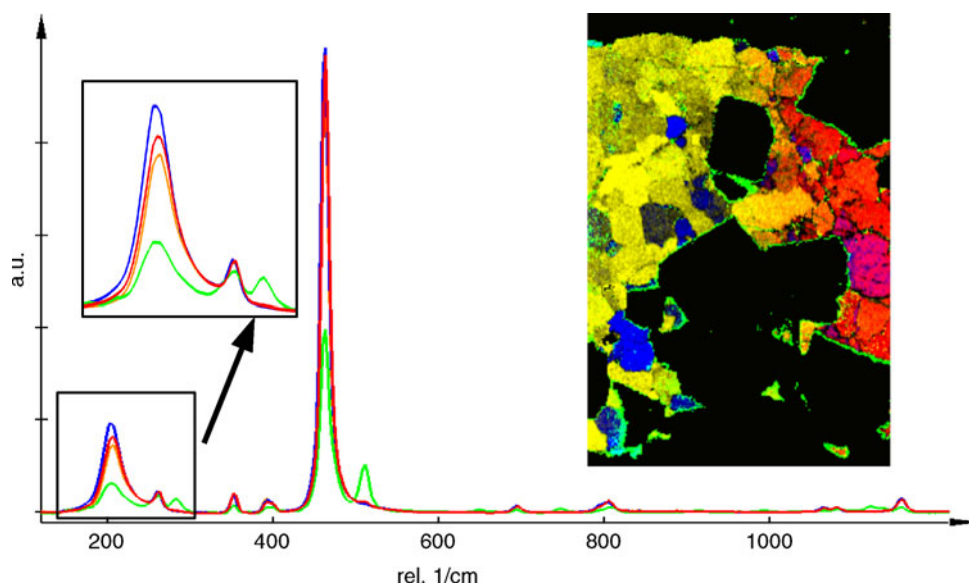
Further evaluation of the data allows the averaging of similar spectra (for which a cluster analysis is often used) and the subtraction of, for example, pure spectra from mixed spectra to extract the spectra of the various components (spectral de-mixing). These spectra can then be used with the basis analysis, where each of the spectra recorded are fitted with a linear combination of the basis spectra. The result of such an analysis is one image for each basis



Raman Microscopy (Confocal), Figure 6 Spectra (d) and spectral images of quartz (a), calcite (b) and fluorite (c) recorded from the diorite (a) and the fracture fillings (b and c). The data were recorded with a WITec alpha500R confocal Raman microscope.



Raman Microscopy (Confocal), Figure 7 DE-mixed and averaged spectra of the various components in the diorite and the adjacent fracture minerals with the combined image on the left.



Raman Microscopy (Confocal), Figure 8 Variations in the quartz phases due to changes in the crystallographic orientation, the crystallinity, and the minerals.

spectra which can then be combined to generate a false color image showing the distribution of all components (Figure 7).

The color-coded Raman image and corresponding spectra in Figure 7 allow for the general assignment of mineral phases and their gross distribution over the scanned area. In addition to the mineralogical context information, organic components were identified, spectrally characterized and located, trapped between two generations of fracture fillings (the hydrothermal fluorite and low temperature calcite, Tullborg et al., 2008; Wallin and Petermann, 1999). This information allowed to infer at which point in time a “deep biosphere” (see entry “*Terrestrial Deep Biosphere*”) was active within these rocks.

Cluster analysis of the data set revealed discrete areas of variation in the mineral phases (Figure 8). This is exemplified by the quartz phase where four distinct regions were identified based on variations in relative peak intensity. Plotting regions of equal intensities of the quartz line at ca. 200 cm^{-1} shows discrete regions in the sample corresponding to each of the identified spectra.

Quartz was selected as an example to highlight the feasibility of color-coded Raman imaging to locate changes of different mineral phases. These changes can likely be attributed to different levels of crystallinity and crystal orientation. It is noteworthy that the discrete phase colored green only occurs at the interface with the plagioclase minerals, which can be attributed to a secondary phase due to the alteration and phase changes of the plagioclases. Since SiO_2 -phases play an important role in biomineralization, this example highlights the potential benefits confocal Raman imaging may provide in understanding the processes and dynamics involved in these processes.

Summary

CRM is a nondestructive analytical technique that merges Raman spectroscopy and confocal microscopy for the visualization of molecular information over a defined sample area. The technique makes use of the Raman effect (Raman, 1928), i.e., the energy shift between exciting and scattered photons which is caused by the excitation (or annihilation) of a molecular vibration. This energy shift is characteristic and therefore a fingerprint for the type and coordination of the molecules involved. By means of CRM, the spatial distribution and association of components in the sample, including organics as well as minerals, can be evaluated from large scale scans in the centimeter range to the finest detail with submicron resolution. This way, CRM may contribute significantly to the understanding of a sample’s chemical composition, and complexity, in geological and geobiological studies.

Bibliography

- Fries, M., and Steele, A., 2010. Raman spectroscopy and confocal Raman imaging in mineralogy and petrography. In Dieing, T., Hollricher, O., and Toporski, J. (eds.), *Confocal Raman Microscopy*, Heidelberg: Springer.
- Hild, S., Marti, O., and Ziegler, A., 2008. Spatial distribution of calcite and amorphous calcium carbonate in the cuticle of the terrestrial crustaceans *Porcellio scaber* and *Armadillidium vulgare*. *Journal of Structural Biology*, **142**, 100–108.
- Ibach, H., and Lüth, H., 2003. *Solid State Physics. An Introduction to Principles of Materials Science*, Berlin: Springer.
- Raman, C., 1928. A new radiation. *Indian Journal of Physics*, **2**, 387.
- Raman, C., and Krishnan, K., 1928. A new type of secondary radiation. *Nature*, **121**, 501.
- Smekal, A. G., 1923. Zur Quantentheorie der Dispersion, *Naturwissenschaften*, **11**, 873–875.

- Tullborg, E.-L., Drake, H., Sandström, B., 2008. Palaeohydrogeology: A method based on fracture mineral studies. *Applied Geochemistry*, **23**, 1881–1897.
- Wallin, B., and Peterman, Z., 1999. Calcite fracture fillings as indicators of palaeohydrology at Laxemar at the Äspö Hard Rock Laboratory, southern Sweden. *Applied Chemistry*, **14**, 953–962.
- Wilson, T., 1990. *Confocal Microscopy*, London: Academic.

Cross-references

[Biosignatures in Rocks](#)
[Terrestrial Deep Biosphere](#)

REDUCTION SPHEROIDS

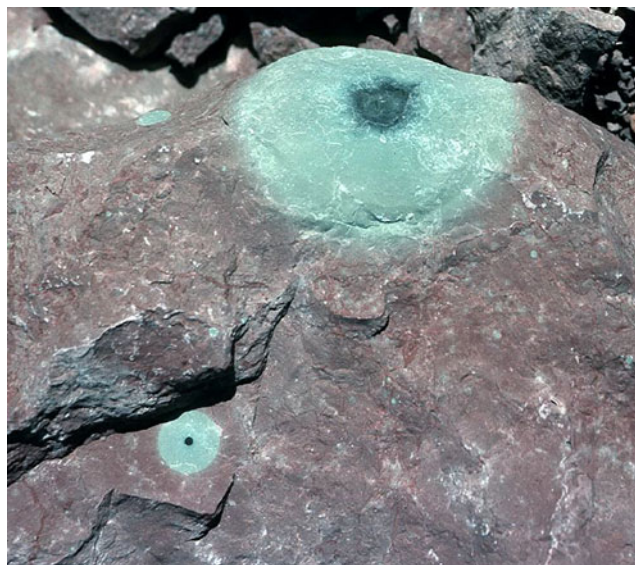
Beda A. Hofmann
 Natural History Museum Bern, Bern, Switzerland

Synonyms

Bleaching haloes; Fish eyes; Radioactive concretions; Radioactive nodules; Reduction mottling; Reduction spots; Reduktionshöfe

Definition

Reduction spheroids are small-scale (diameter: few millimeters to maximum about 20 cm) spheroidal bleached features in red-bed sediments characterized by the absence of hematite and often containing a mineralized core (center, [Figure 1](#)). Reduction spheroids result from local chemical reduction of ferric iron and other elements. Host rocks are marine and continental red beds and, more rarely, altered, hematite-stained crystalline rocks. Reduction spheroids have been described from many occurrences in red beds of widely variable age worldwide: Proterozoic of Canada (Tanton, 1948); Cambro-Silurian marine red beds of the NE USA (Beutner and Charles, 1985); Devonian continental red beds of Scotland (Parnell, 1985); Carboniferous continental red beds of New Brunswick and Nova Scotia, Canada (Dyck and McCorkell, 1983); Permian continental red beds of Germany (Eichhoff and Reineck, 1952; Mempel, 1960; Schreiter, 1930), Switzerland (Hofmann, 1990, 1991), Southwest England (Harrison, 1970; Perutz, 1940), and Cretaceous radiolarian cherts of Oman (Hofmann, 1991). Reduction spheroids characteristically consist of a spheroidal volume of rock from which hematite pigment has been dissolved. Central dark cores often display pronounced concentric zonation. The cores are strongly enriched in numerous redox-sensitive elements (dominant V, but also U, Se, As, Ni, Co, Cu, Ag, Au, PGE, and others). The dominant mineral in reduction spheroid cores is the vanadium mica roscoelite $KV_2AlSi_3O_{10}(OH)_2$. Uraninite is another very common mineral, leading to an often increased radioactivity of reduction spheroids ([Figure 2](#)). Arsenides of Ni, Co, and Cu are also quite common. Radiometric dating of roscoelite, uraninite, and

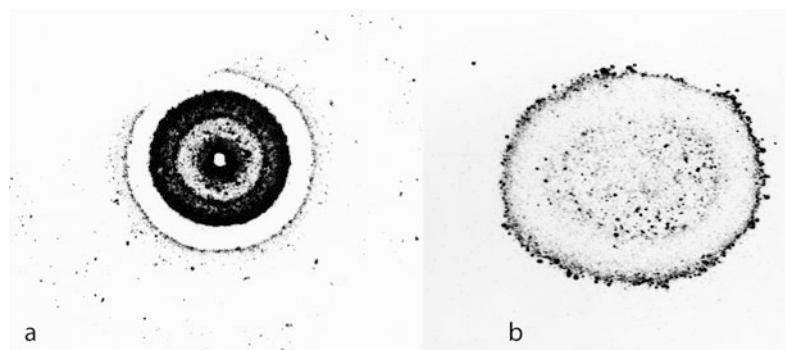


Reduction Spheroids, Figure 1 Reduction spheroids in Triassic red bed (marly dolomite), Chinle Formation, Paradox Valley, Colorado, USA. These reduction spheroids are strongly enriched in V, Cr, and Au. Diameter of larger reduction spheroid is 8 cm.

Pb-depleted haloes indicates late diagenetic ages (Hofmann, 1990; Hofmann and Frei, 1996). Reduction spheroids often occur in large numbers in red beds and can constitute up to several vol% of the rock. Based on textural evidence, such as fracture-bound occurrences and mass-balance calculations (Hofmann, 1991), reduction spheroids cannot be due to detrital organic-rich particles. Instead, the formation must be due to locally catalyzed redox reactions between a mobile reductant and oxidants. The presence of isotopically light sulfides in some cores (Hofmann, 1991), indicating diagenetic sulfate reduction, as well as the need for a local catalyst, strongly favors a microbiological origin of reduction spheroids. Reductants may be mobile hydrocarbons or molecular hydrogen resulting from the radiolysis of pore water (Hofmann, 1992). The formation of reduction spots is most likely due to chemolithotrophic communities. Kerogen-like organic matter is occasionally present at some localities (Curiale et al., 1983; Parnell, 1985) but is lacking at many other occurrences (Hofmann, 1993).

Conclusion

Reduction spheroids are very common in red-bed sediments, and there are strong indications for an origin due to redox reactions between a mobile reductant and oxidized elements locally catalyzed by microbial activity. Reduction spheroids are thus easily visible features resulting from redox processes catalyzed by a deep biosphere of sediments, and represent a type of biosignature that may also be present in oxidized rocks on Mars. Reduction spheroids contain small-scale



Reduction Spheroids, Figure 2 α -Autoradiographs of uranium-enriched reduction spheroid cores, Weiherfeld well, Permian of northern Switzerland. The accumulation of uranium reflects the heterogeneous distribution of reduction activity, consistent with microbial activity. Diameter of radioactive uranium-rich zone is (a) 5 mm and (b) 10 mm.

element enrichments that are in many ways analogous in element inventory, geochemistry, and mineralogy to ore deposits such as unconformity-related and roll-type uranium ores.

Bibliography

- Beutner, E. C., and Charles, E. G., 1985. Large volume loss during cleavage formation, Hamburg sequence, Pennsylvania. *Geology*, **13**, 803–805.
- Curiale, J. A., Bloch, S., Rafalska-Bloch, J., and Harrison, W. E., 1983. Petroleum-related origin for uraniferous organic-rich nodules of southwestern Oklahoma. *AAPG Bulletin*, **67**, 588–608.
- Dyck, W., and McCorkell, R. H., 1983. A study of uranium-rich reduction spheroids in sandstones from Pugwash Harbour, Nova Scotia. *Canadian Journal of Earth Sciences*, **20**, 1738–1746.
- Eichhoff, H. J., and Reineck, H. E., 1952. Uran-Vanadinkerne mit Verfärbungshöfen in Gesteinen. *Neues Jahrbuch für Mineralogie, Monatshefte*, **11/12**, 294–314.
- Harrison, R. K., 1970. Hydrocarbon-bearing nodules from Heysham, Lancashire. *Geological Journal*, **7**, 101–110.
- Hofmann, B. A., 1990. Reduction spheroids from northern Switzerland: mineralogy, geochemistry and genetic models. *Chemical Geology*, **81**, 55–81.
- Hofmann, B. A., 1991. Mineralogy and geochemistry of reduction spheroids in red beds. *Mineralogy and Petrology*, **44**, 107–124.
- Hofmann, B. A., 1992. Isolated reduction phenomena in red beds: a result of porewater radiolysis? In Maest A. S. (ed.), *7th International Symposium on Water-Rock interaction*, Balkema, Park City, Utah, USA, pp. 503–506.
- Hofmann, B. A., 1993. Organic matter associated with mineralized reduction spots in red beds. In Parnell, J., Kucha, H., and Landais, P. (eds.), *Bitumens in Ore Deposits*. Berlin: Springer, pp. 362–378.
- Hofmann, B. A., and Frei, R., 1996. Age constraints of reduction spot formation from Permian red bed sediments, northern Switzerland, inferred from U-Th-Pb systematics. *Schweizerische Mineralogische und Petrographische Mitteilungen*, **76**, 235–244.
- Mempel, G., 1960. Neue Funde von Uran-Vanadiumkernen mit Entfärbungshöfen. *Geologische Rundschau*, **49**, 263–276.
- Parnell, J., 1985. Uranium/rare earth-enriched hydrocarbons in Devonian sandstones, northern Scotland. *Neues Jahrbuch für Mineralogie—Monatshefte*, **H3**, 132–144.
- Perutz, M., 1940. Radioactive nodules from Devonshire, England. *Mineralogische und Petrographische Mitteilungen*, **51**, 141–161.
- Schreiter, R., 1930. Vanadinhaltige Kerne, Bleichungsringe und Bleichungszonen in den Schieferletten des Rotliegenden von Sachsen. *Zeitschrift der Deutschen Geologischen Gesellschaft*, **82**, 41–47.
- Tanton, T. L., 1948. Radioactive nodules in sediments of the Sibley Series, Nipigon, Ontario. *Transactions of the Royal Society of Canada*, **17**(Ser. III, Sec. 4), 69–75.

Cross-references

[Biosignatures in Rocks](#)
[Chemolithotrophy](#)
[Deep Biosphere of Sediments](#)
[Hydrogen](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Radioactivity \(Natural\)](#)

REEFS

Reefs are laterally confined submarine carbonate structures developed by the growth or activity of sessile benthic aquatic organisms, such as corals, bryozoans, sponges, algae, and microbes (Flügel and Kiessling, 2002). See entry “[Carbonate Environments](#)” for further reading and classification.

Bibliography

- Flügel, E., and Kiessling, W., 2002. A new look at ancient reefs. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: SEPM Special Publication Series No. 72, pp. 3–20.

REMINERALIZATION (OF ORGANIC MATTER)

Degradation of organic matter that results in net conversion of organic carbon to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- . See entries "[Carbon \(Organic, Cycling\)](#)" and "[Carbon \(Organic, Degradation\)](#)" for further reading.

RHODOPHYTA

The Rhodophyta, also referred to as "red algae," are a distinct eukaryotic lineage of algae with simple plastids (two membranes, derived from primary endosymbiosis). Rhodophytes have a long geological record since the Mesoproterozoic (1.2 Ga). Today, they encompass about 670 largely marine genera with up to 4,500 species predominating the coastal and continental shelf areas of tropical, temperate, and cold-water regions. Red algae are significant as primary producers and providers of structural habitat for other marine organisms. Calcifying (coralline) red algae play an important role in the primary establishment and maintenance of limestone reefs. For further details, see entry "[Algae \(Eukaryotic\)](#)."

Cross-references

[Algae \(Eukaryotic\)](#)
[Carbonate Environments](#)
[Symbiosis](#)

RNA-WORLD

The term "RNA-World," first suggested by Walter Gilbert in 1986, describes a popular concept on a hypothetical early stage in the origin of life on Earth. Based on discoveries of enzymatic activities in microbial ribonucleic acids (RNA), a system was suggested where primordial RNA molecules accounted for both, the storage of genetic information and catalytic functions necessary to assemble themselves in a primitive self-replicating system. The RNA molecules evolved in self-replicating patterns, using recombination and mutation to explore new functions and to adapt to new niches (Gilbert, 1986). Such a system would not have required the presence of DNA and/or protein enzymes for biochemical reactions. For further reading, please refer to "[Origin of Life](#)."

Bibliography

Gilbert, W., 1986. The RNA world. *Nature*, **319**, 618.

- taxonomy of halobacteriaceae and subcommittee on the taxonomy of Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2975.
- Pearson, E. J., Farrimond, P., and Juggins, S., 2007. Lipid geochemistry of lake sediments from semi-arid Spain: relationships with source inputs and environmental factors. *Organic Geochemistry*, **38**, 1169–1195.
- Piovano, E. L., Ariztegui, D., Bernasconi, S. M., and McKenzie, J. A., 2004. Stable isotopic record of hydrological changes in subtropical Laguna Mar Chiquita (Argentina) over the last 230 years. *The Holocene*, **14**, 525–535.
- Queral, I., Juliá, R., Plana, F., and Bischoff, J. L., 1997. A hydrous Ca-bearing magnesium carbonate from playa lake sediments, Salines Lake, Spain. *American Mineralogist*, **82**, 812–819.
- Rontani, J. -F., and Volkman, J. K., 2005. Lipid characterization of coastal hypersaline cyanobacterial mats from the Camargue (France). *Organic Geochemistry*, **36**, 251–272.
- Saijo, Y., Mitamura, O., and Tanaka, M., 1995. A note on the chemical composition of lake water in the Laguna Amarga, a saline lake in Patagonia, Chile. *International Journal of Salt Lake Research*, **4**, 165–167.
- Taher, A. G., 1999. Inland saline lakes of Wadi El Natrun depression. *International Journal of Salt Lake Research*, **8**, 149–169.
- Taylor, P. L., Hutchinson, L. A., and Muir, M. K., 1980. Heavy metals in the Great Salt Lake, Utah. In Gwynn, J. W. (ed.), *Great Salt Lake A Scientific, Historical and Economic Overview*. Salt Lake City, Utah: Utah Geological and Mineral Survey. Bulletin 116, pp. 195–200.
- Ventosa, A., Nieto, J. J., and Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews*, **62**, 504–544.
- Yechieli, Y., and Ronen, D., 1997. Early diagenesis of highly saline lake sediments after exposure. *Chemical Geology*, **138**, 93–106.
- ZoBell, C. E., 1963. Domain of the marine microbiologist. In Oppenheimer, C. H. (ed.), *Symposium on Marine Microbiology*. Springfield, IL: Charles C. Thomas, pp. 3–24.

Cross-references

Alkalinity
 Archaea
 Bacteria
 Biomarkers (Organic Compound-Specific Isotopes)
 Extreme Environments
 Halobacteria – Halophiles
 Hypersaline Environments
 Microbial Ecology of Submarine Caves
 Microbial Mats
 Pore Waters
 Sediment Diagenesis – Biologically Controlled
 Soda Lakes

SALINITY HISTORY OF THE EARTH'S OCEAN

L. Paul Knauth
 Arizona State University, Tempe, AZ, USA

Definition

Salinity refers to the total quantity of dissolved salts in water and is normally about 35 parts per thousand (‰) in today's ocean. Due to problems with making accurate measurements of total salts, salinity is now normally

expressed as a ratio of the electrical conductivity of the seawater to that of a standard concentration of KCl solution. The concentration of the standard solution is set such that the ratio of the approximate average salinity of the ocean today is equal to 35. The previous concentration value (‰) is thus unnecessary, but it is still widely used to highlight the basic meaning of the term. The relative proportions of the dissolved constituents are approximately constant, so the amount of Cl^- , the major component, is a proxy for salinity. Salinity history refers to past changes in the amount of dissolved salts in sea water over geologic time.

Significance

Salinity is an important environmental variable affecting chemical balances and osmotic pressures in all living cells. It particularly affects metazoans, because the amount of dissolved oxygen available for respiration depends on oxygen solubility that decreases as salinity increases. Besides its direct effects on cellular biochemistry, salinity governs the temperature of maximum density of sea water and thus the global thermohaline circulation that influences ecosystems. Models of geochemical cycles yield no constraints on past salinity levels, and reliable geochemical proxies, isotopic records, or other possible indicators of ancient oceanic salinity are currently unknown. However, Na^+ and Cl^- constitute about 85% of the salt in the sea, so the geologic record of halite deposits allows insights into how ocean salinity may have varied with time.

Causes of salinity changes with time

Early ideas that steady riverine input of salt to the oceans causes salinity to increase with time failed to recognize the geologic processes that intermittently cause salt to be removed from the sea and then later released following tectonic uplift. Nor did they correctly assess the probable initial origin of salt in the sea or consider possible small changes in ocean volume over time. Salt in rivers is derived largely from groundwater dissolution of ancient marine salt beds deposited when sea water evaporated under arid conditions in low latitude, geographically restricted arms of the sea. Deposition of these “saline giants” depletes the ocean in salt and thus lowers salinity. Subsequently, these enormous deposits may be tectonically lifted above sea level. Depending upon elevation, descending groundwaters can eventually dissolve the deposit and debouch into streams or lodge as dense, saline brines deeper in the continental subsurface. In addition to the saline giants, carbonate depositional environments in shallow seas that flooded the continents in past times typically contained vast tidal flats and supratidal areas in which sea water intermittently evaporated. Partially evaporated sea water from these environments as well as their ephemeral salts dissolved by falling rains descended into the subsurface and lodged in sedimentary basins even where large saline giants were absent. Most large

sedimentary accumulations on continents thus have highly saline waters in the deep subsurface. The salts in these brines were also in sea water once and their sequestration on continents thus also served to lower salinity of sea water. The presence of salt beds and brines hundreds of millions of years old illustrates the enormous lengths of time salt extracted from the sea can remain isolated.

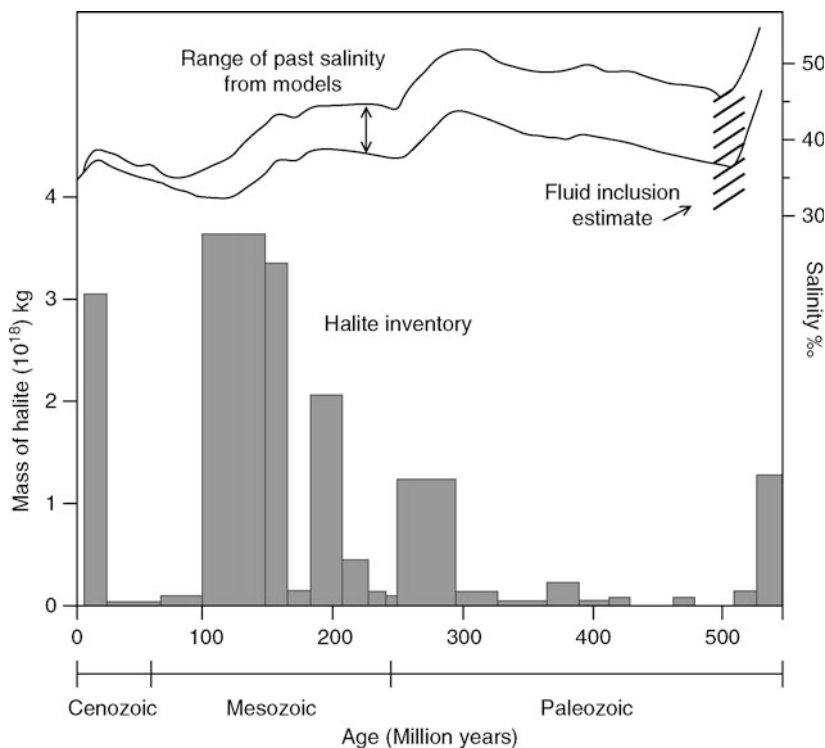
Salinity history for the past 540 Ma

Estimates of the salt inventory in sedimentary rocks have steadily grown as more and more subsurface deposits are discovered. The latest estimate by Hay et al. (2006) confirms that deposition of the saline giants is strikingly episodic (Figure 1). Over half of the salt currently identified was deposited between 100 and 300 million years ago following breakup of the supercontinent Pangea. Presumably, this breakup and subsequent drift of the continental fragments produced the unusual array of geographic, geologic, climatic, and ocean circulation conditions that Sonnenfeld (1984) argued were necessary to produce saline giants. It follows that ocean salinity should have declined during these times of exceptional withdrawal of salts from the sea. The amounts shown in Figure 1 are surely minimal estimates of the amount of salt withdrawn because some significant percentage has been dissolved by groundwaters to become brines in the strata beneath

the salt. Additionally, salt depositional basins are rimmed with clastic deposits adjacent to the surrounding highlands and these are conduits to the subsurface for the prodigious amounts of evaporite brine present when the salt was being deposited. Combined geochemical and isotopic studies have shown that deep basin brines are probably mixtures of partially evaporated sea water and brine created by fresh water dissolution of salt (Knauth, 1988).

Salinity should increase during periods when there are no giant salt extractions underway and when older, uplifted deposits are eroding into the sea. This is the current situation, so the old arguments that the oceans are increasing in salinity via riverine input of salt are understandable. There is no direct evidence yielding the amount of salt returned to the sea at any given past time. Instead, it is necessary to use models for continental sediment erosion that are based on an array of assumptions. Hay et al. (2006) estimate that the return of salt to the sea has varied by a factor of three over the past 540 Ma, reaching maxima in the Upper Cambrian (515 Ma), at the Permian/Triassic boundary (245 Ma), and during the Lower Cretaceous (135 Ma).

Using the inventory of currently sequestered salt and the calculated estimate of salt return, a possible salinity history of sea water for the past 540 Ma can be inferred if the volume history of sea water can be estimated. Fresh basalt on the ocean floors becomes hydrated during subsea



Salinity History of the Earth's Ocean, Figure 1 Salt deposition in the Phanerozoic and estimates of past salinity variations. Ocean salinity declines during deposition of large salt masses. Model estimates by Hay et al. (2006) yield surprisingly high salinity values for the Paleozoic which are not incompatible with values inferred from fluid inclusion measurements by Johnson and Goldstein (1993). Salt masses from estimates by Hay et al. (2006).

weathering and hydrous sedimentary minerals are deposited as the basalt rides tectonic plates to subduction zones. At issue is how much of the hydrated water returns to the surface as it gets progressively heated during subduction. Some models predict a net loss to the mantle over geologic time, so it is not unreasonable to suggest that ocean volumes may have decreased somewhat with time. Combining all these uncertain estimates and models, Hay et al. (2006) have suggested the possible salinity history for the oceans for the past 540 Ma shown in Figure 1. The inferred salinity levels prior to 200 Ma are 50% higher than modern values. Inasmuch as life in the sea is stressed by current salinity values, the idea that Paleozoic life could thrive at such high inferred salinity is likely to be resisted by marine biologists and paleontologists. The amount of brine sequestered during initial deposition and later dissolution remain uncertain and could severely affect such calculated salinity histories. Nevertheless, the provocative Hay et al. (2006) estimates indicate that ocean salinities in the past were likely higher.

Direct salinity measurements in fossil sea water

Tiny fluid inclusions in carbonates or other minerals precipitated directly from ancient seawater may be fossil sea water that could be analyzed for salinity. The difficulty is that the initial precipitates typically undergo dissolution/reprecipitation episodes during subsequent burial and metamorphism. Actual attempts at making this difficult analysis on inferred primary precipitates yield salinities of 31–47‰ at about 500 Ma (Johnson and Goldstein, 1993). These measurements suggest past salinities not too dissimilar from the modern value, but the large error bars do overlap the Hay et al. (2006) estimates (Figure 1). While reliable ancient salinity values remain elusive, it seems demonstrated that significant variations should have occurred in the past 540 Ma and that salinity was likely higher rather than lower in the past.

Salinity history of the early ocean

With regard to deeper time, past salinity can be estimated if it is assumed that the oceans outgassed early in Earth history (Holland, 1978) and that the plate tectonic cycle long ago reached an approximate steady state with regard to hydrosphere cycling through the crust and mantle. Cl^- does not fit readily into any common silicate mineral and thus should have outgassed as HCl along with water following accretion of the Earth. The earliest hydrosphere would thus contain the entire Cl inventory and been at maximum salinity. The only way this high initial salinity can be reduced is by sequestration of salts and brines in the saline giants deposited on long-lived continental cratons. Large continents may have formed shortly after the Earth accreted 4.5 billion years ago (Ga), but recent studies confirm the likelihood that objects in the inner solar system were largely resurfaced by the Late Heavy Bombardment (LHB) at about 3.8–4.0 Ga (Gomes et al., 2005). Continents may have started to reaggregate

following the LHB in the interval between 3.8–2.5 Ga, but the kind of long-lived, continental cratons upon which evaporatively deposited salt and brine could be sequestered following its removal from sea water do not appear until about 2.5 Ga (Rogers, 1996). It follows that all the salt and brine currently lodged on the continents was in the Archean sea following the LHB and prior to the development of these stable cratons.

In the absence of a paleosalinity indicator in the rock record, a reasonable assessment of the earliest salinity could be made if the total amount of salt and brine currently lodged on the continents were known. While the inventory of salt in strata younger than 540 Ma is becoming increasingly better quantified (Figure 1), the amount of salt in older strata is yet to be inventoried. Enormous deposits are known in Neoproterozoic strata (540–800 Ma) across the Middle East, through Pakistan, and into Australia. These apparently accumulated in restricted basins that developed following the breakup of supercontinent Rodinia, analogous to those developed following the breakup of Pangea. The geographic extent of these enormous salt deposits exceeds that of the Cenozoic deposits in the Gulf of Mexico, so it might even be the largest accumulation in Earth history. The amount of subsurface brine associated with the saline giants and with innumerable ancient platform carbonate deposits is also unknown. Land (1995) used estimates of the amount and average salinity of pore fluids to suggest that sequestered brines could hold an amount of salt equal to or greater than that of the rock salt. Adding these amounts of possible total salt and brine currently on the continents back into modern ocean water raises salinity by over a factor of 2. Assuming that the Cl inventory and the oceans were outgassed early in Earth history and have not changed their amounts significantly with time, Archean salinities were therefore much higher and could have exceeded 70‰ (Knauth, 2005).

Implications

The geologic history of salt deposition clearly seems to suggest significant past salinity variations with values possibly much higher than current levels. If so, the impact on biology and biologic evolution must have been significant. An assessment of the paleontologic record in relation to the emerging possible salinity history for the past 540 Ma (Figure 1) remains to be done. The inferred very high salinity of the earliest ocean combined with the probable hot climatic conditions of the early Earth may have limited early marine biologic diversity to the appropriate extremophiles. If the great Neoproterozoic salt deposits were really the first saline giants, the prospect arises that high salinity was prevalent throughout the Precambrian. The great salinity decline likely accompanying deposition of the Neoproterozoic salt combined with declining temperatures associated with probable glaciations may have allowed oxygenation of the ocean for the first time. The Cambrian explosion of life in the sea at 540 Ma may have been, in part, a response of respiring organisms evolving

rapidly in this vast new array of oxygenated marine habitats (Knauth, 2005).

Summary

Salinity is not fixed by any equilibria, feedbacks, or geochemical cycles and appears to have been generally higher in past times. It is apparently a free variable dependent upon the vagaries of intermittent salt (and brine) sequestration on long-lived continental cratons. Ocean salinity declines when the salt and brine accumulate and rises when they are eroded back into the sea. Sequestration seems to have been enhanced following breakup of the two supercontinents in Earth history. These breakups are, in turn, driven by the vagaries of mantle convection. The combination of processes therefore necessary to lower the initial, pre-continent high salinity and to affect the rise and fall of ocean salinity involve a long chain of independent variables. The role of chance and contingency in biologic evolution has long been recognized. The low odds that a rerun of the tape of evolution would yield the same results have been duly noted (Gould, 1990). The same might be said about the evolution of oceanic salinity. Salinity history should therefore probably be added to all those chance occurrences that have likely affected the course of biologic evolution.

Bibliography

- Gomes, R., Levison, H. F., Tsiganis, K., and Morbidelli, A., 2005. Origin of the cataclysmic Late Heavy Bombardment period of the terrestrial planets. *Nature*, **435**, 466–469.
- Gould, S. J., 1990. *Wonderful Life: The Burgess Shale and the Nature of History*. New York: W.W. Norton.
- Hay, W. W., Migdisov, A., Balukhovskiy, A. N., Wold, C. N., Fogel, S., and Soding, E., 2006. Evaporites and the salinity of the ocean during the Phanerozoic: implications for climate, ocean circulation and life. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **240**, 3–46.
- Holland, H. D., 1978. *The Chemistry of the Atmosphere and Oceans*. New York: Wiley.
- Johnson, W. J., and Goldstein, R. H., 1993. Cambrian sea water preserved as inclusions in marine low-magnesium calcite cement. *Nature*, **362**, 335–337.
- Knauth, L. P., 1988. Origin and mixing history of brines, Palo Duro Basin, Texas, USA. *Applied Geochemistry*, **3**, 455–474.
- Knauth, L. P., 2005. Temperature and salinity history of the Precambrian ocean: implications for the course of microbial evolution. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 53–69.
- Land, L. S., 1995. The role of saline formation water in crustal cycling. *Aquatic Chemistry*, **1**, 137–145.
- Rogers, J. J. W., 1996. A history of continents in the past three billion years. *The Journal of Geology*, **104**, 91–107.
- Sonnenfeld, P., 1984. *Brines and Evaporites*. Orlando, FL: Academic Press.

Cross-references

[Critical Intervals in Earth History](#)
[Evaporites](#)
[Hypersaline Environments](#)
[Pore Waters](#)
[Soda Ocean, Hypothesis](#)

SCANNING PROBE MICROSCOPY (INCLUDES ATOMIC FORCE MICROSCOPY)

Michael Hoppert

University of Göttingen, Göttingen, Germany

Synonyms

Atomic force microscopy; Atomic probe microscopy; Force microscopy; Scanning force microscopy

Definition

Scanning probe microscopy uses a nanoscopic probe that is scanned over a solid surface. The interaction between the probe and the surface may be a mechanical or electromagnetic force. The force signal is enhanced and then composed to a force diagram of the surface. Under ideal conditions, an atomic-scale resolution may be achieved.

Introduction

Since their development during the 1970s and early 1980s, scanning probe microscopes (SPMs) have developed into instruments suitable for the analysis of surface topography (and other surface properties) down to near-atomic resolutions. All instruments belonging to this family contain essential components as depicted in [Figure 1](#). In the scanning process, surface material properties are mapped in all three directions. The essential parts of the system are the sharp scanning probe tip, which moves in the *z* direction, a piezoelectric scanner that moves the specimen, or, alternatively, the tip, in the *x/y* directions, and a sensor for tip positioning. Sensor and tip are connected in a feedback loop for the correction of *z* positioning. The *x/y/z* positions are used to generate a topographic image of the specimen. The resolution of SPMs is only limited by the size of the probe tip (or the probe-sample interaction volume). Since the tip apex ends up in one single atom and the piezoelectric actuators execute motions down the range of angstroms, resolutions down to atomic scale are possible. In contrast to electron microscopes, the sample can be observed under ambient conditions, which makes the technique potentially attractive for life science, including geobiological research.

The scanning tunneling microscope (STM)

The scanning tunneling microscope (STM) is the ancestor of all SPMs (Binnig et al., 1982). The specimen needs to be conductive or semiconductive. STM is predominantly used in materials sciences and chemistry. Though not ideally suited for biological specimens, the technique has also been used in life sciences (see, e.g., Guckenberger et al., 1988). The instrument provides a surface topography down to atomic resolution.

STMs use a sharpened conducting tip with a bias voltage (in mV to V-range) applied between the tip and the sample. If the distance between the tip and the sample is large, no current flows. When, however, the tip is brought

S

SALINE LAKES

Carol D. Litchfield
George Mason University, Manassas, VA, USA

Synonyms

Salt Lakes

Definition

Salt: chloride, sulfate, phosphate, carbonate, bicarbonate salts of primarily sodium, magnesium, calcium, and potassium.

Lake: an enclosed body with or without water entering it from a stream, rain, or snow melt.

Ephemeral lake: a lake formed only following rain or snow melt, which dries up during the rest of the year.

Thalassohaline: salts in a lake derived from the evaporation of seawater with the dominant ions of sodium and chloride as found in the ocean.

Athalassohaline: salts in a lake derived from rocks and geological weathering and dominated by magnesium, calcium, and sulfate.

Alkaliphilic lake: also called soda lake. A lake in which the dominant ions are sodium and bicarbonate and carbonate. This causes the lake to have an alkaline pH.

General description of saline lakes

Saline lakes are a natural worldwide phenomenon. They can be found on all continents and in most countries, and they are frequently recognizable by their name – for example, Salt Lick, Salt Lake, Salt Pond, and Salt Marsh. They range in salinity from just above the salt content of sea water (>3.2% salts) to hypersaline lakes (>20% and up to saturation with respect to salts). Examples of saline lakes include, at the lower end, salt lakes in Antarctica, the Caspian Sea, which borders five countries and has

a salinity slightly less than that of seawater, and the Salton Sea in California. At the upper end in salt concentrations are the Dead Sea bordering Israel and Jordan, the soda lakes of North Africa, and Quinghai Lake in China. A general characteristic of saline lakes is that they are terminal lakes, which means that although rivers may flow into them, they have no significant outflow. Therefore, as the water evaporates, the lakes become more salty and in some cases may dry up completely forming salt basins or playas especially in extremely arid environments. In Australia, for example, occasional rains may cause the dry salt basins to become lakes, and these then are called ephemeral salt lakes.

Saline lakes are typically divided into two main types: athalassohaline and thalassohaline. The former refers to lakes which have a different chemical ratio of the main cations from what is found in seawater, while the latter refers to lakes with the cation ratio similar to seawater. [Table 1](#) lists the sodium + potassium ion to calcium + magnesium ion ratios and summarizes the chemical composition of a variety of saline lakes thus showing the wide range of lake types possible.

The data in [Table 1](#) indicate that a subset of the athalassohaline lakes exists. These lakes have a high concentration of carbonate and therefore a high pH. These are the soda lakes or alkaline lakes. They, too, are found worldwide and are described below. No Antarctic lakes are listed in [Table 1](#) because the surface waters are generally only very slightly saline (less than seawater) and it is only with depth, often greater than 10 m, that true saline waters are found. In general, it is the bottom waters that are hypersaline with little turnover to bring the saline waters to the surface. The chemical compositions of Antarctic lakes have been reviewed by Matsubaya et al. (1979).

The reason for the differences in the chemical composition lies in the origins of the various types of lakes. The thalassohaline lakes were generally formed during the

Saline Lakes, Table 1 Examples of the chemical compositions of saline lakes. Concentrations in g/L

Lake name	Location	Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ⁻²	HCO ₃ ⁺	pH	Ratio ^a	References
Small lake near Koombekine	Australia	9.36	0.065	0.077	0.38	15.7	0.67	0	3.0	20.6	Geddes et al. (1981)
Mar Chiquita Lake	Argentina	12.5	0.14	0.35	0.25	16.7	5.1	0.21	8.53	21.1	Martinez (1995)
Laguna Amarga	Chile	24	0.33	0.002	1.7	8.2	38	4.4	9.4	14.3	Saijo et al. (1995)
Great Salt Lake – North Arm	Utah, USA	101	6.9	0.28	8.5	175	22	<0.05	7.9 ^b	12.3	Utah Geol. Survey (unpublished)
Great Salt Lake – South Arm	Utah, USA	59	3.4	0.26	4.6	95	13	<0.05	7.3–8.1 ^b	12.8	Utah Geol. Survey (unpublished)
Dead Sea	Israel	34.3	8.0	18.3	47.1	228.6	0.4	0.3	6.3	0.65	Gavrieli et al. (2005)
Urmia Lake	Iran	88	1.1	1.21	6.6	153	14.2	284	6.75	11.4	Alipour (2006), Ghaheiri et al. (1999)
Mono Lake	California, USA	29.5	1.5	0.004	0.033	17.6	10.3–12.5	30.1	9.8	838	Oren (2002)
Wadi El Natrun	Egypt	279.7	NL ^c	ND ^d	ND	86.4	43.4	5.3	8.9	ND	Taher (1999)
Laguna Colorada	Bolivia	18.1	1.85	NL	NL	33.3	3.84	NL	8.4	ND	Mancinelli (personal communication)
Muskiki	Saskatoon, Canada	62.2	NL	NL	29	11.4	237	1.47	7.9	ND	Javor (1989)
Oygon	Gobi Desert	18.0 ^e		0.02	0.41	16.9	6.25	8.91	NL	41.9	Egorov (1993)
Seawater	Mongolia	11	0.39	0.42	1.32	19.7	2.76	0.145	8.0	6.5	ZoBell (1963)

^aCalculation for this paper: (Na⁺ + K⁺)/(Ca⁺⁺ + Mg⁺⁺)^bTaylor et al. (1980)^cNot listed^dNot determined^eCombined Na⁺ + K⁺

Pleistocene period (Hammer, 1986) and are the result of seawater intrusion before tectonic activity created the mountains which cut the bodies of seawater off from the ocean. Brine springs are formed from the dissolution of salts deposited during evaporation of the seawater. As surface water seeps into the ground, the water percolates downward and eventually encounters the salt, dissolves it, and forms underground saline lakes. These form the basis of often small salty ponds, springs, licks, and rivers.

Athalassohaline lakes are most generally formed by the weathering of rocks by wind, rain, and freshwater and were formed during the Pleistocene period and later by volcanic and faulting activity (Hammer, 1986). These are also terminal lakes, and they are typically close to saturation with respect to salt. They may also be ephemeral – found only occasionally after the rainy season – or permanent containing water year round.

The soda lakes are considered athalassohaline because they were formed from high carbonate rocks due to an absence of rocks of sedimentary origins. Consequently, they have low concentrations of calcium and magnesium ions and high concentrations of carbonate or carbonate complexes (Jones et al., 1998).

Biology of saline lakes

Despite the differences in chemical composition, all saline lakes have certain features in common. In general, fish are not found in lakes with salinities greater than 4‰ (40‰). Two major reasons for this are egg survival (Gerking and Lee, 1980) and loss of osmoregulation (Morris, 1960). One exception is the European sea bass, *Dicentrarchus labrax*, which tolerates salinities up to twice that of seawater (Jensen et al., 1998).

Other eukaryotic organisms are found in the higher salinity lakes, specifically algae, brine flies, and brine shrimp. The diversity of the algae depends on the total salt concentration. At salinities lower than around 10–12‰ salt, a wide variety of diatoms and green algae is found. At salinities greater than 12–15‰ and up to saturation, the main algae are from the genus *Dunaliella*. It is also at these higher salinities that the brine fly and brine shrimp are major biomass contributors. These small eukaryotes provide the food sources for the extensive bird populations that are frequently associated with saline lakes. However, other crustaceans such as the mussel shrimp cannot survive in lakes with salinities greater than 18‰ (Deckker, 1981).

Another group of eukaryotic organisms found throughout the range of saline lakes is the fungi. It is only recently that fungi have been isolated from the higher salinity lakes. The state of our understanding of halotolerant and halophilic fungi was summarized by Frisvad (2005). The presence of protozoans and flagellates in saline lakes has also been reviewed and documented by Hauer and Rogerson (2005) and Cho (2005), respectively.

Just as lower salinities allow for a greater diversity of algae, the same is true for the bacteria. Members of the

Domain *Bacteria* predominate in the salinity range of 3–15‰ salt (Ventosa et al., 1998). Above this, the members of the archaeal kingdom domain, the salt-loving *Halobacteriaceae* become dominant though several members of the domain *Bacteria* grow at these elevated salinities. The predominant *Bacteria* genera in elevated salinities are *Halomonas* and *Salinibacter*. As of August 2007, there were 26 described genera of the haloarchaea and 85 species (Oren et al., 2007), and most, but not all, grow over salinity ranges from approximately 12‰ to close to saturation. Microbes found in most salt lakes are therefore, salt-loving and prefer a circumneutral pH.

Among those haloarchaea genera, the “Natrono-” genera are primarily found in soda lakes. As mentioned above, soda lakes have an alkaline pH; therefore, bacteria which grow under those conditions are considered alkaliphiles (alkaline pH-loving). There are many genera of the kingdom *Bacteria* which also contain genera and species that are alkaliphiles. Thus, all of these bacteria are both salt- and high pH-loving organisms. This distinguishes them from the bacteria described in the previous paragraph.

Halophages are viruses that attack bacteria living in saline lakes. The viruses may incorporate into the bacterial cells or they may cause the lysis of the cells. In either case, halophages, like other bacteriophages, tend to be host-specific and can be found wherever their host organism lives. Dyall-Smith et al. (2005) have recently summarized the state of knowledge about the halophages which are specific for the haloarchaea.

Saline lake sediments

Sediments of saline lakes have been the subject of numerous studies because of the potential for determining stable isotope ratios hence sediment age (Piovano et al., 2004) and using lipid biomarkers (Rontani and Volkman, 2005; Pearson et al., 2007) to indicate the types of biota in the sediments. The exposure history of Dead Sea sediments was determined by examining the various minerals formed within the interstitial waters again improving the understanding of the age and depositional history of saline lake sediments (Yechieli and Ronen, 1997). As the salinity of the overlying water increases, this results in similar increases in the salinity of the interstitial waters within the shallow sediments, and the reverse is also true that decreases in the salinity of the lake due to rainfall or snow melt will decrease the salinity of the pore waters.

These postdepositional changes have also been noted for the alkaline Soda Lake in Washington State in the USA (Mantuani, 1973) and in playa Lake Salines in southeastern Spain (Queralt et al., 1997). Such changes can also affect the biology of the sediments which are anaerobic (low to no oxygen) and are dominated by bacterial mats which can produce sulfides and methane (Miller et al., 1993). These mats are composed of layers of bacteria with the cyanobacteria on the surface and various green and purple sulfur bacteria followed by the sulfate-reducing bacteria that produce the sulfides, and the obligately

anaerobic methane-producing bacteria. More recently, Jioang et al. (2007) have noted, in a core from Lake Chaka on the Tibetan Plateau that the distribution of bacteria within the core is determined by the salinity in the pore waters rather than by the mineralogy, and that the biomass decreased most around 150–200 cm depth and increased slightly at 600 cm with a maximum at 900 cm as determined by phospholipid fatty acid analyses. However, the direct counts remained steady with a slight increase at 900 cm. Most of the organisms identified at these lower depths were anaerobic bacteria, again confirming the importance of oxygen to the types of microorganisms found in any sediment.

Value of saline lakes

Saline lakes are nature preserves and serve as home to extensive permanent and migratory bird populations. Great Salt Lake, Utah, USA, hosts over five million birds annually (and this is true in Africa, Australia, the Caribbean, etc.). They may also act as hatching grounds for flamingoes and other birds at the lower salinities. Thus, saline lakes are important in maintaining the biodiversity of our planet.

A second major value, which is related to the first, is that these environments with their large numbers of microorganisms, brine flies, and brine shrimp provide an important gene pool. Although there are numerous studies on the brine shrimp, very little is known about the brine flies, the algae, or the bacteria found in these lakes. Unknown enzymes, potential antibiotics, drug delivery systems, or mechanisms for adaptation to stress could be present but unknown for lack of research into these areas.

Another value of the lower salinity lakes is their potential for aquaculture. Gamito (1997) successfully showed that the gilthead, *Sparus aurata*, could be grown in the Ria Formosa because of the high benthic productivity (1997). Undoubtedly, there are other fish species that could be successfully grown in the lower salinity saline lakes.

A major economic value of saline lakes is their salt. The ephemeral or dried salt beds are a source of salt to many groups of people. This is especially true in Africa with the Taureg people, and in Tibet which also has salt caravans, in the Atacama region of Chile, in Australia, and in Bolivia to name just a few of the places where salt is harvested from dry saline lake beds.

Bibliography

- Alipour, S., 2006. Hydrogeochemistry of seasonal variation of Urmia Salt Lake, Iran. *Saline Systems*, **2**, 9–28.
- Cho, B. C., 2005. Heterotrophic flagellates in hypersaline waters. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 541–549.
- Decker, P. de., 1981. Ostracods of athalassic saline lakes: a review. *Hydrobiologia*, **81**, 131–144.
- Dyall-Smith, M., Burns, D. G., Camakaris, H. M., Janssen, P. H., Russ, B. E., and Porter, K., 2005. Haloviruses and their hosts. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 553–563.
- Egorov, A. N., 1993. Mongolian salt lakes: some features of their geography, thermal patterns, chemistry and biology. *Hydrobiologia*, **267**, 13–21.
- Frisvad, J. C., 2005. Halotolerant and halophilic fungi and their extrolite production. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 425–439.
- Gamito, S., 1997. Sustainable management of a coastal lagoonal system (Ria Formosa, Portugal): an ecological model for extensive aquaculture. *International Journal of Salt Lake Research*, **6**, 145–173.
- Gavrieli, I., Bein, A., and Oren, A., 2005. The expected impact of the “Peace Conduit” project (The Red Sea-Dead Sea pipeline) on the Dead Sea. *Mitigation and Adaptation Strategies for Global Change*, **10**, 3–21.
- Geddes, M. C., De Decker, P., Williams, W. D., Morton, D. W., and Topping, M., 1981. On the chemistry and biota of some saline lakes in Western Australia. *Hydrobiologia*, **82**, 201–222.
- Gerking, S. D., and Lee, R. M., 1980. Reproductive performance of the desert pupfish (*Cyprinodon n. nevadensis*) in relation to salinity. *Environmental Biology of Fishes*, **5**, 375–378.
- Ghaheri, M., Baghal-Vayjooee, M. H., and Naziri, J., 1999. Lake Urmia, Iran: a summary review. *International Journal of Salt Lake Research*, **8**, 19–22.
- Hammer, U. T., 1986. *Saline Lake Systems of the World*. Boston: Dr. W. Junk, p. 46.
- Hauer, G., and Rogerson, A., 2005. Heterotrophic protozoa from hypersaline environments. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 519–539.
- Javor, B., 1989. *Hypersaline Environments*. New York: Springer.
- Jensen, M. K., Madsen, S. S., and Kristiansen, K., 1998. Osmoregulation and salinity effects on the expression and activity of Na⁺, K⁺-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). *Journal of Experimental Zoology*, **282**, 290–300.
- Jioang, H., Dong, H., Yu, B., Liu, X., Li, Y., Ji, S., and Zhang, C., 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environmental Microbiology*, **9**, 2603–2621.
- Jones, B., Grant, W. D., Duckworth, A. W., and Owenson, G. G., 1998. Microbial diversity of soda lakes. *Extremophiles*, **2**, 191–200.
- Mantuani, M. A., 1973. Postdepositional osmotic adjustments in sediments from Soap Lake, Washington. *Limnology and Oceanography*, **18**, 403–413.
- Martinez, D. E., 1995. Changes in the ionic composition of a saline lake, Mar Chiquita, Province of Córdoba, Argentina. *International Journal of Salt Lake Research*, **4**, 25–44.
- Matsubaya, O., Sakal, H., Toril, T., Burton, H., and Kerry, K., 1979. Antarctic saline lakes – stable isotopic ratios, chemical compositions and evolution. *Geochimica et Cosmochimica Acta*, **43**, 7–25.
- Miller, L. G., Jellison, R., Oremland, R. S., and Culbertson, C. W., 1993. Meromixis in hypersaline Mono Lake, California. 3. Biogeochemical response to stratification and overturn. *Limnology and Oceanography*, **38**, 1040–1051.
- Morris, R. W., 1960. Temperature, salinity, and southern limits of three species of Pacific cottid fishes. *Limnology and Oceanography*, **5**, 175–179.
- Oren, A., 2002. *Halophilic Microorganisms and their Environments*. Dordrecht: Kluwer Academic Publishers.
- Oren, A., Vreeland, H., and Ventosa, A., 2007. International Committee on Systematics of prokaryotes; subcommittee on the

- taxonomy of halobacteriaceae and subcommittee on the taxonomy of Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2975.
- Pearson, E. J., Farrimond, P., and Juggins, S., 2007. Lipid geochemistry of lake sediments from semi-arid Spain: relationships with source inputs and environmental factors. *Organic Geochemistry*, **38**, 1169–1195.
- Piovano, E. L., Ariztegui, D., Bernasconi, S. M., and McKenzie, J. A., 2004. Stable isotopic record of hydrological changes in subtropical Laguna Mar Chiquita (Argentina) over the last 230 years. *The Holocene*, **14**, 525–535.
- Queral, I., Juliá, R., Plana, F., and Bischoff, J. L., 1997. A hydrous Ca-bearing magnesium carbonate from playa lake sediments, Salines Lake, Spain. *American Mineralogist*, **82**, 812–819.
- Rontani, J. -F., and Volkman, J. K., 2005. Lipid characterization of coastal hypersaline cyanobacterial mats from the Camargue (France). *Organic Geochemistry*, **36**, 251–272.
- Saijo, Y., Mitamura, O., and Tanaka, M., 1995. A note on the chemical composition of lake water in the Laguna Amarga, a saline lake in Patagonia, Chile. *International Journal of Salt Lake Research*, **4**, 165–167.
- Taher, A. G., 1999. Inland saline lakes of Wadi El Natrun depression. *International Journal of Salt Lake Research*, **8**, 149–169.
- Taylor, P. L., Hutchinson, L. A., and Muir, M. K., 1980. Heavy metals in the Great Salt Lake, Utah. In Gwynn, J. W. (ed.), *Great Salt Lake A Scientific, Historical and Economic Overview*. Salt Lake City, Utah: Utah Geological and Mineral Survey. Bulletin 116, pp. 195–200.
- Ventosa, A., Nieto, J. J., and Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews*, **62**, 504–544.
- Yechieli, Y., and Ronen, D., 1997. Early diagenesis of highly saline lake sediments after exposure. *Chemical Geology*, **138**, 93–106.
- ZoBell, C. E., 1963. Domain of the marine microbiologist. In Oppenheimer, C. H. (ed.), *Symposium on Marine Microbiology*. Springfield, IL: Charles C. Thomas, pp. 3–24.

Cross-references

[Alkalinity](#)
[Archaea](#)
[Bacteria](#)
[Biomarkers \(Organic Compound-Specific Isotopes\)](#)
[Extreme Environments](#)
[Halobacteria – Halophiles](#)
[Hypersaline Environments](#)
[Microbial Ecology of Submarine Caves](#)
[Microbial Mats](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Soda Lakes](#)

SALINITY HISTORY OF THE EARTH'S OCEAN

L. Paul Knauth
 Arizona State University, Tempe, AZ, USA

Definition

Salinity refers to the total quantity of dissolved salts in water and is normally about 35 parts per thousand (‰) in today's ocean. Due to problems with making accurate measurements of total salts, salinity is now normally

expressed as a ratio of the electrical conductivity of the seawater to that of a standard concentration of KCl solution. The concentration of the standard solution is set such that the ratio of the approximate average salinity of the ocean today is equal to 35. The previous concentration value (‰) is thus unnecessary, but it is still widely used to highlight the basic meaning of the term. The relative proportions of the dissolved constituents are approximately constant, so the amount of Cl^- , the major component, is a proxy for salinity. Salinity history refers to past changes in the amount of dissolved salts in sea water over geologic time.

Significance

Salinity is an important environmental variable affecting chemical balances and osmotic pressures in all living cells. It particularly affects metazoans, because the amount of dissolved oxygen available for respiration depends on oxygen solubility that decreases as salinity increases. Besides its direct effects on cellular biochemistry, salinity governs the temperature of maximum density of sea water and thus the global thermohaline circulation that influences ecosystems. Models of geochemical cycles yield no constraints on past salinity levels, and reliable geochemical proxies, isotopic records, or other possible indicators of ancient oceanic salinity are currently unknown. However, Na^+ and Cl^- constitute about 85% of the salt in the sea, so the geologic record of halite deposits allows insights into how ocean salinity may have varied with time.

Causes of salinity changes with time

Early ideas that steady riverine input of salt to the oceans causes salinity to increase with time failed to recognize the geologic processes that intermittently cause salt to be removed from the sea and then later released following tectonic uplift. Nor did they correctly assess the probable initial origin of salt in the sea or consider possible small changes in ocean volume over time. Salt in rivers is derived largely from groundwater dissolution of ancient marine salt beds deposited when sea water evaporated under arid conditions in low latitude, geographically restricted arms of the sea. Deposition of these “saline giants” depletes the ocean in salt and thus lowers salinity. Subsequently, these enormous deposits may be tectonically lifted above sea level. Depending upon elevation, descending groundwaters can eventually dissolve the deposit and debouch into streams or lodge as dense, saline brines deeper in the continental subsurface. In addition to the saline giants, carbonate depositional environments in shallow seas that flooded the continents in past times typically contained vast tidal flats and supratidal areas in which sea water intermittently evaporated. Partially evaporated sea water from these environments as well as their ephemeral salts dissolved by falling rains descended into the subsurface and lodged in sedimentary basins even where large saline giants were absent. Most large

- Goldmann, W. H., Galneder, R., Ludwig, M., Xu, W., Adamson, E. D., Wang, N., and Ezzell, R. M., 1998. Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Experimental Cell Research*, **239**, 235–242.
- Grude, O., Hammiche, A., Pollock, H., Bentley, A. J., Walsh, M., Martin, F. L., and Fullwood, N. J., 2007. Near-field photothermal microspectrometry for adult stem-cell identification and characterization. *Journal of Microscopy*, **228**, 366–372.
- Guckenberger, R., Kösslinger, C., Gatz, R., Breu, H., Levai, W., and Baumeister, W., 1988. A scanning tunneling microscope (STM) for biological applications. Design and performance. *Ultramicroscopy*, **25**, 795–802.
- Haupt, B. J., Pelling, A. E., and Horton, M. A., 2006. Integrated confocal and scanning probe microscopy for biomedical research. *The Scientific World Journal*, **6**, 1609–1618.
- Hildebrand, M., Doktycz, M. J., and Allison, D. P., 2008. Application of AFM in understanding biomineral formation in diatoms. *Pflügers Archiv-European Journal of Physiology*, **456**, 127–137.
- Krottil, H.-U., Stifter, T., Waschipky, H., Weishaupt, K., Hild, S., and Marti, O., 1999. Pulsed force mode: a new method for the investigation of surface properties. *Surface and Interface Analysis*, **27**, 336–340.
- Lehenkari, P. P., and Horton, M. A., 1999. Single integrin molecule adhesion forces in intact cells measured by atomic force microscopy. *Biochemical and Biophysical Research Communications*, **259**, 645–650.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Schewanella* and α -FeOOH. *Science*, **292**, 1360–1363.
- Murdfeld, T., Fischer, U. C., and Fuchs, H., 1996. Acoustic and dynamic force microscopy with ultrasonic probes. *Journal of Vacuum Science and Technology B*, **14**, 877–881.
- Overney, R. M., Meyer, E., Frommer, J., Brodbeck, D., Lüthi, R., Howald, L., Güntherodt, H.-J., Fujihira, M., Takano, H., and Gotoh, Y., 1992. Friction measurements on phase-separated thin films with a modified atomic force microscope. *Nature*, **359**, 133–134.
- Price, D. M., Reading, M., Hammiche, A., and Pollock, H. M., 1999. Micro-thermal analysis: scanning thermal microscopy and localised thermal analysis. *International Journal of Pharmaceutics*, **192**, 85–96.
- Rotsch, C., Braet, F., Wisse, E., and Radmacher, M., 1997. AFM imaging and elasticity measurements on living rat liver macrophages. *Cell Biology International*, **21**, 685–696.
- Troyon, M., Wang, Z., Pastre, D., Lei, H. N., and Hazotte, A., 1997. Force modulation microscopy for the study of stiff materials. *Nanotechnology*, **8**, 163–171.
- Yip, C. M., 2001. Atomic force microscopy of macromolecular interactions. *Current Opinion in Structural Biology*, **11**, 567–572.

Cross-references

[Raman Microscopy \(Confocal\)](#)

SEDIMENT DIAGENESIS – BIOLOGICALLY CONTROLLED

Kurt O. Konhauser¹, Murray K. Gingras¹, Andreas Kappler²

¹University of Alberta, Edmonton, Alberta, Canada

²University of Tübingen, Tübingen, Germany

Definition

The biological processes that modify unconsolidated sediment after initial deposition and burial.

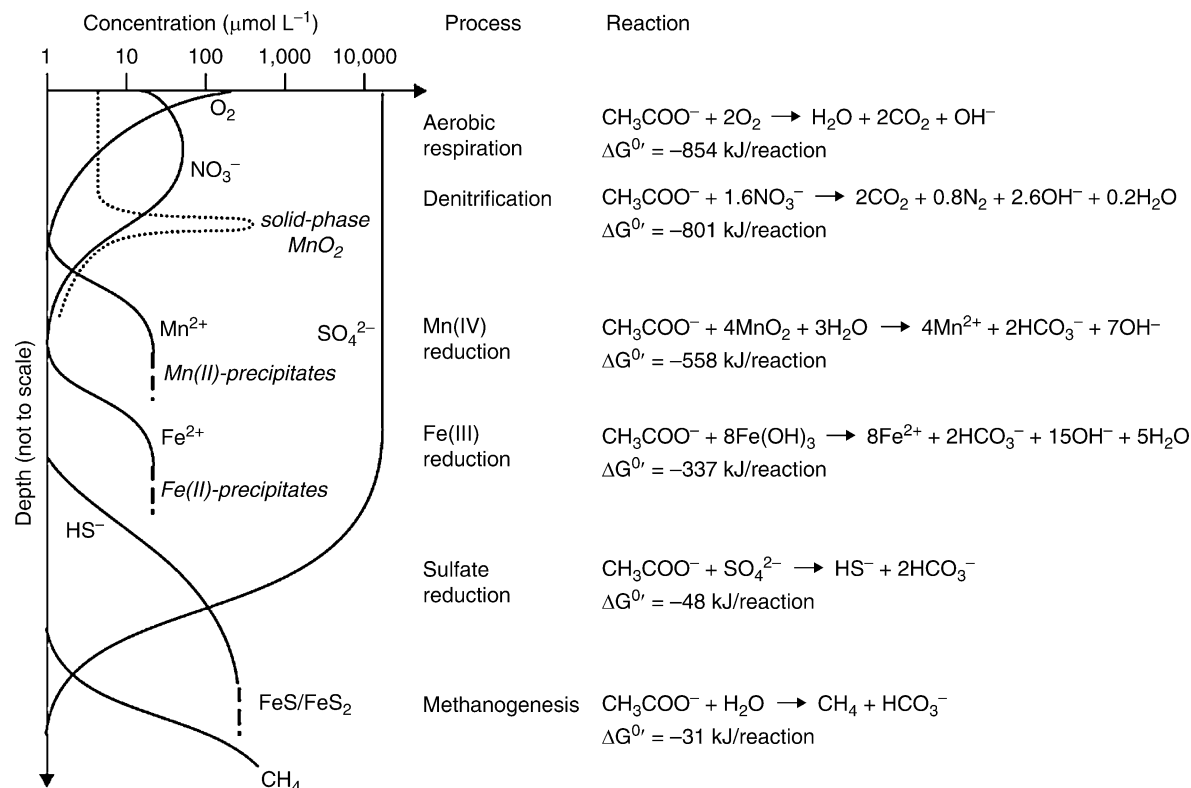
Overview

Throughout the sediment column, up to the temperature threshold of life, microbial activity plays an integral role in diagenesis. It is widely recognized that through their various chemoheterotrophic pathways, microorganisms are ultimately responsible for the conversion of organic carbon to CO₂ and CH₄ at temperatures <100°C. Some aerobic bacteria use hydrolytic enzymes to break down complex molecules into simple monomers such as sugars, amino acids, and fatty acids that they can then utilize, while most anaerobes are restricted to simple organic compounds (e.g., acetate, lactate, H₂) that are the by-products of fermentation (Lovley and Chapelle, 1995). Typically, the more labile materials (e.g., proteins, carbohydrates) are degraded in near-surface sediments on time scales of days to years, and more refractory materials (e.g., lipids) are broken down deeper in the sediment on time scales of decades, while the most resistant materials (lignins) are transformed only on timescales of thousands to millions of years. The amount of refractory material left after microbial attack will depend on the nature of the primary producers and the degree of processing that has taken place in the river catchment area or oceanic water column. In the end, for example, <1% of the original material buried into marine sediment may ultimately contribute to the sedimentary organic geochemical record (Emerson and Hedges, 1988).

Pore water and mineralogical changes during diagenesis are directly related to bacterial reduction of soluble (O₂, NO₃[−], SO₄^{2−}, CO₂) or solid-phase (MnO₂ and ferric oxyhydroxides) components in the sediment. The terminal electron accepting processes (TEAPs) that occur at any given layer depends on oxidants that are available and, in the situation when multiple electron acceptors are present (as in the uppermost sediment layers), on the free energy yield of the specific reaction. Thus, the decomposition of freshly deposited organic material in sediments proceeds in a continuous sequence of redox reactions, with the most electropositive oxidants being consumed at, or near the surface, and progressively energetically poorer oxidants being consumed at depth until the labile organic fraction is exhausted and the deeper sediments are left with a composition very different from that of the sediments originally deposited (Figure 1). Inorganic by-products of chemoheterotrophy (e.g., HCO₃[−], Mn²⁺, Fe²⁺, NH₄⁺, NO₂[−], HS[−], HPO₃^{2−} and CH₄) seldom accumulate. Physical and macrofaunal processes will cause the net transport of these reduced species from the deeper layers toward the sediment surface, where residing anaerobic and aerobic chemolithoautotrophic bacteria use them as metabolic reactants. Alternatively, their presence in pore waters may trigger important abiotic reactions between the solid and dissolved phases leading to secondary mineral formation, i.e., cementation.

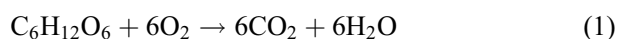
Oxic zone reactions

The sediment–water interface is the site of most intense heterotrophic activity. Aerobic respiration (Reaction 1) is



Sediment Diagenesis – Biologically Controlled, Figure 1 Idealized pore water and solid-phase profiles based on the successive utilization of terminal electron acceptors during the decomposition of marine sedimentary organic matter. (From Konhauser 2007.)

the first used metabolic pathway for the degradation of organic matter, not only because it has the greatest energy yield per amount of organic carbon oxidized, but the aerobes are equipped with a full suite of extracellular enzymes capable of degrading complex organic polymers into simpler substrates (e.g., cellulose into glucose).



$$\Delta G^0 = -2872 \text{ kJ/mol glucose or } -240 \text{ kJ/2e}^-$$

It is estimated that on a global scale, the majority of all particulate organic carbon (POC) deposited is aerobically respired; the percentage varies among different marine environments, with aerobic respiration accounting for >90% of the organic carbon oxidized in deep sea sediments (e.g., Bender and Heggie, 1984), while O_2 is the TEA in only 45% of coastal marine settings and freshwater lakes (e.g., Jones, 1985).

In marine sediment, one of the primary factors controlling the patterns above is the composition of the organic material and the rate at which it is deposited. Plankton-derived organic matter, rich in lipids and cellulose, exhibit significantly higher reactivities than land-derived organic material consisting of vascular plants rich in lignins (Hedges et al., 1988). Thus, in coastal settings, a significant fraction of POC may be relatively difficult

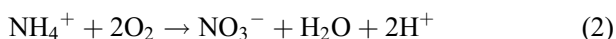
to decompose. Furthermore, under the high deposition rates characteristic of coastal settings (1 mm year^{-1}), O_2 diffusion is very shallow and the oxic zone may only extend down a few millimeters in depth, although physical stirring of surface sediments by waves and currents, circulation of overlying water through animal burrows that are connected to the sediment–water interface (known as irrigation), and bioturbation of surface sediments may locally increase the depth to several centimeters (e.g., Hammond et al., 1985). Therefore, with high accumulation rates, freshly deposited organic matter passes through the oxic zone on the orders of 1–10s of years, leaving a significant amount of carbon intact to support fermentative and anaerobic heterotrophic populations (Canfield, 1993). By contrast, the influx rate of POC and nutrients decreases with distance from land and water depth. This results in a progressive diminishment of macrofaunal and planktonic species, as well as the benthic bacterial populations, leading to low organic matter accumulation rates in the deep sea. Coupled with the low sedimentation rates associated with the open ocean ($<0.01 \text{ mm year}^{-1}$), the organic fraction will remain exposed to oxygenated waters for thousands of years, leading to almost complete oxidation by O_2 – only the most refractory fractions will remain available for other TEAs in the deeper sediments (Murray and Grundmanis, 1980).

O₂ is also consumed by a vast number of chemolithoautotrophic bacteria that metabolize upwardly diffusing reductants (such as HS⁻, NH₄⁺, Fe(II)). Therefore, as reduced metabolites from anaerobic respiratory pathways diffuse upward, a fraction of the oxygen consumed in the surface layers is diverted away from aerobic respiration, toward reoxidation of the reduced species. As a consequence, the depth of O₂ penetration correspondingly decreases. In fact, some diagenesis models show that the amount of O₂ partitioned to organic carbon oxidation in coastal sediments can be <1%, with nitrification or hydrogen sulfide oxidation comprising the major O₂ sinks (e.g., Blackburn and Blackburn, 1993).

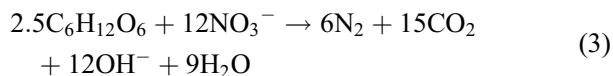
Suboxic zone

Nitrification–denitrification

Nitrogen cycling in aquatic sediments is relatively complex, with nitrate pore water concentrations governed by the balance between nitrification in the oxic zone and denitrification in the suboxic zone. The amount of nitrification is regulated by the concentration of pore water ammonium (NH₄⁺), which largely depends on the C:N ratio of the sedimentary organic carbon being oxidized, and the amount of O₂ penetration (Blackburn and Blackburn, 1993). In the presence of O₂, ammonium is first oxidized to nitrite by ammonium-oxidizing bacteria (e.g., *Nitrosomonas* sp.), and the nitrite is then further oxidized to nitrate by nitrite oxidizers (e.g., *Nitrobacter* sp.). Collectively, these processes (Reaction 2) cause a subsurface peak of nitrate in the pore water (Mortimer et al., 1999).



The opposing processes in marine N-cycling is denitrification (loss of NO₃⁻ to N₂) and nitrate ammonification (loss of NO₃⁻ to NH₄⁺). Denitrifiers most closely resemble aerobic respirers in that they are capable of completely degrading complex organic matter to carbon dioxide (Reaction 3).

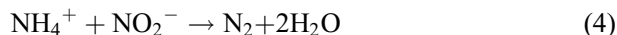


$$\Delta G^0 = -2715 \text{ kJ/mol glucose or } -226 \text{ kJ/2e}^-$$

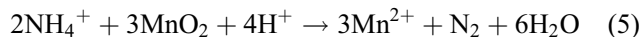
In coastal sediments (and lakes), these bacteria reduce nitrate to negligible levels within 1–10 cm of the sediment–water interface. No nitrate occurs below this depth except where burrows, lined with nitrifying bacteria, irrigate oxygenated water to depths well below the oxic–suboxic boundary (Hansen et al., 1981). In deep sea sediment, the vertical zone of all terminal electron acceptors (TEAs) are expanded, and for nitrate, concentrations may not become negligible until depths of >1 m. Denitrification fluxes range over several orders of magnitude in marine sediments, between values as low as 1 μmol m⁻² day⁻¹ in deep sea sediments (Bender and Heggie, 1984)

and over 1 mmol m⁻² day⁻¹ in some estuarine and coastal waters (e.g., Devol, 1991).

The flux of N₂ escaping from sediments is often higher than predicted biological or inorganic denitrification rates based solely on pore water nitrate concentrations. This means that in the sediments, excess N₂ is being formed independent of a nitrate intermediate. One way this occurs is through the anammox process, where shunting nitrogen directly from ammonium to N₂ (Reaction 4) can promote ammonium deficiencies in sediments where this process plays a key role in nitrogen cycling (Thamdrup and Dalsgaard, 2002).

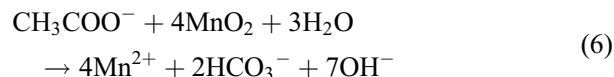


Ammonium can also react inorganically with MnO₂ to form N₂ instead of nitrate (Reaction 5). The Mn(II) formed in this reaction is biologically oxidized by Mn(II)-oxidizing bacteria (see below), with O₂ as the TEA, generating more reactive MnO₂ to continue the oxidation of the soluble ammonium (Luther et al., 1997). Field evidence suggests that this reaction can outcompete the direct oxidation of NH₄⁺ by O₂ to NO₃⁻ in Mn-rich coastal sediments, and in doing so, potentially account for 90% of N₂ formation. In essence, this process short-circuits the traditionally considered nitrification–denitrification process.



Manganese cycling

Detrital mineral phases that survive transport and deposition are for the most part an unreactive component of the bottom sediment. The oxides and hydroxides of manganese and iron are the exceptions. These phases are stable under the oxygenated conditions encountered in the water column, but they quickly become unstable after burial below the oxic layers of sediment. Following denitrification, reduction of Mn(IV) oxides (typically written as MnO₂) to dissolved Mn²⁺ becomes the most energy-efficient bacterial respiratory process (Reaction 6).



$$\Delta G^0 = -558 \text{ kJ/mol acetate or } -139 \text{ kJ/2e}^-$$

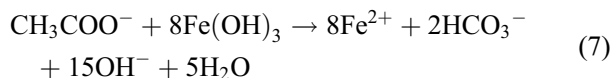
In most sediments, the limited amount of manganese (and iron) oxides deposited in sediment, as well as the slower rates of burial of these oxides compared to the downward diffusion of O₂, NO₃⁻ and SO₄²⁻, generally makes their reduction of minor importance (<10%) in terms of the amount of total organic carbon oxidized (Burdige, 1993). There are, however, exceptions to this generalization, i.e., some lakes and marine hydrothermal settings where high rates of metal oxide reduction are driven by a combination of relatively high metal inputs and active macrofaunal activity that mix suboxic sediment with

oxygenated bottom waters, thereby reoxidizing dissolved Mn^{2+} (and Fe^{2+}) to MnO_2 or $\text{Fe}(\text{OH})_3$ (Aller, 1990).

In typical marine sediments, the concentration of Mn^{2+} is negligible at the sediment surface, but at some depth between the NO_3^- maximum and zero NO_3^- , it begins to increase toward its maximum values. Despite forming near the base of the nitrate reduction zone, its concentration is never highest at that depth because the $\text{Mn}(\text{II})$ that diffuses upward is biologically reoxidized and precipitated as fresh MnO_2 at the sediment surface, suggesting that the TEA can be either nitrate or oxygen (Froelich et al., 1979). A characteristic feature of $\text{Mn}(\text{II})$ oxidation is the upward convexity of the pore water Mn^{2+} profile and the discrete layer of MnO_2 forming at the base of the oxic zone (recall Figure 1). By contrast, downward diffusion of $\text{Mn}(\text{II})$, and its reaction with HCO_3^- , most commonly results in the formation of manganese carbonates, such as rhodochrosite (MnCO_3).

Iron cycling

Below the zone of dissimilatory manganese reduction, and at the depth of complete nitrate removal from pore waters, is where $\text{Fe}(\text{III})$ reduction takes place (Reaction 7). Ferrihydrite [$\text{Fe}(\text{OH})_3$] is the least crystalline, most reactive, and easily reducible iron oxide phase, with rates of $\text{Fe}(\text{III})$ reduction in sediment declining rapidly with depth as these poorly crystalline phases become depleted (Lovley and Phillips, 1986).



$$\Delta G^0 = -337 \text{ kJ/mol acetate or } -84 \text{ kJ}/2\text{e}^-$$

Despite the lower reactivity of crystalline phases, in sediment where ferrihydrite has either been depleted or where crystalline iron oxides are inherently more abundant, the latter can serve as significant source of reducible iron that couples the oxidation of buried organic material. According to experimental observations, $\text{Fe}(\text{III})$ -reducing microorganisms developed three different strategies to cope with the difficulty of transferring electrons from the cell to the surface of a barely soluble electron acceptor (see Lovley et al., 2004 for review). First, physical contact between the cell surface and ferric iron allows direct delivery of electrons. Second, iron chelators increase the solubility of $\text{Fe}(\text{III})$ and hence, alleviate the need for $\text{Fe}(\text{III})$ reduction. Third, electron-shuttling compounds transfer electrons from the cell to $\text{Fe}(\text{III})$ oxide surface without the necessity of physical contact between cells and mineral. Considering the complexity of natural environments and the wealth of microbial capabilities, it is not surprising that different organisms, as well as single organisms, developed different strategies in order to reduce diverse $\text{Fe}(\text{III})$ compounds under varying conditions. For example, some evidence indicates that *Geothrix fermentans* produce and release both $\text{Fe}(\text{III})$ chelators and electron

shuttles (Nevin and Lovely, 2002). Furthermore, studies on *Geobacter* sp. indicate that different cellular compounds are involved in reduction of dissolved $\text{Fe}(\text{III})$ -citrate and barely soluble ferrihydrite (e.g., Leang et al., 2005).

The reduction of ferric iron minerals increases the concentration of $\text{Fe}(\text{II})$ in suboxic sediment pore waters, with a peak in concentration at the boundary between the $\text{Fe}(\text{III})$ and sulfate reduction zones. Some of this ferrous iron may diffuse upward to be reoxidized to ferric hydroxide inorganically by either NO_2^- , MnO_2 , or O_2 (e.g., Myers and Nealson, 1988), and it is likely that in some lakes with near-surface sediment, phototrophic $\text{Fe}(\text{II})$ oxidation should take place. Some $\text{Fe}(\text{II})$ also precipitates as mineral phases. In iron-rich sediments, the high reactivity of Fe^{2+} and the general availability of HCO_3^- in suboxic and anoxic pore waters tend to cause ferrous iron to precipitate quickly as micritic cement in the form of siderite (FeCO_3). By contrast, when sulfate reduction rates exceed $\text{Fe}(\text{III})$ reduction rates (see below), enough HS^- is produced to react preferentially with any ferrous iron to precipitate iron monosulfide minerals instead (FeS has lower solubility than FeCO_3). Therefore, the precise juxtaposition of iron reduction and sulfate reduction in marine sediments controls the formation of siderite and/or pyrite (e.g., Coleman, 1985). The formation of vivianite [$\text{Fe}_3(\text{PO}_4)_3$] in many ways resembles that of siderite, namely $\text{Fe}(\text{II})$ concentrations must exceed those of HS^- . It also requires that soluble phosphate is made available through oxidation of organic matter, the dissolution of phosphorous-bearing solid phases, or through reduction of phosphorous-adsorbing $\text{Fe}(\text{III})$ oxides (Krom and Berner, 1980).

Anoxic zone

Sulfate reduction

Below the suboxic layers is a highly reducing, oxygen-free zone under which sulfate reduction predominates. In marine sediments, this is the principal process by which simple fermentation products are oxidized (Reaction 8), accounting for approximately 50% of the carbon oxidation in coastal marine sediments (Jørgensen, 1982), but <10% in deep sea and freshwater environments (Bender and Heggie, 1984; Jones, 1985).



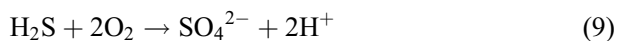
$$\Delta G^0 = -49 \text{ kJ/mol acetate or } -12 \text{ kJ}/2\text{e}^-$$

Its relative importance in marine environments compared to freshwater environments is based simply on the higher concentrations of dissolved SO_4^{2-} (~28 mM) at the sediment–water interface, some 50 times greater than the combined sum of all other electron acceptors with higher electrode potentials. Moreover, external electron acceptors that yield more energy than SO_4^{2-} typically disappear within the first few centimeters of sediment depth, leaving sulfate as the dominant TEA for most of the sediment column (D'Hondt et al., 2002).

The rates of dissimilatory sulfate reduction are proportional to the quantity and reactivity of organic matter entering the anoxic sediments – the greater the amount of aerobic decomposition, the less labile organic matter remains available for sulfate reduction, and the more refractory the residual material becomes (Westrich and Berner, 1984). As a result of natural variations in the above, sulfate reduction rates in marine sediments can vary by six orders of magnitude, with the highest in rapidly deposited coastal and lagoonal sediments, where it occurs just below the sediment–water interface ($>1 \text{ mM cm}^{-2} \text{ year}^{-1}$) and at the lowest levels in deep sea sediments, where rates may be $<10^{-5} \text{ mM cm}^{-2} \text{ year}^{-1}$ (Canfield, 1993).

The product of sulfate reduction is hydrogen sulfide, and as mentioned above, can lead to the formation of iron sulfide minerals. The amount and reactivity of the detrital iron phases dictate the amount of pyrite that will form, commonly referred to as the “degree of pyritization.” In slowly accumulating sediments (i.e., the deep sea), little labile organic matter is buried, sulfate reduction rates are slow, less HS^- is available for reaction with detrital iron, so less ferric iron is eventually converted to pyrite. Since the saturation state of the pore waters with respect to iron monosulfides controls the pore water concentrations of dissolved iron and sulfide, limited HS^- means that pore water Fe(II) will increase in concentration. By contrast, in rapidly accumulating sediments, more labile organics are buried, near-surface sulfate reduction rates are high, HS^- is abundant, and more of it is available for reaction with dissolved Fe(II) and solid-phase ferric iron (Berner and Raiswell, 1983). Nonetheless, not all ferric iron phases are pyritized because some ferric iron minerals react only very slowly with dissolved sulfide, and complete pyritization becomes impossible in the time span over which sulfate reduction occurs in the sediment (Canfield et al., 1992).

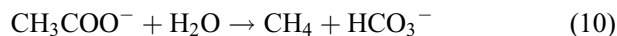
Only a fraction of the HS^- that forms via bacterial sulfate reduction is actually precipitated as pyrite sulfur (Berner, 1982). Instead, some 90% of the HS^- is reoxidized to sulfate. Although O_2 , NO_3^- , and Mn(IV)- or Fe(III)-oxides are the ultimate oxidants in sedimentary systems, there exists a dynamic S-subcycle that involves a number of sulfur intermediate products, with thiosulfate appearing to be the most significant (Jørgensen, 1990). In addition, some of the HS^- that ultimately escapes the thio-sulfate subcycle provides a useable source of reducing power for various chemolithoautotrophs, most of which gain energy from oxidizing sulfide, with O_2 as the TEAP (Reaction 9).



Methanogenesis

The terminal step in the anaerobic degradation of organic material is methanogenesis (Reactions 10 and 11). As the least energetically favorable process of organic

degradation, the reaction product, methane (CH_4), actually stores a major part of the energy available for aerobically respiring species, i.e., methanotrophs.



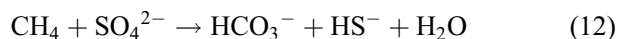
$$\Delta G^0 = -31 \text{ kJ/reaction}$$



$$\Delta G^0 = -34 \text{ kJ/reaction}$$

On average, methanogenesis is responsible for 5–10 times less organic carbon degradation than sulfate reduction, and it is only important in sediments where a significant amount of relatively fresh organic material is delivered rapidly enough to pass through the sulfate reduction zone that it takes on any prominence (Canfield, 1993). Indeed, methane does not accumulate in sediments until more than 90% of the dissolved sulfate has been reduced (Martens and Berner, 1974). This is unsurprising given that the growth of methanogens is inhibited by SRBs, because the latter have a higher affinity for the oxidizable substrates than do the former (Lovley et al., 1982). In other words, methanogens prosper when subsurface activity of SRBs is high and pore water SO_4^{2-} becomes diminished.

The CH_4 generated by methanogens in the underlying sediment subsequently diffuses upward toward the sulfate-rich sediments, where it serves as an electron donor in the sulfate–methane transition zone (SMTZ) (Reaction 12).



In some marginal marine sites, it has been estimated that nearly 100% of the downward SO_4^{2-} flux and the upward CH_4 flux can go toward anaerobic CH_4 oxidation. Comparison of these fluxes to open ocean sites even suggests that anaerobic methane oxidation might be the dominant sink for sulfate in marine sediments relative to organic carbon oxidation (D'Hondt et al., 2002). The diffusive flux of CH_4 upward is usually inadequate to completely reduce pore water sulfate, and hence, accumulate above the SMTZ. Yet, it has been observed in some rapidly depositing shallow water sediments that methane saturates pore waters and ultimately may escape the sediment by bubble ebullition, without complete oxidation by sulfate (Martens and Klump, 1984).

Sediment hydrogen concentrations

As discussed above, the dominant terminal electron accepting pathways in sediments are generally segregated into distinct zones based on the potential thermodynamic yield of the various metabolic processes. Yet, on a purely thermodynamic basis, reactions yielding less energy should also take place as long as they are energetically favorable. Instead, the segregation can be more accurately explained by competition between different types of

microorganisms for electron donors, such as the fermentation products H_2 and acetate (Lovley and Klug, 1986). Accordingly, microorganisms utilizing electropositive TEAs effectively maintain H_2 concentrations below the capabilities of microorganisms using TEAs that yield less energy, and it is only when the electropositive TEA becomes depleted that the next most electropositive TEA controls the H_2 concentrations in the pore water system (Lovley and Goodwin, 1988). In pore waters, this translates into distinct H_2 concentration gradients for each particular metabolism and a threshold level below which H_2 cannot be further metabolized. When nitrate and manganese reductions are the dominant terminal electron accepting pathways, pore water hydrogen concentrations are extremely low (<0.05 nM), with increasing levels through iron reduction (0.2 nM), sulfate reduction (1–1.5 nM), and methanogenesis (7–10 nM). Acetate concentrations follow a similar pattern (Chapelle and Lovley, 1992).

This pattern of H_2 and acetate competition can be ascribed to the physiological capabilities of the microorganisms growing in the sediments. Microorganisms that use amorphous ferric hydroxide as their TEA can metabolize H_2 or acetate to concentrations lower than those that can be utilized by sulfate reducers, and the sulfate reducers can metabolize the same substrates to concentrations below those usable by methanogens (Lovley and Phillips, 1987). These findings are consistent with observations of sediments where sulfate is not reduced, and methane is not produced until the reducible ferric iron minerals have been converted to Fe(II). Given the pattern above, it is not surprising then that the availability of NO_3^- or Mn(IV) oxides diminishes the magnitude of Fe(III) reduction in suboxic sediment, and under such conditions, some of the Fe(III) reducers may even switch over to nitrate or manganese reduction when H_2 becomes limiting for them under their normal mode of growth (Lovley and Phillips, 1988; diChristina, 1992).

Macroscopic biological influence on diagenesis

On the seafloor, recently deposited sediment is subject to grazing, burrowing, and particle reworking by relatively large animals moving over the surface of the sediment and the more numerous small organisms and microorganisms living within the upper layers of sediment. This influence may extend several decimeters (and even meters) below the sediment–water interface, but their effect is largely concentrated in the uppermost 20 cm. The benthos are typically inhibited when (1) the particulate organic supply is too low; (2) when the sediment is too fine-grained and compacted to provide sufficient space for them to live, (3) when O_2 levels reach values <0.2 mg l^{-1} , and (4) when strong ebb and current flows quickly redistribute sediment for burrows to become established (e.g., Van Cappellen and Gaillard, 1996).

Burrowing organisms biomechanically and geochemically alter sediments – a process generally referred to as

bioturbation – in spatially intricate ways. Biomechanical modification includes the redistribution of grain sizes and the modification of grain size and sediment cohesiveness. It also impacts on the organic properties of the sediment because grazing causes greater disintegration of intact organic remains, leading to higher surface areas for microbial oxidation, while burrowing mixes fresh organic matter with older, more decomposed materials, causing enhanced degradation of the latter. Ingestion of refractory organic carbon may lead to excretion of more labile fecal pellets (Lee, 1992). The style of biomechanical deformation can be variable depending on the intrusive process employed by the burrowing animal. In clastic sediments, the redistribution of sediment grain sizes strongly influences the arrangement of permeability pathways, thereby exerting a control on the flux of pore water near, and within, preserved burrows. Through abrasion and ingestion, carbonate sediments are prone to (biogenic) grain-size reduction. This provides a more reactive medium for diagenetic fluids to react with.

The biochemical modification of sediment is the result of local compositional heterogeneity imposed by biogenic structures. This includes an increase in the surface area at the sediment–water interface, which in turn, increases the rate and amount of chemical exchange between the sediment, the pore water, and the water column. For instance, irrigation of worm burrow tubes with oxygenated seawater results in increased downward and lateral diffusion of O_2 into surrounding sediment and concomitantly the loss of sulfur from the sediment by oxidation of pyrite and enhanced diffusive loss of HS^- (Bernier and Westrich, 1985). Subsurface flushing with oxygenated waters also influences nitrification, Mn(II)- and Fe(II)-oxidation patterns. In the case of iron cycling, as long as ferric iron persists, phosphate remains adsorbed to the oxide surface and is thus unavailable to the pore waters. Meanwhile, the incorporation of concentrated (and typically labile) organic material in the form of mucus or feces not only provides an oxidizable substrate for the microbial communities, but also leads to concentration of metals in, and adjacent to, burrow linings as sorbed cations or as metal oxyhydroxide, sulfide, or phosphate coatings (Over, 1990). These metals may then serve as nucleation sites for authigenic mineral formation, which provides a variable chemical stock for diagenetic fluids to interact with (i.e., dolomitization, cementation).

Collectively, the imposition of burrows at the sediment–water interface perturbs the generally accepted geochemical zonations within (subaqueous) sediments, proffering a complex distribution of geochemical zones (Aller, 1980).

Summary

Microorganisms are integral to the chemical and physical changes sediment undergoes during the process of diagenesis. Through various chemoheterotrophic pathways, microorganisms are ultimately responsible for the

conversion of organic carbon to CO_2 (or CH_4) through the coupled reduction of dissolved (O_2 , NO_3^- , SO_4^{2-} , CO_2) or Fe(III)/Mn(IV) oxyhydroxides in the sediment. The inorganic by-products of chemoheterotrophy (HCO_3^- , Mn^{2+} , Fe^{2+} , NH_4^+ , NO_2^- , HS^- , HPO_4^{2-} , CH_4) are either transported from deeper sediment toward the seafloor, where residing chemolithoautotrophic bacteria use them as reactants in their metabolism, or their presence in pore waters may trigger secondary mineral precipitation and cement formation. Macrofauna also contribute to diagenetic reactions through their bioturbating activity. This includes the direct effects of burrowing and mixing of fresh sediment with older sediment, grazing and disintegration of intact organic remains, and the excretion of labile fecal pellets. Indirectly, they influence the solid-phase and pore water properties of sediment by increasing the transport of redox-active elements across the sediment–water interface.

Bibliography

- Aller, R. C., 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. *Geochimica et Cosmochimica Acta*, **44**, 1955–1965.
- Aller, R. C., 1990. Bioturbation and manganese cycling in hemipelagic sediments. *Philosophical Transactions of the Royal Society of London*, **331**, 51–68.
- Bender, B., and Heggge, D. T., 1984. Fate of organic carbon reaching the deep sea: a status report. *Geochimica et Cosmochimica Acta*, **48**, 977–986.
- Berner, R. A., 1982. Burial of organic carbon and pyrite sulphur in the modern ocean: its geochemical and environmental significance. *American Journal of Science*, **282**, 451–473.
- Berner, R. A., and Raiswell, R., 1983. Burial of organic carbon and pyrite sulfur in sediments over Phanerozoic time: a new theory. *Geochimica et Cosmochimica Acta*, **47**, 855–862.
- Berner, R. A., and Westrich, J. T., 1985. Bioturbation and the early diagenesis of carbon and sulphur. *American Journal of Science*, **285**, 193–206.
- Blackburn, T. H., and Blackburn, N. D., 1993. Coupling of cycles and global significance of sediment diagenesis. *Marine Geology*, **113**, 101–110.
- Burdige, D. J., 1993. The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Reviews*, **35**, 249–284.
- Canfield, D. E., 1993. Organic matter oxidation in marine sediments. In Wollast, R., Chou, L., and Mackenzie, F. (eds.), *Interactions of C, N, P and S Biogeochemical Cycles and Global Change*. Berlin: Springer, pp. 333–363.
- Canfield, D. E., Raiswell, R., and Bottrell, S., 1992. The reactivity of sedimentary iron minerals toward sulfide. *American Journal of Science*, **292**, 659–683.
- Chapelle, F. H., and Lovley, D. R., 1992. Competitive exclusion of sulfate reduction by Fe(III)-reducing bacteria: a mechanism for producing discrete zones of high-iron ground water. *Ground Water*, **30**, 29–36.
- Coleman, M. L., 1985. Geochemistry of diagenetic non-silicate minerals. Kinetic considerations. *Philosophical Transactions of the Royal Society of London*, **315**, 39–56.
- Devol, A. H., 1991. Direct measurement of nitrogen gas fluxes from continental shelf sediments. *Nature*, **349**, 319–321.
- D'Hondt, S., Rutherford, S., and Spivack, A. J., 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science*, **295**, 2067–2070.
- diChristina, T. J., 1992. Effects of nitrate and nitrite on dissimilatory iron reduction by *Shewanella putrefaciens* 200. *Journal of Bacteriology*, **174**, 1891–1896.
- Emerson, S., and Hedges, J. I., 1988. Processes controlling the organic carbon content of open ocean sediments. *Paleoceanography*, **3**, 621–634.
- Froelich, P. N. et al., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic. Suboxic diagenesis. *Geochimica et Cosmochimica Acta*, **43**, 1075–1090.
- Hammond, D. E., Fuller, C., Harmon, D., Hartman, B., Korosec, M., Miller, L. G., Rea, R., Warren, S., Berelson, W., and Hager, S. W., 1985. Benthic fluxes in San Francisco Bay. *Hydrobiologia*, **129**, 69–90.
- Hansen, J. I., Henriksen, K., and Blackburn, T. H., 1981. Seasonal distribution of nitrifying bacteria and rates of nitrification in coastal marine sediments. *Microbial Ecology*, **7**, 297–304.
- Hedges, J. I., Clark, W. A., and Cowie, G. L., 1988. Fluxes and reactivities of organic matter in a coastal marine bay. *Limnology and Oceanography*, **33**, 1137–1152.
- Jones, J. G., 1985. Microbes and microbial processes in sediments. *Philosophical Transactions of the Royal Society of London*, **315**, 3–17.
- Jørgensen, B. B., 1982. Mineralization of organic matter in the seabed – the role of sulfate reduction. *Nature*, **296**, 643–645.
- Jørgensen, B. B., 1990. A thiosulphate shunt in the sulfur cycle of marine sediments. *Science*, **249**, 152–154.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Publishing.
- Krom, M. D., and Berner, R. A., 1980. Adsorption of phosphate in anoxic marine sediments. *Limnology and Oceanography*, **25**, 797–806.
- Leang, C., Adams, L. A., Chin, K. J., Nevin, K. P., Methé, B. A., Webster, J., Sharma, M. L., and Lovley, D. R., 2005. Adaptation to disruption of the electron transfer pathway for Fe(III) reduction in *Geobacter sulfurreducens*. *Journal of Bacteriology*, **187**, 5918–5926.
- Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochimica et Cosmochimica Acta*, **56**, 3323–3335.
- Lovley, D. R., and Chapelle, F. H., 1995. Deep subsurface microbial processes. *Reviews of Geophysics*, **33**, 365–381.
- Lovley, D. R., and Goodwin, S., 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochimica et Cosmochimica Acta*, **52**, 2993–3003.
- Lovley, D. R., and Klug, M. J., 1986. Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments. *Geochimica et Cosmochimica Acta*, **50**, 11–18.
- Lovley, D. R., and Phillips, E. J. P., 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Microbiology*, **51**, 683–689.
- Lovley, D. R., and Phillips, E. J. P., 1987. Competitive mechanisms for inhibition of sulphate reduction and methane production in the zone of ferric iron reduction in sediments. *Applied and Environmental Microbiology*, **53**, 2636–2641.
- Lovley, D. R., and Phillips, E. J. P., 1988. Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiology Journal*, **6**, 145–155.
- Lovley, D. R., Dwyer, D. F., and Klug, M. J., 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Applied and Environmental Microbiology*, **43**, 1373–1379.

- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Luther, G. W., III, Sundby, B., Lewis, B. L., Brendel, P. J., and Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle. Alternative pathways to dinitrogen. *Geochimica et Cosmochimica Acta*, **61**, 4043–4052.
- Martens, C. S., and Berner, R. A., 1974. Methane production in the interstitial waters of sulfate-depleted sediments. *Science*, **185**, 1167–1169.
- Martens, C. S., and Klump, J. V., 1984. Biogeochemical cycling in an organic-rich coastal marine basin. 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. *Geochimica et Cosmochimica Acta*, **48**, 1987–2004.
- Mortimer, R. J. G., Davey, J. T., Krom, M. D., Watson, P. G., Frickers, P. E., and Clifton, R. J., 1999. The effect of macrofauna on pore-water profiles and nutrient fluxes in the intertidal zone of the Humber Estuary. *Estuarine, Coastal and Shelf Science*, **48**, 683–699.
- Murray, J. W., and Grundmanis, V., 1980. Oxygen consumption in pelagic marine sediments. *Science*, **209**, 1527–1530.
- Myers, K. H., and Neelson, K. H., 1988. Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochimica et Cosmochimica Acta*, **52**, 2727–2732.
- Nevin, K. P., and Lovley, D. R., 2002. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Applied and Environmental Microbiology*, **68**, 2294–2299.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Thamdrup, B., and Dalsgaard, T., 2002. Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology*, **68**, 1312–1318.
- Van Cappellen, P., and Gaillard, J. F., 1996. Biogeochemical dynamics in aquatic sediments. In Lichtner, P. C., Steefel, C. I., and Oelkers, E. H. (eds.), *Reactive Transport in Porous Media*. Reviews in Mineralogy. Washington: Mineralogical Society of America, Vol. 34, pp. 335–376.
- Westrich, J. T., and Berner, R. A., 1984. The role of sedimentary organic matter in bacterial sulphate reduction: the G model tested. *Limnology and Oceanography*, **29**, 236–249.

Cross-references

Aerobic Metabolism
 Anaerobic Oxidation of Methane with Sulfate
 Biogeochemical Cycles
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cold Seeps
 Deep Biosphere of Sediments
 Deep Biosphere of the Oceanic Deep Sea
 Fe(III)-Reducing Prokaryotes
 Hydrogen
 Iron Sulfide Formation
 Methane Oxidation (Aerobic)
 Methanogens
 Microbial Degradation
 Pore Waters
 Siderite
 Sulfate-Reducing Bacteria
 Sulfur Cycle

SELENIUM

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Selenium, element 34, is a metalloid in row VIB of the periodic table. The main oxidation states are selenide (−2), elemental selenium (0), selenite (+4), and selenate (+6). Selenate and selenite are found in the oxic zone, while Se(0) is more abundant in the anoxic zone. Although hydrogen selenide may be produced by microorganisms, it is highly toxic. Se(−II) is more often found as organoselenium in the form of selenoproteins. The common methylated species (e.g., dimethylselenide, dimethyldiselenide) are volatile. Selenium can be a substitute for sulfur and is often found as metal selenide (e.g., Clausthalite, Crookesite). Selenium occurs in eight stable isotopes, the most common of which are Se80 (~50%) and Se78 (~24%), and nine radioactive isotopes.

The microbial oxidation of selenium is a slow process, whereas the dissimilatory reduction of selenate via selenite to Se(0) is rapid (Dowdle and Oremland, 1998). Selenate and selenite may also be methylated, and Se(0) can be further reduced to selenide (HSe^{−1}). Se(0) is insoluble, and bacteria form “nanospheres” of roughly uniform diameter (0.2–0.3 μm) (Oremland et al., 2004). These nanospheres can occur inside the cytoplasm or on the outside of the cell wall and may exhibit measurable isotope fractionation (⁸⁰Se/⁷⁶Se of −8.0‰ to −12.4‰) (Herbel et al., 2000). Selenium may be assimilated via the sulfur assimilation pathway.

Selenium is an essential element primarily needed for the synthesis of selenocysteine and selenomethionine (as reviewed in Stolz et al., 2006). Selenocysteine (Sec, U) is the 21st amino acid (encoded by UGA) and is found in a number of enzymes (e.g., glycine reductase, formate dehydrogenase). Sec requires four specific gene products: SelA, selenocysteine synthase; SelB, a special translation factor; SelC, a Sec-specific tRNA (tRNA^{Sec}); and SelD, the selenophosphate synthase. SelB recognizes the alternative coding of UGA for selenocysteine aided by a stem loop structure in the mRNA. The selenocysteine insertion sequence, or SECIS, is found within the 3′ untranslated region of the mRNA in archaea and eukaryotes and immediately downstream of the UGA codon in bacteria.

Over 20 species of selenate respiring prokaryotes are known and include members of the Crenarchaeota, low and high G + C Gram-positive bacteria, Halanaerobacter, and beta-, gamma-, and epsilon-proteobacteria; however, only a few species respire selenite. The respiratory selenate reductase (Ser) from *Thauera selenatis* is a heterotrimer (SerA, SerB, SerC) and belongs to the

- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Luther, G. W., III, Sundby, B., Lewis, B. L., Brendel, P. J., and Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle. Alternative pathways to dinitrogen. *Geochimica et Cosmochimica Acta*, **61**, 4043–4052.
- Martens, C. S., and Berner, R. A., 1974. Methane production in the interstitial waters of sulfate-depleted sediments. *Science*, **185**, 1167–1169.
- Martens, C. S., and Klump, J. V., 1984. Biogeochemical cycling in an organic-rich coastal marine basin. 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. *Geochimica et Cosmochimica Acta*, **48**, 1987–2004.
- Mortimer, R. J. G., Davey, J. T., Krom, M. D., Watson, P. G., Frickers, P. E., and Clifton, R. J., 1999. The effect of macrofauna on pore-water profiles and nutrient fluxes in the intertidal zone of the Humber Estuary. *Estuarine, Coastal and Shelf Science*, **48**, 683–699.
- Murray, J. W., and Grundmanis, V., 1980. Oxygen consumption in pelagic marine sediments. *Science*, **209**, 1527–1530.
- Myers, K. H., and Neelson, K. H., 1988. Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochimica et Cosmochimica Acta*, **52**, 2727–2732.
- Nevin, K. P., and Lovley, D. R., 2002. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Applied and Environmental Microbiology*, **68**, 2294–2299.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Thamdrup, B., and Dalsgaard, T., 2002. Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology*, **68**, 1312–1318.
- Van Cappellen, P., and Gaillard, J. F., 1996. Biogeochemical dynamics in aquatic sediments. In Lichtner, P. C., Steefel, C. I., and Oelkers, E. H. (eds.), *Reactive Transport in Porous Media*. Reviews in Mineralogy. Washington: Mineralogical Society of America, Vol. 34, pp. 335–376.
- Westrich, J. T., and Berner, R. A., 1984. The role of sedimentary organic matter in bacterial sulphate reduction: the G model tested. *Limnology and Oceanography*, **29**, 236–249.

Cross-references

Aerobic Metabolism
 Anaerobic Oxidation of Methane with Sulfate
 Biogeochemical Cycles
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cold Seeps
 Deep Biosphere of Sediments
 Deep Biosphere of the Oceanic Deep Sea
 Fe(III)-Reducing Prokaryotes
 Hydrogen
 Iron Sulfide Formation
 Methane Oxidation (Aerobic)
 Methanogens
 Microbial Degradation
 Pore Waters
 Siderite
 Sulfate-Reducing Bacteria
 Sulfur Cycle

SELENIUM

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Selenium, element 34, is a metalloid in row VIB of the periodic table. The main oxidation states are selenide (−2), elemental selenium (0), selenite (+4), and selenate (+6). Selenate and selenite are found in the oxic zone, while Se(0) is more abundant in the anoxic zone. Although hydrogen selenide may be produced by microorganisms, it is highly toxic. Se(−II) is more often found as organoselenium in the form of selenoproteins. The common methylated species (e.g., dimethylselenide, dimethyldiselenide) are volatile. Selenium can be a substitute for sulfur and is often found as metal selenide (e.g., Clausthalite, Crookesite). Selenium occurs in eight stable isotopes, the most common of which are Se80 (~50%) and Se78 (~24%), and nine radioactive isotopes.

The microbial oxidation of selenium is a slow process, whereas the dissimilatory reduction of selenate via selenite to Se(0) is rapid (Dowdle and Oremland, 1998). Selenate and selenite may also be methylated, and Se(0) can be further reduced to selenide (HSe^{−1}). Se(0) is insoluble, and bacteria form “nanospheres” of roughly uniform diameter (0.2–0.3 μm) (Oremland et al., 2004). These nanospheres can occur inside the cytoplasm or on the outside of the cell wall and may exhibit measurable isotope fractionation (⁸⁰Se/⁷⁶Se of −8.0‰ to −12.4‰) (Herbel et al., 2000). Selenium may be assimilated via the sulfur assimilation pathway.

Selenium is an essential element primarily needed for the synthesis of selenocysteine and selenomethionine (as reviewed in Stolz et al., 2006). Selenocysteine (Sec, U) is the 21st amino acid (encoded by UGA) and is found in a number of enzymes (e.g., glycine reductase, formate dehydrogenase). Sec requires four specific gene products: SelA, selenocysteine synthase; SelB, a special translation factor; SelC, a Sec-specific tRNA (tRNA^{Sec}); and SelD, the selenophosphate synthase. SelB recognizes the alternative coding of UGA for selenocysteine aided by a stem loop structure in the mRNA. The selenocysteine insertion sequence, or SECIS, is found within the 3′ untranslated region of the mRNA in archaea and eukaryotes and immediately downstream of the UGA codon in bacteria.

Over 20 species of selenate respiring prokaryotes are known and include members of the Crenarchaeota, low and high G + C Gram-positive bacteria, Halanaerobacter, and beta-, gamma-, and epsilon-proteobacteria; however, only a few species respire selenite. The respiratory selenate reductase (Ser) from *Thauera selenatis* is a heterotrimer (SerA, SerB, SerC) and belongs to the

DMSO reductase family of molybdenum enzymes. Two different methyltransferases have been identified. Bacterial thiopurine methyltransferase converts selenite and selenocysteine to dimethylselenide and dimethyldiselenide. MmtA (the calicheamicin-like methyltransferase) produces dimethylselenide and dimethyldiselenide from selenite and selenocysteine (as reviewed in Stolz et al., 2006).

Bibliography

- Dowdle, P. R., and Oremland, R. S., 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environmental Science & Technology*, **32**, 3749–3755.
- Herbel, M. J., Johnson, T. M., Oremland, R. S., and Bullen, T. D., 2000. Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochimica et Cosmochimica Acta*, **64**, 3701–3709.
- Oremland, R. S., Herbel, M. J., Switzer Blum, J., Langley, S., Beveridge, T. J., Sutto, T., Ajayan, P. M., Ellis, A., and Curran, S., 2004. Structural and spectral features of selenium nanospheres formed by Se-respiring bacteria. *Applied and Environmental Microbiology*, **70**, 52–60.
- Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

SHALES

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

Historically, the term shale has been used to describe fine-grained sedimentary rocks of largely terrigenous derivation, dominated by mud-size particles (0.06 mm and smaller) and containing appreciable quantities (several 10%) of clay minerals. The definition of the term has been a subject of debate (Potter et al., 2005) because of the closely allied terms mud and clay for which competing definitions may emphasize grain size, mineralogy (clay dominated), or physical properties (plasticity). In place of shale, the term mudstone is also used widely and describes the same compositional spectrum of sedimentary rocks. Because shales span the clay–silt boundary, a good many rocks that are identified as siltstones in the literature also qualify as shales (and vice versa). Although the term shale traditionally had a “terrigenous” connotation, shales that accumulated in sediment starved settings may to more than 50% consist of microbially induced early diagenetic cements (calcite, dolomite, ferroan carbonates, silica, authigenic clays, and iron sulfides), organic matter, and biogenic debris (fish bones, microfossils) upon close inspection. One could argue that the term “biogenic” would be more appropriate for these rocks.

Thus, categorizing shales is by no means a trivial exercise and the various classification schemes in existence (e.g., Ingram, 1953; Dunbar and Rodgers, 1957; Folk,

1965; Picard, 1971; Lewan, 1979; Blatt et al., 1980; Lundegard and Samuels, 1980; Potter et al., 1980; Stow and Piper, 1984; Macquaker and Adams, 2003) leave much to be desired. Many fine-grained sedimentary rocks contain variable amounts of a fine-grained carbonate component and it is common practice to use the terms shale and mudstone for rocks with less than 50% carbonate (Potter et al., 2005). Beyond that arbitrary cut-off the terms marl, micrite, limestone, and dolostone are most commonly used. The classification scheme proposed by Macquaker and Adams (2003), however, also includes fine-grained materials of non-terrigenous origin. The books by Potter et al. (1980, 2005) and Schieber et al. (1998) provide further information on this, as well as other topics concerning shales.

Fundamentally, shales span such a large compositional and textural spectrum that a comprehensive and meaningful classification will only be possible when we can build on a much larger body of case studies than currently available. At present it is probably pointless to agonize over the actual names given to shale types encountered in a given study. What matters primarily is that the rocks be dominantly fine grained, and that we report as accurately as possible their composition, texture, and fabric. The latter two are indispensable for meaningful descriptions of shales because they convey key insights into their transport and deposition (Schieber, 1999a; Schieber et al., 2007).

Significance of shales for geobiology

Although still understudied when compared to sandstones and carbonates (Schieber and Zimmerle, 1998), shales nonetheless are geologically important for multiple reasons. They constitute by volume two thirds of the sedimentary rock record and once examined systematically will contribute much to a better understanding of Earth history and the evolution of life. Because overall they accumulate slower than sandstones or carbonates, as much as 90% of geologic time recorded in sedimentary rocks may be preserved in shales.

Shales are an excellent matrix for fossil preservation, and contained fossils are comparatively easy to extract for study. Because their low permeability limits chemical exchange and access by oxygen, metastable skeletal minerals (e.g., aragonite) and even soft tissues may be preserved. It is not by accident that many of the world's most scientifically significant sites of fossil preservation (Fossil Lagerstätten; Seilacher et al., 1985) are found in shale successions. Notable examples are the Cambrian Burgess Shale (Morris, 1998), the Devonian Hunsrück Slate (Bartels et al., 1998), the Jurassic Posidonia Shale (Kauffman, 1978), and the Messel oil shale deposit (Wuttke, 1983). Preservation of fossils carries much valuable information, particularly in the case of marine shales. Fossils are useful because taphonomy (the study of organismal decay over time), spatial distribution of fossils, fossil diagenesis, morphological attributes of fossils

DMSO reductase family of molybdenum enzymes. Two different methyltransferases have been identified. Bacterial thiopurine methyltransferase converts selenite and selenocysteine to dimethylselenide and dimethyldiselenide. MmtA (the calicheamicin-like methyltransferase) produces dimethylselenide and dimethyldiselenide from selenite and selenocysteine (as reviewed in Stolz et al., 2006).

Bibliography

- Dowdle, P. R., and Oremland, R. S., 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environmental Science & Technology*, **32**, 3749–3755.
- Herbel, M. J., Johnson, T. M., Oremland, R. S., and Bullen, T. D., 2000. Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochimica et Cosmochimica Acta*, **64**, 3701–3709.
- Oremland, R. S., Herbel, M. J., Switzer Blum, J., Langley, S., Beveridge, T. J., Sutto, T., Ajayan, P. M., Ellis, A., and Curran, S., 2004. Structural and spectral features of selenium nanospheres formed by Se-respiring bacteria. *Applied and Environmental Microbiology*, **70**, 52–60.
- Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

SHALES

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

Historically, the term shale has been used to describe fine-grained sedimentary rocks of largely terrigenous derivation, dominated by mud-size particles (0.06 mm and smaller) and containing appreciable quantities (several 10%) of clay minerals. The definition of the term has been a subject of debate (Potter et al., 2005) because of the closely allied terms mud and clay for which competing definitions may emphasize grain size, mineralogy (clay dominated), or physical properties (plasticity). In place of shale, the term mudstone is also used widely and describes the same compositional spectrum of sedimentary rocks. Because shales span the clay–silt boundary, a good many rocks that are identified as siltstones in the literature also qualify as shales (and vice versa). Although the term shale traditionally had a “terrigenous” connotation, shales that accumulated in sediment starved settings may to more than 50% consist of microbially induced early diagenetic cements (calcite, dolomite, ferroan carbonates, silica, authigenic clays, and iron sulfides), organic matter, and biogenic debris (fish bones, microfossils) upon close inspection. One could argue that the term “biogenic” would be more appropriate for these rocks.

Thus, categorizing shales is by no means a trivial exercise and the various classification schemes in existence (e.g., Ingram, 1953; Dunbar and Rodgers, 1957; Folk,

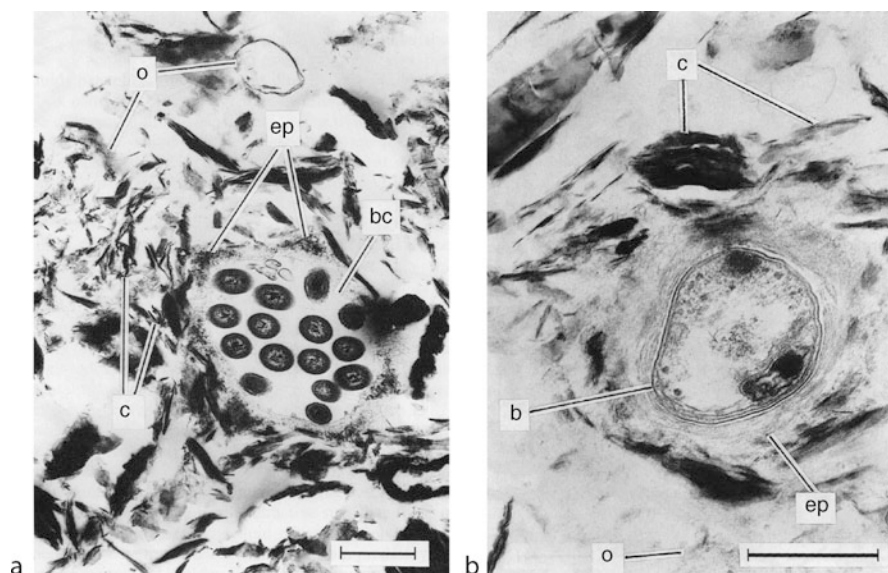
1965; Picard, 1971; Lewan, 1979; Blatt et al., 1980; Lundegard and Samuels, 1980; Potter et al., 1980; Stow and Piper, 1984; Macquaker and Adams, 2003) leave much to be desired. Many fine-grained sedimentary rocks contain variable amounts of a fine-grained carbonate component and it is common practice to use the terms shale and mudstone for rocks with less than 50% carbonate (Potter et al., 2005). Beyond that arbitrary cut-off the terms marl, micrite, limestone, and dolostone are most commonly used. The classification scheme proposed by Macquaker and Adams (2003), however, also includes fine-grained materials of non-terrigenous origin. The books by Potter et al. (1980, 2005) and Schieber et al. (1998) provide further information on this, as well as other topics concerning shales.

Fundamentally, shales span such a large compositional and textural spectrum that a comprehensive and meaningful classification will only be possible when we can build on a much larger body of case studies than currently available. At present it is probably pointless to agonize over the actual names given to shale types encountered in a given study. What matters primarily is that the rocks be dominantly fine grained, and that we report as accurately as possible their composition, texture, and fabric. The latter two are indispensable for meaningful descriptions of shales because they convey key insights into their transport and deposition (Schieber, 1999a; Schieber et al., 2007).

Significance of shales for geobiology

Although still understudied when compared to sandstones and carbonates (Schieber and Zimmerle, 1998), shales nonetheless are geologically important for multiple reasons. They constitute by volume two thirds of the sedimentary rock record and once examined systematically will contribute much to a better understanding of Earth history and the evolution of life. Because overall they accumulate slower than sandstones or carbonates, as much as 90% of geologic time recorded in sedimentary rocks may be preserved in shales.

Shales are an excellent matrix for fossil preservation, and contained fossils are comparatively easy to extract for study. Because their low permeability limits chemical exchange and access by oxygen, metastable skeletal minerals (e.g., aragonite) and even soft tissues may be preserved. It is not by accident that many of the world's most scientifically significant sites of fossil preservation (Fossil Lagerstätten; Seilacher et al., 1985) are found in shale successions. Notable examples are the Cambrian Burgess Shale (Morris, 1998), the Devonian Hunsrück Slate (Bartels et al., 1998), the Jurassic Posidonia Shale (Kauffman, 1978), and the Messel oil shale deposit (Wuttke, 1983). Preservation of fossils carries much valuable information, particularly in the case of marine shales. Fossils are useful because taphonomy (the study of organismal decay over time), spatial distribution of fossils, fossil diagenesis, morphological attributes of fossils



Shales, Figure 1 (a) TEM micrograph of modern mud from the continental slope off northern California at a burial depth of 5 cm. Illustrates the spatial relationships of a colony of bacteria (bc), its extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c) and organic matter (o). Scale bar represents 1 μm . (b) TEM micrograph of a modern mud from Eckernförde Bay, Germany. Shows the relationships between bacteria (b), their extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c), and organic matter (o). Scale bar represents 0.5 μm . (From Ransom et al., 1999.)

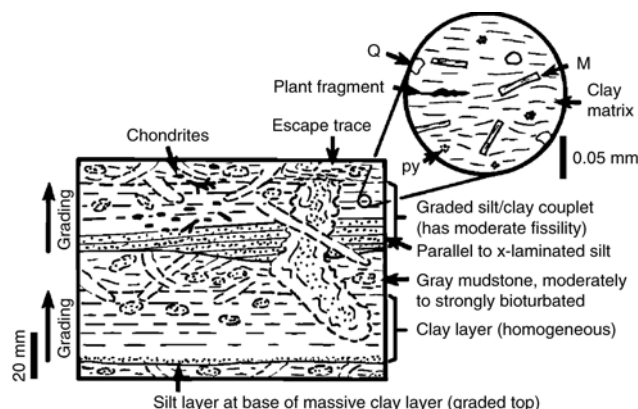
(e.g., reflecting adaptation to substrate conditions), fossil assemblages and comparison to modern counterparts, and paleocommunities (paleoecology) all can provide a wealth of information about the deposition of a shale succession (e.g., Allison, 1988; Brett and Allison, 1998).

Freshly deposited muds, the sediments destined to become shales after compaction, have an enormous internal surface area. Depending on the assumptions about particle geometry and water content, one can readily estimate that a liter of watery mud should have an internal surface area on the order of several hundred square meters (e.g., Fenchel, 1970). That is enough space to provide habitat for trillions (10^{12}) of microbial cells (Figure 1).

Deposition of shales

The sedimentology of shales has made progress over the past decades (Potter et al., 2005), and sedimentologists are gradually learning to appreciate the utility of the many small scale sedimentary structures observable in shales (e.g., Schieber, 1989, 1990a,b, 1999a; O'Brien and Slatt, 1990). When studied in polished slabs and petrographic thin sections, a wealth of information about depositional conditions and history can be gleaned from most shales (Figure 2).

Laminae are the most typically observed sedimentary feature in shales. They show a large range in thickness and lamination styles (even, discontinuous, lenticular, wrinkled etc.), and these may represent conditions that include quiet settling, sculpting of the sediment surface by bottom currents, and growth of microbial mats



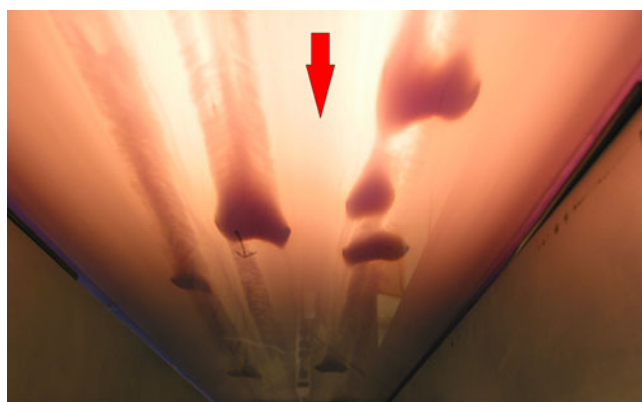
Shales, Figure 2 Line drawing that summarizes features observed in a mudstone from the late Devonian Sonyea Group in New York. (From Schieber 1999a.) M = mica; Q = quartz; py = pyrite.

respectively (Schieber, 1986, 1999b). Internal lamina features, e.g., grading (a), random clay orientation (b), preferred clay orientation (c), sharp basal contacts (d), and sharp top contacts (e) may be interpreted as indicative of (a) event-sedimentation (e.g., floods, storms, turbidites), (b) flocculation or sediment trapping by microbial mats, (c) settling from dilute suspension, (d) current flow and erosion prior to deposition, and (e) current flow and erosion/reworking after deposition (Schieber, 1990a,b). Due to somewhat larger grain size, silt laminae are the most readily observed lamina type in shales and may imply

somewhat more energetic conditions. Examples are density currents (grading, fading ripples), storm reworking and transport (graded rhythmites), wave winnowing (fine even laminae with scoured bases), and bottom currents (silt layers with sharp bottom and top). Gradual compositional changes between, e.g., clay and silt dominated laminae are another commonly observed feature, and are suggestive of continuous (although slow) deposition, possibly from deltaic sediment plumes and shifting nepheloid flows.

For many years conventional wisdom has held that parallel laminae in shales are the result of settling from slow moving or still suspensions, and the common assumption was that this reflects distal deposition in comparatively deep water with only minor current activity. Recent flume experiments, however, have demonstrated conclusively that muds can be deposited from swift moving currents that are competent enough to move sand in bedload (Schieber et al., 2007). This occurs because muds have a strong tendency to flocculate, regardless of exact particle mineralogy and water composition. The floccules travel in bedload and form migrating ripples (Figure 3) that build up a contiguous mud bed if sediment supply is sufficient. In spite of accumulation via lateral accretion, after compaction these muds have a parallel laminated appearance, just like many shales in the rock record (Figure 4; Schieber et al., 2007). Obviously, it will be difficult from now on to ascribe a quiet, deep water setting to a laminated shale without corroborating evidence (e.g., paleoecology, trace fossils).

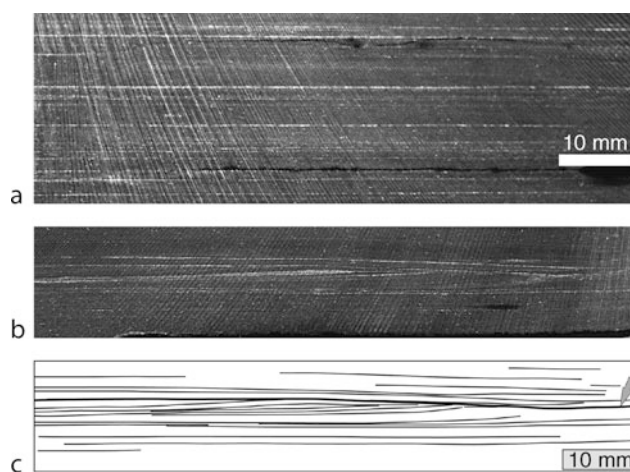
A variety of other small scale sedimentary features also occurs in shales, including mudcracks, load casts, flame structures, dewatering structures, graded rhythmites (Reineck and Singh, 1980), cross-lamination, loop structures (Cole and Picard, 1975), bioturbation (Wetzel and Uchmann, 1998), fossil concentrations and lags, all of



Shales, Figure 3 Migrating ripples of flocculated kaolinite in seawater, photographed through the bottom of a flume. Width of flume channel is 25 cm, flow direction indicated by red arrow. (From Schieber et al., 2007.) The ribbed "tails" behind the ripples are the eroded remains of foreset laminae.

which carry information about conditions of sedimentation. Among the more subtle sedimentary features are clay-filled mud cracks, brecciation due to desiccation, and sands or conglomerates that consist entirely of shale particles (Schieber, 1985). The latter can, for example, form as a result of soil erosion (pedogenic particles; Nanson et al., 1986; Rust and Nanson, 1989), erosion of cracked mud crusts, and submarine scouring of mud substrates by strong currents.

Biologic agents may produce microbial laminae and protection of mud surfaces from erosion (e.g., Schieber, 1986; O'Brien, 1990), or may manifest themselves as bioturbation and via destruction of primary fabrics (Wetzel and Uchmann, 1998). Infaunal activity that took place early in depositional history, when the muds had a high water content, may be exceedingly subtle and hard to detect after compaction (Schieber, 2003). In many instances of bioturbation sufficient proportions of primary features survive and can still be interpreted in terms of depositional processes. Careful examination of bioturbation features can provide additional information about substrate firmness, event deposits (escape traces), and rates of deposition. Fecal pellets and pelletal fabrics are another by-product of organic activity, and they are probably more abundant in mudstones and shales than commonly recognized (Pryor, 1975; Potter et al., 1980; Cuomo and Rhoads, 1987). Best seen in thin section, pellets typically differ from the shale matrix with respect to texture, color, and organic content. They range in size from 0.2–2 mm, are generally of elliptical outline, and



Shales, Figure 4 (a) Parallel-laminated black shale, New Albany Shale, Devonian, Indiana. Lighter laminae are silt enriched (the steeply inclined and slightly curved lines are saw marks). (b) A sample from the same core interval as seen in (a). In the center, we see inclined (to the left) truncated laminae, forming the outline of a compacted mud-dominated ripple. (c) Tracing of silt laminae visible in (b). Arrow marks an internal erosion surface. This is a fossil equivalent of migrating mud ripples observed in flume experiments. (From Schieber et al., 2007.)

flattened by compaction. If matrix and pellets are similar in composition and composed of particles of similar grain size, pellet identification can be challenging. Pellets of benthic versus planktonic organisms may be differentiated on the basis of composition (Cuomo and Bartholomew, 1991; Schieber, 1994).

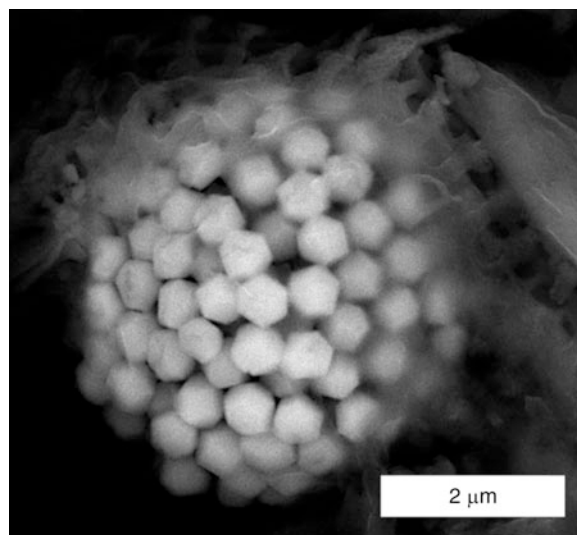
Early diagenesis of shales

It is due to the abundance of endosedimentary microbes (Figure 1) that surficial muds are very active sediments from a geochemical perspective. Much of the initially buried organic material is remineralized in these water-rich sediments (80–90% initial water content), prompting the description of surficial muds as “fluidized bed reactors” (Aller, 1998). The microbial breakdown of buried organic matter requires terminal electron acceptors (oxidizing agents) and due to limitations by diffusion, overall abundance, and differences in energy yield for the various oxidation reactions, we observe a systematic exhaustion of available electron acceptors (e.g., oxygen, nitrate, iron (III), manganese (IV), sulfate, carbon dioxide) as we move downwards from the sediment-water interface. For these reasons closely comparable microbial decay profiles (Brett and Allison, 1998; Curtis et al., 2000) are observed in many marine sediments. For a detailed description of these microbial decay profiles, see Chapters *Sediment Diagenesis – Biologically Controlled* and *Pore Waters*.

A good number of diagenetic minerals, such as pyrite (Figure 5), are readily explained by these decay-reaction schemes (Brett and Allison, 1998; Curtis et al., 2000; Allison, 1988). For others that are not, associated pH shifts can nonetheless be instrumental for their precipitation. For example, early diagenetic silica (quartz) precipitation is not uncommon in shales (Figure 6). In Phanerozoic shales it is derived from the dissolution of biogenic opal (radiolarian, diatoms, sponge spicules) and fills pore spaces that range from tens of microns to as much as a millimeter in size (Figure 7). Quite frequently these pore fills (in situ quartz) are mistaken for detrital quartz (Schieber, 1996; Schieber et al., 2000).

If phosphorous is abundant in the initial mud, such as due to accumulation of phosphatic skeletal material, phosphatic concretions, ranging in size from less than a mm to more than 100 mm's, may form (Figure 8). An early diagenetic phosphate matrix seems to be an excellent medium for preserving minute details of soft tissues, such as in the Neoproterozoic Doushantuo Formation of China (Shen et al., 2000).

Due to the rapid and systematic expulsion of pore waters and decrease of porosity during compaction, early diagenetic minerals in shales are preferentially preserved. For this reason, shales contain a rich record of geochemical processes that relate closely to the chemistry of the overlying ocean waters and paleoceanographic conditions. When studied systematically, the authigenic component of shales holds the promise to capture a snapshot of seawater and/or porewater chemistry. With the necessary

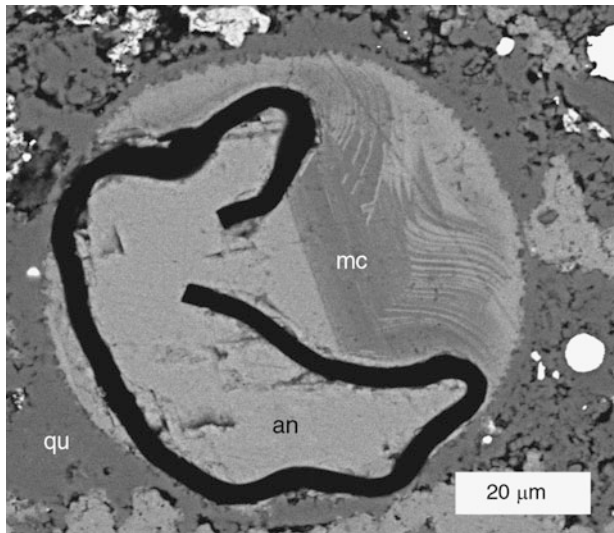


Shales, Figure 5 Pyrite framboid from bottom muds of the Santa Barbara Basin. Note regular arrangement of euhedral subcrystals. The sediment layer in which this framboid occurs is only a few years old. Pyrite formation is associated with the activity of anaerobic bacteria that utilize seawater sulfate (SO_4) as an oxygen source and generate hydrogen sulfide as a byproduct. They are among the most common and familiar of early diagenetic mineral formations in shales.

age constraints, this may enable us to develop a much more detailed record of ocean geochemical changes than currently available.

Conclusion

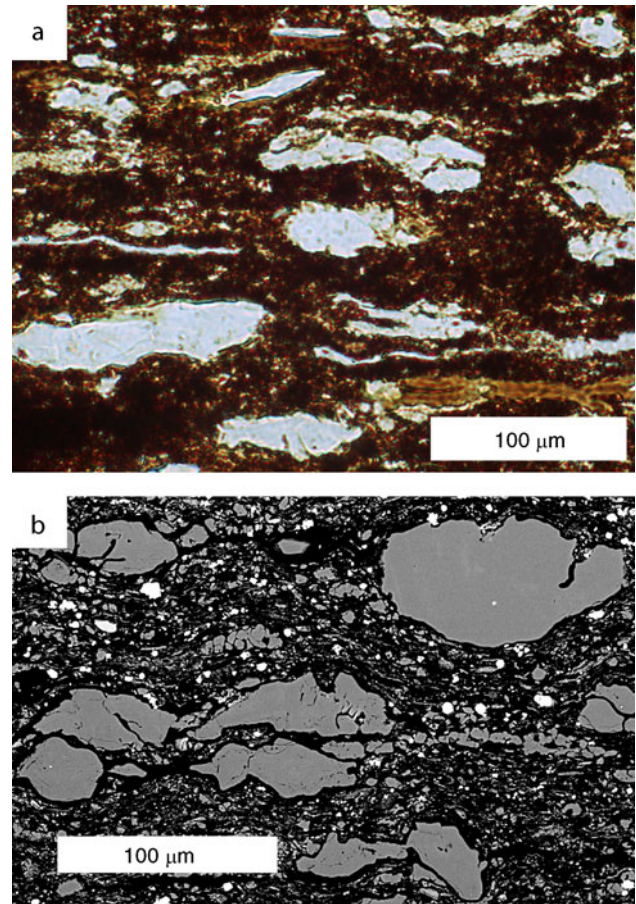
Shales result from the complex interplay of a range of geologic variables and processes, and there are no easy answers and no “one size fits all” models. Although these rocks have an (undeserved) reputation for being drab, uniform, and uninteresting, the repay the investigative challenges they pose by being a rich storehouse of information about the geologic past. Sedimentological study includes recognition and tracing of facies changes, sedimentary features, shale fabrics, erosion surfaces and internal stratigraphy, as well as information that can be derived from interbedded non-shale lithologies. Paleontological studies may provide data on paleo-oxygenation, primary production, substrate conditions, paleocurrents, bathymetry, and paleosalinity (Schieber et al., 1998). Petrographic investigations provide the basic inventory of shale constituents, and may yield clues about provenance, compaction history, chemical conditions, sedimentary processes (via small scale sedimentary structures), and the origin and maturation of organic matter (Taylor et al., 1998). Chemical analysis of major and trace elements can provide information on provenance (Roser and Korsch, 1986), and a range of proxies for paleo-oxygenation (Jones and Manning, 1994). Organic geochemistry can furnish information on the source of organic matter, its dispersal,



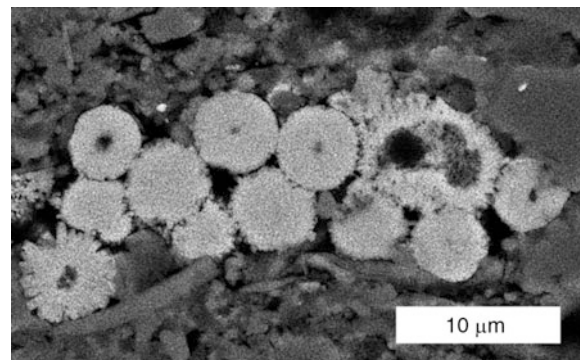
Shales, Figure 6 SEM photomicrograph (backscatter image) of a pore space in the Devonian New Albany Shale (Indiana, USA), illustrating suboxic to anoxic diagenesis. Light gray mineral **an** is ankerite (Fe-Mg-Ca-carbonate) that initially grew in the interior of a *Tasmanites* cyst (dark black line), a locally reducing and anoxic microenvironment. Very early growth is indicated by the uncompacted *Tasmanites* wall and the overall circular growth of carbonate (**an** + **mc**) cement. Outside of the protected microenvironment of the cyst we see growth of manganoan calcite (**mc**, darker gray), indicating suboxic conditions in the sediment and oxygenated bottom waters. (From Calvert et al., 1996.) The carbonate cement is surrounded by fine crystalline quartz (**qu**), derived from dissolution of radiolarian opal. (From Schieber et al., 2000). The image suggests that pore waters were initially alkaline, allowing for precipitation of carbonates and dissolution of biogenic opal, and that this was followed by a lowering of pH that led to precipitation of quartz. This image illustrates the level of insight one can derive from careful petrographic study of early diagenetic cements.

and maturation (Engel and Macko, 1993). Certain organic molecules, such as photosynthetic pigments, may survive as “carbon skeletons” (biomarkers), and may identify the original source of diagenetically altered organic matter and potential indicators of water column anoxia (Brassell, 1992).

Fundamentally, shales do not give up their secrets easily. The tools are at hand, however, to extract a wide range of data from them and allow for multiple avenues of inquiry (Schieber et al., 1998). Interpretations that rely on just one perspective (e.g., petrography, fabric study, trace element geochemistry, or organic geochemistry) typically do not match conclusions derived from a different line of inquiry. Shales need to be investigated in a multidisciplinary way and at multiple scales because of the complex interplay of variables that produces them. Conclusions derived from microscopic features must be in agreement with insights coming out of basin scale studies, as well as with findings from all scales in between.



Shales, Figure 7 Authigenic quartz (chalcedony) in the late Devonian Chattanooga Shale (Kentucky, USA). (a) Photomicrograph in transmitted light. The clear/bright grains are chalcedonic infills of algal cysts (*Tasmanites*). (b) SEM image (backscatter) from the same specimen, showing more clearly the rounded-lobate outlines of the quartz grains and the enclosing cyst walls (black rim). For a detailed discussion of these features see Schieber (1996) and Schieber et al. (2000).



Shales, Figure 8 SEM photomicrograph (backscatter image) of an example of early diagenetic phosphate precipitation. The image shows what appears to be a colony of phosphate encrusted bacteria (Mid-Proterozoic Bijaigarh Shale, India).

Bibliography

- Aller, R. C., 1998. Mobile deltaic and continental shelf muds as suboxic, fluidized bed reactors. *Marine Chemistry*, **61**, 143–155.
- Allison, P. A., 1988. The role of anoxia in the decay and mineralization of proteinaceous macrofossils. *Paleobiology*, **14**, 139–154.
- Bartels, C., Briggs, D. E. G., and Brassell, G., 1998. *The Fossils of the Hunsrück Slate - Marine Life in the Devonian*. Cambridge: Cambridge University Press, p. 309.
- Blatt, H., Middleton, G., and Murray, R., 1980. *Origin of Sedimentary Rocks*. Englewood Cliffs: Prentice Hall, 782 p.
- Brassell, S. C., 1992. Biomarkers in sediments, sedimentary rocks and petroleum. In Pratt, L. M., Brassell, S. C., and Comer, J. B. (eds.), *Geochemistry of Organic Matter in Sediments and Sedimentary Rocks*. SEPM Short Course Notes 27. Tulsa: Society for Sedimentary Geology, pp. 29–72.
- Brett, C., and Allison, P. A., 1998. Paleontological approaches to the environmental interpretation of marine mudrocks. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology, Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, pp. 301–349.
- Calvert, S. E., Bustin, R. M., and Ingall, E. D., 1996. Influence of water column anoxia and sediment supply on the burial and preservation of organic carbon in marine shales. *Geochimica et Cosmochimica Acta*, **60**, 1577–1593.
- Cole, R. D., and Picard, M. D., 1975. Primary and secondary sedimentary structures in oil shale and other fine-grained rocks, Green River Formation (Eocene), Utah and Colorado. *Utah Geology*, **2**, 49–67.
- Cuomo, M. C., and Bartholomew, P. R., 1991. Pelletal black shale fabrics: their origin and significance. In Tyson, R. V., and Pearson, T. H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. London: Geological Society, Special Publication No. 58, pp. 221–232.
- Cuomo, M. C., and Rhoads, D. C., 1987. Biogenic sedimentary fabrics associated with pioneering polychaete assemblages: modern and ancient. *Journal of Sedimentary Petrology*, **57**, 537–543.
- Curtis, C. D., Cope, J. C. W., Plant, D., and Macquaker, J. H. S., 2000. "Instantaneous" sedimentation, early microbial sediment strengthening and a lengthy record of chemical diagenesis preserved in Lower Jurassic ammonitiferous concretions from Dorset. *Journal of the Geological Society, London*, **157**, 165–172.
- Dunbar, C. O., and Rodgers, J., 1957. *Principles of Stratigraphy*. New York: Wiley, 356 p.
- Engel, M. H., and Macko, S. A., 1993. *Organic Geochemistry: Principles and Applications*. New York: Plenum Press, 861 p.
- Fenchel, T., 1970. Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. *Limnology and Oceanography*, **15**, 14–20.
- Folk, R. L., 1965. *Petrology of Sedimentary Rocks*. Austin: Hemphill's Bookstore, 170 p.
- Ingram, R. L., 1953. Fissility in mudrocks. *Geological Society of America Bulletin*, **65**, 869–878.
- Jones, B., and Manning, D. A. C., 1994. Comparisons of geochemical indices used for the interpretation of paleoredox conditions in ancient mudstones. *Chemical Geology*, **111**, 111–129.
- Kauffman, E. G., 1978. Benthic environments and paleoecology of the Posidonienschiefer (Toarcian). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **157**, 18–36.
- Lewan, M. D., 1979. Laboratory classification of very fine-grained sedimentary rocks. *Geology*, **6**, 745–748.
- Lundegard, P. D., and Samuels, N. D., 1980. Field classification of fine-grained sedimentary rocks. *Journal of Sedimentary Petrology*, **50**, 781–786.
- Macquaker, J. H. S., and Adams, A. E., 2003. Maximizing information from fine-grained sedimentary rocks: an inclusive nomenclature for mudstones. *Journal of Sedimentary Research*, **73**, 735–744.
- Morris, S. C., 1998. *The Crucible of Creation: The Burgess Shale and the Rise of Animals*. Oxford: Oxford University Press, 276 p.
- Nanson, C. G., Rust, B. R., and Taylor, G., 1986. Coexistent mud braids and anastomosing channels in an arid-zone river: Cooper Creek, central Australia. *Geology*, **14**, 175–178.
- O'Brien, N. R., 1990. Significance of lamination in Toarcian (Lower Jurassic) shales from Yorkshire, Great Britain. *Sedimentary Geology*, **67**, 25–34.
- O'Brien, N. R., and Slatt, R. M., 1990. *Argillaceous Rock Atlas*. New York: Springer, 141 p.
- Picard, M. D., 1971. Classification of fine grained sedimentary rocks. *Journal of Sedimentary Petrology*, **41**, 179–195.
- Potter, P. E., Maynard, J. B., and Pryor, W. A., 1980. *Sedimentology of Shale*. New York: Springer, 303 p.
- Potter, P. E., Maynard, J. B., and Depetris, P. J., 2005. *Mud and Mudstones*. New York: Springer, 297 p.
- Pryor, W. A., 1975. Biogenic sedimentation and alteration of argillaceous sediments in shallow marine environments. *Geological Society of America Bulletin*, **86**, 1244–1254.
- Ransom, B., Bennett, R. H., Baerwald, R., Hulbert, M. H., and Burkett, P. J., 1999. In situ conditions and interactions between microbes and minerals in fine-grained marine sediments; a TEM microfabric perspective. *American Mineralogist*, **84**, 183–192.
- Reineck, H. E., and Singh, I. B., 1980. *Depositional Sedimentary Environments*. Berlin: Springer, 549 p.
- Roser, B. P., and Korsch, R. J., 1986. Determination of tectonic setting of sandstone-mudstone suites using SiO₂ content and K₂O/Na₂O ratio. *Journal of Geology*, **94**, 635–650.
- Rust, B. R., and Nanson, G. C., 1989. Bedload transport of mud as pedogenic aggregates in modern and ancient rivers. *Sedimentology*, **36**, 291–306.
- Schieber, 1985. *The Relationship between Basin Evolution and Genesis of stratiform Sulfide Horizons in Mid-Proterozoic Sediments of Central Montana (Belt Supergroup)*. PhD dissertation, University of Oregon, Eugene, 811 p.
- Schieber, J., 1986. The possible role of benthic microbial mats during the formation of carbonaceous shales in shallow Proterozoic basins. *Sedimentology*, **33**, 521–536.
- Schieber, J., 1989. Facies and origin of shales from the Mid-Proterozoic Newland Formation, Belt basin, Montana, U.S.A. *Sedimentology*, **36**, 203–219.
- Schieber, J., 1990a. Sedimentary structures, textures, and depositional settings of shales from the Lower Belt Supergroup, Mid-Proterozoic, Montana, U.S.A. In Bennett, R. H., Bryant, W. R., and M. H. Hulbert (eds.), *Microstructure of Fine-Grained Sediments*, New York, Springer, pp. 101–108.
- Schieber, J., 1990b. Significance of styles of epicontinental shale sedimentation in the Belt basin, Mid-Proterozoic of Montana, U.S.A. *Sedimentary Geology*, **69**, 297–312.
- Schieber, J., 1994. Reflection of deep vs shallow water deposition by small scale sedimentary features and microfabrics of the Chattanooga Shale in Tennessee. *Canadian Society of Petroleum Geologists, Memoir*, **17**, 773–784.
- Schieber, J., 1996. Early diagenetic silica deposition in algal cysts and spores: a source of sand in black shales? *Journal of Sedimentary Research*, **66**, 175–183.
- Schieber, J., 1999a. Distribution and deposition of mudstone facies in the Upper Devonian Sonyea Group of New York. *Journal of Sedimentary Research*, **69**, 909–925.
- Schieber, J., 1999b. Microbial mats in terrigenous clastics: the challenge of identification in the rock record. *Palaios*, **14**, 3–12.
- Schieber, J., 2003. Simple gifts and hidden treasures – implications of finding bioturbation and erosion surfaces in black shales. *The Sedimentary Record*, **1**, 4–8.
- Schieber, J., Krinsley, D., and Riciputi, L., 2000. Diagenetic origin of quartz silt in mudstones and implications for silica cycling. *Nature*, **406**, 981–985.

- Seilacher, A., Reif, W. E., Westphal, F., Riding, R., Clarkson, E. N. K., and Whittington, H. B., 1985. Sedimentological, Ecological and Temporal Patterns of Fossil Lagerstätten [and Discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Vol. **311**, No. 1148, Extraordinary Fossil Biotas: Their Ecological and Evolutionary Significance (Oct. 17, 1985), 5–24.
- Schieber, J., and Zimmerle, W., 1998. The history and promise of shale research. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology, Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, pp. 1–10.
- Schieber, J., Zimmerle, W., and Sethi, P. (eds.), 1998. *Shales and Mudstones*. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vols. 1 and 2.
- Schieber, J., Southard, J. B., and Thaisen, K. G., 2007. Accretion of mudstone beds from migrating floccule ripples. *Science*, **318**, 1760–1763.
- Shen, Y., Schidlowski, M., and Chu, X. L., 2000. Biogeochemical approach to understanding phosphogenic events of the terminal Proterozoic to Cambrian. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **158**, 99–108.
- Stow, D. A. V., and Piper, D. J. W., 1984. Deep-water fine-grained sediments: facies models. In Stow, D. A. V., and Piper, D. J. W. (eds.), *Fine-Grained Sediments: Deep-Water Processes and Facies*. London: Geological Society, Special Publication No. 15, 611–645.
- Taylor, G. H., Teichmüller, M., Davis, A., Diessel, C. F. K., Littke, R., and Robert, P., 1998. *Organic Petrology*. Stuttgart: Borntraeger, 704 p.
- Wetzel, A., and Uchmann, A., 1998. Biogenic sedimentary structures in mudstones – an overview. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, p. 351–369.
- Wuttke, M., 1983. 'Weichteil-Erhaltung' durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus en Ölschiefern der 'Grube Messel' bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

Biofilms and Fossilization
 Black Shales
 Iron Sulfide Formation
 Mat-Related Sedimentary Structures

SHEWANELLA

Nadia-Valérie Quéric
 University of Göttingen, Göttingen, Germany

Classification

Bacteria – Proteobacteria – Gammaproteobacteria – Alteromonadales – Shewanellaceae (Ivanova et al., 2004), fam. nov. – *Shewanella* (MacDonell and Colwell, 1985), gen. nov.

Definition

Assumed as one of the first metabolic processes of ancient microbial life (prior to oxygen, nitrate, and sulfate respiration), microbial iron reduction is still of particular interest

in recent geobiological systems. The efficiency of iron reducing microorganisms in utilizing structural or crystalline solid-phase Fe^{3+} oxide minerals as electron acceptors with respect to nutrient availability and pH is still matter of discussion; this type of metabolism, however, is an ubiquitous phenomenon, applied by a wide range of species throughout the Archaea and Bacteria (Weber et al., 2006 and references therein). Members of the family *Geobacteraceae* and the genus *Shewanella* are among the most significant and common studied organisms with regard to dissimilatory iron reduction and oxidation of organic matter in soils, sediments, and groundwater.

According to the surface complexation model (Dzombak and Morel, 1990), Warren and Ferris (1998) report on an equilibrium between ferric iron sorption and precipitation reactions on bacterial surfaces, emphasizing iron-reducing bacteria like *Shewanella* as highly reactive key players in pristine and contaminated waters (Pedersen and Albinsson, 1991).

Actually including 50 recognized species (List of prokaryotic names with standing in nomenclature: <http://www.bacterio.cict.fr/>), the genus *Shewanella* features Gram-negative, facultatively anaerobic, dissimilatory metal-reducing, rod-shaped organisms. Due to their highly diverse physiology, they inhabit a wide range of redox-stratified environments varying in nutrient composition, salinity, temperature, redox potential, and barometric pressure (marine and freshwater, surface, ground and deep waters, sediment, mud and soils), but also comprise symbiotic, epibiotic, and pathogenic species (Venkateswaran et al., 1998; Hau and Gralnick, 2007 and references therein). Under anaerobic conditions, most *Shewanella* species can produce hydrogen sulfide when grown on thiosulfate or polysulfide, and also can grow on lactate, pyruvate, formate, and on amino acids (Nealson and Scott, 2006 and references therein). For respiratory growth, they can use oxygen as well as a variety of different terminal electron acceptors, including nitrate, elemental sulfur, fumarate, dimethyl sulfoxide, and metals like Mn^{4+} , Fe^{3+} , As^{5+} , Cr^{6+} , Co^{3+} , Tc^{7+} , and U^{6+} (Nealson and Myers, 1992; Haas et al., 2001; Guha et al., 2003; Saltikov et al., 2003). As diffusion across the cell wall is not possible for metals in their solid form, metal-reducing *Shewanella* species specifically interact with the mineral surface to facilitate the electron transfer process by active cell adhesion in combination with a unique shuttle-system of proteins reaching from the cytoplasm to the outer membrane (Lower, 2001; Korenevsky and Beveridge, 2007; Fredrickson et al., 2008 and references therein).

Near-surface habitats may get limited in oxygen but not in minerals like iron oxyhydroxides (ferrihydrite, goethite, and hematite), in large part consisting of Fe^{3+} (Urrutia et al., 1998). In case of dissimilatory metal-reducing bacteria like *Shewanella*, Fe^{3+} is reductively dissolved during oxidation of carbon substrates, affecting processes like the biogeochemical cycle of iron and phosphorus, the oxidation of natural and anthropogenic carbon sources, and

- Seilacher, A., Reif, W. E., Westphal, F., Riding, R., Clarkson, E. N. K., and Whittington, H. B., 1985. Sedimentological, Ecological and Temporal Patterns of Fossil Lagerstätten [and Discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Vol. **311**, No. 1148, Extraordinary Fossil Biotas: Their Ecological and Evolutionary Significance (Oct. 17, 1985), 5–24.
- Schieber, J., and Zimmerle, W., 1998. The history and promise of shale research. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology, Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, pp. 1–10.
- Schieber, J., Zimmerle, W., and Sethi, P. (eds.), 1998. *Shales and Mudstones*. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vols. 1 and 2.
- Schieber, J., Southard, J. B., and Thaisen, K. G., 2007. Accretion of mudstone beds from migrating floccule ripples. *Science*, **318**, 1760–1763.
- Shen, Y., Schidlowski, M., and Chu, X. L., 2000. Biogeochemical approach to understanding phosphogenic events of the terminal Proterozoic to Cambrian. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **158**, 99–108.
- Stow, D. A. V., and Piper, D. J. W., 1984. Deep-water fine-grained sediments: facies models. In Stow, D. A. V., and Piper, D. J. W. (eds.), *Fine-Grained Sediments: Deep-Water Processes and Facies*. London: Geological Society, Special Publication No. 15, 611–645.
- Taylor, G. H., Teichmüller, M., Davis, A., Diessel, C. F. K., Littke, R., and Robert, P., 1998. *Organic Petrology*. Stuttgart: Borntraeger, 704 p.
- Wetzel, A., and Uchmann, A., 1998. Biogenic sedimentary structures in mudstones – an overview. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, p. 351–369.
- Wuttke, M., 1983. 'Weichteil-Erhaltung' durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus en Ölschiefern der 'Grube Messel' bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

Biofilms and Fossilization
 Black Shales
 Iron Sulfide Formation
 Mat-Related Sedimentary Structures

SHEWANELLA

Nadia-Valérie Quéric
 University of Göttingen, Göttingen, Germany

Classification

Bacteria – Proteobacteria – Gammaproteobacteria – Alteromonadales – Shewanellaceae (Ivanova et al., 2004), fam. nov. – *Shewanella* (MacDonell and Colwell, 1985), gen. nov.

Definition

Assumed as one of the first metabolic processes of ancient microbial life (prior to oxygen, nitrate, and sulfate respiration), microbial iron reduction is still of particular interest

in recent geobiological systems. The efficiency of iron reducing microorganisms in utilizing structural or crystalline solid-phase Fe^{3+} oxide minerals as electron acceptors with respect to nutrient availability and pH is still matter of discussion; this type of metabolism, however, is an ubiquitous phenomenon, applied by a wide range of species throughout the Archaea and Bacteria (Weber et al., 2006 and references therein). Members of the family *Geobacteraceae* and the genus *Shewanella* are among the most significant and common studied organisms with regard to dissimilatory iron reduction and oxidation of organic matter in soils, sediments, and groundwater.

According to the surface complexation model (Dzombak and Morel, 1990), Warren and Ferris (1998) report on an equilibrium between ferric iron sorption and precipitation reactions on bacterial surfaces, emphasizing iron-reducing bacteria like *Shewanella* as highly reactive key players in pristine and contaminated waters (Pedersen and Albinsson, 1991).

Actually including 50 recognized species (List of prokaryotic names with standing in nomenclature: <http://www.bacterio.cict.fr/>), the genus *Shewanella* features Gram-negative, facultatively anaerobic, dissimilatory metal-reducing, rod-shaped organisms. Due to their highly diverse physiology, they inhabit a wide range of redox-stratified environments varying in nutrient composition, salinity, temperature, redox potential, and barometric pressure (marine and freshwater, surface, ground and deep waters, sediment, mud and soils), but also comprise symbiotic, epibiotic, and pathogenic species (Venkateswaran et al., 1998; Hau and Gralnick, 2007 and references therein). Under anaerobic conditions, most *Shewanella* species can produce hydrogen sulfide when grown on thiosulfate or polysulfide, and also can grow on lactate, pyruvate, formate, and on amino acids (Nealson and Scott, 2006 and references therein). For respiratory growth, they can use oxygen as well as a variety of different terminal electron acceptors, including nitrate, elemental sulfur, fumarate, dimethyl sulfoxide, and metals like Mn^{4+} , Fe^{3+} , As^{5+} , Cr^{6+} , Co^{3+} , Tc^{7+} , and U^{6+} (Nealson and Myers, 1992; Haas et al., 2001; Guha et al., 2003; Saltikov et al., 2003). As diffusion across the cell wall is not possible for metals in their solid form, metal-reducing *Shewanella* species specifically interact with the mineral surface to facilitate the electron transfer process by active cell adhesion in combination with a unique shuttle-system of proteins reaching from the cytoplasm to the outer membrane (Lower, 2001; Korenevsky and Beveridge, 2007; Fredrickson et al., 2008 and references therein).

Near-surface habitats may get limited in oxygen but not in minerals like iron oxyhydroxides (ferrihydrite, goethite, and hematite), in large part consisting of Fe^{3+} (Urrutia et al., 1998). In case of dissimilatory metal-reducing bacteria like *Shewanella*, Fe^{3+} is reductively dissolved during oxidation of carbon substrates, affecting processes like the biogeochemical cycle of iron and phosphorus, the oxidation of natural and anthropogenic carbon sources, and

biocorrosion. The respiratory reduction of metals turns these organisms to key players in groundwater ecology, as they contribute to the mobilization of occasionally harmful metals (e.g., arsenic) from the solid to the soluble phase (Malasarn et al., 2008).

Bibliography

- Dzombak, D. A., and Morel, F. M. M., 1990. *Surface Complex Modelling: Hydrous Ferric Oxide*. New York: Wiley Interscience.
- Fredrickson, J. K., Romine, M. F., Beliaev, A. S., Auchtung, J. M., Driscoll, M. E., Gardner, T. S., Nealson, K. H., Osterman, A. L., Pinchuk, G., Reed, J. L., Rodionov, D. A., Rodrigues, J. L. M., Saffarini, D. A., Serres, M. H., Spormann, A. M., Zhulin, I. B., and Tiedje, J. M., 2008. Towards environmental systems biology of *Shewanella*. *Nature Reviews Microbiology*, **6**(8), 592–603.
- Guha, H., Jayachandran, K., and Maurrasse, F., 2003. Microbiological reduction of chromium(VI) in presence of pyrolusite-coated sand by *Shewanella alga* Simidu ATCC 55627 in laboratory column experiments. *Chemosphere*, **52**, 175–183.
- Haas, J. R., Dichristina, T. J., and Wade, R., 2001. Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. *Chemical Geology*, **180**(1–4), 33–54.
- Hau, H. H., and Gralnick, J. A., 2007. Ecology and biotechnology of the genus *Shewanella*. *Annual Review of Microbiology*, **61**(1), 237–258.
- Ivanova, E. P., Flavier, S., and Christen, R., 2004. Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family *Alteromonadaceae* and proposal of *Pseudoalteromonadaceae* fam. nov., *Colwelliaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Idiomarinaceae* fam. nov. and *Psychromonadaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, **54**(5), 1773–1788.
- Korenevsky, A., and Beveridge, T. J., 2007. The surface physicochemistry and adhesiveness of *Shewanella* are affected by their surface polysaccharides. *Microbiology*, **153**(6), 1872–1883.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Shewanella* and alpha-FeOOH. *Science*, **292**(5520), 1360–1363.
- MacDonell, M. T., and Colwell, R. R., 1985. Phylogeny of the *Vibrionaceae* and recommendation for two new genera, *Listonella* and *Shewanella*. *Systematics and Applied Microbiology*, **6**, 171–182.
- Malasarn, D., Keeffe, J. R., and Newman, D. K., 2008. Characterization of the arsenate respiratory reductase from *Shewanella* sp. Strain ANA-3. *Journal of Bacteriology*, **190**(1), 135–142.
- Nealson, K. H., and Myers, C. R., 1992. Microbial reduction of manganese and iron: new approaches to carbon cycling. *Applied and Environmental Microbiology*, **58**, 439–443.
- Nealson, K. H., and Scott, J. H., 2006. Ecophysiology of the genus *Shewanella*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.). *The Prokaryotes: A Handbook on the Biology of Bacteria*, Proteobacteria: Gamma Subclass. New York: Springer-Verlag, Vol. 6, pp. 1133–1151.
- Pedersen, K., and Albinsson, Y., 1991. Effect of cell number, pH and lanthanide concentration on the sorption of promethium by *Shewanella putrefaciens*. *Radiochimica Acta*, **54**, 91–95.
- Saltikov, C. W., Cifuentes, A., Venkateswaran, K., and Newman, D. K., 2003. The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella* species strain ANA-3. *Applied and Environmental Microbiology*, **69**, 2800–2809.
- Urrutia, M. M., Roden, E. E., Fredrickson, J. K., and Zachara, J. M., 1998. Microbial and surface chemistry controls on reduction of

synthetic Fe(III) oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga*. *Geomicrobiology Journal*, **15**(4), 269–291.

Venkateswaran, K., Dollhopf, M. E., Aller, R., Stackebrandt, E., and Nealson, K. H., 1998. *Shewanella amazonensis* sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds. *International Journal of Systematic Bacteriology*, **48**(3), 965–972.

Warren, L. A., and Ferris, F. G., 1998. Continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Weber, K. A., Achenbach, L. A., and Coates, J. D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology*, **4**(10), 752–764.

Cross-references

[Banded Iron Formations](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Microbial Biomineralization](#)
[Microbial-Metal Binding](#)

SIDERITE

Volker Thiel
 University of Göttingen, Göttingen, Germany

Siderite is an iron carbonate mineral (FeCO_3 , 48% Fe) belonging to the calcite group (hexagonal crystal system). Magnesium and manganese may substitute for the iron. Siderite crystals are yellow to dark brown in color, rhombohedral in shape, and with curved and striated faces, and also occur in masses. Siderite is commonly found in bedded sedimentary iron ores, including banded iron formations (BIFs, see entry [Banded Iron Formations](#)), and in hydrothermal veins. It is also a common diagenetic mineral, forming at shallow burial depths in shales and sandstones where it tends to form concretions. The diagenetic formation of siderite strongly depends on the availability of Fe^{2+} ions in pore waters (see entry [Pore Waters](#)) and is thus a phenomenon of suboxic to anoxic environments (Berner, 1971). Diagenetic siderite precipitation has been linked to microbially mediated reactions, specifically to the release of ferrous (Fe^{2+}) iron due to dissimilatory iron reduction performed by Fe^{3+} -reducing prokaryotes (see entry [Fe\(III\)-Reducing Prokaryotes](#)) such as *Shewanella* (Roh et al., 2003; Hansel et al., 2003; see entry [Shewanella](#)). Microbially assisted siderite precipitation appears to preferentially occur in environments where the rate of bacterial iron reduction exceeds the rate of sulfate reduction performed by sulfate-reducing bacteria (Pye et al., 2006; see entry [Sulfate-Reducing Bacteria](#)). In sulfidogenic sediments, iron is largely precipitated as iron sulfide rather than as siderite (see entry [Iron Sulfide Formation](#)).

Bibliography

- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.

biocorrosion. The respiratory reduction of metals turns these organisms to key players in groundwater ecology, as they contribute to the mobilization of occasionally harmful metals (e.g., arsenic) from the solid to the soluble phase (Malasarn et al., 2008).

Bibliography

- Dzombak, D. A., and Morel, F. M. M., 1990. *Surface Complex Modelling: Hydrous Ferric Oxide*. New York: Wiley Interscience.
- Fredrickson, J. K., Romine, M. F., Beliaev, A. S., Auchtung, J. M., Driscoll, M. E., Gardner, T. S., Nealson, K. H., Osterman, A. L., Pinchuk, G., Reed, J. L., Rodionov, D. A., Rodrigues, J. L. M., Saffarini, D. A., Serres, M. H., Spormann, A. M., Zhulin, I. B., and Tiedje, J. M., 2008. Towards environmental systems biology of *Shewanella*. *Nature Reviews Microbiology*, **6**(8), 592–603.
- Guha, H., Jayachandran, K., and Maurrasse, F., 2003. Microbiological reduction of chromium(VI) in presence of pyrolusite-coated sand by *Shewanella alga* Simidu ATCC 55627 in laboratory column experiments. *Chemosphere*, **52**, 175–183.
- Haas, J. R., Dichristina, T. J., and Wade, R., 2001. Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. *Chemical Geology*, **180**(1–4), 33–54.
- Hau, H. H., and Gralnick, J. A., 2007. Ecology and biotechnology of the genus *Shewanella*. *Annual Review of Microbiology*, **61**(1), 237–258.
- Ivanova, E. P., Flavier, S., and Christen, R., 2004. Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family *Alteromonadaceae* and proposal of *Pseudoalteromonadaceae* fam. nov., *Colwelliaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Idiomarinaceae* fam. nov. and *Psychromonadaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, **54**(5), 1773–1788.
- Korenevsky, A., and Beveridge, T. J., 2007. The surface physicochemistry and adhesiveness of *Shewanella* are affected by their surface polysaccharides. *Microbiology*, **153**(6), 1872–1883.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Shewanella* and alpha-FeOOH. *Science*, **292**(5520), 1360–1363.
- MacDonell, M. T., and Colwell, R. R., 1985. Phylogeny of the *Vibrionaceae* and recommendation for two new genera, *Listonella* and *Shewanella*. *Systematics and Applied Microbiology*, **6**, 171–182.
- Malasarn, D., Keeffe, J. R., and Newman, D. K., 2008. Characterization of the arsenate respiratory reductase from *Shewanella* sp. Strain ANA-3. *Journal of Bacteriology*, **190**(1), 135–142.
- Nealson, K. H., and Myers, C. R., 1992. Microbial reduction of manganese and iron: new approaches to carbon cycling. *Applied and Environmental Microbiology*, **58**, 439–443.
- Nealson, K. H., and Scott, J. H., 2006. Ecophysiology of the genus *Shewanella*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.). *The Prokaryotes: A Handbook on the Biology of Bacteria*, Proteobacteria: Gamma Subclass. New York: Springer-Verlag, Vol. 6, pp. 1133–1151.
- Pedersen, K., and Albinsson, Y., 1991. Effect of cell number, pH and lanthanide concentration on the sorption of promethium by *Shewanella putrefaciens*. *Radiochimica Acta*, **54**, 91–95.
- Saltikov, C. W., Cifuentes, A., Venkateswaran, K., and Newman, D. K., 2003. The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella* species strain ANA-3. *Applied and Environmental Microbiology*, **69**, 2800–2809.
- Urrutia, M. M., Roden, E. E., Fredrickson, J. K., and Zachara, J. M., 1998. Microbial and surface chemistry controls on reduction of

synthetic Fe(III) oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga*. *Geomicrobiology Journal*, **15**(4), 269–291.

Venkateswaran, K., Dollhopf, M. E., Aller, R., Stackebrandt, E., and Nealson, K. H., 1998. *Shewanella amazonensis* sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds. *International Journal of Systematic Bacteriology*, **48**(3), 965–972.

Warren, L. A., and Ferris, F. G., 1998. Continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Weber, K. A., Achenbach, L. A., and Coates, J. D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology*, **4**(10), 752–764.

Cross-references

[Banded Iron Formations](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Microbial Biomineralization](#)
[Microbial-Metal Binding](#)

SIDERITE

Volker Thiel
 University of Göttingen, Göttingen, Germany

Siderite is an iron carbonate mineral (FeCO_3 , 48% Fe) belonging to the calcite group (hexagonal crystal system). Magnesium and manganese may substitute for the iron. Siderite crystals are yellow to dark brown in color, rhombohedral in shape, and with curved and striated faces, and also occur in masses. Siderite is commonly found in bedded sedimentary iron ores, including banded iron formations (BIFs, see entry [Banded Iron Formations](#)), and in hydrothermal veins. It is also a common diagenetic mineral, forming at shallow burial depths in shales and sandstones where it tends to form concretions. The diagenetic formation of siderite strongly depends on the availability of Fe^{2+} ions in pore waters (see entry [Pore Waters](#)) and is thus a phenomenon of suboxic to anoxic environments (Berner, 1971). Diagenetic siderite precipitation has been linked to microbially mediated reactions, specifically to the release of ferrous (Fe^{2+}) iron due to dissimilatory iron reduction performed by Fe^{3+} -reducing prokaryotes (see entry [Fe\(III\)-Reducing Prokaryotes](#)) such as *Shewanella* (Roh et al., 2003; Hansel et al., 2003; see entry [Shewanella](#)). Microbially assisted siderite precipitation appears to preferentially occur in environments where the rate of bacterial iron reduction exceeds the rate of sulfate reduction performed by sulfate-reducing bacteria (Pye et al., 2006; see entry [Sulfate-Reducing Bacteria](#)). In sulfidogenic sediments, iron is largely precipitated as iron sulfide rather than as siderite (see entry [Iron Sulfide Formation](#)).

Bibliography

- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.

- Hansel, C. M., Benner, S. G., Neiss, J., Dohnalkova, A., Kukkadapu, R. K., and Fendorf, S., 2003. Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. *Geochimica et Cosmochimica Acta*, **67**, 2977–2992.
- Pye, K., Dicksin, J. A. D., Schiavon, N., Coleman, M. L., and Cox, M., 2006. Formation of siderite–Mg–calcite–iron sulphide concretions in intertidal marsh and sandflat sediments, north Norfolk, England. *Sedimentology*, **37**, 325–343.
- Roh, Y., Zhang, C. L., Vali, H., Lauf, R. J., Zhou, J., and Phelps, T. J., 2003. Biogeochemical and environmental factors in Fe biomineralization: magnetite and siderite formation. *Clays and Clay Minerals*, **51**, 83–95.

Cross-references

Banded Iron Formations
Carbonates
Fe(III)-Reducing Prokaryotes
Iron Sulfide Formation
Pore Waters
Shewanella
Sulfate-Reducing Bacteria

SIDEROPHORES

Stephan M. Kraemer
University of Vienna, Vienna, Austria

Definition

Siderophores are low molecular weight iron-specific organic ligands that are exuded by iron-limited organisms as part of a high-affinity iron acquisition strategy.

Introduction

Iron is a nutrient to almost all known organisms. Even though iron is the fourth most abundant element on earth, the acquisition of this nutrient poses a serious challenge to organisms in many natural environments. A particularly iron-depleted system is the photic zone of the marine water column (Kraemer et al., 2005). Here, soluble iron is removed by biological uptake and subsequent sinking of the biomass below the mixed zone. In ocean areas with particularly low iron concentrations relative to other nutrients in the photic zone, the primary productivity is limited by the low bioavailability of iron. This marine iron limitation may affect the global climate by limiting the efficiency of the marine carbon pump. However, even in soils that contain abundant iron-bearing mineral phases, iron acquisition poses a challenge to microbial and plant life. Indeed, the low solubility and dissolution rates of iron-bearing minerals in calcareous soils are responsible for large annual agricultural losses by plant iron limitation (iron chlorosis) (Kraemer et al., 2006).

The ability of organisms to scavenge iron from their surroundings is prerequisite to controlling intracellular iron concentrations within the physiologically required range (homeostasis). An efficient mechanism of iron

uptake involves the exudation of siderophores that are designed to bind iron(III) in the extracellular space with a sufficient affinity and specificity to ensure the formation of Fe(III)-siderophore complexes even in the presence of high concentrations of other competing cations and in the appropriate pH range. This high-affinity iron uptake strategy is used by bacteria (including cyanobacteria) in marine, freshwater, and terrestrial environments, by fungi and some plant species. Other iron acquisition mechanisms include the acidification of the environment, the reduction of Fe(III) coupled to Fe(II) uptake, or the secretion of other ligands (e.g., citrate) with lower affinity for iron. High-affinity iron uptake involving siderophores is generally the most efficient system in environments with extremely low iron availability.

Siderophore structure and properties

Siderophores are not defined by their chemical structure, but by their biological function. Several hundreds of distinct and structurally diverse siderophores are known. Siderophores are low molecular mass (<1,500 Dalton) organic ligands, typically chelating iron in hexadentate octahedral coordination, although siderophores with lower denticity are known. Iron is coordinated by hard ligating groups including hydroxamate, catecholate, carboxylate, and hydroxycarboxylate groups (Figure 1). The nature of the ligating groups and the optimization of the overall siderophore structure are responsible for their outstanding affinity for iron. For example, desferrioxamine B (DFO-B), a siderophore produced by the soil bacterium *Streptomyces pilosus* forms an iron complex with a 1:1 stability constant logK of 32.02 (Martell et al., 2001). The affinity of microbial siderophores for iron binding is much higher than for competing ions that are often found in high concentrations in the extracellular environment. For example, DFO-B does not, to measurable extent, coordinate with calcium in seawater or in pore water of calcareous soils (Kraemer et al., 2005, 2006). Most siderophores excreted by terrestrial organisms are highly water soluble, allowing the diffusion of siderophores toward insoluble iron sources. This is prerequisite to their function as an iron shuttle overcoming the necessity for the organisms to directly contact the iron source (Kraemer et al., 2006). However, amphiphilic siderophores possessing fatty acid tails have been isolated from cultures of distinct genera of marine bacteria (Martinez and Butler, 2007) and pathogenic bacteria. The inhibition of ligand diffusion away from individual bacteria by anchoring of amphiphilic siderophores in the plasma membrane seems to be advantageous in marine systems where bacterial abundances are low.

Graminaceous plant species, including agriculturally important crops, such as barley, wheat, and corn, are the only plant species known to possess a high-affinity iron uptake system. They exude a distinct class of iron-specific ligands with aminocarboxylate and hydroxycarboxylate functional groups, the so-called phytosiderophores or

Cross-references

Bacteria
 Biofilms
 Leptothrix
 Microbial Biomineralization
 Microbial-Metal Binding
 Ores, Microbial Precipitation and Oxidation
 Subsurface Filamentous Fabrics
 Terrestrial Deep Biosphere

METALS, ACQUISITION BY MARINE BACTERIA

Alison Butler, Vanessa V. Homann
 University of California, Santa Barbara, CA, USA

Definition

Oceanic bacteria produce siderophores to acquire iron. Given that the iron concentration in surface ocean water is very low, siderophores produced by marine bacteria are tailored to the physical and chemical constraints of the marine environment.

Introduction

Microbial iron uptake

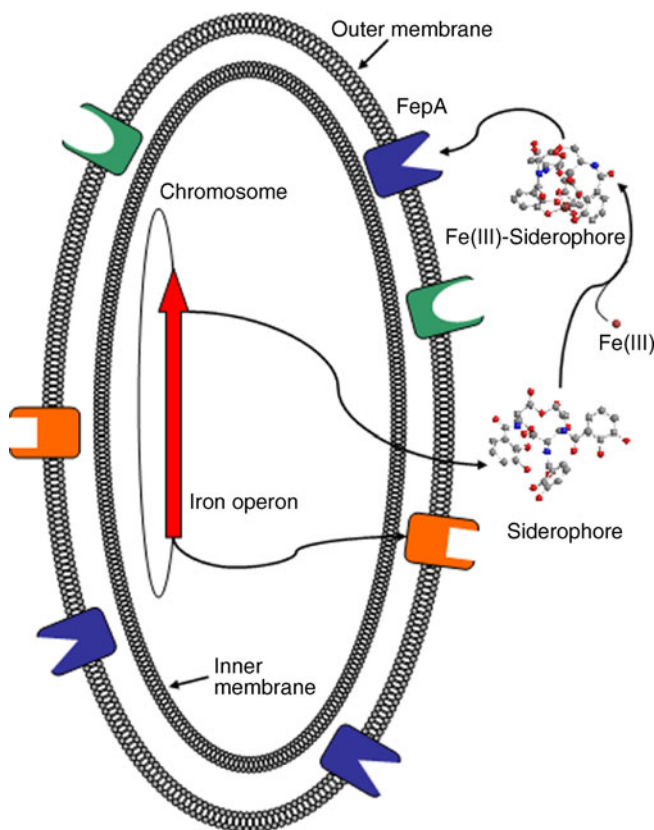
Virtually, all bacteria require iron for growth. Bacteria growing under aerobic conditions utilize a variety of pathways to acquire iron, but under conditions of iron starvation, bacteria often produce siderophores (Crosa et al., 2004). Siderophores are low molecular weight, chelating compounds produced by bacteria to facilitate iron(III) uptake (Crosa et al., 2004; Kraemer et al., 2005; Butler, 2007). The Fe(III)-siderophore complex is recognized by a specific outer membrane protein receptor, which catalyzes the transport of the Fe(III)-siderophore complex across the outer membrane (Figure 1).

The study of marine microbial siderophores is relatively new (Butler and Martin, 2005), yet certain characteristic structural features have already begun to emerge: suites of amphiphilic siderophores and photoreactive Fe(III)-siderophores.

Marine Siderophores

Amphiphilic marine siderophores

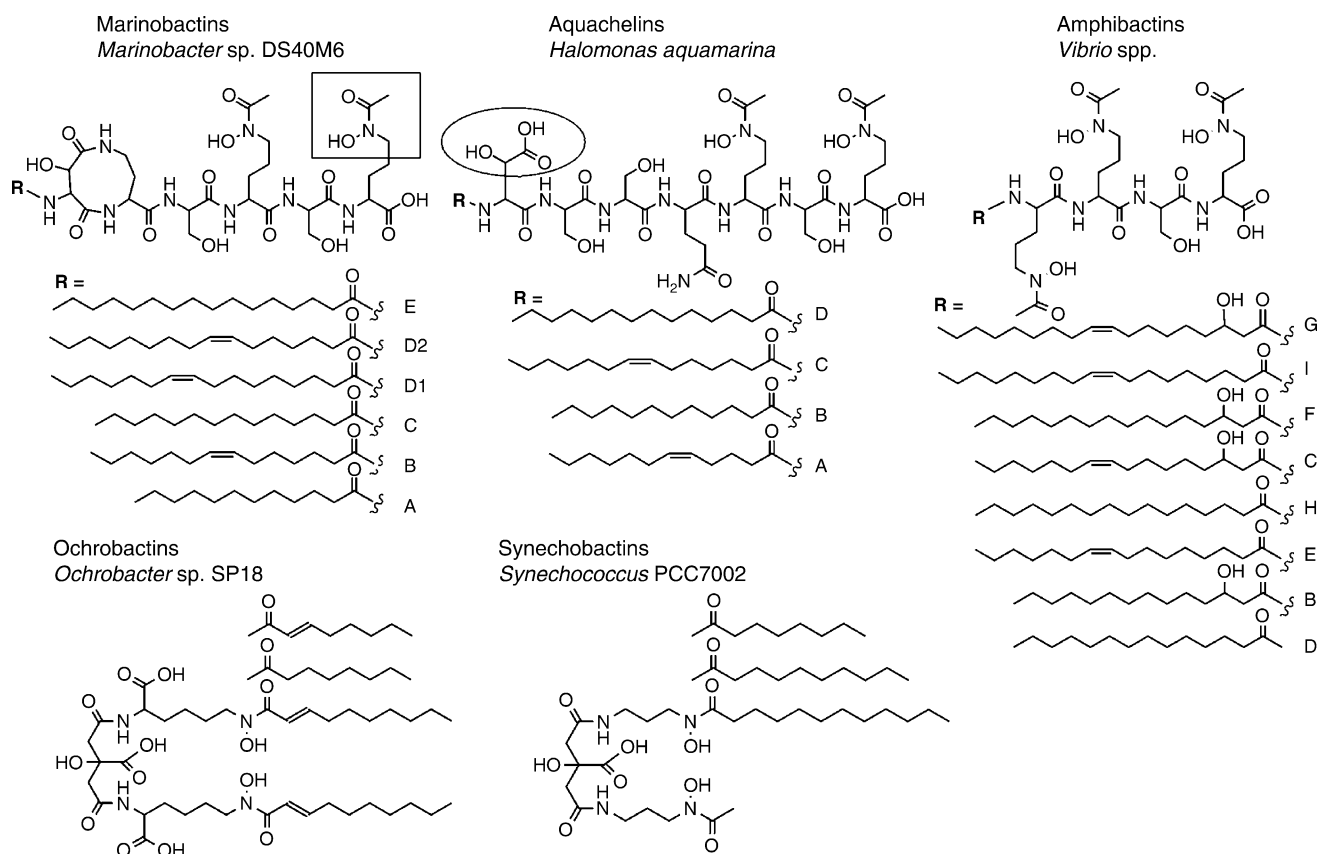
One characteristic of marine siderophores is the predominance of suites of amphiphilic siderophores, comprised of a hydrophilic head group, which coordinates with Fe(III), and one of a series of hydrophobic fatty acid appendages, which confers amphiphilicity (Figure 2). Two types of suites of amphiphilic siderophores are produced by marine bacteria. The aquachelins, marinobactins, and amphibactins are amphiphilic peptidic siderophores produced by distinct genera of marine bacteria (Figure 2) (Martinez et al., 2000, 2003). The relative amphiphilicity of each of these siderophores is



Metals, Acquisition by Marine Bacteria, Figure 1 Siderophore-mediated iron uptake by a bacterium.

defined by both the number of amino acids in the peptide fraction, compared to the fatty acid chain length (e.g., C12–C18). The amphibactins with only four amino acids and relatively long C18 fatty acids are the most hydrophobic of the amphiphilic peptide siderophores. As a result, they remain cell associated and are extracted from the bacterial pellet during the isolation process (Martinez et al., 2003). In contrast, the marinobactins and aquachelins, with five and six amino acids in the head group and a predominance of the shorter fatty acids, are more hydrophilic and are thus isolated from the supernatant following centrifugation of the bacterial culture to pellet the cells (Martinez et al., 2000).

The synechobactins and ochrobactins (Figure 2) are different suites of amphiphilic siderophores, in which the head group is based on citric acid, as opposed to a peptide. The head group is thus smaller than in the peptidic amphiphilic siderophores. The fatty acids are also shorter than observed for the peptidic amphiphilic siderophores. Nevertheless, the ochrobactins are quite hydrophobic as a result of two fatty acid appendages; these siderophores remain associated with the bacterial pellet (Martin et al., 2006). The synechobactins, with



Metals, Acquisition by Marine Bacteria, Figure 2 Amphiphilic marine siderophores. Marinobactins (Martinez et al., 2000); aquachelins (Martinez et al., 2000); amphibactins (Martinez et al., 2003); ochrobactins (Martin et al., 2006); synechobactins (Ito and Butler, 2005). The boxed portion shows the hydroxamic acid group. The circled portion shows the α -hydroxyacid in β -hydroxyaspartic acid.

only one fatty acid appendage, partition between the culture supernatant and the bacterial pellet (Ito and Butler, 2005).

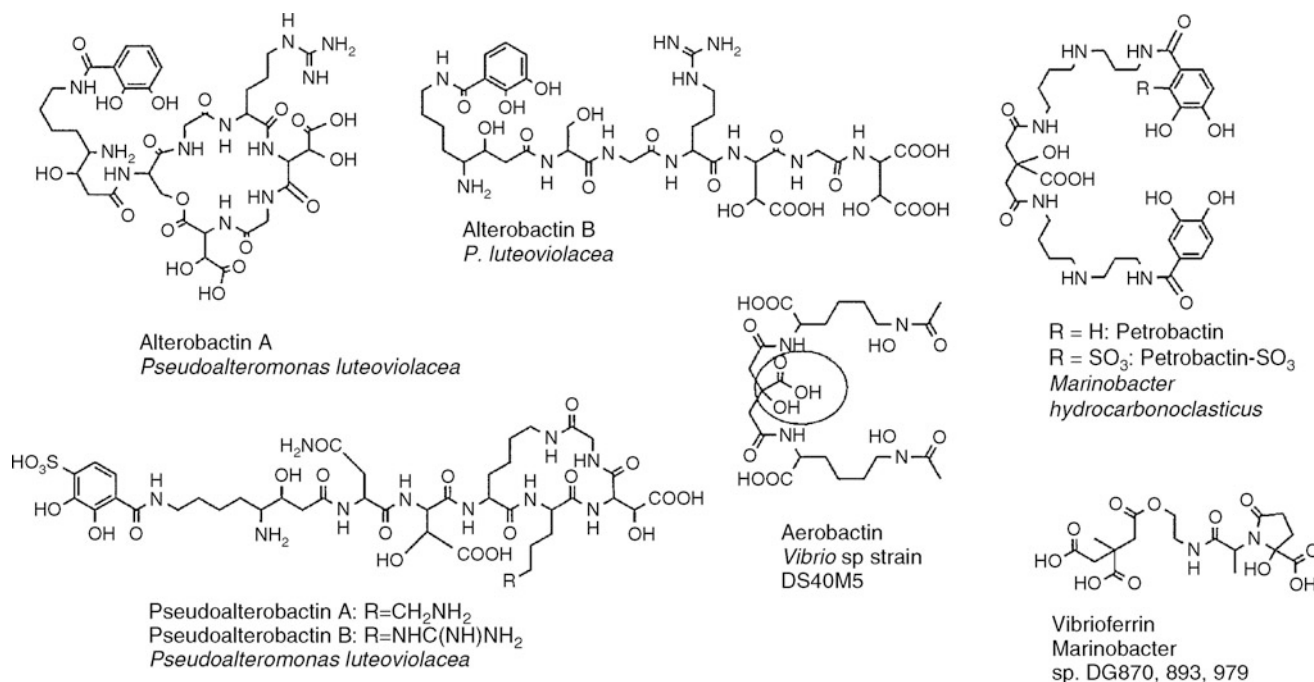
Photoreactive Fe(III)-siderophores

The other characteristic of marine siderophores is the predominance of α -hydroxycarboxylic acid moieties in the form of β -hydroxyaspartic acid or citric acid (Figures 2 and 3).

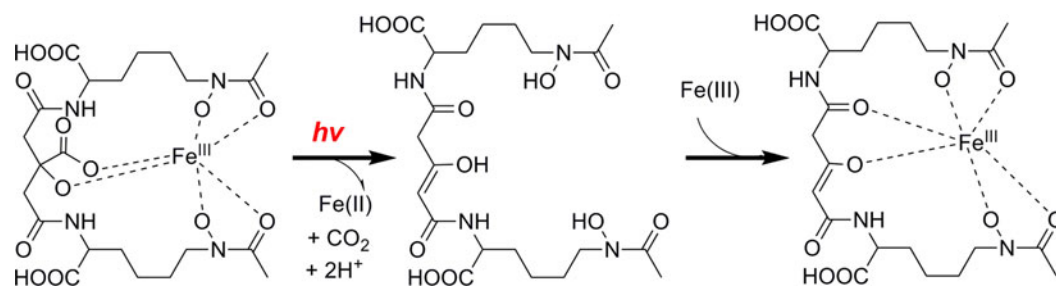
The Fe(III)-complex of an α -hydroxycarboxylic acid moiety is photoreactive in UV light, including natural sunlight, which is a condition experienced by the mixed layer of the upper 30–40 m of the ocean. Upon UV photolysis of the Fe(III)-siderophores containing citric acid, such as the ochrobactins (Martin et al., 2006), the synechobactins (Ito and Butler, 2005), the petrobactins (Bergeron et al., 2003; Hickford et al., 2004), and aerobactin (marine *Vibrio* sp. DS40M5) (Küpper et al., 2006; Haygood et al., 1993), citrate is oxidized to 3-ketoglutarate and Fe(III) is reduced to Fe(II). The resulting ligand photoproduct

retains the ability to coordinate Fe(III) with remarkably high stability constant as a result of the 3-ketoglutarate moiety and the remaining hydroxamic acid groups (Figure 4, for aerobactin (Küpper et al., 2006).

Other marine siderophores such as the aquachelins, the marinobactins, the alterobactins, and the pseudoalterobactins contain β -hydroxyaspartic acid as the α -hydroxycarboxylic acid group. UV photolysis of the Fe(III)-aquachelins results in ligand oxidation, specifically loss of β -hydroxyaspartic acid, as well as reduction of Fe(III) to Fe(II) (Barbeau et al., 2001). This peptide photoproduct retains the two hydroxamate groups and thus the ability to coordinate with Fe(III), although the conditional stability constant is somewhat reduced (Barbeau et al., 2001). Only catalytic amount of Fe(III) is required to effect the complete oxidation of the aquachelins in aerobic conditions, because the Fe(II) that is produced is oxidized back to Fe(III) and thus can coordinate with another apo aquachelin. The ferric complexes of the alterobactins A and B and the marinobactins are also photoreactive (Barbeau et al., 2003), although the



Metals, Acquisition by Marine Bacteria, Figure 3 Marine siderophores with α -hydroxycarboxylic acid groups. The aquachelins, marinobactins, ochrobactins, and synechobactins shown in Figure 2 also contain α -hydroxycarboxylic acid moiety. Alterobactins (Reid et al., 1993); pseudoalterobactins (Kano et al., 2003); aerobactin (Haygood et al., 1993); petrobactin (Bergeron et al., 2003); petrobactin-SO₃ (Hickford et al., 2004); vibrioferriin (Amin et al., 2007). The circled portion shows the α -hydroxyacid in citric acid.



Metals, Acquisition by Marine Bacteria, Figure 4 Reaction products in the photolysis of Fe(III)-aerobactin. (Adapted from Küpper et al., 2006).

structures of the oxidized ligands have not been completed yet. The ferric complexes of pseudoalterobactins A and B, which are chemically related to the alterobactins (Kano et al., 2003), would also be expected to be photoreactive.

Summary

The preponderance of suites of amphiphilic siderophores and of α -hydroxycarboxylic-acid-containing siderophores produced by marine bacteria suggests that these properties have evolved as common iron acquisition strategies for

marine bacteria, although details of the biological advantages conferred by these molecular properties are yet to be elucidated.

Bibliography

- Amin, S. A., Küpper, F. C., Green, D. H., Harris, W. R., and Carrano, C. J., 2007. Boron binding by a siderophore isolated from marine bacteria associated with the toxic dinoflagellate *Gymnodinium catenatum*. *Journal of the American Chemical Society*, **129**, 478–479.
- Barbeau, K., Rue, E. L., Bruland, K. W., and Butler, A., 2001. Photochemical cycling of iron in the surface ocean

- mediated by microbial iron(III)-binding ligands. *Nature*, **413**, 409–413.
- Barbeau, K., Rue, E. L., Trick, C. G., Bruland, K. W., and Butler, A., 2003. The photochemical reactivity of siderophores produced by marine heterotrophic bacteria and cyanobacteria, based on characteristic iron(III)-binding groups. *Limnology and Oceanography*, **48**, 1069–1078.
- Bergeron, R. J., Huang, G., Smith, R. E., Bharti, N., McManis, J. S., and Butler, A., 2003. Total synthesis of petrobactin. *Tetrahedron*, **59**, 2007–2014.
- Butler, A., 2007. Siderophores. In Bertini, I., Gray, H. B., and Valentine, J. S. (eds.), *Biological Inorganic Chemistry: Structure and Reactivity (Metal Transport and Storage Proteins section)*. Sausalito: University Science Books, (Chap. VIII.3), pp. 151–156.
- Butler, A., and Martin, J. D., 2005. The marine biogeochemistry of iron. In Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), *“Biogeochemical Cycles,” Metal Ions in Biological Systems*. New York: M. Dekker, Vol. 43, pp. 21–46.
- Crosa, J. H., Mey, A. R., and Payne, S. M., 2004. *Iron Transport in Bacteria*. Washington, DC: ASM, p. 499.
- Haygood, M. J., Holt, P., and Butler, A., 1993. Aerobactin production by a planktonic marine *Vibrio* sp. *Limnology and Oceanography*, **38**, 1091–1097.
- Hickford, S. J. H., Küpper, F. C., Zhang, G., Carrano, C. J., Blunt, J. W., and Butler, A., 2004. Petrobactin sulfonate, a new siderophore produced by the marine bacterium *Marinobacter hydrocarbonoclasticus*. *Journal of Natural Products*, **67**, 1897–1899.
- Ito, Y., and Butler, A., 2005. Structure of synechobactins, new siderophores of the marine cyanobacterium *Synechococcus* sp. PCC 7002. *Limnology and Oceanography*, **50**, 1918–1923.
- Kanoh, K., Kamino, K., Leleo, G., Adachi, K., and Shizuri, Y., 2003. Pseudoalterobactin A and B, new siderophores excreted by marine bacterium *Pseudoalteromonas* sp. KP20–4. *The Journal of Antibiotics*, **56**, 871–875.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Biogenic ligands and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry: Molecular Geomicrobiology*, **59**, 53–76.
- Küpper, F. C., Carrano, C. J., Kuhn, J. U., and Butler, A., 2006. Photoreactivity of iron(III)-aerobactin: Photoproduct structure and iron(III) coordination. *Inorganic Chemistry*, **45**, 6026–6033.
- Martin, J. D., Ito, Y., Homann, V. V., Haygood, M. G., and Butler, A., 2006. Structure and membrane affinity of new amphiphilic siderophores produced by *Ochrobactrum* sp. SP18. *Journal of Biological Inorganic Chemistry*, **11**(5), 633–641.
- Martinez, J. S., Zhang, G. P., Holt, P. D., Jung, H. T., Carrano, C. J., Haygood, M. G., and Butler, A., 2000. Self-assembling amphiphilic siderophores from marine bacteria. *Science*, **287**, 1245–1247.
- Martinez, J. S., Carter-Franklin, J. N., Mann, E. L., Martin, J. D., Haygood, M. G., and Butler, A., 2003. Structure and membrane affinity of a new suite of amphiphilic siderophores produced by a marine bacterium. *Proceedings of the National Academy of Sciences of the United States of America*, **100**(7), 3754–3759.
- Reid, R. T., Live, D. H., Faulkner, D. J., and Butler, A., 1993. A siderophore from a marine bacterium with an exceptional ferric ion stability constant. *Nature*, **366**, 455–457.

Cross-references

Bacteria
Cyanobacteria
Fe(II)-Oxidizing Prokaryotes
Metalloenzymes
Siderophores

METEORITICS

Mark A. Sephton

Imperial College London, London, UK

Definition

Meteorites are fragments of extraterrestrial material that fall on the Earth's surface. Most meteorites are parts of asteroids propelled into Earth-crossing orbits by relatively recent collisions in the asteroid belt, initiated by the gravitational effects of Jupiter's orbit. Relatively small numbers of meteorites originate from larger objects such as the Moon and Mars. The Earth acquires 10^2 to 10^3 t of such material each day, but only 1% or less arrives in pieces large enough for identification and recovery (Dodd, 1981). The surface of the meteorite usually melts and emits a glowing tail and a trail of smoke, but the interior of the meteorite is unaffected by passage through the Earth's atmosphere and remains at the temperature of interplanetary space.

Collection of meteorites

“Falls” are meteorites that have been observed to fall and were subsequently collected; “finds” are meteorites that were not seen to fall. Before the 1970s, the total number of known meteorites amounted to less than 1,000 falls and 1,706 authenticated finds. Since then, it has been recognized that certain parts of the Earth's surface preserve and concentrate meteorites. To date, tens of thousands of meteorites have been collected from the Antarctic ice. Similar numbers of meteorite fragments have also been collected from hot deserts. Most recently, telescope and camera network surveys have produced meteorites with photographed fireballs allowing the calculation of their orbits and assignment to a particular source within the solar system (Bland et al., 2009).

Chondrites

About 85% of the meteorites observed to fall on the Earth are chondrites, conglomerate rocks with an overall elemental composition similar to the Sun, if the loss of the most volatile elements is ignored. When their near solar makeup is considered alongside radiometric ages of 4.56×10^9 years, it becomes clear that chondrites are rocks formed during, or shortly after, the birth of the Sun.

The record in chondrites

Their status as some of the earliest formed solids in the solar system combined with a lack of subsequent processing ensures that chondrites represent a valuable record of extraterrestrial environments. Contributing regions include interstellar space, the solar nebula and the asteroidal meteorite parent bodies.

- Hansel, C. M., Benner, S. G., Neiss, J., Dohnalkova, A., Kukkadapu, R. K., and Fendorf, S., 2003. Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. *Geochimica et Cosmochimica Acta*, **67**, 2977–2992.
- Pye, K., Dicksin, J. A. D., Schiavon, N., Coleman, M. L., and Cox, M., 2006. Formation of siderite–Mg–calcite–iron sulphide concretions in intertidal marsh and sandflat sediments, north Norfolk, England. *Sedimentology*, **37**, 325–343.
- Roh, Y., Zhang, C. L., Vali, H., Lauf, R. J., Zhou, J., and Phelps, T. J., 2003. Biogeochemical and environmental factors in Fe biomineralization: magnetite and siderite formation. *Clays and Clay Minerals*, **51**, 83–95.

Cross-references

Banded Iron Formations
Carbonates
Fe(III)-Reducing Prokaryotes
Iron Sulfide Formation
Pore Waters
Shewanella
Sulfate-Reducing Bacteria

SIDEROPHORES

Stephan M. Kraemer
University of Vienna, Vienna, Austria

Definition

Siderophores are low molecular weight iron-specific organic ligands that are exuded by iron-limited organisms as part of a high-affinity iron acquisition strategy.

Introduction

Iron is a nutrient to almost all known organisms. Even though iron is the fourth most abundant element on earth, the acquisition of this nutrient poses a serious challenge to organisms in many natural environments. A particularly iron-depleted system is the photic zone of the marine water column (Kraemer et al., 2005). Here, soluble iron is removed by biological uptake and subsequent sinking of the biomass below the mixed zone. In ocean areas with particularly low iron concentrations relative to other nutrients in the photic zone, the primary productivity is limited by the low bioavailability of iron. This marine iron limitation may affect the global climate by limiting the efficiency of the marine carbon pump. However, even in soils that contain abundant iron-bearing mineral phases, iron acquisition poses a challenge to microbial and plant life. Indeed, the low solubility and dissolution rates of iron-bearing minerals in calcareous soils are responsible for large annual agricultural losses by plant iron limitation (iron chlorosis) (Kraemer et al., 2006).

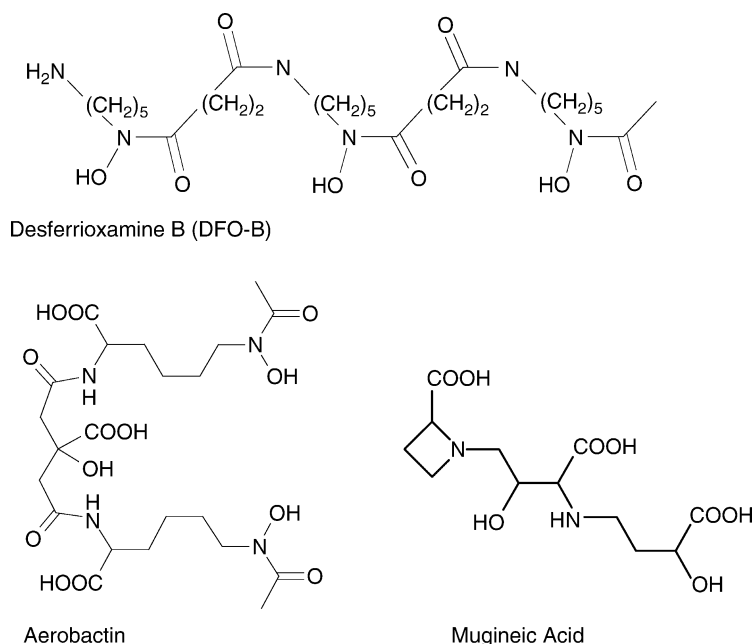
The ability of organisms to scavenge iron from their surroundings is prerequisite to controlling intracellular iron concentrations within the physiologically required range (homeostasis). An efficient mechanism of iron

uptake involves the exudation of siderophores that are designed to bind iron(III) in the extracellular space with a sufficient affinity and specificity to ensure the formation of Fe(III)-siderophore complexes even in the presence of high concentrations of other competing cations and in the appropriate pH range. This high-affinity iron uptake strategy is used by bacteria (including cyanobacteria) in marine, freshwater, and terrestrial environments, by fungi and some plant species. Other iron acquisition mechanisms include the acidification of the environment, the reduction of Fe(III) coupled to Fe(II) uptake, or the secretion of other ligands (e.g., citrate) with lower affinity for iron. High-affinity iron uptake involving siderophores is generally the most efficient system in environments with extremely low iron availability.

Siderophore structure and properties

Siderophores are not defined by their chemical structure, but by their biological function. Several hundreds of distinct and structurally diverse siderophores are known. Siderophores are low molecular mass (<1,500 Dalton) organic ligands, typically chelating iron in hexadentate octahedral coordination, although siderophores with lower denticity are known. Iron is coordinated by hard ligating groups including hydroxamate, catecholate, carboxylate, and hydroxycarboxylate groups (Figure 1). The nature of the ligating groups and the optimization of the overall siderophore structure are responsible for their outstanding affinity for iron. For example, desferrioxamine B (DFO-B), a siderophore produced by the soil bacterium *Streptomyces pilosus* forms an iron complex with a 1:1 stability constant logK of 32.02 (Martell et al., 2001). The affinity of microbial siderophores for iron binding is much higher than for competing ions that are often found in high concentrations in the extracellular environment. For example, DFO-B does not, to measurable extent, coordinate with calcium in seawater or in pore water of calcareous soils (Kraemer et al., 2005, 2006). Most siderophores excreted by terrestrial organisms are highly water soluble, allowing the diffusion of siderophores toward insoluble iron sources. This is prerequisite to their function as an iron shuttle overcoming the necessity for the organisms to directly contact the iron source (Kraemer et al., 2006). However, amphiphilic siderophores possessing fatty acid tails have been isolated from cultures of distinct genera of marine bacteria (Martinez and Butler, 2007) and pathogenic bacteria. The inhibition of ligand diffusion away from individual bacteria by anchoring of amphiphilic siderophores in the plasma membrane seems to be advantageous in marine systems where bacterial abundances are low.

Graminaceous plant species, including agriculturally important crops, such as barley, wheat, and corn, are the only plant species known to possess a high-affinity iron uptake system. They exude a distinct class of iron-specific ligands with aminocarboxylate and hydroxycarboxylate functional groups, the so-called phytosiderophores or



Siderophores, Figure 1 Chemical structures of the phytosiderophore mugineic acid and of microbial siderophores desferrioxamine B (DFO-B) and aerobactin.

mugineic acids (Takagi, 1976; Takagi et al., 1984) (Figure 1).

Uptake of Fe(III)-siderophore complexes

Gram-negative bacteria bind siderophore complexes by a receptor in the outer membrane followed by shuttling to the cytoplasmic membrane. Gram-positive bacteria have a receptor anchored to the cytoplasmic membrane. The complexes are then delivered to the cytoplasm by a transmembrane protein (ABC-transporter) (Andrews et al., 2003). Similarly, graminaceous plants possess a high-affinity uptake system specific for iron(III)-phytosiderophore complexes (Curie et al., 2001). Some microorganisms are known to be endowed with uptake systems for iron complexes of siderophores that are not produced by the same organism.

The intracellular release of iron from internalized siderophores is promoted by either hydrolysis of the siderophore or reduction of ferric iron to ferrous iron followed by competitive sequestration of the iron (Miethke and Marahiel, 2007). The biosynthesis and exudation of siderophores as well as the expression of their uptake systems are highly regulated as a response to iron limitation (Hantke, 2001).

Siderophore concentrations in natural environments

The exudation of siderophores is a costly strategy in terms of cellular nutrient and energy budgets, particularly in systems with low bacterial densities such as oligotrophic marine systems (Völker and Wolf-Gladrow, 1999).

Therefore, it is not surprising that observed siderophore concentration in the environment do not exceed iron concentrations required for efficient uptake. Powell et al. (1980) have estimated hydroxamate siderophore concentrations in soil solutions to be between 10 and 100 nM. Essen et al. (2006) found up to 2 and 12 nM of the hydroxamate siderophores ferrichrome and ferricrocin, respectively, in soil solutions of podzolic forest soils. However, the quantitative analysis of siderophores in natural waters remains challenging due to their structural diversity and low concentrations. For example, siderophores have not been detected unambiguously in ocean water even though iron complexes with conditional stability constants comparable to siderophore complexes in the nanomolar and subnanomolar concentration range have been observed by voltammetric techniques (Rue and Bruland, 1995). Römheld (1991) has estimated that phytosiderophore concentrations in the rhizosphere can reach local concentrations of up to 1 mM in the soil solution.

Sources of iron

The soluble iron concentrations in oxic soils or surface waters are limited by the solubility of iron oxides, iron hydroxides, or hydrous ferric oxide. In the neutral to slightly alkaline pH range corresponding to the pH of calcareous soil solutions and ocean water, their solubility reaches a minimum below 1 nM (Liu and Millero, 2002). The presence of iron complexing organic ligands can increase the iron solubility. Thus, iron complexes with low molecular weight organic ligands, fulvic and humic acids, as well as iron-bearing mineral phases (including

primary silicates and pedogenic iron oxides) are the most important iron sources for high-affinity uptake systems (Kraemer, 2004). Prerequisite for iron sequestration from these sources are the superior affinity of siderophores for iron compared to the stability of the iron complexes or minerals that serve as iron source and reasonably fast rates of the associated ligand exchange or dissolution reactions.

Siderophore promoted dissolution of iron oxides and iron-bearing silicates

Siderophores promote iron sequestration from iron oxides (Kraemer et al., 1999) and iron-bearing primary (e.g., hornblende [Liermann et al., 2000]) and secondary silicates [e.g., kaolinite (Rosenberg and Maurice, 2003)]. The effect of siderophores on iron dissolution rests on their ability to increase the solubility of iron and to accelerate dissolution rates. It has been shown that bacterial and plant siderophores promote iron oxide dissolution in the dark by a ligand-controlled dissolution mechanism, where the rate of dissolution is a linear function of the adsorbed siderophore concentration (Kraemer et al., 1999; Reichard et al., 2005). This mechanism is important in high-affinity iron acquisition by graminaceous plants. Considering the low concentrations of microbial siderophores observed in natural systems, their main function seems to be to increase solubility of iron oxides, generating thermodynamic driving force for other dissolution mechanisms (Cheah et al., 2003; Reichard et al., 2007).

Photochemistry of siderophore complexes

Soluble iron complexes of siderophores possessing α -hydroxycarboxylate ligating groups photolyze in seawater under irradiation by sunlight, resulting in oxidation of the ligand and formation of transient Fe(II) that is rapidly reoxidized (Barbeau, 2006). Both the Fe(II) and iron bound to the oxidized ligand are bioavailable to phototrophic phytoplankton that cannot take up Fe(III)-siderophore complexes. Moreover, siderophores promote photoreductive dissolution processes that tend to be faster than ligand-promoted dissolution reactions (Borer et al., 2005). These photochemical processes may be of key importance for algal and bacterial iron acquisition in marine systems.

Formation of siderophore complexes with other metals and their uptake

A prerequisite for the efficiency of the high-affinity iron uptake system is the ability of organisms to discriminate against uptake of other (potentially deleterious) metal ions. The selectivity of the uptake system is ensured by the high specificity of siderophores for iron binding relative to most cations in solution and by the specific recognition of iron(III)-siderophore complexes by receptors at the cell surfaces. However, some exceptions to the selectivity of iron binding and uptake are known. Siderophores have a high affinity for metal ions with an equal or higher charge to radius ratio than Fe(III). For example, the

siderophore DFO-B forms a Pu(IV) complex with a 1:1 stability constant that is orders of magnitude higher than the corresponding iron complex (Boukhalfa et al., 2007). Moreover, slow uptake of Pu(IV)-siderophore complexes by the high-affinity iron uptake system of *Microbacterium flavescens* has been observed (John et al., 2001). The formation of plutonium-siderophore complexes and their bacterial uptake illustrate that the selectivity for a metal ion other than iron does not necessarily imply that the metal ion is actively targeted by the high-affinity uptake system for the purpose of nutrition. Also, the siderophore DFO-B has a high selectivity for the trivalent manganese ion (Duckworth and Sposito, 2005). Manganese in this redox state is not commonly observed in the absence of the siderophores, but the shifted redox potential of the complex stabilizes Mn(III) under conditions that are found in natural systems. In order to appreciate the effect that siderophores may have on the geochemistry of metal ions other than iron, it is important to keep in mind the extremely low siderophore concentrations measured in natural systems (vide supra).

Uptake of phytosiderophore complexes of Fe(II), Ni(II), Zn(II), Cu(II), Mn(II), and Cd(II) by maize (*Zea mays*) has been observed (Schaaf et al., 2004), and it has been controversially discussed if their uptake by the high-affinity iron uptake system is regulated or fortuitous. A high-affinity uptake system also seems to promote the uptake of molybdenum and vanadium by the nitrogen fixing soil bacterium *Azotobacter vinelandii* (Bellenger et al., 2008). Methanotrophic bacteria use a high-affinity uptake system for copper acquisition involving the exudation of Cu(I) specific organic ligands, the so-called chalcophores. Chalcophores have a distinctly different architecture compared to siderophores involving nitrogen and sulfur as ligands (Kim et al., 2004).

Conclusions

The key role of siderophores in marine and terrestrial biological iron acquisition is well established. However, a thorough and quantitative understanding of their effect on processes involved in, e.g., global carbon cycling or crop production requires a better understanding of how siderophores influence the availability and distribution of iron among prokaryotic and eukaryotic organisms in complex ecosystems. New observations of siderophore photochemistry, their amphiphilic properties and their mineral surface chemistry as well as observations of iron speciation in natural systems are important steps in this direction. Furthermore, recent work has shown that nutrient acquisition involving the exudation of ion-specific ligands is not unique to high-affinity iron acquisition. Discoveries of other similar nutrient acquisition systems may reshape our thinking about the effect of trace metal biogeochemistry on the microbial ecology of oligotrophic natural systems and vice versa. Finally, the unique properties of siderophores and other ion-specific biogenic ligands continue to inspire technical applications in fields such as pollutant remediation or biomedicine.

Bibliography

- Andrews, S. C., Robinson, A. K., and Rodriguez-Quinones, F., 2003. Bacterial iron homeostasis. *FEMS Microbiology Ecology*, **27**, 215–237.
- Barbeau, K., 2006. Photochemistry of organic iron(III) complexing ligands in oceanic systems. *Photochemistry and Photobiology*, **82**, 1505–1516.
- Bellenger, J. P., Wichard, T., Kustka, A. B., and Kraepiel, A. M. L., 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience*, **1**, 243–246.
- Borer, P., Sulzberger, B., Reichard, P. U., and Kraemer, S. M., 2005. Effect of siderophores on the light-induced dissolution of colloidal iron(III)(hydr)oxides. *Marine Chemistry*, **93**, 179–193.
- Boukhalfa, H., Reilly, S. D., and Neu, M. P., 2007. Complexation of Pu(IV) with the natural siderophore Desferrioxamine B and the redox properties of Pu(IV)(siderophore) complexes. *Inorganic Chemistry*, **46**, 1018–1026.
- Cheah, S. F., Kraemer, S. M., Cervini-Silva, J., and Sposito, G., 2003. Steady-state dissolution kinetics of goethite in the presence of desferrioxamine B and oxalate ligands: implications for the microbial acquisition of iron. *Chemical Geology*, **198**, 63–75.
- Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S. L., Briat, J. -F., and Walker, E. L., 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature*, **409**, 346–349.
- Duckworth, O. W., and Sposito, G., 2005. Siderophore-manganese (III) interactions. I. Air-oxidation of manganese(II) promoted by desferrioxamine B. *Environmental Science Technology*, **39**, 6037–6044.
- Essen, S. A., Bylund, D., Holmstrom, S. J. M., Moberg, M., and Lundstrom, U. S., 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *Biometals*, **19**, 269–282.
- Hantke, K., 2001. Iron and metal regulation in bacteria. *Current Opinion in Microbiology*, **4**, 172–177.
- John, S. G., Ruggiero, C. E., Hersman, L. E., Tung, C. S., and Neu, M. P., 2001. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). *Environmental Science Technology*, **35**, 2942–2948.
- Kim, H. J., Graham, D. W., DiSpirito, A. A., Alterman, M. A., Galeva, N., Larive, C. K., Asunskis, D., and Sherwood, P. M. A., 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science*, **305**, 1612–1615.
- Kraemer, S. M., 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science*, **66**, 3–18.
- Kraemer, S. M., Cheah, S. F., Zapf, R., Xu, J. D., Raymond, K. N., and Sposito, G., 1999. Effect of hydroxamate siderophores on Fe release and Pb(II) adsorption by goethite. *Geochimica et Cosmochimica Acta*, **63**, 3003–3008.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Siderophores and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry*, **59**, 53–76.
- Kraemer, S. M., Crowley, D., and Kretzschmar, R., 2006. Siderophores in plant iron acquisition: geochemical aspects. *Advances in Agronomy*, **91**, 1–46.
- Liermann, L. J., Kalinowski, B. E., Brantley, S. L., and Ferry, J. G., 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta*, **64**, 587–602.
- Liu, X. W., and Millero, F. J., 2002. The solubility of iron in seawater. *Marine Chemistry*, **77**, 43–54.
- Martell, A. E., Smith, R. M., and Motekaitis, R. J., 2001. NIST critically selected stability constants of metal complexes database. Gaithersburg MD: NIST. http://www.nist.gov/srd/WebGuide/Critical/46_8.htm.
- Martinez, J. S., and Butler, A., 2007. Marine amphiphilic siderophores: marinobactin structure, uptake, and microbial partitioning. *Journal of Inorganic Biochemistry*, **101**, 1692–1698.
- Miethke, M., and Marahiel, M. A., 2007. Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews*, **71**, 413–451.
- Powell, P. E., Cline, G. R., Reid, C. P. P., and Szaniszlo, P. J., 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature*, **287**, 833–834.
- Reichard, P. U., Kraemer, S. M., Frazier, S. W., and Kretzschmar, R., 2005. Goethite dissolution in the presence of phytosiderophores: rates, mechanisms, and the synergistic effect of oxalate. *Plant and Soil*, **276**, 115–132.
- Reichard, P. U., Kretzschmar, R., and Kraemer, S. M., 2007. Dissolution mechanisms of goethite in the presence of siderophores and organic acids. *Geochimica et Cosmochimica Acta*, **71**, 5635–5650.
- Römheld, V., 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in gramineous species – an ecological approach. *Plant Soil*, **130**, 127–134.
- Rosenberg, D. R., and Maurice, P. A., 2003. Siderophore adsorption to and dissolution of kaolinite at pH 3 to 7 and 22 degrees C. *Geochimica et Cosmochimica Acta*, **67**, 223–229.
- Rue, E. L., and Bruland, K. W., 1995. Complexation of iron(III) by natural organic-ligands in the central North Pacific as determined by a new competitive ligand equilibration adsorptive cathodic stripping voltammetric method. *Marine Chemistry*, **50**, 117–138.
- Schaaf, G., Erenoglu, B., and von Wiren, N., 2004. Physiological and biochemical characterization of metal-phytosiderophore transport in graminaceous species. *Soil Science and Plant Nutrition*, **50**, 989–995.
- Takagi, S. I., 1976. Naturally occurring iron-chelating compounds in oat-root and rice-root washings. I. Activity measurement and preliminary characterization. *Soil Science and Plant Nutrition*, **22**, 423–433.
- Takagi, S., Nomoto, K., and Takemoto, T., 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *Journal Of Plant Nutrition*, **7**, 469–477.
- Völker, C., and Wolf-Gladrow, D. A., 1999. Physical limits on iron uptake mediated by siderophores or surface reductases. *Marine Chemistry*, **65**, 227–244.

Cross-references

Gallionella
 Leptothrix
 Metals, Acquisition by Marine Bacteria
 Microbial Biomineralization
 Microbial-Metal Binding

SILICA BIOMINERALIZATION, SPONGES

Hermann Ehrlich
 Dresden University of Technology, Dresden, Germany

Synonyms

Biosilicification, Silication

Definition

Biosilicification: The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges,

- spicules in the marine demosponge *Suberites domuncula*. *Journal of Biological Chemistry*, **281**(17), 12001–12009.
- Schulze, F. E., 1886. *Über den Bau und das System der Hexactinelliden*. Berlin: Royal Prussian Academy of Sciences, pp. 3–97.
- Schulze, F. E., 1904. Hexactinellida. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **4**, 1–266.
- Schwartz, K., 1973. A bound form of silicon in glucosaminoglycans and polyuronides. *Proceedings of National Academy of Sciences of the United States of America*, **70**, 1608–1612.
- Shimizu, K., and Morse, D. E., 2000. The biological and biomimetic synthesis of silica and other polysiloxanes. In Baeuerlein, E. (ed.), *Biomimetalization: From Biology to Biotechnology and Medical Application*. Wiley, Weinheim, pp. 207–219.
- Shimizu, K., Cha, J. N., Stucky, G. D., and Morse, D. E., 1998. Silicatein a: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6234–6238.
- Simpson, T. L., 1984. *The Cell Biology of Sponges*. New York: Springer, pp. 1–660.
- Simpson, T. L., 1989. Silicification processes in sponges: *Geodia asters* and the problem of morphogenesis of spicule shape. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomimetalization in Plants and Animals*. New York: Kluwer, pp. 125–136.
- Simpson, T. L., 1990. Recent data on pattern of silicification and the origin of monaxons from tetraxons. In Rützler, K. (ed.), *New Perspectives in Sponge Biology. 3rd International Conference on the Biology of Sponges*, 1985. Washington: Smithsonian Institution Press, pp. 264–272.
- Skinner, H. C. W., 2005. Biominerals. *Mineralogical Magazine*, **69**(5), 621–641.
- Sollas, W. J., 1888. Report on Tetractinellida collected by H.M.S. challenger during the years 1873–1876. *Report of the Scientific Results of the Voyage of the H.M.S. Challenger. Zoology*, **5**, 1–458.
- Tabachnick, K. R., 1991. Adaptation of the Hexactinellid sponges to deep-sea life. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 378–386.
- Tabachnick, K. R., 2002. Family Monorhaphididae Ijima, 1927. In Hooper, N. A. V., and Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1264–1266.
- Tabachnick, K. R., and Reisswig, H. M., 2002. Dictionary of Hexactinellida. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1224–1229.
- Uriz, M.-J., 2003. Biology of silica deposition of sponges. *Microscopical Research and Technology*, Wiley-Liss. California, **62**(4), 277–381.
- Uriz, M.-J., 2006. Mineral skeletogenesis in sponges. *Canadian Journal of Zoology*, **84**, 322–356.
- Uriz, M.-J., Turon, X., Becero, M. A., and Agell, G., 2003. Siliceous spicules and skeletal frameworks in sponges: origin, diversità, ultrastructural patterns, and biological functions. *Microscopical Research and Technology*, **62**, 279–299.
- Vosmaer, G. C. J., and Wijsman, H. P., 1905. On the structure of some Siliceous Spicules of Sponges. I. The styli of *Tethya lyncurium*. *Koninklijke Nederlandsche Akademie van Wetenschappen Proceedings*, **8**, 15–28.
- Weaver, J. C., and Morse, D. E., 2003. Molecular biology of demosponge axial filaments and their roles in biosilicification. *Microscopical Research and Technology*, **62**(4), 356–367.
- Weaver, J. C., Pietrasanta, I., Hedin, N., Chmelka, B. F., Hansma, P. K., and Morse, D. E., 2003. Nanostructural features of demosponge biosilica. *Journal of Structural Biology*, **144**, 271–281.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–27.
- Weissenfels, N., 1989. *Biologie und Mikroskopische Anatomie der Süßwasserschwämme (Spongillidae)*. Stuttgart: Fischer.
- Weissenfels, N., and Landschoff, H. W., 1977. Bau und Funktion des Süßwasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die Entwicklung der monaxialen SiO₂-Nadeln in Sandwich-Kulturen, *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenese der Tiere*, **98**, 355–371.

Cross-references

[Biogeochemical Cycles](#)
[Critical Intervals in Earth History](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Origins of the Metazoa](#)
[Reefs](#)
[Sponges \(Porifera\) and Sponge Microbes](#)

SILICOFLAGELLATES

Silicoflagellates are planktonic, microscopic algae with siliceous skeletons, characterized by their simple geometries and remarkable variability. Taxonomically, they belong to different classes of the Stramenopiles and possess complex plastids derived from secondary endosymbiosis (see entry [Symbiosis](#)). Silicoflagellate skeletons may have considerable potential as environmental indicators and are common in fossil deposits, dating back to the middle Cretaceous (ca. 120 Ma), with species diversity reaching a peak in the Miocene (ca. 23–25 Ma). Today, only a single genus *Dictyocha* with approximately five recognized species exists. For more details, please refer to entry ["Algae \(Eukaryotic\)"](#).

SINTER

Robin W. Renaut¹, Brian Jones²
¹University of Saskatchewan, Saskatoon, SK, Canada
²University of Alberta, Edmonton, Alberta, Canada

Synonyms

Silica sinter; Siliceous sinter

Definition

Sinter: A sedimentary rock primarily composed of silica that is precipitated from hot waters at the vents of high-temperature (high-enthalpy) hot springs and geysers, and from cooled waters on their surrounding discharge aprons.

Geyserite. A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some high-temperature springs.

Some banded and laminated types of nonmarine carbonates (calcite or aragonite), including spring travertines and speleothems, have also been termed “sinter” or “calc-sinter.” We suggest that the geological term “sinter” should be restricted to *siliceous* deposits precipitated from silica-rich waters discharged at hot springs and geysers.

Introduction

Sinters are deposits of silica precipitated by hot waters discharged at the vents of hot springs and geysers. Most sinters are precipitated as noncrystalline opal-A, but they change to quartz during diagenesis. They form almost exclusively in high-enthalpy geothermal fields where the underground reservoir temperatures exceed $\sim 220^{\circ}\text{C}$, and so are most common in volcanic and tectonically active regions. Most geysers discharge from vents lined with sinter. Weed (1889) was among the first to discuss the role of microorganisms in the formation of sinter. After his pioneering work, very little scientific study was done on these rocks until Allen (1934) described sinters at Yellowstone. Walter (1976) undertook petrographic study of sinters, including stromatolitic types. Rimstidt and Cole (1983) made one of the first detailed studies of the chemistry of sinter formation. Krumbein and Werner (1983), followed later by Cady and Farmer (1996) and Jones and Renaut (1996), described, illustrated, and interpreted silicified microbes in recent sinters. Since then, interest in these unusual rocks has increased significantly, and more than 140 studies of sinters have been published during the past decade. The possible role of bacteria in silica precipitation has been a focus of much interest. Many filamentous and coccoid microbes preserved in recent sinters resemble the microfossils found in Precambrian quartzose cherts.

Morphology of sinter deposits

Most sinter is gray to white in color, but the addition of trace elements can produce vividly colored deposits such as the orange sinters found around the edge of Champagne Pool, Waiotapu, New Zealand (Jones and Renaut, 2003, Figure 2b). The main types of sinter deposit are *mounds*, which form around the vents of many geysers, *pool-rim dams* that precipitate around the margins of hot-spring pools, and various types of *terraces* and *platforms* that form downslope from the vent(s) on the spring or geyser discharge apron. Sinter mounds (Figure 1a) develop where aqueous silica precipitates from fluids that overflow the margins of a geyser vent or small-diameter point-sourced spring, or from silica that precipitates from air-borne spray. Sinter mounds can be columnar, domal, or broadly conical with slopes that range from nearly vertical to low angled ($<10^{\circ}$). Mounds vary from decimeters to meters in height and up to several meters in basal diameter. Many factors control mound morphology including the

original shape and dimensions of the vent, the volume and type of discharge (i.e., geyser versus hot spring activity), the original slope, climate (e.g., predominant wind direction), and water chemistry (e.g., silica concentration). Mound morphology can vary with time as the sinter deposits build up or the pattern or volume of discharge changes.

Pool-rim dams (Figure 1b) form where silica precipitates around the edges of hot-spring pools and some (fountain-type) geysers. The discharged water either flows across the lowest parts of the dam or escapes through erosional notches. Nested concentric pool-rim dams, each with different elevations, may form when mean water level in the pool changes because of external (e.g., hydrostatic) controls.

Water discharged from hot springs either flows across the land surface forming a “discharge apron” or it may flow directly into the local (nonthermal) drainage system. Outflow waters that mix with river or lake waters can be diluted below saturation with respect to amorphous silica, so that any sinter deposits are restricted to the vent area. If undiluted, however, the thermal outflow will cool progressively downslope leading to more silica precipitation. Sinter terraces (Figure 1c–f) commonly form where silica-laden waters flow across slopes, precipitating sheets of silica, as they cool and (or) evaporate. Each terrace is composed of a rimstone dam and pool. In scale, they range from miniterraces a few centimeters across, to large terraces tens of meters across, with dams several meters high. Some terrace pools temporarily pond water, but much of the outflow crosses terrace surfaces as largely unconfined sheets a few millimeters deep. Where several active vents lie near each other, their terraces may merge to form extensive flat sinter platforms.

Silica precipitated from hot waters also forms many small features including oncoids and pisoids (geyser eggs), which typically accrete in shallow pools near geyser vents; silica stalactites, which grow below overhanging terraces; and many small-scale ornate forms of geyserite on proximal sinter terraces (Figure 1e and g). Stromatolites develop locally in ponds or spring pools in the outflow system, usually where water temperature is $<70^{\circ}\text{C}$.

The thickest, most extensive sinter deposits form in neutral to alkaline waters (pH 7–9.5). The sinters that form in acid to neutral waters (pH 2–7) tend to be thin (millimeter to decimeter rather than meter thick). They lack some of the complex ornamentation of sinters forming in more alkaline fluids (Figure 1h), but commonly show spicular and bladed morphologies, and tend to be glassier in appearance than sinters forming in alkaline waters.

Microorganisms and sinter

Petrographic and scanning electron microscope (SEM) studies of sinter commonly reveal abundant silicified remains of microbes including coccoid and filamentous bacteria, eukaryotic algae, diatoms, fungi, and

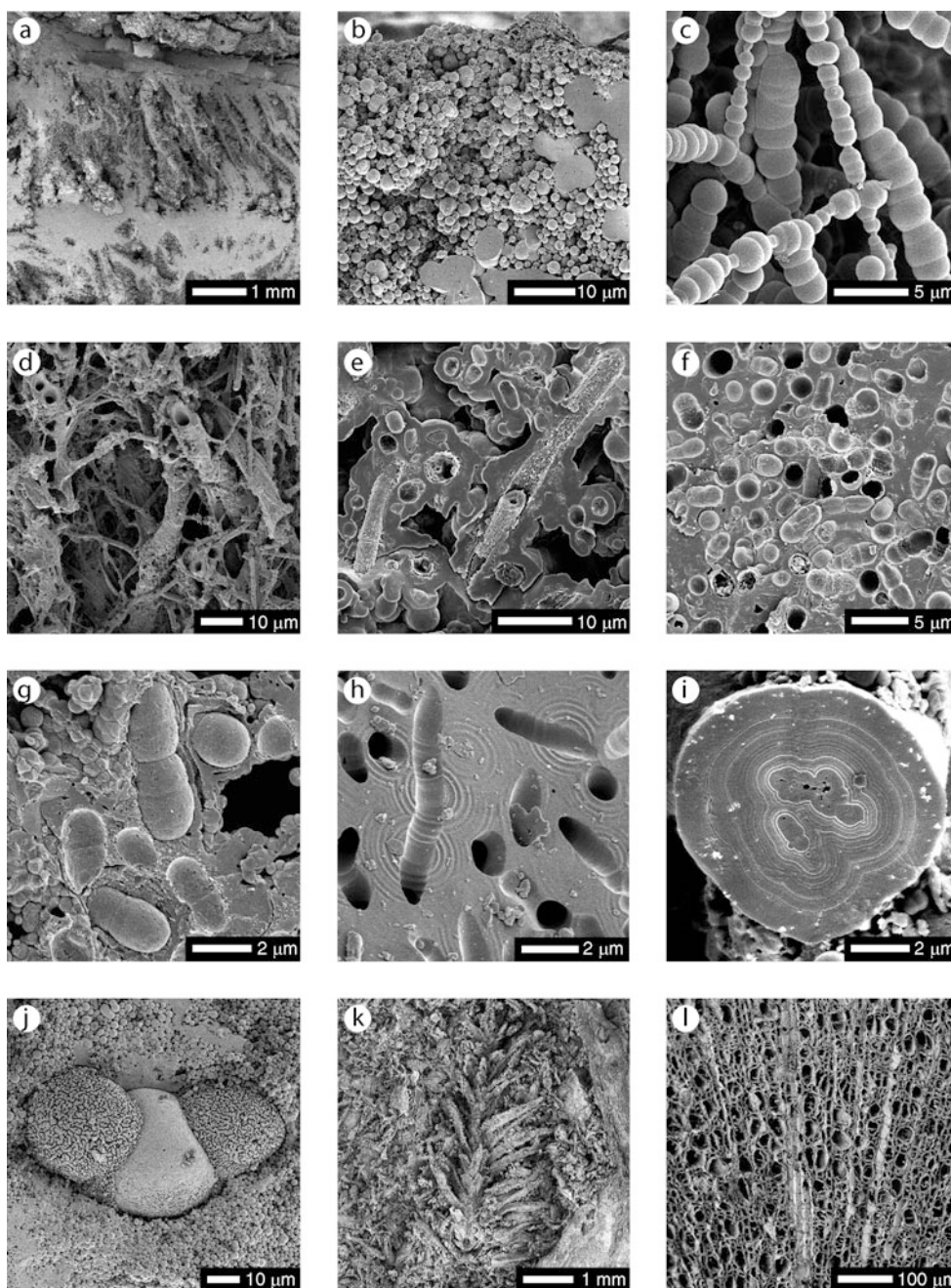


Sinter, Figure 1 Field photographs of sinter deposits. (a) Sinter mound of White Dome Geyser, Yellowstone National Park. (b) Pool-rim dam at Ngararatuatara hot spring, Whakarewarewa, New Zealand. (c) Rimstone dam at El Tatio geothermal field, Chile. Coated grains (oncoids) are strewn on the terrace at the base of the dam. (d) Sinter terraces covered by cyanobacterial mats on the discharge apron of Iodine Pool geyser, Waimangu, New Zealand. (e) Geyserite deposits around the vents of Anemone Geyser, Yellowstone National Park. (f) Sinter miniterraces on the surface of Primrose Terrace, Waiotapu, New Zealand. (g) Ornate geyserite deposits near a geyser vent at El Tatio geothermal field, Chile. (h) Thin platy sinter precipitated by acidic waters on the southern shore of Hot Pools Lake, Rotokawa, New Zealand. The sinter, which was probably precipitated by capillary processes, shows desiccation(?) cracks partly filled by silica cements.

extracellular polymeric substances (EPS) (Figure 2). Near spring vents and on many terraces where waters have cooled to $<70^{\circ}\text{C}$, microbial mats commonly cover the substrate and can become encrusted and (or) replaced by opaline silica. Bunches of current-aligned, partially silicified, filamentous cyanobacteria (stringers) are present in some channels. If hydrothermal discharge is ephemeral and the terrace surfaces dry out periodically, microbial mats may be absent. However, biofilms, some thinly

(millimeter thick) coated by opaline silica, and endoliths may still be present either at or just below the terrace surface, including sites very close to active vents.

Microbial fossils are preserved in most settings from vent to distal discharge apron. In waters $>75^{\circ}\text{C}$, nonphotosynthetic prokaryotes, including heterotrophic and chemolithotrophic bacteria and archaea, are present, although their silicified remains are often difficult to identify. Cyanobacteria and photosynthetic bacteria



Sinter, Figure 2 SEM photomicrographs of silicified biota in sinters from New Zealand (a–j) and Iceland (k, l). (a) Laminae formed of low-porosity opal-A alternating with laminae formed of (sub)erect filamentous microbes. (b) Opal-A spheres that formed in water column, settled to floor, and accumulated in pocket between erect microbial filaments. (c) Silicified microbes encased by opal-A spheres of variable diameters. (d) Large-diameter filamentous microbes (probably *Calothrix*) surrounded by small-diameter filaments (probably *Phormidium*). (e) Large-diameter filamentous microbes (probably *Calothrix*) and associated coccoid microbes (probably *Synechococcus*) encased by opal-A. (f) Dense accumulation of *Synechococcus* held in opal-A matrix. (g) Enlarged view of *Synechococcus* showing specimens in process of binary fusion. (h) Naturally etched surface showing microbes in groundmass formed of amalgamated opal-A spheres; etching has highlighted laminae in spheres showing their growth patterns. (i) Transverse cross section through silicified filamentous microbe – natural etching has highlighted the growth laminae in the opal-A. (j) Silicified pollen grain from a pine tree held in matrix of opal-A spheres. (k) Silicified bryophyte. (l) Transverse cross section through silicified twig showing perfect preservation of cells. Sample locations: a–c, h–j from discharge apron of Waikite Geyser, Whakarewarewa geothermal area, New Zealand; d–g from geyser system at Tokaanu, New Zealand; k and l from discharge apron of Geysir, Haukadalur, Iceland.

commonly inhabit waters from ~ 65 – 75°C (e.g., *Synechococcus*, *Chloroflexus*), with a wider range of cyanobacteria at temperatures $<65^\circ\text{C}$ (e.g., *Phormidium*, *Spirulina*, *Anabaena*, *Oscillatoria*, *Calothrix*, and others). Eukaryotic algae colonize substrates at temperatures $<60^\circ\text{C}$ and diatoms at $<45^\circ\text{C}$. Diatoms, fungi, and *Cyanidium* spp. are often the dominant fossil microbes in sinters precipitated from acidic fluids. Macrophytes, including grasses, wetland species, and trees and their root systems, may also be submerged by hot spring fluids and encrusted or replaced by amorphous silica.

Mineralogy and petrology

Most sinter is precipitated as noncrystalline opaline silica (opal-A: $\text{SiO}_2 \cdot n\text{H}_2\text{O}$) that can contain up to ~ 20 wt.% H_2O ; less commonly, chalcedony is a primary mineral. Although sinters precipitated from neutral and alkaline fluids are commonly monomineralic, silica precipitated from acidic fluids is commonly accompanied by kaolinite, ferric oxyhydroxides, alunite, jarosite, pyrite, and gypsum.

Sinters exhibit a wide range of textures and fabrics. Geyserites tend to be laminated, columnar, or stromatolitic, and generally have low primary porosity. Sintors that form distally from the vent are more diverse and include bedded and laminated facies that form on terraces, commonly with in situ silicified microbial mats, fenestral porosity, and intraclasts reworked from silicified mats upslope. Early cementation by opaline silica commonly masks the original fabrics, producing apparently massive sinter, but slight differences in the water content of the precipitated opal-A can help differentiate primary textures and cements (Jones and Renaut, 2004).

During diagenesis, the opal-A transforms to opal-CT, moganite, and ultimately quartz. The opal-A to opal-CT transformation can be rapid (Lynne et al., 2006; Jones and Renaut, 2007) and proceeds mainly by dissolution–reprecipitation reactions.

Origin of sinter

Silica solubility increases with increasing temperature, pH, and pressure, and can be influenced by other factors such as the presence of certain ions (e.g., Al, Fe) and salinity. Most silica precipitation at hot springs has been attributed to the cooling of thermal fluids that have equilibrated with respect to quartz, chalcedony, or volcanic glass at depth, and then rise rapidly to the surface, emerging as hot water supersaturated with respect to amorphous silica (White et al., 1956; Rimstidt and Cole, 1983; Fournier, 1985).

Much of the dissolved silica is present in its monomeric form (H_4SiO_4). As saturation increases, silica polymerization occurs through reactions that involve linear, cyclic, and three-dimensional forms of polymeric silica (Iler, 1979). In strongly supersaturated fluids, including some spring pools, nuclei grow by Ostwald ripening into nanospheres and microspheres (10 nm–1 μm) and may

form colloidal particle suspensions (sols). Continued growth of spheres can destabilize the sols and fine sediment settles from suspension. If the pH falls or the electrolyte concentrations increase (e.g., by evaporation), suspended particles may flocculate, forming aggregates that eventually sink to the substrate. In both cases, the silica sediment then undergoes cementation to form sinter. At sites of evaporative concentration, microspheres may coagulate to form gels. In contrast, at relatively low levels of supersaturation, dissolved silica precipitates mainly by heterogeneous nucleation upon existing substrates or suspended particles.

Evaporative concentration can induce silica precipitation at hot springs in semiarid and arid regions (e.g., Kenya Rift), although evaporation is usually less effective than cooling in humid environments. However, evaporation is an important mechanism on subaerial sites of periodic wetting including outflow terraces between geyser eruptions and sites receiving airborne spray. Seasonal freezing of geothermal outflow can also induce opal-A precipitation and agglomeration by “cryogelling,” which concentrates silica in residual fluid as ice is formed (Channing and Butler, 2007).

The pH and salinity also affect amorphous silica solubility. Nucleation and polymerization are inhibited in acidic waters because monomeric silica is slow to deprotonate (Fournier, 1985; Mountain et al., 2003), which explains the thin sinter development around acid hot springs. At $\text{pH} > \sim 9$, silica dissociates into other species, increasing the solubility. A rapid decrease in pH of a highly alkaline, silica-saturated thermal fluid might induce amorphous silica to precipitate on contact with neutral lake waters or groundwaters or if the fluid is acidified by microbes.

The waters that flow from most geysers and many hot springs in the geothermal fields of Iceland, New Zealand, Kamchatka, Chile, and Yellowstone, are strongly supersaturated with respect to amorphous silica so should precipitate silica abiotically upon rapid atmospheric cooling. Physical processes control the gross morphology of most sinter deposits, whereas chemical processes control the silica precipitation mechanisms. There has been much debate about whether or not microbes mediate silica precipitation and silicification, particularly in waters where homogeneous nucleation does not occur. Microbes can provide reactive surface ligands that adsorb aqueous silica from solution, and should therefore promote heterogeneous nucleation by reducing activation energy barriers. Three main mechanisms have been proposed: hydrogen bonding, cation bridging by metals (especially Fe), and electrostatic interactions (Phoenix et al., 2003; Lalonde et al., 2005; Konhauser, 2007, pp. 156–160). Although consensus has not yet been attained, most recent field studies (e.g., Jones et al., 2004) and laboratory experiments (e.g., Fein et al., 2002; Yee et al., 2003) suggest that the role of microbes in silica precipitation is largely incidental, especially in strongly supersaturated fluids. At lower levels of supersaturation, they may provide organic

substrates for heterogeneous nucleation. Bacteria (including cyanobacteria), however, control microfabric development in many types of sinter, over a wide range of scales and fluid temperatures (Jones and Renaut, 2003).

Fossil sinter

Fossil sinters, also termed paleosinters, are increasingly being recognized in the geological record. They commonly form “caprocks” to some epithermal mineral deposits (e.g., Guido et al., 2002) but are rarely a target of study. Two exceptions are the early Devonian Rhynie and Windyfield cherts of Aberdeenshire, Scotland (Trewin, 1994), and the Devonian–Carboniferous sinters of the Drummond Basin, Australia (Walter et al., 1996).

Conclusions

By mass, sinters are trivial compared to most sedimentary rocks, but their rarity belies their importance. Hot spring sinters form in one of the few environments where silica precipitation and silicification processes can be studied at the Earth’s surface, another environment being highly alkaline lakes. Consequently, they will remain important in studying how microbes and plants are silicified, both in modern environments and the geological record.

Bibliography

- Allen, E. T., 1934. The agency of algae in the deposition of travertine and silica from thermal waters. *American Journal of Science*, **27**, 373–389.
- Cady, S. L., and Farmer, J. D., 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In Bock, G. R., and Goode, J. A. (eds.), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*. Ciba Foundation Symposium no. 202, Chichester, UK: Wiley, pp. 150–173.
- Channing, A., and Butler, I. B., 2007. Cryogenic opal-A deposition from Yellowstone hot springs. *Earth and Planetary Science Letters*, **257**, 121–131.
- Fein, J. B., Scott, S., and Rivera, N., 2002. The effect of Fe on Si adsorption by *Bacillus subtilis* cell walls: insights into the non-metabolic bacterial precipitation of silicate minerals. *Chemical Geology*, **182**, 265–273.
- Fournier, R. O., 1985. The behavior of silica in hydrothermal solutions. In Berger, B. R., and Bethke, P. M. (eds.), *Geology and Geochemistry of Epithermal Systems*, Reviews in Economic Geology. El Paso, TX: Society of Economic Geologists, Vol. 2, pp. 45–61.
- Guido, D., de Barrio, R., and Schalamuk, I., 2002. La Marciana Jurassic sinter—implications for exploration for epithermal precious-metal deposits in Deseado Massif, southern Patagonia, Argentina. *Applied Earth Science*, **111**, 106–113.
- Iler, R. K., 1979. *The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry*. New York: Wiley-Interscience.
- Jones, B., and Renaut, R. W., 1996. Influence of thermophilic bacteria on calcite and silica precipitation in hot springs with water temperatures above 90°C: evidence from Kenya and New Zealand. *Canadian Journal of Earth Sciences*, **33**, 72–83.
- Jones, B., and Renaut, R. W., 2003. Hot spring and geyser sinters: the integrated product of precipitation, replacement, and deposition. *Canadian Journal of Earth Sciences*, **40**, 1549–1569.

- Jones, B., and Renaut, R. W., 2004. Water content of opal-A: implications for the origin of laminae in geyserite and sinter. *Journal of Sedimentary Research*, **74**, 117–128.
- Jones, B., and Renaut, R. W., 2007. Microstructural changes accompanying the opal-A to opal-CT transition: new evidence from the siliceous sinters of Geysir, Haukadalur, Iceland. *Sedimentology*, **54**, 921–948.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society, London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial–silica interactions in Icelandic hot spring sinter: possible analogues for some Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–433.
- Krumbein, W. E., and Werner, D., 1983. The microbial silica cycle. In Krumbein, W. E. (ed.), *Microbial Geochemistry*. London: Blackwell, pp. 125–157.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A. L., and Ferris, F. G., 2005. The experimental silicification of Aquificales and their role in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Lynne, B. Y., Campbell, K. A., Perry, R. S., Moore, J., and Browne, P. R. L., 2006. Acceleration of sinter diagenesis in an active fumarole, Taupo Volcanic Zone, New Zealand. *Geology*, **34**, 749–752.
- Mountain, B. W., Benning, L. G., and Boerema, J., 2003. Experimental studies on New Zealand hot spring sinters: rates of growth and trace metal incorporation. *Canadian Journal of Earth Sciences*, **40**, 1643–1667.
- Phoenix, V. R., Konhauser, K. O., and Ferris, F. G., 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe–Si systems: implications for microbial silicification in hot springs. *Canadian Journal of Earth Sciences*, **40**, 1669–1678.
- Rimstidt, J. D., and Cole, D. R., 1983. Geothermal mineralization I: the mechanism of formation of the Beowawe, Nevada, siliceous sinter deposit. *American Journal of Science*, **283**, 861–875.
- Trewin, N. H., 1994. Depositional environments and preservation of biota in the Lower Devonian hot springs of Rhynie, Aberdeenshire, Scotland. *Philosophical Transactions of the Royal Society of Edinburgh, Earth Sciences*, **84**, 433–442.
- Walter, M. R., 1976. Geyserites of Yellowstone National Park: an example of abiogenic “stromatolites”. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 87–112.
- Walter, M. R., Des Marais, D., Farmer, J. D., and Hinman, N. W., 1996. Lithofacies and biofacies of mid-Paleozoic thermal spring deposits in the Drummond basin, Queensland, Australia. *Palaaios*, **11**, 497–518.
- Weed, W. H., 1889. Formation of travertine and siliceous sinter by the vegetation of hot springs. *US Geological Survey 9th Annual Report (for 1887–1888)*, pp. 613–676.
- White, D. E., Brannock, W. W., and Murata, K. J., 1956. Silica in hot-spring waters. *Geochimica et Cosmochimica Acta*, **10**, 27–59.
- Yee, N., Phoenix, V. R., Konhauser, K. O., Benning, L. G., and Ferris, F. G., 2003. The effect of cyanobacteria on silica precipitation at neutral pH: implications for bacterial silicification in geothermal hot springs. *Chemical Geology*, **199**, 83–90.

Cross-references

Cherts
 Hot Springs and Geysers
 Hydrothermal Environments, Fossil
 Hydrothermal Environments, Terrestrial
 Silica Biomineralization, Sponges

SKELETON

Biologically, a skeleton is a hard or rigid framework that could provide protection and support in many types of organisms. Most of skeletons are mineralized, but some may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, not including hydroskeletons. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites. Endoskeletons are internal hard parts (typical of many vertebrates). Most of endoskeletons are generally surrounded by skin and musculature. See entry "[Animal Skeletons, Advent](#)" for further reading.

SMALL SHELLY FOSSILS

Small shelly fossils (SSF, also "Small Shelly Fauna") is a non-taxonomic term, first used by Matthews and Missarzhevsky in the title of their review paper in 1975, to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites. See entries "[Animal Skeletons, Advent](#)" and "[Critical Intervals in Earth History](#)" for further reading.

SNOWBALL EARTH

Paul F. Hoffman
Harvard University, Cambridge, MA, USA

Synonyms

Cryochron; Global glaciation; Hard snowball; Ice-albedo catastrophe; Snowball (in geology); White Earth

Definition

Snowball Earth is a climate in which the oceans and most land areas are permanently covered by glacial ice. As less sunlight is absorbed by ice than by water or land, a critical area of ice will cause surface temperatures to fall below freezing everywhere, resulting in a snowball Earth. This arguably occurred near the beginning and end of the Proterozoic eon. Snowball Earths self-destruct after millions of years due to the buildup of atmospheric carbon dioxide of volcanic and metamorphic origin, which could not be converted into organic matter or limestone. Biological evolution is a postulated cause and consequence of snowball episodes.

History of the concept

Sedimentary deposits of glacial origin were first described from the late Proterozoic (Neoproterozoic) of Scotland in 1871 and from the early Proterozoic (Paleoproterozoic) of Canada in 1907. By 1940, it was apparent that the Neoproterozoic glaciation(s) had been more extreme than those of the Pleistocene and at least equal in severity to the late Paleozoic glaciations of Gondwanaland. In the 1960s, the occurrence of glacial deposits within thick successions of nearly pure carbonate strata suggested that Neoproterozoic ice sheets had entered what were normally the warmest parts of the globe. This was confirmed in the 1980–1990s by reliable paleomagnetic data showing that ice sheets had reached the ocean close to the paleoequator in the Neoproterozoic of Australia and Paleoproterozoic of South Africa (Evans, 2003). Moreover, evidence of marine geochemical anomalies concurrent with glaciation came increasingly to light in the form of sedimentary Fe_2O_3 and MnO_2 ore deposits, large excursions in stable carbon and sulfur isotopic compositions of minerals precipitated from seawater, and distinctive post-glacial "cap" carbonate sediments that rest directly on the terminal glacial deposit at all latitudes (Hoffman and Schrag, 2002). Neoproterozoic glacial deposits are now known from 22 different paleocontinents and microcontinents, and Paleoproterozoic deposits from 6.

The fraction of incoming solar radiation absorbed by ocean water under cloudless skies is 0.9, but for continuous ice it is only 0.4–0.6 and as low as 0.1 if the ice is covered by fresh snow. In the 1950–1960s, calculations by meteorologists of zonal average surface temperatures at different latitudes, assuming radiative energy balance and incorporating crude parameterizations of the radiative effects of clouds, meridional heat transport, and positive feedbacks due to the reflectivity (albedo) of ice, suggested that a lowering of solar luminosity by a few percent would result in a globe-encircling glaciation, or "white" Earth (North, 1990). If the luminosity is incrementally lowered, polar ice caps expand until their edges reach a critical latitude, beyond which ice advances rapidly to the equator without any additional luminosity reduction, or even with increased luminosity. This is due to runaway ice-albedo feedback – more ice reflects more radiation, creating more ice. (The same calculations indicated that a small increase in radiative forcing, due to anthropogenic CO_2 emissions for example, could cause a sudden loss of polar sea ice at a critical $p\text{CO}_2$ threshold.) These sensational findings greatly stimulated the nascent field of climate modeling, but proponents were puzzled how a "white" Earth disaster had been averted given that solar luminosity has increased by 25–30% over geological time (the so-called faint young Sun paradox). They assumed (incorrectly, as it turned out) that such an occurrence would extinguish all life – deep-sea chemoautotrophs and polar extremophiles were then unknown – and would be permanent because of the very large increase (~25%) in radiative forcing required to overwhelm a "white" Earth's high albedo.

As meteorologists assumed that a “white” Earth never occurred, they did not think to consult geologists, who remained oblivious to their findings.

In 1981, planetary scientists (Walker et al., 1981) proposed that Earth’s climate is modulated within the habitable range by a negative climate feedback resulting from the temperature-dependence of silicate-rock weathering. In the geochemical cycle of carbon, this is the means by which atmospheric CO_2 (dissolved in rainwater) is converted into bicarbonate ion (HCO_3^-), which dissolved in runoff makes its way to the ocean, where it is removed from circulation by the burial of carbonate sediments and organic matter. The feedback operates on a geological timescale ($\sim 10^6$ year) and causes the climate system to slowly seek whatever atmospheric CO_2 level, and hence climate, that is needed to balance the rate of CO_2 removal by silicate weathering with the rate of CO_2 emitted by volcanoes and metamorphic reactions. Walker et al. (1981) suggested that this feedback had prevented a “white” Earth disaster but noted that if one did occur, the consequent reduction of weathering rates would lead to an inexorable increase in atmospheric CO_2 as plate tectonics and therefore of CO_2 emissions would continue unabated. With the present emission rate and no CO_2 consumption, $p\text{CO}_2$ would increase to ~ 1.0 bar after 20 Myr. At some critical $p\text{CO}_2$, the “white” Earth would self-destruct violently due to ice-albedo and other positive feedbacks, leaving a transient aftermath in which high $p\text{CO}_2$, low surface albedo, and the recently deglaciated surface would conspire to cause intense weathering, exponentially lowering atmospheric $p\text{CO}_2$. The hysteresis loop in CO_2 versus planetary albedo means that a “white” Earth would be long-lived (10^6 – 10^7 year) but swift to melt (10^3 – 10^4 year), giving rise to a “greenhouse” transient (10^5 – 10^6 year). These are geologically testable predictions. But as the paleomagnetic evidence for global glaciation was not secure until 1986 (Evans, 2000), consideration of a self-reversing “white” Earth as a model for the geological record was delayed.

It was the paleomagnetist Joseph L. Kirschvink who first applied these concepts to low-latitude Neoproterozoic (Kirschvink, 1992) and Paleoproterozoic (Kirschvink et al., 2000) glaciations. He related the synglacial Fe_2O_3 and MnO_2 ore deposits to ocean stagnation and anoxia, caused by ice cover, which allowed the metals to be transported widely in solution in reduced form, and be precipitated upon deglaciation when the ocean was again ventilated. He predicted the global occurrence of sediments recording the abrupt climate switch at glacial terminations, later recognized as cap carbonates (Hoffman et al., 1998b). He speculated that an unusual preponderance of low-latitude continents (a situation that never occurred after 500 Ma) raised the planetary albedo to the critical threshold. He named the phenomenon “snowball” Earth as that is how the globe would appear from outer space. The name tacitly recognizes that the hydrological cycle, although greatly diminished on the cold solid

surface, would still be weakly maintained by sublimation and precipitation, not of snowflakes, but of tiny hexagonal snow prisms due to the global average surface temperature of around -50°C (Furukawa and Wettlaufer, 2007).

Number, age, and duration of snowballs

At least three snowball episodes are inferred from the geological record, two in the Cryogenian Period (~ 850 – 635 Ma) of the Neoproterozoic era (1,000–551 Ma), and one or more in the first half of the Paleoproterozoic era (2,500–1,600 Ma). The 1.5×10^9 -year interval between older and younger snowballs is curiously devoid of glacial deposits. The timing of the youngest snowball is the best constrained and of the oldest the most poorly known. This is primarily a function of differences in their respective areas of preservation. Termination of the youngest snowball occurred in 635 Ma, coincident with the boundary between the Cryogenian and Ediacaran Periods (Knoll et al., 2006b). The age is based on ultra-precise (0.5 Myr uncertainty) U-Pb geochronology of primary zircons from volcanic ash-falls in Namibia and South China (Halverson, 2006). Sections in other regions are correlated with these based on synde glacial “cap” carbonates (see below), which are uniquely distinctive in many areas. The onset of the snowball is loosely constrained. It is younger than 654.5 ± 3.8 Ma in South China, based on ion-microprobe U-Pb geochronology (Zhang et al., 2008). The maximum duration of this snowball was 23 Myr.

The timing of the older Cryogenian snowball is weakly constrained (Halverson, 2006): its onset is younger than 726 ± 1 Ma in Oman and 725 ± 10 Ma in South China, and its termination occurred before 667 ± 5 Ma in the western USA. In Oman, strata within the glacial succession are no older than 713 ± 1 Ma. Interregional correlation of glacial strata is more uncertain than for the younger Cryogenian glaciation. There is fragmentary evidence for an earlier Cryogenian glaciation of presumed regional scale ~ 750 Ma in central and southern Africa, while a mid-Ediacaran glaciation of short duration occurred in eastern Newfoundland, Canada, at 582 Ma (Knoll et al., 2006b), with possible correlatives in western and northern Europe, central Asia, Western Australia, and southwestern Brazil.

Paleoproterozoic glacial deposits in the western Transvaal, South Africa, are interfingering with volcanic lavas erupted near sea level at $11 \pm 5^\circ$ paleolatitude (Evans et al., 1997) approximately 2.22 Ga (10^9 year before present) (Kopp et al., 2005). The duration of the low-latitude glaciation is unknown. Three distinct glaciations occurred between 2.45 and 2.22 Ga in the Huronian succession of Ontario, Canada, and correlative glacial deposits occurred in Wyoming (USA), Quebec (Canada) and possibly Finland (Ojakangas, 1988). The paleolatitudes of these deposits being unknown, they are assumed to represent regional-scale glaciations.

Paleogeographic reconstructions and paleoclimatic indicators in bounding strata

At the time of the Cryogenian snowballs, many continents were in low paleolatitudes and few if any were near the poles (Trindade and Macouin, 2007). This is consistent with cold climates because a preponderance of low-latitude continents maximizes the rate of atmospheric CO₂ consumption by silicate weathering. It was also a time of fragmentation and dispersal of the long-lived (1.0–0.8 Ga) supercontinent Rodinia (Li et al., 2008). Continental fragmentation also enhances silicate weathering by bringing land closer to the source of moisture. Cryogenian paleogeography contrasted with that of the late Paleozoic ice ages, when a supercontinent (Pangea) had just formed and the glaciated lands (Gondwanaland) lay in a polar region. Little is known of early Paleoproterozoic paleogeography, but there are suggestions that they accompanied the fragmentation of land-masses consolidated in the late Archean eon (2.7–2.5 Ga).

Cryogenian glacial deposits are found within thick successions of shallow-water carbonate rocks in Namibia, California, Arctic Alaska, and southern Mongolia (Halverson et al., 2005). This is paradoxical because non-skeletal carbonates form preferentially in the warmest parts of the surface ocean on account of their “reverse” solubility. Throughout Earth history, thick carbonate successions formed only at latitudes less than 35° (Ziegler et al., 1984; Opdyke and Wilkinson, 1990). Their range did not change between warmer and colder climates because their distribution depends on the relative not the absolute temperature. Thus the meridional temperature gradient has never been reversed due to high orbital obliquity (Evans, 2006), as has been proposed to account for low-latitude glaciation (Schmidt and Williams, 1995). If glaciers reached the ocean in the warmest areas of the world, then the colder areas must also have been frozen.

Sedimentology of the glaciogenic deposits

The sedimentology and inferentially the depositional processes of Proterozoic and Phanerozoic glacial deposits are quite similar (Boulton and Deynoux, 1981; Hambrey and Harland, 1981). Those more interested in the rocks themselves than in their distribution have a hard time believing that Proterozoic ice ages were different in character. They range from terrestrial lodgement, moraine and outwash (sandur) complexes to glaciomarine grounding-line wedges and fans, and proglacial suspension fallout with or without sediment gravity flows and ice-rafted debris (Kellerhals and Matter, 2003).

Shaped and striated clasts, subglacial pavements with grooves and chatter marks, and stratigraphic relations suggestive of ice streams (currents of fast-flowing ice within an ice sheet) all attest to dynamic glacial action (Hoffman, 2005). At first it was thought that dynamic ice sheets were inconsistent with a snowball because the frozen ocean could not resupply them with snow. However, climate models suggest that net sublimation of subtropical

sea-ice would supply ice sheets with up to centimeters of ice-equivalent annually, meaning that ice sheets would thicken sufficiently to generate dynamic ice streams within a few 10⁵ year, a fraction of the estimated snowballs duration (Donnadieu et al., 2003; Pollard and Kasting, 2004). Accordingly, the evidence for dynamic ice sheets is not incompatible with a frozen ocean.

On the Otavi carbonate platform of Namibia, the younger Cryogenian glaciation left a continuous grounding-line prism situated 500–1,000 m vertically below the rim of the platform (Hoffman, 2005). This is quite unlike any Quaternary ice grounding line in polar seas, which typically switched between the inner and outer shelf. The grounding-line prism in Namibia is directly overlain by a synde glacial “cap” dolostone (see below) deposited above prevailing wave-base, the only shallow-water deposit at such depth on the slope (Hoffman et al., 2007). This indicates glacioeustatic changes of >500 m, consistent with an average ice-sheet thickness of >1.1 km over all continents. An important implication of this finding is that shelves and platforms were above sea level during most of the glacial interval, and any glaciomarine deposits found there could only have been deposited when the deglaciation was well-advanced. The nature of such deposits has often been cited as inconsistent with snowball conditions (e.g., wave action, phototrophism, ice-rafting, etc.). This is true; by the time they were deposited, the snowball no longer existed.

Associated marine geochemical anomalies

Fe₂O₃- and MnO₂-ore formations

During the Fe-ore exploration boom following World War II, it was learned that the only “banded iron-formations” (BIF) younger than 1.9 Ga are intimately and exclusive associated with Cryogenian glacial deposits (Klein, 2005). Extensive ore-grade deposits of Fe₂O₃ (±MnO₂) were found in SE Australia, SW Brazil, NW Canada, and Namibia. The Australian and Canadian deposits are in the older Cryogenian assemblage, the Brazilian ones are in the younger(?) Cryogenian assemblage, and the Namibian deposits are in both(?). As only reduced Fe(II) is soluble in seawater, Martin (1964) attributed “this peculiar combination of sediments” to “oxygen deficiency in stagnating bottom waters caused by an ice cover.” Canfield and Raiswell (1999) pointed out that decreased continental runoff during a snowball would lower the influx of sulfate to the ocean, which after bacterial sulfate reduction, removes dissolved Fe(II) from seawater in the form of FeS₂ (pyrite). Lowering the influx of sulfate therefore raises the concentration of Fe(II). The large sea-level fall associated with a snowball also raises the Fe:S flux ratio in mid-ocean ridge hydrothermal vent fluids on account of the drop in hydrostatic pressure (Kump and Seyfried, 2005).

Kirschvink (1992) suggested that the dissolved Fe(II) was oxidized to insoluble Fe(III) at snowball terminations, consistent with the stratigraphic position of the Australian

BIF near the top of the glacial sequence. Elsewhere, BIF occurs as sporadic lenses within glaciomarine sequences and Fe oxidation may have been caused by discharging of oxic subglacial meltwater along the grounding lines of ice shelves.

The 2.22-Ga Makganyene glaciation in South Africa ended with outpourings of mafic lava and the deposition of major Fe- and Mn-ore deposits. The latter are the world's largest and imply the presence of abundant molecular O_2 (not required for Fe oxidation) ~ 0.22 Gyr after atmospheric O_2 first rose above 10^{-6} PAL (present atmospheric level) according to mass-independent sulfur isotope fractionation data (Farquhar et al., 2000). The youngest pre-Cryogenian BIFs are limited to collisional (foredeep) basins (1.89 Ga) around the Superior craton of North America, unrelated to glaciation.

"Cap" carbonates

"Cap" carbonates are highly continuous, meter- to decameter-thick layers of relatively pure carbonate lying sharply without significant hiatus on top of the last glacial deposits of a snowball (Hoffman and Schrag, 2002; Shields, 2005). They typically extend far beyond the actual glacial deposits at the same stratigraphic horizon.

The best developed cap carbonates are those associated with the youngest snowball deglaciation in 635 Ma: they are global in extent and sedimentologically unique in the entire stratigraphic column (Allen and Hoffman, 2005; Shields, 2005; Hoffman et al., 2007). They begin with a layer of pale gray, often pinkish, micro- and macropeloidal dolostone ($Ca_{0.5}Mg_{0.5}CO_3$). Its global average thickness is ~ 20 m and it was laid down diachronously, mainly above storm wave-base, during glacioeustatic flooding of continental margins and inland seas (Hoffman et al., 2007). Assuming that strong positive climate feedbacks (e.g., ice albedo, ice elevation, CO_2 degassing, gas-hydrate destabilization) drove snowball deglaciation on timescales < 10 kyr, cap dolostone accumulation rates were > 2 mm year $^{-1}$.

The dolostone contains anomalous sedimentary features such as giant wave ripples (Allen and Hoffman, 2005) and stromatolite biostromes containing strictly vertical tubes of internal sediment with meniscus-like laminae (Corsetti and Grotzinger, 2005). The top of the dolostone layer in some areas is marked by a continuous crust of primary seafloor $BaSO_4$ (barite) cement in the form of digitate rosettes (pseudostromatolites) with internal growth lamellae (Hoffman and Schrag, 2002). The barite crust and other redox-sensitive geochemical (Hurtgen et al., 2006) and biomolecular (Elie et al., 2007) proxies indicate that an oxic-anoxic interface within the water column intersected the seafloor at this horizon. In subsiding areas, the dolostone is overlain by deeper water shales or limestones, locally with layer-upon-layer of seafloor cements in the form of macroscopic crystal fans originally composed of aragonite, the orthorhombic polymorph of $CaCO_3$ (James et al., 2001). In

non-subsiding areas, the dolostone layer ends at a subaerial exposure surface heavily disrupted by products of phreatic and vadose diagenesis (Hoffman and Schrag, 2002; Shields et al., 2007).

The nature and extent of the 635-Ma cap carbonate indicates an ocean in which the mixed layer and thermocline waters were highly oversaturated with respect to $(CaMg)CO_3$ and $CaCO_3$ respectively. Sources of alkalinity responsible for the anomalous saturation state include the weathering of carbonate and silicate rock powder and debris exposed by deglaciation (Higgins and Schrag, 2003; Anderson, 2007), anaerobic oxidation of CH_4 released by melting of permafrost in organic-rich shelf sediments glacioeustatically exposed during the snowball (Kennedy et al., 2001; Jiang et al., 2003), and anaerobic respiration at depth of organic matter produced in surface waters newly exposed to sunlight (Font et al., 2006). It was suggested (Kennedy, 1996; Ridgwell et al., 2003) that the 635-Ma cap carbonate can be explained by analogy with the "coral reef hypothesis" of Quaternary glacial-interglacial cycles (Berger, 1982), in which alkalinity builds up in deep waters during glacial stages because of the loss of shelf area, where carbonate is normally sequestered without passage through corrosive deep waters. This mechanism presupposes continued carbonate production in open ocean surface waters during the glaciation, for which evidence is lacking.

The cap carbonate ending the older Cryogenian snowball is different in character but comparable in thickness to the one just described, as well as in its sharp basal contact. Lithologically, it is usually a dark, fetid, limestone or dolostone, locally rhodochrosite ($MnCO_3$), deposited below storm wave-base. Former aragonite seafloor cements are prominent in some areas, notably in eastern Brazil (Vieira et al., 2007). In Namibia, there are hundreds of meters of continuous sublittoral microbialaminates with distinctive roll-up structures (Hoffman et al., 1998a). Missing altogether is the transgressive cap dolostone characteristic of the 635-Ma deglaciation. Carbonate production evidently did not begin until after deglaciation was complete, suggesting a weaker alkalinity flux into the ocean or a lower saturation state when the deglaciation began.

Cap carbonates a few centimeters to decimeters thick occur locally above the mid-Ediacaran (582 Ma) and early Cryogenian (~ 750 Ma) glacial deposits, believed to represent regional-scale glaciations.

In the Paleoproterozoic, the middle Huronian glacial formation has an impure cap limestone up to 17 m thick, the only carbonate unit in a terrigenous clastic succession up to 12 km thick (Bekker et al., 2005). The low-latitude Makganyene glaciation (~ 2.22 Ga) in South Africa has a possible cap carbonate (Mooidrai Formation) known only from subsurface bore holes (Kirschvink et al., 2000).

Post-glacial isotopic anomalies

Cap carbonates are typically depleted in ^{13}C (i.e., $\delta^{13}C < 0.0\text{‰}$ PDB) and detailed studies of expanded

sections of the 635-Ma cap carbonate in different areas show large negative isotopic excursions (Halverson et al., 2005). Excursions are deviations over time in a positive (higher $\delta^{13}\text{C}$) or negative direction, followed by a return to previous values. The excursion begins $\sim 0\text{‰}$ at the base of the oldest (deepest) cap dolostone, descends through the cap dolostone to a low of -6‰ in the basal limestones (after a $\sim 1.5\text{‰}$ step-function across the dolostone–limestone transition), and then rises slowly back to $\sim 0\text{‰}$. Its stratigraphic thickness depends on the sedimentation rate: on the Otavi platform, Namibia, it is ~ 400 m thick but only ~ 50 m thick on the deeper slope sections (formerly the ice grounding-line zone). The origin of the excursion is disputed (e.g., Kennedy et al., 2001; Higgins and Schrag, 2003).

The same cap carbonate hosts negative excursions in $\delta^{11}\text{B}$, interpreted as an expression of greatly elevated $p\text{CO}_2$ (Kasemann et al., 2005), and $\delta^{34}\text{S}$ of carbonate-associated sulfate (CAS), interpreted as oxidized sulfidic snowball waters (Hurtgen et al., 2006). The negative $\delta^{34}\text{S}$ excursion is followed by a positive one with lows and highs of 15 and 45‰, respectively. The positive values (the highest in Earth history) are attributed by a rapid increase in isotopic fractionation between sedimentary sulfate and pyrite, without a compensatory decline in the fractional burial rate of pyrite (Halverson and Hurtgen, 2007).

The older Cryogenian cap carbonate preserves only the rising leg of the post-glacial isotopic excursions, reflecting stratigraphic truncation (non-deposition) during glacioeustatic flooding. Surface waters became strongly enriched ($\delta^{13}\text{C} > 4\text{‰}$) again soon after carbonate production resumed (Yoshioka et al., 2003). Paired negative and positive $\delta^{34}\text{S}_{\text{CAS}}$ excursions resemble those above the younger snowball but data are fewer (Hurtgen et al., 2002).

The two known Paleoproterozoic cap carbonates are also depleted in ^{13}C (Kirschvink et al., 2000; Bekker et al., 2005), but lack local isotopic context as they occur in clastic-dominated successions. A major positive $\delta^{13}\text{C}$ excursion (Lomagundi excursion) appears to begin before age-equivalents of the Makganyene glaciation in North America (Bekker et al., 2006).

Pre-glacial isotopic anomalies

Both Cryogenian snowballs were preceded by long ($>10^7$ year) intervals of consistent isotopic enrichment ($\delta^{13}\text{C} \geq 5\text{‰}$ PDB), followed by Myr-long excursions to much depleted values ($\delta^{13}\text{C} \leq -5\text{‰}$), variably truncated by subglacial erosion. There is no accompanying $\delta^{34}\text{S}$ excursion. The excursions largely predate sea-level falls due to ice-sheet growth. Schrag et al. (2002) suggest that the prolonged isotopic enrichment reflects high fractional organic burial due to rapid sediment burial and expanded suboxic zones on preponderantly low-latitude continental margins. They propose that this situation ultimately led to a slow rise in atmospheric CH_4 due to methanogenesis in

organic-rich sediments. The rise in CH_4 is held responsible for the negative $\delta^{13}\text{C}$ excursion (Halverson et al., 2002). Silicate weathering feedback would lower $p\text{CO}_2$ in response to a slow rise in CH_4 , causing the climate to become more unstable because of its greater sensitivity to CH_4 concentration and the absence of buffering of its concentration by the ocean. Greater stochastic variability in a cold climate increases the risk of exceeding the critical threshold for runaway ice-albedo feedback. Unusual stromatolite and oolite development during the pre-glacial negative $\delta^{13}\text{C}$ excursions could reflect a rise in pH caused by the proposed lowering of $p\text{CO}_2$. The methane substitution model (Schrag et al., 2002) may not be viable, however, because it depends on an unrealistic residence time for CH_4 in the Cryogenian atmosphere of $>10^5$ year. Unless their timing around 1 Myr before the onsets of both Cryogenian snowballs is a coincidence, or merely wrong, the negative $\delta^{13}\text{C}$ excursions should have some bearing on snowball causation.

Climate modeling

Climate models are used to simulate the conditions under which a snowball could initiate, develop over time, and deglaciate. Ideally, an atmospheric general circulation model (AGCM) would be coupled to a dynamic ocean model, a dynamic ice-sheet model, a dynamic sea-ice model, and an interactive model of the geochemical cycle of carbon. In reality, this is computationally unattainable in the foreseeable future. To begin with, full-blown AGCMs are typically run to equilibrium over ~ 100 model years, which are ~ 4 orders of magnitude less than the adjustment time for the geochemical carbon cycle. Therefore, atmospheric CO_2 concentrations are either prescribed at various levels, providing “snapshots” under arbitrary conditions, or else a greatly simplified AGCM must be employed.

Snowball climate models must be adjusted for ambient Proterozoic conditions. Solar luminosity was $\sim 7\%$ lower than present in the Cryogenian and 16–20% lower at 2.22 Ga (Crowley and North, 1991). The Earth’s rotation rate was $\sim 10\%$ higher in the Cryogenian (Williams, 2000) and faster by an unknown amount in the Paleoproterozoic. And Proterozoic continents had higher albedos in the absence of forest cover. These factors all tend to lower the global mean surface temperature and would have resulted in compensatory higher levels of atmospheric CO_2 (Walker et al., 1981).

Modeling suggests that while snowball initiation is resisted by ocean dynamics (Bendtsen, 2002; Poulsen and Jacob, 2004), it is assisted by atmospheric Hadley convection (Bendtsen, 2002), flowage of ice-shelf ice (Goodman and Pierrehumbert, 2003), and wind-driven sea ice (Lewis et al., 2007). Both the thickness of tropical sea ice and the CO_2 level required for deglaciation are highly sensitive to the prescribed ice albedos (Warren et al., 2002; Pollard and Kasting, 2005; Lewis et al., 2006; Le Hir et al., 2007). The role of clouds is complex

and is a major source of uncertainty in the models (Pierrehumbert, 2005).

Theories of causation

In addition to the methane substitution model described above, a number of other astronomical, oceanographic, geodynamic, and biological theories have been proposed to account for low latitude glaciation. Astronomical theories include solar variations (Harland, 1964), large orbital obliquity (Williams, 1975), ice-ring collapses (Sheldon, 1984), impact ejecta (Bendtsen and Bjerrum, 2002; Fawcett and Boslough, 2002), and passage through giant molecular clouds (Pavlov et al., 2005). The large obliquity hypothesis is the most testable and contested of these models (Néron de Surgy and Laskar, 1997; Williams et al., 1998; Pais et al., 1999; Maloof et al., 2002; Levrard and Laskar, 2003). Oceanographic theories include enhanced carbonate burial (Roberts, 1971), intermittent ocean stagnation (Kaufman et al., 1993; Grotzinger and Knoll, 1995), glacioeustatic hypsometry change (Ridgwell et al., 2003), and enhanced organic burial (Kaufman et al., 1997). Geodynamic theories involve continental distribution (Kirschvink, 1992; Schrag et al., 2002), supercontinent breakup (Donnadieu et al., 2004), flood-basalt weathering (Goddéris et al., 2003), and true polar wander (Li et al., 2004). Biological theories include the evolution of biocatalyzed weathering (Carver and Vardavas, 1994), methane destruction (Pavlov et al., 2000, 2003; Catling et al., 2001; Claire et al., 2006), methane substitution (Halverson et al., 2002; Schrag et al., 2002), and the evolution of oxygenic photosynthesis (Kopp et al., 2005). As the ultimate cause of the Quaternary ice ages remains obscure, it is not surprising that the “why” question for Proterozoic snowballs is undecided. Different snowball may have had different causes, but whatever their cause, it was an uncommon occurrence.

Snowballs and the history of oxygenation

There is increasing evidence for stepwise increases in environmental oxygenation near the beginning and end of the Proterozoic eon, roughly contemporaneous with the inferred snowballs. This is the empirical motivation for causative theories involving the oxidative destruction of reduced “greenhouse” gases (Pavlov et al., 2000, 2003; Catling et al., 2001; Kopp et al., 2005; Claire et al., 2006). To make a climatic impact, changes in oxygenation must occur rapidly ($<10^6$ year), or else silicate weathering feedback will cause compensatory adjustments in $p\text{CO}_2$, largely nullifying the effect on radiative forcing.

Mass-independent sulfur isotope fractionation data indicate that atmospheric O_2 concentrations remained below 10^{-6} PAL (present atmospheric level) until ~ 2.45 Ga, close to the first Huronian glaciation and ~ 230 Myr before the Makganyene snowball. The same data suggest the possibility that O_2 remained very low

(but $>10^{-6}$ PAL) during the intervening interval. However, deposition of the succeeding Kalahari MnO_2 -ore formation required at least transient O_2 concentrations >5 orders of magnitude higher than this, possibly caused by a photosynthetic “bloom” and resultant organic burial in the snowball aftermath (Kirschvink et al., 2000) or by H_2O_2 (hydrogen peroxide) released by the melting of snowball ice (Liang et al., 2006).

There is increasing evidence that the deep oceans remained anoxic, but sulfidic not ferrous, during most of the 1.5 Gyr ice-age gap between the Makganyene and the Cryogenian snowballs (Scott et al., 2008). Mass-dependent S-isotope data imply that seawater sulfate concentrations remained low (<4 mM) between the Cryogenian snowballs (Hurtgen et al., 2002), but rose rapidly to near modern (29 mM) levels in the early Ediacaran (Halverson and Hurtgen, 2007). This rise in marine sulfate, presumably caused by increased atmospheric $p\text{O}_2$, appears to follow rather than lead the end-Cryogenian snowball. However, the response time between oxygenation and methane destruction is much shorter than between oxygenation and growth of the marine sulfate reservoir, so an increase in oxygenation to trigger the snowball is not ruled out by these data.

Implications for evolutionary biology

Eukaryotic survival

The younger Phanerozoic glaciations had little impact on the course of biological evolution as measured by rates of origination or extinction of taxa: only the Hirnantian (latest Ordovician) glaciation ~ 445 Ma was accompanied by major mass extinctions. Snowballs, however, presented qualitatively different challenges to existing biota, if they actually occurred. For millions of years, mean annual surface temperatures at the equator would have resembled those of Antarctica today. Areas where sunlight and liquid water coexisted were severely limited, hot springs and zones of perpetual crack development where mobile sea ice encountered fixed rocky substrates. With permanent ice cover, seawater would be starved of oxygen, becoming anoxic due to sinks for oxygen in the ocean. It would become progressively more acidic due to submarine volcanic CO_2 emissions and air–sea gas exchange through cracks at rates lower than the oxygen consumption rate. The geochemical response of the ocean to these afflictions is complex, but seawater pH would probably be lowered by 2.0–2.5 pH units (Le Hir et al., 2008). Deep-sea vent biota would be adversely affected by reduced Eh and pH gradients between hydrothermal fluids and seawater. With low overall productivity, nutrient levels would be high, except for fixed nitrogen due to denitrification. Upon deglaciation, a “bloom” of primary producers would occur, contingent upon nitrate availability. The bloom might be delayed because meltwater injection and surface warming would combine to produce an unusually stable density stratification (Shields, 2005), temporarily suppressing nutrient-upwelling from depth. A 635-Ma

cap carbonate in southwestern Brazil preserves a molecular record of this bloom in the form of an unusual assemblage of biomarkers pointing to a low-diversity eukaryotic flora dominated by red algae and a prokaryotic biota rich in green sulfur bacteria (Elie et al., 2007). The latter imply anoxic waters at the base of the photic zone and the former are consistent with low levels of illumination as exists, for example, in a turbid water column.

No biologist doubts that prokaryotic life, bacteria and archaea, could survive a snowball cryochron and its aftermath. Some do question if eukaryotic algae could survive (but see Vincent et al., 2004). As several extant clades of eukaryotic algae evolved before the Cryogenian, their survival causes some to doubt the existence of snowballs (Knoll, 2003). This is the attraction of more benign but less physically plausible climate states like “slushball” Earth, wherein continents are glaciated at all latitudes (satisfying geological observations) but half of the ocean remains ice-free (Peltier et al., 2004). However, the slushball solution provides no satisfactory explanation for synglacial Fe- and Mn-ore deposits (Klein, 2005), for cap carbonates (Pollard and Kasting, 2005) or for the multimillion-year duration of Cryogenian glaciations inferred from stratigraphic subsidence analysis (Hoffman and Schrag, 2002).

The only fossil record that is sufficiently continuous to bear directly on the evolutionary impact of snowballs is the record of organic-walled, acid-resistant microfossils representing a variety of eukaryotic algae, protists, and possibly fungi (Knoll et al., 2006a). Their Proterozoic record is characterized by extremely low rates of turnover (extinctions and originations) of taxa, roughly 100× slower than in the Ediacaran and Phanerozoic, following the last snowball (Peterson et al., 2005). The sparser macrofossil record is also consistent with slow turnover. Taxonomic diversity drops precipitously during the interval that includes the Cryogenian snowballs (Knoll et al., 2006a), with the inevitable result that it is little studied by palynologists. There have been no systematic studies to determine if the interval of very low diversity actually begins at the first Cryogenian snowball. The microfossil record effectively starts only after the Paleoproterozoic snowball (Knoll, 2003).

Evolutionary innovation

Since the mid-twentieth century, geologists have speculated that Neoproterozoic glaciation and its aftermath was somehow responsible for the advent of multicellular animals, the first fossils of which were known to occur not too far above the glacial deposits in many areas (Harland and Rudwick, 1964). (Note this was not the advent of multicellularity *per se*, which occurred earlier in algae (Knoll, 2003).) In the 1990s, with improvements of radiometric dating, this connection appeared less likely: the canonical Cambrian “explosion” in diversity of animal taxa with hard parts did not occur until ~530–520 Ma and

the oldest macrofossils arguably attributable to metazoans, the enigmatic Ediacara biota, appear ~580 Ma. This is >100 Myr and 55 Myr, respectively, after the last snowball ended in 635 Ma. In contrast, molecular divergence in modern animals, calibrated by known branching points in the geological record (so-called molecular “clocks”), gave estimates of animal origins much older than the Cryogenian snowballs.

However, recent evidence from microfossils and molecular fossils appears to put the connection back in play once again. In South China, remarkable cysts enclosing metazoan diapause eggs and embryos extend down to strata deposited within ~3 Myr (632 Ma) of the last snowball termination in the same area (Yin et al., 2007). In Oman, molecular fossils (24-isopropylcholesterol) believed to be diagnostic of sponges are found down to and even within correlative glacial deposits (Love et al., 2009). In addition, new molecular “clock” analyses place the origin of eumetazoa more in line with the new fossil evidence (Peterson and Butterfield, 2005), while the molecular signature of animal radiation indicates a geologically instantaneous event (Rokas et al., 2005).

How might the environmental stress of a snowball and its aftermath have contributed to revolutionary biological innovation? One idea involves the dual role of heat-shock protein 90 (HSP90) in suppressing the expression of mutant signal transduction proteins and in promoting the proper folding of stress-damaged proteins (Baker, 2006). Under extreme temperatures, HSP90 is diverted from the former role in favor of the latter, allowing accumulated cryptic mutations to be phenotypically expressed and therefore subject to natural selection. Another possibility is that spatial isolation of biological refugia in a snowball favored the evolution of altruism, a basic requirement for intercellular cooperation, by promoting kin selection and eliminating non-altruistic invaders (Boyle et al., 2007).

The evolutionary impact of the Paleoproterozoic snowball(s) is even more conjectural as there is no detailed fossil evidence for synchronicity. Nevertheless, it has been suggested that horizontal gene transfer between taxa at levels higher than at the species level was triggered by environmental catastrophes, and that these gene exchanges were responsible for such macroevolutionary innovations of possible Paleoproterozoic age as thermophilic and photosynthetic bacteria, and the eukaryotic cell (Hartman, 2002). Molecular fossil evidence for an Archean (pre-snowball) origin for oxygenic photosynthesis and for eukaryotes has been presented (Brocks et al., 1999; Summons et al., 1999); however, more recent studies suggest that the “biomarkers” in question are less phylogenetically diagnostic than earlier believed (Fischer et al., 2005; Kopp et al., 2005; Rashby et al., 2007). Finally, the surprising finding that the highest 18S rRNA gene diversity in extant protists and the richest in ancestral lineages occurs in Arctic communities (Stoeck et al., 2007) suggests the possibility that snowballs figured prominently in their evolution.

Conclusion

Important new evidence for extreme atmospheric carbon dioxide concentrations during and after the Marinoan snowball Earth has recently been obtained from mass-independent oxygen isotope ratios in sulfate minerals (Bao et al., 2008, 2009).

Summary

Virtually all continents were covered by large ice sheets during two discrete intervals of the Cryogenian Period (between 725 and 635 Ma). The multimillion-year duration of the glacial intervals, the deposition of syn-glacial Fe₂O₃ and MnO₂ sedimentary ores in different areas and of post-glacial “cap” carbonates at all latitudes support the hypothesis that the world ocean was also ice-covered. These episodes represent a climate state unknown on Earth since 635 Ma, or in the 1.5 Gyr before 725 Ma. Climate models indicate that the pan-glacial climates, popularly known as snowball Earth, were the result of runaway ice-albedo feedback, and that they were reversed by infra-red radiative forcing due to the accumulation of atmospheric CO₂ of volcanic-metamorphic origin, which could not be removed because of the absence of liquid precipitation when surface temperatures were below freezing everywhere. New microfossil and molecular biomarker evidence opens up the possibility that the origin of multicellular animals was related to these climate shocks. Evidence for glaciation at low paleolatitudes around 2.22 Ga, also associated with sedimentary Fe and Mn ores, suggests the existence of snowball states possibly related to the initial rise of atmospheric O₂.

Bibliography

- Allen, P. A., and Hoffman, P. F., 2005. Extreme winds and waves in the aftermath of a Neoproterozoic glaciation. *Nature*, **433**, 123–127.
- Anderson, S. P., 2007. Biogeochemistry of glacial landscape systems. *Annual Reviews of Earth and Planetary Sciences*, **35**, 375–399.
- Baker, M. E., 2006. The genetic response to snowball Earth: role of HSP90 in the Cambrian explosion. *Geobiology*, **4**, 11–14.
- Bao, H., Lyons, J. R., and Zhou, C., 2008. Triple oxygen isotope evidence for elevated CO₂ levels after a Neoproterozoic glaciation. *Nature*, **452**, 504–506.
- Bao, H., Fairchild, I. J., Wynn, P. M., and Spoetl, C., 2009. Stretching the envelope of past surface environments: Neoproterozoic glacial lakes from Svalbard. *Science*, **323**, 119–122.
- Bekker, A., Kaufman, A. J., Karhu, J. A., and Eriksson, K. A., 2005. Evidence for Paleoproterozoic cap carbonates in North America. *Precambrian Research*, **137**, 167–206.
- Bekker, A., Karhu, J. A., and Kaufman, A. J., 2006. Carbon isotope record for the onset of the Lomagundi carbon isotope excursion in the Great Lakes area, North America. *Precambrian Research*, **148**, 145–180.
- Bendtsen, J., 2002. Climate sensitivity to changes in solar insolation in a simple coupled climate model. *Climate Dynamics*, **18**, 595–609.
- Bendtsen, J., and Bjerrum, C. J., 2002. Vulnerability of climate on Earth to sudden changes in insolation. *Geophysical Research Letters*, **29**, 10.1029/2002GL014829.
- Berger, W. H., 1982. Increase of carbon dioxide in the atmosphere during deglaciation: the coral reef hypothesis. *Naturwissenschaften*, **69**, 87–88.
- Boulton, G. S., and Deynoux, M., 1981. Sedimentation in glacial environments and the identification of tills and tillites in ancient sedimentary sequences. *Precambrian Research*, **15**, 397–422.
- Boyle, R. A., Lenton, T. M., and Williams, H. T. P., 2007. Neoproterozoic ‘snowball Earth’ glaciations and the evolution of altruism. *Geobiology*, **5**(4), 337–349.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1026.
- Canfield, D. E., and Raiswell, R., 1999. The evolution of the sulfur cycle. *American Journal of Science*, **299**, 697–723.
- Carver, J. H., and Vardavas, I. M., 1994. Precambrian glaciations and the evolution of the atmosphere. *Annales Geophysicae*, **12**, 674–682.
- Catling, D. C., Zahnle, K. J., and McKay, C. P., 2001. Biogenic methane, hydrogen escape, and the irreversible oxidation of early Earth. *Science*, **293**, 839–843.
- Claire, M. W., Catling, D. C., and Zahnle, K. J., 2006. Biogeochemical modelling of the rise in atmospheric oxygen. *Geobiology*, **4**, 239–269.
- Corsetti, F. A., and Grotzinger, J. P., 2005. Origin and significance of tube structures in Neoproterozoic post-glacial cap carbonates: example from Noonday Dolomite, Death Valley, United States. *Palaos*, **20**, 348–363.
- Crowley, T. J., and North, G. R., 1991. *Paleoclimatology*. New York: Oxford University Press.
- Donnadieu, Y., Fluteau, F., Ramstein, G., Ritz, C., and Besse, J., 2003. Is there a conflict between the Neoproterozoic glacial deposits and the snowball Earth interpretation: an improved understanding with numerical modeling. *Earth and Planetary Science Letters*, **208**, 101–112.
- Donnadieu, Y., Goddérès, Y., Ramstein, G., Nédélec, A., and Meert, J., 2004. A ‘snowball Earth’ climate triggered by continental break-up through changes in runoff. *Nature*, **428**, 303–306.
- Elie, M., Nogueira, A. C. R., Nédélec, A., Trindade, R. I. F., and Kenig, F., 2007. Biodiversity collapse and red algal bloom in the aftermath of the Marinoan Snowball Earth. *Terra Nova*, **19**, 303–308.
- Evans, D. A. D., 2000. Stratigraphic, geochronological, and paleomagnetic constraints upon the Neoproterozoic climatic paradox. *American Journal of Science*, **300**, 347–433.
- Evans, D. A. D., 2003. A fundamental Precambrian–Phanerozoic shift in earth’s glacial style? *Tectonophysics*, **375**, 353–385.
- Evans, D. A. D., 2006. Proterozoic low orbital obliquity and axial-dipolar geomagnetic field from evaporite palaeolatitudes. *Nature*, **444**, 51–55.
- Evans, D. A., Beukes, N. J., and Kirschvink, J. L., 1997. Low-latitude glaciation in the Palaeoproterozoic era. *Nature*, **386**, 262–266.
- Farquhar, J., Bao, H., and Thieme, M., 2000. Atmospheric influence of Earth’s earliest sulfur cycle. *Science*, **289**, 756–758.
- Fawcett, P. J., and Boslough, M. B. E., 2002. Climatic effects of an impact-induced equatorial debris ring. *Journal of Geophysical Research*, **107**, 10.1029/2001JD001230.
- Fischer, W. W., Summons, R. E., and Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. *Geobiology*, **3**, 33–40.
- Font, E., Nédélec, A., Trindade, R. I. F., Macouin, M., and Charrière, A., 2006. Chemostratigraphy of the Neoproterozoic Mirassol d’Oeste cap dolostones (Mato Grosso, Brazil): an alternative model for Marinoan cap dolostone formation. *Earth and Planetary Science Letters*, **250**, 89–103.

- Furukawa, Y., and Wettlaufer, J. S., 2007. Snow and ice crystals. *Physics Today*, **60**, 70–71.
- Godd  ris, Y., Donnadi  u, Y., N  d  lec, A., Dupr  , B., Dessert, C., Grard, A., Ramstein, G., and Francois, L. M., 2003. The Sturtian ‘snowball’ glaciation: fire and ice. *Earth and Planetary Science Letters*, **211**, 1–12.
- Goodman, J., and Pierrehumbert, R. T., 2003. Glacial flow of floating marine ice in ‘Snowball Earth’. *Journal of Geophysical Research*, **108**, (C10): 10.1029/2002JC001471.
- Grotzinger, J. P., and Knoll, A. H., 1995. Anomalous carbonate precipitates: Is the Precambrian the key to the Permian? *Pala  os*, **10**, 578–596.
- Halverson, G. P., 2006. A Neoproterozoic chronology. In Xiao, S., and Kaufman, A. J. (eds.), *Neoproterozoic Geobiology and Paleobiology*. Dordrecht: Springer, pp. 231–271.
- Halverson, G. P., and Hurtgen, M. T., 2007. Ediacaran growth of the marine sulfate reservoir. *Earth and Planetary Science Letters*, **263**, 32–44.
- Halverson, G. P., Hoffman, P. F., Schrag, D. P., and Kaufman, J. A., 2002. A major perturbation of the carbon cycle before the Ghaub glaciation (Neoproterozoic) in Namibia: prelude to snowball Earth? *Geophysics, Geochemistry, Geosystems*, **3**, 10.1029/2001GC000244.
- Halverson, G. P., Hoffman, P. F., Schrag, D. P., Maloof, A. C., and Rice, A. H. N., 2005. Toward a Neoproterozoic composite carbon-isotope record. *Geological Society of America Bulletin*, **117**, 1181–1207.
- Hambrey, M. J., and Harland, W. B., 1981. *Earth’s Pre-Pleistocene Glacial Record*. London: Cambridge University Press.
- Harland, W. B., 1964. Evidence of late Precambrian glaciation and its significance. In Nairn, A. E. M. (ed.), *Problems in Palaeoclimatology*. London: Interscience, pp. 115–118.
- Harland, W. B., and Rudwick, M. J. S., 1964. The great infra-Cambrian ice age. *Scientific American*, **211**(2), 28–36.
- Hartman, H., 2002. Macroevolution, catastrophe and horizontal transfer. In Syvanen, M., and Kado, C. I. (eds.), *Horizontal Gene Transfer*, 2nd edn. San Diego: Academic, pp. 411–415.
- Higgins, J. A., and Schrag, D. P., 2003. Aftermath of a snowball Earth. *Geophysics, Geochemistry, Geosystems*, **4**, 10.1029/2002GC000403.
- Hoffman, P. F., 2005. On Cryogenian (Neoproterozoic) ice-sheet dynamics and the limitations of the glacial sedimentary record. *South African Journal of Geology*, **108**, 557–576.
- Hoffman, P. F., and Schrag, D. P., 2002. The snowball Earth hypothesis: testing the limits of global change. *Terra Nova*, **14**, 129–155.
- Hoffman, P. F., Kaufman, J. A., and Halverson, G. P., 1998a. Comings and goings of global glaciations on a Neoproterozoic carbonate platform in Namibia. *GSA Today*, **8**, 1–9.
- Hoffman, P. F., Kaufman, J. A., Halverson, G. P., and Schrag, D. P., 1998b. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Hoffman, P. F., Halverson, G. P., Domack, E. W., Husson, J. M., Higgins, J. A., and Schrag, D. P., 2007. Are basal Ediacaran (635 Ma) post-glacial ‘cap dolostones diachronous? *Earth and Planetary Science Letters*, **258**, 114–131.
- Hurtgen, M. T., Arthur, M. A., Suits, N. S., and Kaufman, A. J., 2002. The sulfur isotopic composition of Neoproterozoic seawater sulfate: implications for a snowball Earth? *Earth and Planetary Science Letters*, **203**, 413–429.
- Hurtgen, M. T., Halverson, G. P., Arthur, M. A., and Hoffman, P. F., 2006. Sulfur cycling in the aftermath of a 635-Ma snowball glaciation: evidence for a syn-glacial sulfidic deep ocean. *Earth and Planetary Science Letters*, **245**, 551–570.
- James, N. P., Narbonne, G. M., and Kyser, T. K., 2001. Late Neoproterozoic cap carbonates: Mackenzie Mountains, northwestern Canada: precipitation and global glacial meltdown. *Canadian Journal of Earth Sciences*, **38**, 1229–1262.
- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopic evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Kasemann, S. A., Hawkesworth, C. J., Prave, A. R., Fallick, A. E., and Pearson, P. N., 2005. Boron and calcium isotope composition in Neoproterozoic carbonate rocks from Namibia: evidence for extreme environmental change. *Earth and Planetary Science Letters*, **231**, 73–86.
- Kaufman, A. J., Jacobsen, S. B., and Knoll, A. H., 1993. The Vendian record of Sr and C isotopic variations in seawater: implications for tectonic and paleoclimate. *Earth and Planetary Science Letters*, **120**, 409–430.
- Kaufman, A. J., Knoll, A. H., and Narbonne, G. M., 1997. Isotopes, ice ages, and terminal Proterozoic earth history. *Proceedings of the National Academy of Sciences (USA)*, **94**, 6600–6605.
- Kellerhals, P., and Matter, A., 2003. Facies analysis of a glaciomarine sequence, the Neoproterozoic Mirbat Sandstone Formation, Sultanate of Oman. *Eclogae Geologicae Helveticae*, **96**, 49–70.
- Kennedy, M. J., 1996. Stratigraphy, sedimentology, and isotopic geochemistry of Australian Neoproterozoic postglacial cap dolostones: deglaciation, $\delta^{13}\text{C}$ excursions, and carbonate precipitation. *Journal of Sedimentary Research*, **66**, 1050–1064.
- Kennedy, M. J., Christie-Blick, N., and Sohl, L. E., 2001. Are Proterozoic cap carbonates and isotopic excursions a record of gas hydrate destabilization following Earth’s coldest intervals? *Geology*, **29**, 443–446.
- Kirschvink, J. L., 1992. Late Proterozoic low-latitude glaciation: the snowball Earth. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 51–52.
- Kirschvink, J. L., Gaidos, E. J., Bertani, L. E., Beukes, N. J., Gutzmer, J., Maepa, L. N., and Steinberger, R. E., 2000. Paleoproterozoic snowball Earth: extreme climatic and geochemical global change and its biological consequences. *Proceedings of the National Academy of Sciences (USA)*, **97**, 1400–1405.
- Klein, C., 2005. Some Precambrian banded iron-formations (BIFs) from around the world: their age, geologic setting, mineralogy, metamorphism, geochemistry, and origin. *American Mineralogist*, **90**, 1473–1499.
- Knoll, A. H., 2003. *Life on a Young Planet*. Princeton: Princeton University Press.
- Knoll, A. H., Javaux, E. J., Hewitt, D., and Cohen, P., 2006a. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society, London, Series B*, **361**, 1023–1038.
- Knoll, A. H., Walter, M. R., Narbonne, G. M., and Christie-Blick, N., 2006b. The Ediacaran Period: a new addition to the geologic time scale. *Lethaia*, **39**, 13–30.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A., and Nash, C. Z., 2005. The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences (USA)*, **102**, 11131–11136.
- Kump, L. R., and Seyfried, W. E. Jr., 2005. Hydrothermal Fe fluxes during the Precambrian: effect of low oceanic sulfate concentrations and low hydrostatic pressure on the composition of black smokers. *Earth and Planetary Science Letters*, **235**, 654–662.
- Le Hir, G., Ramstein, G., Donnadi  u, Y., and Godd  ris, Y., 2008. Scenario for the evolution of atmospheric $p\text{CO}_2$ during a snowball Earth. *Geology*, **36**, 47–50.
- Le Hir, G., Ramstein, G., Donnadi  u, Y., and Pierrehumbert, R. T., 2007. Investigating plausible mechanisms to trigger a deglaciation from a hard snowball Earth. *Comptes Rendus Geoscience*, **339**, 274–287.

- Lévraud, B., and Laskar, J., 2003. Climate friction and the Earth's obliquity. *Geophysical Journal International*, **154**, 970–990.
- Lewis, J. P., Weaver, A. J., and Eby, M., 2006. Deglaciating the snowball Earth: sensitivity to surface albedo. *Geophysical Research Letters*, **33**, L23604, doi: 10.1029/2006GL027774.
- Lewis, J. P., Weaver, A. J., and Eby, M., 2007. Snowball versus slushball Earth: dynamic versus nondynamic sea ice? *Journal of Geophysical Research*, **112**, C11014, doi: 10.1029/2006JC004037.
- Li, Z. X., Evans, D. A. D., and Zhang, S., 2004. A 90° spin on Rodinia: possible causal links between the Neoproterozoic supercontinent, superplume, true polar wander and low-latitude glaciation. *Earth and Planetary Science Letters*, **220**, 409–421.
- Li, Z. X., Bogdanova, S. V., Collins, A. S., Davidson, A., De Waele, B., Ernst, R. E., Fitzsimons, I. C. W., Fuck, R. A., Gladkochub, D. P., Jacobs, J., Karlstrom, K. E., Lu, S., Natapov, L. M., Pease, V., Pisarevsky, S. A., Thrane, K., and Vernikovsky, V., 2008. Assembly, configuration, and break-up history of Rodinia: a synthesis. *Precambrian Research*, **160**, 179–210.
- Liang, M. C., Hartman, H., Kopp, R. E., Kirschvink, J. L., and Yung, Y. L., 2006. Production of hydrogen peroxide in the atmosphere of the Snowball Earth and the origin of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences (USA)*, **103**(50), 18896–18899.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–722.
- Maloof, A. C., Kellogg, J. B., and Anders, A. M., 2002. Neoproterozoic sand wedges: crack formation in frozen soils under diurnal forcing during a snowball Earth. *Earth and Planetary Science Letters*, **204**, 1–15.
- Martin, H., 1964. Observations concerning the problem of the late Precambrian glacial deposits in South West Africa (in German with English summary). *Geologische Rundschau*, **54**, 115–127.
- Néron de Surgy, O., and Laskar, J., 1997. On the long term evolution of the spin of the Earth. *Astronomy and Astrophysics*, **318**, 975–989.
- North, G. R., 1990. Multiple solutions in energy balance climate models. *Palaeogeography, Palaeoclimatology, Palaeoecology Global and Planetary Change Section*, **82**, 225–235.
- Ojakangas, R. W., 1988. Glaciation: an uncommon “mega-event as a key to intracontinental and intercontinental correlation of early Proterozoic basin fill, North America and Baltic cratons. In Kleinspehn, K. L., and Paola, C. (eds.), *New Perspectives in Basin Analysis*. New York: Springer, pp. 431–444.
- Opdyke, B. N., and Wilkinson, B. H., 1990. Paleolatitude distribution of Phanerozoic marine ooids and cements. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **78**, 135–148.
- Pais, M. A., Le Mouél, J. L., Lambeck, K., and Poirier, J. P., 1999. Late Precambrian paradoxical glaciation and obliquity of the Earth – a discussion of dynamical constraints. *Earth and Planetary Science Letters*, **174**, 155–171.
- Pavlov, A. A., Hurtgen, M. T., Kasting, J. F., and Arthur, M. A., 2003. Methane-rich Proterozoic atmosphere? *Geology*, **31**, 87–90.
- Pavlov, A. A., Kasting, J. F., Brown, L. L., Rages, K. A., and Freedman, R., 2000. Greenhouse warming by CH₄ in the atmosphere of early Earth. *Journal of Geophysical Research*, **105**, 11,981–11,990.
- Pavlov, A. A., Toon, O. B., Pavlov, A. K., Bally, J., and Pollard, D., 2005. Passing through a giant molecular cloud: “Snowball” glaciations produced by interstellar dust. *Geophysical Research Letters*, **32**, L03705, 10.1029/2004GL021890.
- Peltier, W. R., Tarasov, L., Vettoretti, G., and Solheim, L. P., 2004. Climate dynamics in deep time: modeling the “snowball bifurcation” and assessing the plausibility of its occurrence. In Jenkins, G. S., McMenamin, M. A. S., McKey, C. P., and Sohl, L. (eds.), *The Extreme Proterozoic: Geology, Geochemistry, and Climate*. Geophysical Monograph. Washington: American Geophysical Union, Vol. 146, pp. 107–124.
- Peterson, K. J., and Butterfield, N. J., 2005. Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences (USA)*, **102**, 9547–9552.
- Peterson, K. J., McPeck, M. A., and Evans, D. A. D., 2005. Tempo and mode of early animal evolution: inferences from rocks, Hox, and molecular clocks. *Paleobiology*, **31**, 36–55.
- Pierrehumbert, R. T., 2005. Climate dynamics of a hard snowball Earth. *Journal of Geophysical Research*, **110**, D01111, 10.1029/2004JD005162.
- Pollard, D., and Kasting, J. F., 2004. Climate-ice sheet simulations of Neoproterozoic glaciation before and after collapse to Snowball Earth. In Jenkins, G. S., McMenamin, M. A. S., McKey, C. P., and Sohl, L. (eds.), *The Extreme Proterozoic: Geology, Geochemistry, and Climate*. Geophysical Monograph. Washington: American Geophysical Union, Vol. 146, pp. 91–105.
- Pollard, D., and Kasting, J. F., 2005. Snowball Earth: a thin-ice solution with flowing glaciers. *Journal of Geophysical Research*, **110**, C07010: 10.1029/2004JC002525.
- Poulsen, C., and Jacob, R., 2004. Factors that inhibit snowball Earth simulation. *Paleoceanography*, **19**, PA4021, doi: 10.1029/2004PA001056.
- Rashby, S. E., Sessions, A. L., Summons, R. E., and Newman, D. K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of the National Academy of Sciences (USA)*, **104**, 15099–15104.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Roberts, J. D., 1971. Late Precambrian glaciation: an anti-greenhouse effect? *Nature*, **234**, 216.
- Rokas, A., Krüger, D., and Carroll, S. B., 2005. Animal evolution and the molecular signature of radiations compressed in time. *Science*, **310**, 1933–1938.
- Schmidt, P. W., and Williams, G. E., 1995. The Neoproterozoic climatic paradox: equatorial paleolatitude for Marinoan glaciation near sea level in South Australia. *Earth and Planetary Science Letters*, **134**, 107–124.
- Schrag, D. P., Berner, R. A., Hoffman, P. F., and Halverson, G. P., 2002. On the initiation of a snowball Earth. *Geophysics, Geochemistry, Geosystems*, **3**, 10.1029/2001GC000219.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X., and Anbar, A. D., 2008. Tracing stepwise oxygenation of the Proterozoic biosphere. *Nature*, **452**, 456–460.
- Sheldon, R. P., 1984. Ice-ring origin of the Earth's atmosphere and hydrosphere and Late Proterozoic–Cambrian hypothesis. *Geological Survey of India Special Publication*, **17**, 17–21.
- Shields, G. A., 2005. Neoproterozoic cap carbonates: a critical appraisal of existing models and the plumeworld hypothesis. *Terra Nova*, **17**, 299–310.
- Shields, G. A., Deynoux, M., Strauss, H., Paquet, H., and Nahon, D., 2007. Barite-bearing cap dolostone of the Taoudéni Basin, northwest Africa: sedimentary and isotopic evidence for methane seepage after a Neoproterozoic glaciation. *Precambrian Research*, **154**, 209–235.
- Stoeck, T., Kasper, J., Bunge, J., Leslin, C., Ilyin, V., and Epstein, S., 2007. Protistan diversity in the Arctic: a case of palaeoclimate shaping of modern biodiversity? *PloS ONE*, **2**(8), e728. doi: 10.1371/journal.pone.0000728.

- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Trindade, R. I. F., and Macouin, M., 2007. Paleolatitude of glacial deposits and paleogeography of Neoproterozoic ice ages. *Comptes Rendus Geoscience*, **339**, 200–211.
- Vieira, L. C., Trindade, R. I. F., Nogueira, A. C. R., and Ader, M., 2007. Identification of a Sturtian cap carbonate in the Neoproterozoic Sete Lagoas carbonate platform, Bambuí Group, Brazil. *Comptes Rendus Geoscience*, **339**, 240–258.
- Vincent, W. F., Mueller, D. R., and Bonilla, S., 2004. Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology*, **48**, 103–112.
- Walker, J. C. G., Hays, P. B., and Kasting, J. F., 1981. A negative feedback mechanism for the long-term stabilization of Earth's surface temperature. *Journal of Geophysical Research*, **86** (C10), 9776–9782.
- Warren, S. G., Brandt, R. E., Grenfell, T. C., and McKay, C. P., 2002. Snowball Earth: ice thickness on the tropical ocean. *Journal of Geophysical Research*, **107**(C10), 10.1029/2001JC001123.
- Williams, G. E., 1975. Late Precambrian glacial climate and the Earth's obliquity. *Geological Magazine*, **112**, 441–544.
- Williams, G. E., 2000. Geological constraints on the Precambrian history of Earth's rotation and the Moon's orbit. *Reviews of Geophysics*, **38**(1), 37–59.
- Williams, D. M., Kasting, J. F., and Frakes, L. A., 1998. Low-latitude glaciation and rapid changes in the Earth's obliquity explained by obliquity–oblateness feedback. *Nature*, **396**, 453–455.
- Yin, L., Zhu, M., Knoll, A. H., Yuan, X., Zhang, J., and Hu, J., 2007. Doushantuo embryos preserved inside diapause egg cysts. *Nature*, **446**, 661–663.
- Yoshioka, H., Asahara, Y., Tojo, B., and Kawakami, S., 2003. Systematic variations in C, O, and Sr isotopes and elemental concentrations in Neoproterozoic carbonates in Namibia: implications for a glacial to interglacial transition. *Precambrian Research*, **124**, 69–85.
- Zhang, S., Jiang, G., and Han, Y., 2008. The age of the Nantuo Formation and Nantuo glaciation in South China. *Terra Nova*, **20**, 289–294.
- Ziegler, A. M., Hulver, M. L., Lottes, A. L., and Schmachtenberg, W. F., 1984. Uniformitarianism and paleoclimates: inferences from the distribution of carbonate rocks. In Brenchley, P. J. (ed.), *Fossils and Climate*. New York: Wiley, pp. 3–25.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Bacteria
 Banded Iron Formations
 Biomarkers (Molecular Fossils)
 Breakup of Rodinia
 Cap Carbonate
 Carbon Cycle
 Carbonate Environments
 Critical Intervals in Earth History
 Dolomite, Microbial
 Endosymbiosis
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Isotopes (Methods)
 Lateral Gene Transfer
 Methane Oxidation (Aerobic)
 Microbialites, Modern
 Origins of the Metazoa
 Stromatolites

SODA LAKES

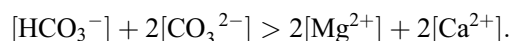
Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The term “soda lake” designates a class of lakes with waters showing an excess of the total alkalinity ($TA \cong [HCO_3^-] + 2[CO_3^{2-}]$, i.e., the sum of the charges of the bicarbonate ion plus carbonate ion) over the charges of the alkaline earth ions magnesium and calcium:



When such water is evaporated, the high $[CO_3^{2-}]$ concentrations will cause a rise in pH and eventually, Na carbonates precipitate. These are soda (or natron, $Na_2CO_3 \cdot 10H_2O$, monoclinic), trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$, monoclinic), and thermonatrite ($Na_2CO_3 \cdot H_2O$, orthorhombic). They are commercially used in the glass-, textile-, paint-, soap- and metal-industry, mined worldwide (Garret, 1992).

Occurrence

Soda lakes occur worldwide, albeit in very specific zones: they seem to be associated with active tectonic and volcanic zones (Figure 1). Most of the famous soda lakes occur in East Africa along the East African rift (e.g., Kraml and Bull, 1998/1999). Here, the succession of soda lakes left deeply buried soda deposits that also serve as the source of natro-carbonatite lavas (Ol Doinyo Lengai Stratovolcano, Tanzania). Another well-known group of alkaline lakes, some fossil such as Searles Lake, occur in the western USA. Less well known are lakes on the Altiplano, or in Australia, Afghanistan, Turkey, and other places around the world. So far no comprehensive list of soda lakes has apparently been compiled.

Setting

However, there seem to be two different types of lakes that acquire soda chemistry: most of the larger lakes are depressions in endorheic regions closed toward exorheic runoff by tectonic movements or by volcanic dams. The ratio between the tributary area and lake surface area is large. The largest soda lake on the Earth is Lake Van (Van Gölü) in eastern Anatolia (e.g., Kempe et al., 1991). Table 1 gives its morphometric data; it is (because Lake Aral dried up) by volume the third largest closed basin lake on Earth. Lake Van occupies the former headwaters of the Euphrates before it was dammed by an eruption of the Nemrut volcano.

The other class of soda lakes occur in volcanic craters or calderas. They typically do not have any surface tributaries and their ratio between recharge area and lake surface area is small. We have discovered the alkaline nature of several crater lakes, including Satonda Crater,

- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Trindade, R. I. F., and Macouin, M., 2007. Paleolatitude of glacial deposits and paleogeography of Neoproterozoic ice ages. *Comptes Rendus Geoscience*, **339**, 200–211.
- Vieira, L. C., Trindade, R. I. F., Nogueira, A. C. R., and Ader, M., 2007. Identification of a Sturtian cap carbonate in the Neoproterozoic Sete Lagoas carbonate platform, Bambui Group, Brazil. *Comptes Rendus Geoscience*, **339**, 240–258.
- Vincent, W. F., Mueller, D. R., and Bonilla, S., 2004. Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology*, **48**, 103–112.
- Walker, J. C. G., Hays, P. B., and Kasting, J. F., 1981. A negative feedback mechanism for the long-term stabilization of Earth's surface temperature. *Journal of Geophysical Research*, **86** (C10), 9776–9782.
- Warren, S. G., Brandt, R. E., Grenfell, T. C., and McKay, C. P., 2002. Snowball Earth: ice thickness on the tropical ocean. *Journal of Geophysical Research*, **107**(C10), 10.1029/2001JC001123.
- Williams, G. E., 1975. Late Precambrian glacial climate and the Earth's obliquity. *Geological Magazine*, **112**, 441–544.
- Williams, G. E., 2000. Geological constraints on the Precambrian history of Earth's rotation and the Moon's orbit. *Reviews of Geophysics*, **38**(1), 37–59.
- Williams, D. M., Kasting, J. F., and Frakes, L. A., 1998. Low-latitude glaciation and rapid changes in the Earth's obliquity explained by obliquity–oblateness feedback. *Nature*, **396**, 453–455.
- Yin, L., Zhu, M., Knoll, A. H., Yuan, X., Zhang, J., and Hu, J., 2007. Doushantuo embryos preserved inside diapause egg cysts. *Nature*, **446**, 661–663.
- Yoshioka, H., Asahara, Y., Tojo, B., and Kawakami, S., 2003. Systematic variations in C, O, and Sr isotopes and elemental concentrations in Neoproterozoic carbonates in Namibia: implications for a glacial to interglacial transition. *Precambrian Research*, **124**, 69–85.
- Zhang, S., Jiang, G., and Han, Y., 2008. The age of the Nantuo Formation and Nantuo glaciation in South China. *Terra Nova*, **20**, 289–294.
- Ziegler, A. M., Hulver, M. L., Lottes, A. L., and Schmachtenberg, W. F., 1984. Uniformitarianism and paleoclimates: inferences from the distribution of carbonate rocks. In Brenchley, P. J. (ed.), *Fossils and Climate*. New York: Wiley, pp. 3–25.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Bacteria
 Banded Iron Formations
 Biomarkers (Molecular Fossils)
 Breakup of Rodinia
 Cap Carbonate
 Carbon Cycle
 Carbonate Environments
 Critical Intervals in Earth History
 Dolomite, Microbial
 Endosymbiosis
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Isotopes (Methods)
 Lateral Gene Transfer
 Methane Oxidation (Aerobic)
 Microbialites, Modern
 Origins of the Metazoa
 Stromatolites

SODA LAKES

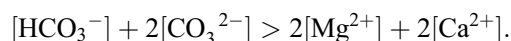
Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The term “soda lake” designates a class of lakes with waters showing an excess of the total alkalinity ($TA \cong [HCO_3^-] + 2[CO_3^{2-}]$, i.e., the sum of the charges of the bicarbonate ion plus carbonate ion) over the charges of the alkaline earth ions magnesium and calcium:



When such water is evaporated, the high $[CO_3^{2-}]$ concentrations will cause a rise in pH and eventually, Na carbonates precipitate. These are soda (or natron, $Na_2CO_3 \cdot 10H_2O$, monoclinic), trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$, monoclinic), and thermonatrite ($Na_2CO_3 \cdot H_2O$, orthorhombic). They are commercially used in the glass-, textile-, paint-, soap- and metal-industry, mined worldwide (Garret, 1992).

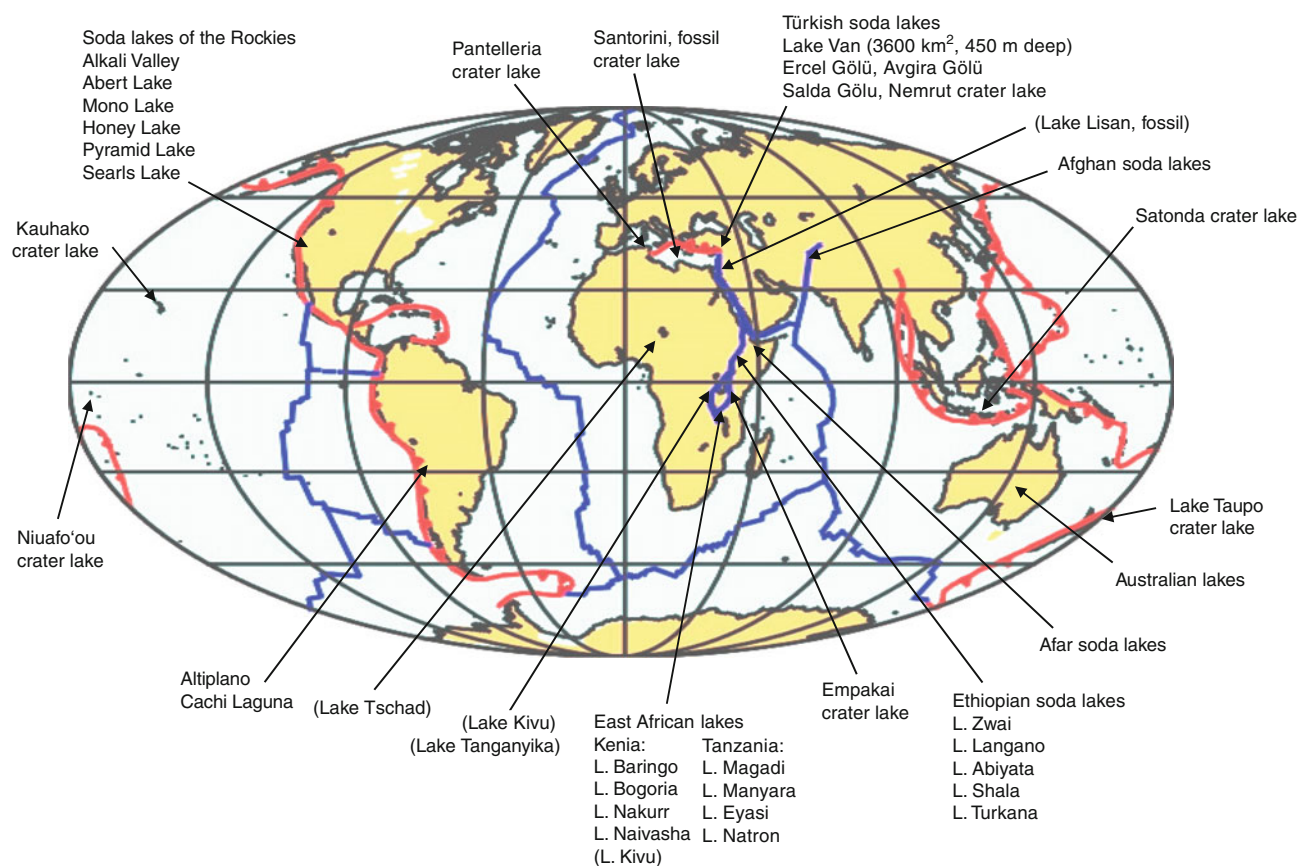
Occurrence

Soda lakes occur worldwide, albeit in very specific zones: they seem to be associated with active tectonic and volcanic zones (Figure 1). Most of the famous soda lakes occur in East Africa along the East African rift (e.g., Kraml and Bull, 1998/1999). Here, the succession of soda lakes left deeply buried soda deposits that also serve as the source of natro-carbonatite lavas (Ol Doinyo Lengai Stratovolcano, Tanzania). Another well-known group of alkaline lakes, some fossil such as Searles Lake, occur in the western USA. Less well known are lakes on the Altiplano, or in Australia, Afghanistan, Turkey, and other places around the world. So far no comprehensive list of soda lakes has apparently been compiled.

Setting

However, there seem to be two different types of lakes that acquire soda chemistry: most of the larger lakes are depressions in endorheic regions closed toward exorheic runoff by tectonic movements or by volcanic dams. The ratio between the tributary area and lake surface area is large. The largest soda lake on the Earth is Lake Van (Van Gölü) in eastern Anatolia (e.g., Kempe et al., 1991). Table 1 gives its morphometric data; it is (because Lake Aral dried up) by volume the third largest closed basin lake on Earth. Lake Van occupies the former headwaters of the Euphrates before it was dammed by an eruption of the Nemrut volcano.

The other class of soda lakes occur in volcanic craters or calderas. They typically do not have any surface tributaries and their ratio between recharge area and lake surface area is small. We have discovered the alkaline nature of several crater lakes, including Satonda Crater,



Soda Lakes, Figure 1 The global distribution of some of the major soda lakes or soda lake groups and their relation to margins of tectonic plates.

Soda Lakes, Table 1 Lake Van morphometric data

Total basin area	16,096 km ²
Total tributary area	12,574 km ²
Lake surface	3,522 km ²
Lake volume	576 km ³
Lake maximum depth	451 m
Annual water exchange	3.78 km ³
Altitude of lake level	1,648 m a.s.l.

Source: Kempe and Kazmierczak (2003); Compiled from Reimer (1995)

Indonesia (Kempe and Kazmierczak, 1993), Niuafo'ou Caldera Lake, Tonga (Kazmierczak and Kempe, 2006), and Kauhako Crater Lake, Molokai, Hawaii (unpublished data and Donachie et al., 2004). Other soda lakes occupy the Empakai Crater in Kenya, the crater in Pantelleria, and possibly a score of other craters that have not been investigated as yet. Even Santorini, Greece, must have had an alkaline crater lake for some period prior to the famous Minoan eruption that blew out the stromatolites of that lake.

Chemistry

Soda lakes can reach pH in excess of 10.5 and alkalinities of >150 meq/l. Some of the most alkaline water bodies are Lake Magadi (Eugster, 1986) in Kenya and Mono Lake in California (e.g., Bischoff et al., 1993). Lake Van has a pH of 9.7 and an alkalinity of 151 meq/l (Kempe et al., 1991; Reimer et al., 2009). These are examples of highly alkaline lakes. But there are soda lakes “in the making,” i.e., lakes that obey the basic condition of $TA > (Mg + Ca)$ but have not acquired higher salinities. One of them is the crater lake of the Nemrut Volcano itself that has dammed Lake Van. In Table 2 the water of Lake Van is compared with that of the Nemrut Lake.

The Table lists the main parameters that are used to characterize natural waters, all in all the two lakes are not very different in their respective surface and bottom samples, i.e., they are relatively well mixed. First of all the conductivity gives a rough measure of the amount of total salts dissolved, which is in seawater about 35 g/l, in Lake Van it is about 22 g/l, and in Nemrut Lake only about 440 mg/l, i.e., Nemrut Lake is what would be classified as a “fresh water” lake. But this is only one of the criteria: when calculating the soda lake criterion

(i.e., $TA/(Mg + Ca)$) we find 16.65 and 16.85 for Lake Van (0 and 440 m, respectively), 3.11 and 3.18 for Lake Nemrut (10 and 150 m, respectively) and 0.018 for seawater. This ratio tells us that Lake Van is a typical soda lake with >16 times as much alkalinity as alkaline earth ions, but that also Lake Nemrut is a soda lake with >3 times higher alkalinity than alkaline earth ions; whereas seawater has much more Mg and Ca than dissolved carbonates (i.e., >54 times). We also see that the chemical

composition of both lakes do not differ much with depth, apart from the pH in Nemrut Crater that drops considerably. Lake Van has a very high pH as expected from its high alkalinity. But Nemrut crater lake has – at the surface – the same slightly alkaline pH as seawater, not identifying it (yet) as a soda lake. When from these data (and temperature not given here) the CO_2 pressure of the water (PCO_2) is calculated (using PHREEQE; Parkhurst et al., 1990) and compared to the PCO_2 of the atmosphere of

Soda Lakes, Table 2 Chemical composition of Lake Van, the largest contemporary soda lake, compared to Nemrut Crater Lake, a “soda lake in the making” and to modern seawater

Lake/Depth	pH	PCO_2 ppmv	Alkal. meq/l	Cl meq/l	SO_4 meq/l	Na meq/l	Mg meq/ l	Ca meq/l	Cond $\mu S/cm$	SI_{Cc}	SI_{Ara}	SI_{Dol}
Seawater at surface	8.24	363	2.33	546	56.6	468	106.2	20.6	ca. 48,000	0.59	0.44	1.96
Van 3 m	9.74	309	151.2	160	48.9	338	8.870	0.210	26,000	1.14	0.99	4.09
Van 440 m	9.88	257	155.6	166	50.9	348	9.06	0.174	26,700	1.06	0.91	3.81
Nemrut 10 m	8.27	1,240	4.61	0.43	0.22	3.67	0.52	0.96	456	0.37	0.22	0.43
Nemrut 150 m	6.89	28,600	4.77	0.45	0.25	3.80	0.53	0.97	469	–1.11	–1.27	–2.59
Same degassed	8.73	380	4.77	0.45	0.25	3.80	0.53	0.97	–	1.30	1.15	2.25

Source: From Kempe and Kazmierczak (2003); data see Reimer (1995); (Reimer et al., 2009)

Soda Lakes, Table 3 List of modern soda lakes with $CaCO_3$ precipitation in comparison with modern oceanic conditions

Site	Minerals dominating	Saturation index	Kind of $CaCO_3$ precipitate	Source
North Sea 1986	Calcite	0.375–0.904 Cc mean of 122 samples: 0.567	Biomineralization of coccolithophorids	Pegler and Kempe, 1988
Bahamas banks	Aragonite	0.47–0.6 Ara	Extremely low rates (if any) of aragonite (not the reason of whittings)	Morse et al., 2002
Satonda crater lake	Aragonite, Calcite	0.98 Cc, 0.84 Ara	Biomineralization by red algae and cyanobacterial sheaths	Kempe and Kazmierczak, 1993; Kazmierczak et al., 2004
Lake Van	Aragonite, Calcite	1.14 at 0 m to 1.06 at 440 m Cc, 0.99–0.91 Ara 4.09–3.81 Dol	Permineralization of cyanobacterial sheaths, inorganic chemical gardens, river plume whiting, summer whittings	Kempe et al., 1991; Reimer, 1995; Kazmierczak et al., 2004; Reimer et al., 2008
Lake Tanganyika	High-Mg Calcite	0.96 Cc	Five zones of massive thrombolites down to 50 m	Kempe and Kazmierczak, 1990 and citations therein
Andros Island	Low-Mg Calcite	Upon degassing and evaporation 1.14 Cc	Inland lakes with weakly calcified cyanobacterial and algal mats	Same as above
Walker Lake	Low-Mg Calcite	1.11 Cc, 0.95 Ara, 3.90 Dol	Small cabbage-like stromatolites up to 4 cm high, built by filamentous green alga and oscillatorian and coccoidal cyanobacteria.	Same as above; Kempe and Kazmierczak, 1997
Mono Lake Figure 2 top	Aragonite (Ikaite)	1.24 Cc, 1.09 Ara, 3.74 Dol	Large tufa columns at former groundwater outlets	Kempe and Kazmierczak, 1997; Bischoff et al., 1993
Pyramid Lake Figure 2 bottom	Calcite	1.36 Cc, 1.21 Ara, 3.89 Dol	Huge towers and mounds at former groundwater outlets	Benson, 1994; Arp et al., 1999
Niuafou, crater lake	Aragonite	0.65 Cc, 0.50 Ara, 2.65 Dol	Head-like stromatolites built by coccoid and filamentous cyanobacteria	Kazmierczak and Kempe, 2006
Kauhako crater lake	Calcite	0.97 Cc, 0.82 Ara	Calcified sheaths of filamentous cyanobacteria	Kempe, unpublished

Cc = calcite; Ara = aragonite; Dol = dolomite

380 ppm, then we find that the crater lake has a much higher PCO_2 than the other samples, indicating that it receives volcanic CO_2 from subsurface springs, while Lake Van does not.

PHREEQE also allows for calculating the state of saturation of water with respect to minerals. The saturation index (SI) is defined as the logarithm of the ion activity product divided by the solubility constant of a given mineral (here, for calcite as an example):

$$SI_{\text{calcite}} = \log([Ca^{2+}] * [CO_3^{2-}] / K_{\text{calcite}}).$$

The SI yields negative values if water is undersaturated, zero if saturated, and positive values if supersaturated. The results (Table 2) show that all samples, with the exception of the deep Nemrut sample, are oversaturated with respect to calcite, aragonite, and dolomite. But when the deep Nemrut sample is numerically degassed, it will also show high carbonate supersaturation (last line in Table 2). The main difference between Lake Van and seawater is that the carbonate supersaturation (which is >10 times its saturation) of Lake Van allows for in situ precipitation of aragonite, while in seawater, even though supersaturated, no spontaneous precipitation occurs. Rather, the supersaturation of seawater is kept low by the multitude of enzymatically biomineralizing organisms that are generally absent in soda lakes due to the high alkalinity.

Soda lake microbialites

Those of the mature soda lakes that reach high supersaturation values (i.e., above $\approx SI\ 0.8$, Kempe and Kazmierczak, 1990) produce cyanobacterial microbialites. Table 3 lists

a few of them for which sufficient geochemical data are available to calculate saturation. The water analysis available for Andros Island originally do not show supersaturation, but the permineralizing cyanobacterial mats grow in ephemeral very shallow nonmarine inland lakes that apparently sustain growth only after an extensive evaporative phase. In case of one of the Niuafu'ou caldera lakes (Vai Lahi), the current water composition also does not sustain in situ permineralization, and the stromatolites discovered there stopped growing about 200 years ago. Overall, soda lakes represent the best sites to study modern stromatolites, their structure, microbial ecosystem, and growth rates. Many of them have shown to produce structures strikingly similar to those of Precambrian microbialites.

Genesis of soda lakes

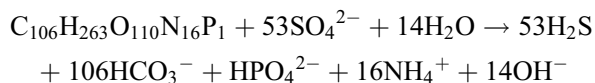
These data presented here and in the cited papers show that soda chemistry can arise in various climatic, geologic, and morphologic settings. We find water bodies that are soda lakes in the making that will eventually acquire mature soda chemistry, such as Nemrut Crater Lake, a lake that originated only a few hundred years ago after the last eruption of the Nemrut caldera. Other crater lakes, such as Lake Taupo in New Zealand, will never reach this state, since they have an outlet. Astonishing is also the fact that Lake Kivu and Lake Tanganyika (both with an outlet) have soda lake characteristics (Soda Lake Index for Tanganyika: 1.5) and (at least in Lake Tanganyika) grow microbialites.

It has long been stated that the type of chemistry is dependent on the proportions of the ions in the waters



Soda Lakes, Figure 2 Calcareous tufa towers in Mono Lake (*top*) and Pyramid Lake (*bottom*). These towers originally formed under water at inlets of groundwater.

delivered to it (Garrels and Mackenzie, 1967). Eugster and Hardie (1978) constructed flow diagrams that show the various possibilities of brine evolution. In case of soda lakes, the criterion is the ratio of TA/(Mg + Ca). From the distributions of the soda lakes along the plate boundaries it appears that this ratio most likely arises in areas with fresh volcanic rocks. Here, biogenic CO₂ and/or (see Lake Nemrut) volcanic CO₂ can react with fresh silicates, mobilizing Na, K, Mg, and Ca, in the necessary proportions. Ongoing weathering within the crater lakes and their hydrothermal system (as in the case of Niuafu'ou), or continuing evaporation in the terminal lakes will then lead to mature soda lake chemistry and to a CaCO₃ supersaturation of around SI = 1 that will sustain microbialite growth. However, this is only a very broad picture. When looking at Satonda and Kauhako crater lakes, we find involvement of a second process that causes the alkalinity to rise. This is sulfate reduction. Both lakes are marine-derived lakes, i.e., are filled originally with sulfate-rich seawater. Microbial disintegration of organic matter must replace the charge of the sulfate ion with that of bicarbonate ions according to



This pathway of alkalinity production in anaerobic water bodies was coined “alkalinity pump” (Kempe, 1990; Kempe and Kazmierczak, 1994), supplementing the shift toward a soda chemistry, where sulfate is available.

Summary

Soda lake waters show an excess of the total alkalinity over the charges of the alkaline earth ions magnesium and calcium ($[\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] > 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}]$). They are characterized by high pH values (increasing with evaporation) and, eventually, precipitation of Na carbonates. Mature soda lakes that reach high supersaturation values with respect to calcite, aragonite, and dolomite (above $\approx$ SI 0.8) may produce cyanobacterial microbialites. The ubiquitous soda lakes in areas with fresh volcanic rocks and high weathering potential and the microbialites found therein gave rise to the *Soda Ocean Hypothesis* (see separate entry), stating that the early ocean, by following simple geological rules, should have been highly alkaline, possibly similar to present Lake Van.

Bibliography

- Arp, G., Thiel, V., Reimer, A., Michaelis, W., and Reitner, J., 1999. Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. *Sedimentary Geology*, **126**, 159–196.
- Benson, L. V., 1994. Carbonate deposition, Pyramid Lake subbasin, Nevada: I. Sequence of formation and elevational distribution of carbonate deposits (tufas). *Palaeogeography, Palaeoecology, Palaeoclimatology*, **109**, 55–87.
- Bischoff, J. L., Stine, S., Rosenbauer, R. J., Fitzpatrick, J. A., and Stafford, T. W., 1993. Ikaite precipitation by mixing of shoreline springs and lake water, Mono Lake, California, USA. *Geochimica et Cosmochimica Acta*, **57**, 3855–3865.
- Donachie, S. P., Hou, S., Lee, K. S., Riley, C. W., Pikina, A., Kempe, S., Gregory, T. G., Bossuyt, A., Boerema, J., Liu, J., Freitas, T. A., Malahoff, A., and Alam, M., 2004. The Hawaiian Archipelago: a microbial diversity hotspot. *Microbial Ecology*, **48**, 509–520.
- Eugster, H. P., 1986. Lake Magadi, Kenya: a model for rift valley hydrochemistry and sedimentation? In Frostick, L. E. et al., (eds.), *Sedimentation in the African Rifts*. London: Geological Society, Special Publications 25, pp. 177–189.
- Eugster, H. P., and Hardie, L. A., 1978. Lake Magadi, Kenya: a model for rift valley hydrochemistry and sedimentation? In Lerman, A. (ed.), *Physics and Chemistry of Lakes*, New York: Springer, pp. 237–293.
- Garrels, R. M., and Mackenzie, F. T., 1967. Origin of the chemical composition of some springs and lakes. In equilibrium concepts of natural water systems. *American Chemical Society Advances in Chemistry*, **67**, 222–242.
- Garret, D. E., 1992. *Natural Soda Ash, Occurrences, Processing, and Use*. New York: Van Nostrand Reinhold.
- Kazmierczak, J., and Kempe, S., 2006. Modern analogues of Precambrian stromatolites from caldera lakes of Niuafu'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kazmierczak, J., Kempe, S., and Altermann, W., 2004. Microbial origin of Precambrian carbonates: lessons from modern analogues. In Eriksson, P., Altermann, W., Nelson, D., Mueller, W., and Catuneanu, O. (eds.), *The Precambrian Earth: Tempos and Events*. Amsterdam: Elsevier, pp. 545–564.
- Kempe, S., 1990. Alkalinity: the link between anaerobic basins and shallow water carbonates? *Naturwissenschaften*, **77**, 426–427.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In Ittekkot, V. A., Kempe, S., Michaelis, W., and Spitz, A. (eds.), *Facets of Modern Biogeochemistry*, (Festschrift for E.T. Degens). Berlin: Springer-Verlag, pp. 255–278.
- Kempe, S., and Kazmierczak, J., 1993. Satonda Crater Lake, Indonesia: Hydrogeochemistry and biocarbonates. *Facies*, **28**, 1–32.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Bulletin de l'Institut océanographique, Monaco, no. spec. 13, 61–117.
- Kempe, S., and Kazmierczak, J., 1997. A terrestrial model for an alkaline martian hydrosphere. *Planetary and Space Science*, **45**, 1493–1499.
- Kempe, S., and Kazmierczak, J., 2003. Modern soda lakes: model environments for an early alkaline ocean. In Müller, T., and Müller, H. (eds.), *Modelling in Natural Sciences; Design, Validation and Case Studies*. Berlin: Springer-Verlag, pp. 309–322.
- Kempe, S., Kazmierczak, J., Landmann, G., Konuk, T., Reimer, A., and Lipp, A., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kraml, M., and Bull, A., 1998/1999. Sodaseen im Ostafrikanischen Graben – ihre Entstehung und Bedeutung. *Berichte der Naturforschenden Gesellschaft zu Freiburg i. Br.*, **88/89**, 85–118.
- Morse, J. W., Gledhill, W. K., and Millero, F. J., 2002. CaCO₃ precipitation kinetics in waters from the Grand Bahama Bank: implications for the relationship between hydrochemistry and whittings. *Abstracts of the 6th International Symposium on the Geochemistry of Earth's Surface*, May 20–24, 2002, Honolulu, Hawaii, pp. 138–140.

- Parkhurst, D. L., Thorstenson, D. C., and Plummer, L. N., 1990. PHREEQE – A computer program for geochemical calculation. (Conversion and upgrade of the prime version of PHREEQE to IBM PC-compatible systems by Tirisanni, J. V., Glynn, P. D.,) US Geological Survey and Water Research Reports 80–96, 197 pp.
- Pegler, K., and Kempe, S., 1988. The carbonate system of the North Sea: determination of alkalinity and TCO_2 and calculation of PCO_2 and Sical (Spring 1986). In Kempe, S., Liebezeit, G., Dethlefsen, V., and Harms, U. (eds.), *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications to Fisheries Biology*. Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, SCOPE/UNEP Sonderband, **65**, pp. 35–87.
- Reimer, A., 1995. *Hydrochemie und Geochemie der Sedimente und Porenwasser des hochalkalinen Van Sees in der Osttürkei*, Faculty of Geosciences. Unpublished PhD Theses, University of Hamburg, 136 pp.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, Eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Ocean Hypothesis
 Tufa, Freshwater

SODA OCEAN HYPOTHESIS

Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The “soda ocean hypothesis” (SOH) stands for the concept of an early (i.e., in essence Precambrian) alkaline or even highly “alkaline ocean,” in analogy to the chemistry of the present-day “soda lakes.”

Soda ocean hypothesis (SOH)

The SOH has been advanced in biology (e.g., Snyder and Fox, 1975) for biochemical reasons before it was developed in earth sciences for geochemical reasons (Kempe and Degens, 1985; Kempe et al., 1989; Kempe and Kazmierczak, 1994).

In *biology*, the SOH rests on the observations that certain reactions considered essential for biogenesis would be favored by alkaline conditions (e.g., Abelson, 1966). One of those is the experimental observation that peptide bonds are more stable in alkaline than in acidic environments (e.g., Dose and Rauchfuss, 1972).

In *earth sciences*, the SOH rests on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes. These arguments are in short:

1. A CO_2 -rich atmosphere in the presence of water would react vigorously with the highly meta-stable, widespread, fine-grained glassy silicates from impacts and volcanic eruptions as would be present during the period of the terminal cataclysm in the Hadean. Carbonic acid weathering of silicates (“Urey Reaction”) will liberate cations in equivalence to the consumed carbonic acid, producing bicarbonate and carbonate as anions.
2. Continental silicate weathering today (aided by high soil PCO_2 due to biological activity; $\text{PCO}_2 = \text{CO}_2$ Pressure) consumes annually $0.15 \cdot 10^9 \text{ t C}$ (Hartmann et al., 2009); thus today’s volume of atmospheric CO_2 would be consumed in about 5,000 years by the Urey Reaction. This rate suggests that a high PCO_2 atmosphere is not stable on geological timescales.
3. Other primordial acids, such as HCl , have much less mass and therefore the primordial ocean must have had TA (total alkalinity $\equiv [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] > [\text{Cl}^-]$).
4. Sulfur-based acids were not available in larger quantities since not enough free oxygen was in the system to allow formation of either sulfurous or sulfuric acids.
5. The entire mass of carbon available in crustal compartments (i.e., $65.5 \cdot 10^{21} \text{ gC}$) as carbonates and organic carbon today could in principle have been dissolved as alkali carbonates in the oceans - as long as H_2O and CO_2 were degassed at similar rates - because this would amount to $48.5 \text{ gC/kg H}_2\text{O}$ while Na_2CO_3 is solvable at $53 \text{ gC/kg H}_2\text{O}$ (Kempe and Degens, 1985).
6. Observations of modern soda lakes show that their tributaries have inputs of $[\text{Na}^+] + [\text{K}^+] > [\text{Cl}^-] + [\text{SO}_4^{2-}]$ and $[\text{Ca}^{2+}] + [\text{Mg}^{2+}] < [\text{TA}]$ (Garrels and Mackenzie, 1967). When evaporated these waters will become alkaline because Ca and Mg carbonates (and sulfates in modern settings) will reach saturation first and Na and K carbonates will stay in solution causing increasingly higher alkalinity and pH. Soda lakes (i.e., lakes with an excess in Na-carbonates) almost exclusively occur today in or near volcanic regions. The early ocean (as well as possible primordial oceans on other planets such as Mars; Kempe and Kazmierczak, 1997) could have reacted the same way, storing CO_2 as TA as quickly as CO_2 was degassed from the mantle or deposited on Earth by comets.

These arguments are based on simple acid–base reactions but have several *derivatives*:

1. The atmosphere could not have had a high enough PCO_2 to counterbalance the lower insolation of the “faint early sun”.
2. In alkaline waters SiO_2 can be kept in solution in high concentrations (at least in the primordial seas where

- Parkhurst, D. L., Thorstenson, D. C., and Plummer, L. N., 1990. PHREEQE – A computer program for geochemical calculation. (Conversion and upgrade of the prime version of PHREEQE to IBM PC-compatible systems by Tirisanni, J. V., Glynn, P. D.,) US Geological Survey and Water Research Reports 80–96, 197 pp.
- Pegler, K., and Kempe, S., 1988. The carbonate system of the North Sea: determination of alkalinity and TCO_2 and calculation of PCO_2 and Sical (Spring 1986). In Kempe, S., Liebezeit, G., Dethlefsen, V., and Harms, U. (eds.), *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications to Fisheries Biology*. Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, SCOPE/UNEP Sonderband, **65**, pp. 35–87.
- Reimer, A., 1995. *Hydrochemie und Geochemie der Sedimente und Porenwasser des hochalkalinen Van Sees in der Osttürkei*, Faculty of Geosciences. Unpublished PhD Theses, University of Hamburg, 136 pp.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, Eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Ocean Hypothesis
 Tufa, Freshwater

SODA OCEAN HYPOTHESIS

Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The “soda ocean hypothesis” (SOH) stands for the concept of an early (i.e., in essence Precambrian) alkaline or even highly “alkaline ocean,” in analogy to the chemistry of the present-day “soda lakes.”

Soda ocean hypothesis (SOH)

The SOH has been advanced in biology (e.g., Snyder and Fox, 1975) for biochemical reasons before it was developed in earth sciences for geochemical reasons (Kempe and Degens, 1985; Kempe et al., 1989; Kempe and Kazmierczak, 1994).

In *biology*, the SOH rests on the observations that certain reactions considered essential for biogenesis would be favored by alkaline conditions (e.g., Abelson, 1966). One of those is the experimental observation that peptide bonds are more stable in alkaline than in acidic environments (e.g., Dose and Rauchfuss, 1972).

In *earth sciences*, the SOH rests on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes. These arguments are in short:

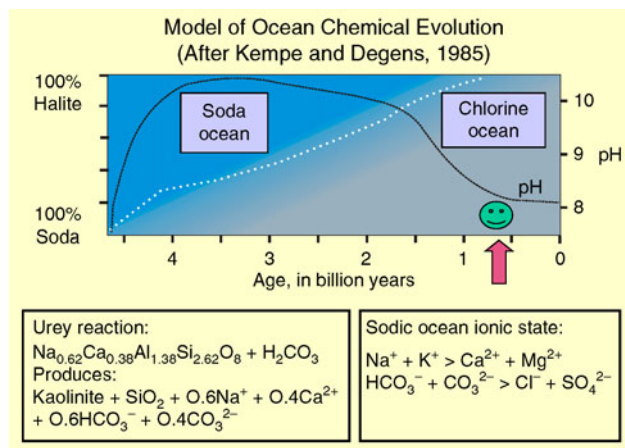
1. A CO_2 -rich atmosphere in the presence of water would react vigorously with the highly meta-stable, widespread, fine-grained glassy silicates from impacts and volcanic eruptions as would be present during the period of the terminal cataclysm in the Hadean. Carbonic acid weathering of silicates (“Urey Reaction”) will liberate cations in equivalence to the consumed carbonic acid, producing bicarbonate and carbonate as anions.
2. Continental silicate weathering today (aided by high soil PCO_2 due to biological activity; $\text{PCO}_2 = \text{CO}_2$ Pressure) consumes annually $0.15 \cdot 10^9 \text{ t C}$ (Hartmann et al., 2009); thus today’s volume of atmospheric CO_2 would be consumed in about 5,000 years by the Urey Reaction. This rate suggests that a high PCO_2 atmosphere is not stable on geological timescales.
3. Other primordial acids, such as HCl , have much less mass and therefore the primordial ocean must have had TA (total alkalinity $\equiv [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] > [\text{Cl}^-]$).
4. Sulfur-based acids were not available in larger quantities since not enough free oxygen was in the system to allow formation of either sulfurous or sulfuric acids.
5. The entire mass of carbon available in crustal compartments (i.e., $65.5 \cdot 10^{21} \text{ gC}$) as carbonates and organic carbon today could in principle have been dissolved as alkali carbonates in the oceans - as long as H_2O and CO_2 were degassed at similar rates - because this would amount to $48.5 \text{ gC/kg H}_2\text{O}$ while Na_2CO_3 is solvable at $53 \text{ gC/kg H}_2\text{O}$ (Kempe and Degens, 1985).
6. Observations of modern soda lakes show that their tributaries have inputs of $[\text{Na}^+] + [\text{K}^+] > [\text{Cl}^-] + [\text{SO}_4^{2-}]$ and $[\text{Ca}^{2+}] + [\text{Mg}^{2+}] < [\text{TA}]$ (Garrels and Mackenzie, 1967). When evaporated these waters will become alkaline because Ca and Mg carbonates (and sulfates in modern settings) will reach saturation first and Na and K carbonates will stay in solution causing increasingly higher alkalinity and pH. Soda lakes (i.e., lakes with an excess in Na-carbonates) almost exclusively occur today in or near volcanic regions. The early ocean (as well as possible primordial oceans on other planets such as Mars; Kempe and Kazmierczak, 1997) could have reacted the same way, storing CO_2 as TA as quickly as CO_2 was degassed from the mantle or deposited on Earth by comets.

These arguments are based on simple acid–base reactions but have several *derivatives*:

1. The atmosphere could not have had a high enough PCO_2 to counterbalance the lower insolation of the “faint early sun”.
2. In alkaline waters SiO_2 can be kept in solution in high concentrations (at least in the primordial seas where

- silica-sequestering organisms such as sponges, diatoms, silicoflagellates, and radiolarians were absent).
3. The higher the TA becomes, the lower the concentration of the free Ca-ion will become because of the fact that the IAP (the ion activity product) governing CaCO_3 solubility, i.e., $\text{IAP} = [\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}]$ is dominated by the carbonate ion.
 4. In the absence of biologically controlled biomineralization (as was typical for the entire Precambrian until, at the onset of the Cambrian, enzymatic biocalcification evolved) the ocean must have been of a very high supersaturation, since the spontaneous precipitation of CaCO_3 -minerals is kinetically inhibited in natural settings. Saturation is most commonly expressed as the saturation index (SI), i.e., the quotient between the IAP and the solubility product of the mineral (K_{Mineral}). $\text{SI} = \log \text{IAP}/K_{\text{Mineral}}$. A survey of nonenzymatic carbonate CaCO_3 -precipitating environments (Kempe and Kazmierczak, 1990; Kazmierczak and Kempe, 2006) shows that these environments have an SI of +0.8 to +1 (i.e., a 10-times supersaturation) while the modern ocean has a much lower supersaturation (North Sea $\text{SI}_{\text{calcite}}$ average summer 1986 = 0.567 ± 0.096 ; Pegler and Kempe, 1988) because it is controlled by enzymatic reactions and not by inorganic precipitation. Dolomite can be supersaturated to $\text{SI} = 4$, i.e., 10,000 times its solubility product in soda lakes (e.g., Kempe and Kazmierczak, 2007). This fact, i.e., the higher carbonate mineral supersaturation of the primordial ocean is today accepted widely (e.g., Grotzinger, 1990; Arp et al., 2001).

Because the soda ocean has vanished and the present day halite ocean prevails, there must have been a transition between the two (Figure 1). This transition must have been driven by a process with a long time constant. This process



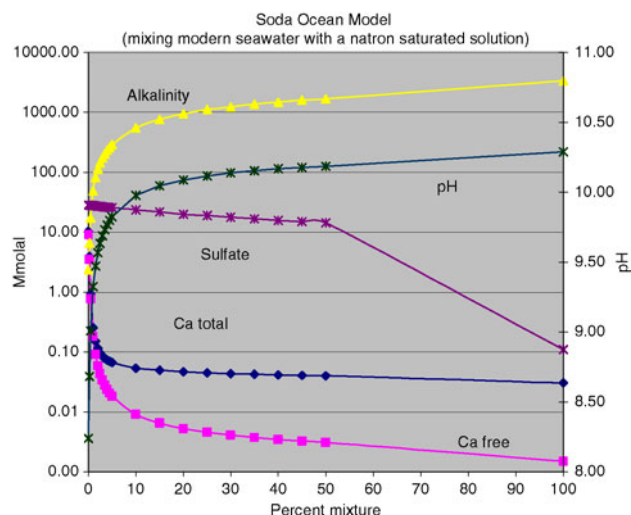
Soda Ocean Hypothesis, Figure 1 Simple representation of the timing of the rise and fall of the soda ocean due to the Urey reaction. The happy face marks the onset of multicellularity triggered by a massive increase of the free calcium ion towards the end of the Precambrian.

is thought to be the subduction of seawater with marine crust and sediments in subduction zones and the consecutive formation of continental crust. About 1 km^3 of seawater is subducted per year today. Any compound dissolved in the ocean has therefore a half-life of about 1 billion years, depending on the rate of subduction. Thus the soda ocean could in fact have prevailed for several billion years before being gradually diminished. According to the soda ocean model (Kempe and Degens, 1985), Na and K would have been transferred into feldspars of continental granodiorites and the carbon would have been deposited as CaCO_3 and kerogen on the continents where they are stored for longer times than deep sea deposits that are lost to subduction within less than 100 million years. The chloride subducted would be recycled as volcanic HCl, in a cycle quicker than continental weathering.

Consequences for biogenesis

The alkaline model has also a series of consequence for *biogenesis* and the evolution of life in the first 3.5 billion years of Earth history.

1. Thermodynamic calculations show that in highly alkaline solutions (up to the saturation of Na_2CO_3), the free $[\text{Ca}^{2+}]$ can be as low as $4 \cdot 10^{-7} \text{ mol}$ (Kazmierczak and Kempe, 2004), while, due to ion-pairs, total Ca is significantly higher. In the model calculation shown in Figure 2 seawater (i.e., a solution with high chloride and sulfate) was mixed with the natron-saturated solution based on the dilute water from Nemrut Crater Lake (-10 m), a “soda lake in the making” (see entry “*Soda Lakes*” Table 3) that leads to a free $[\text{Ca}^{2+}]$ of $1.5 \cdot 10^{-6} \text{ mol}$. Very low environmental Ca^{2+} concentrations are favorable for biogenesis because cells have to maintain a $[\text{Ca}^{2+}] < 10^{-6} \text{ mol}$ in their cytosol (e.g., Kretsinger, 1983; Carafoli, 1987). Otherwise, the proteins would be denaturalized and lose their ability to function. Thus the formation of life in an acidic and consequentially high $[\text{Ca}^{2+}]$ environment is even more difficult to understand.
2. The only macroscopic fossils throughout the Precambrian (with the exception of the Ediacara fauna and some problematic biostructures) are microbialites (stromatolites, thrombolites), biosedimentary structures produced by colonies of microorganisms. The majority of researchers accept that they have formed by the permineralization of the EPS of biofilms dominated by cyanobacteria (e.g., Riding, 2000; Altermann et al., 2006). This form of CaCO_3 -precipitation is nonenzymatic and the cyanobacteria do not instrumentalize these precipitates for functions. The high CaCO_3 -supersaturation caused by the alkaline marine condition is thus an environment in which these microbialites could have thrived easily. Today soda-lake microbialites are formed by the same processes (e.g., in Lake Van, Kempe et al., 1991; López-García et al., 2005, in Mono Lake, and in Niufo’ou caldera lakes, Kazmierczak and Kempe, 2006) while today’s



Soda Ocean Hypothesis, Figure 2 PHREEQUE mixing experiment of modern seawater (*left*) with the natron saturated Nemrut Crater Lake water (see Table 2 in entry “*Soda Lakes*”) (*right*), illustrating the possible transition of an alkaline ocean (*right*) to one dominated by halite and gypsum (*left*). After linear mixing of constituents, the solutions are adjusted to have a calcite saturation index of +1 and a $p\text{CO}_2$ of 3.5 (roughly the present atmospheric PCO_2). Note that the sharp drop in Ca concentration is accompanied by only a slight increase in alkalinity. Note also that the sulfate concentration would describe an exponential curve between 50 and 100% if subsamples were calculated for that section of the mixing experiment.

marine stromatolites are apparently growing predominantly by trapping and binding of allogene particles (e.g., Ginsburg, 1991).

- For three billion years cyanobacteria seem to be among the primary photosynthetic organisms. Most modern cyanobacteria are alkalophiles, suggesting that their evolution proceeded in alkaline environments.
- The development of eukaryotic organisms in the late Palaeoproterozoic may have also been triggered by the early ocean conditions: The gradual increase in the $[\text{Ca}^{2+}]$ ambient concentration due to the slow transition between the soda and halite dominated oceans, could have caused the geochemical stress that might have influenced the evolution of a cell nucleus by protecting the genetic material against the disruptive Ca^{2+} -influx.
- And even the onset of multicellular organisms may have been triggered by the increasing $[\text{Ca}^{2+}]$. Experiments with sponges suggest that adhesion of cells can be triggered by an increase in $[\text{Ca}^{2+}]$ in the environment (e.g., Kretsinger, 1977). Thus the appearance of faunas in Ediacara times may be the indication that the soda ocean was near its demise.
- The biomineralization that appears in many different taxa at the turn of the Precambrian/Cambrian is, in view of the SOH, a detoxification reaction to the

rapidly rising $[\text{Ca}^{2+}]$ when the ocean, as TA decreases (e.g., from 10.3 to 2.38 meq/l as in present seawater), goes through a sort of titration endpoint triggering a >10-fold increase in $[\text{Ca}^{2+}]$ (Kempe and Kazmierczak, 1994). Measurements of Ca in fluid inclusions seem to give this facet of the SOH credibility (Brennan et al., 2004; Petrychenko et al., 2005).

Counterarguments

Some arguments against the SOH were brought forward. These, however, do not really disprove the SOH:

- Models of early Earth often prescribe a long-lasting high PCO_2 -atmosphere to keep the oceans from freezing. However, it may also be argued that the lack of free oxygen in the oceans and atmosphere could have allowed for higher methane concentrations that prevented permanent freezing of Earth.
- Möller and Bau (1993), Bau and Möller (1994) state that the water of Lake Van has a positive cerium anomaly but Precambrian carbonates (or BIFs) do not. However, these authors compare water with sediments. Measurements showed that Lake Van sediments do not exhibit the same anomaly as the water body (own data, unpublished).

Outlook

The *acceptance of the SOH* in the literature is mixed. Some authors agree with it in essence (Arp et al., 2001), others accept certain aspects (such as the high CaCO_3 -supersaturation; Grotzinger and Kasting, 1993; Riding, 2000; or Mg/Ca ratios; Ries et al., 2008), and some doubt its relevance and follow own modeling lines (e.g., Morse and Mackenzie, 1998). Many papers deal with questions of the rising oxygen and sulfate, redox-reactions or certain sedimentary problems like BIF-formation or microbialite (stromatolite) structures without recurring to the overall chemical evolution of the ocean. Although the majority of researchers tend to ignore the SOH, it found entrance into several textbooks of general geology (Degens, 1989; Einsele, 1992; Bahlburg and Bretkreuz, 1998). Digital modeling appears to be not of much help - apart from stating thermodynamic conditions correctly - since the results depend on the adopted boundary conditions of the Hadean and Archean environment, which are as yet not well defined. For example, when prescribing certain PCO_2 values to avoid freezing of oceans because of the faint early sun, then no highly alkaline ocean can evolve. Future research will have to show which one of the many models explains the past of our planet and its geological and biological evolution best.

Summary

Resting on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes, the soda ocean hypothesis (SOH) states that the Precambrian ocean was alkaline, with a total alkalinity $> \text{Ca} + \text{Mg}$

and a high supersaturation of carbonate minerals (SI ca. 1). The soda ocean was lost slowly by subduction of seawater and replaced by the present halite-dominated ocean toward the end of the Precambrian. This facilitated the development of multicellularity and biomineralization as detoxification reactions of life against the rising Ca-concentrations. The SOH is also consistent with the widespread occurrence of stromatolites and cherts in the Precambrian.

Bibliography

- Abelson, P. H., 1966. Chemical events on the primitive Earth. *Proceedings of the National Academy of Sciences USA*, **55**, 1365–1372.
- Altermann, W., Kazmierczak, J., Oren, A., and Wright, D. T., 2006. Cyanobacterial calcification and its rock-building potential during 3.5 billion years of earth history. *Geobiology*, **4**, 147–166.
- Arp, G., Reimer, A., and Reiter, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Bahlburg, G., and Breitzkreuz, C., 1998. *Grundlagen der Geologie*. Stuttgart: F. Enke.
- Bau, M., and Möller, P., 1994. Präkambrische chemisch-sedimentäre Mineralisationen. *Geowissenschaften*, **12**, 333–336.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Carafoli, E., 1987. Intracellular calcium homeostasis. *Annual Review of Biochemistry*, **56**, 395–433.
- Degens, E. T., 1989. *Perspectives on Biogeochemistry*. Berlin: Springer-Verlag.
- Dose, K., and Rauffuss, H., 1972. On the electrophoretic behavior of thermal polymers of amino acids. In Rohlfsing, D. L., Oparin, A. I. (eds.), *Molecular Evolution: Prebiological and Biological*. New York: Plenum Press, pp. 1–199.
- Einsele, G., 1992. *Sedimentary Basins*. Berlin: Springer-Verlag.
- Garrels, R. M., and Mackenzie, F. T., 1967. Origin of the chemical composition of some springs and lakes. In *Equilibrium Concepts of Natural Water Systems. Advances in Chemistry*, 67. American Chemical Society, pp. 222–242.
- Ginsburg, R. N., 1991. Controversies about stromatolites: Vices and virtues. In Müller, D. W., McKenzie, J. A., and Weissert, H. (eds.), *Controversies in Modern Geology*. London: Academic Press, pp. 25–36.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. In Knoll, A. H., and Ostrom, J. H. (eds.), *Proterozoic Evolution and Environments*, American Journal of Science (P. E. Cloud Special Volume), 290-A, pp. 80–104.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Hartmann, J., Kempe, S., Dürr, H. H., and Jansen, N., 2009. Global CO₂-consumption by chemical weathering: What is the contribution of highly active weathering regions? *Global and Planetary Change*, **69**, 185–194.
- Kazmierczak, J., and Kempe, S., 2004. Calcium build-up in the Precambrian sea: A major promoter in the evolution of eukaryotic life. In Seckbach, J. (ed.), *Origins, Evolution and Biodiversity of Microbial Life*. Dordrecht: Kluwer, pp. 329–345.
- Kazmierczak, J., and Kempe, S., 2006. Modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In Ittekkot, V. A., Kempe, S., Michaelis, W., and Spitz, A. (eds.), *Facets of Modern Biogeochemistry* Festschrift for E.T. Degens on occasion of his 60th birthday, Berlin: Springer-Verlag, pp. 255–278.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Monaco: Bulletin de l'Institut océanographique, no. spec. 13, pp. 61–117.
- Kempe, S., and Kazmierczak, J., 1997. A terrestrial model for an alkaline martian hydrosphere. *Planetary and Space Science*, **45**, 1493–1499.
- Kempe, S., and Kazmierczak, J., 2003. Modern soda lakes: Model environments for an early alkaline ocean. In Müller, T., and Müller, H. (eds.), *Modelling in Natural Sciences; Design, Validation and Case Studies*. Berlin: Springer-Verlag, pp. 309–322.
- Kempe, S., and Kazmierczak, J., 2007. Hydrochemical key to the genesis of calcareous non-laminated and laminated cyanobacterial microbialites. In Seckbach, J. (ed.), *Algae and Cyanobacteria in Extreme Environments*. Berlin: Springer-Verlag, pp. 241–264.
- Kempe, S., and Pegler, K., 1991. Sinks and sources of CO₂ in coastal seas: the North Sea. *Tellus*, **43B**, 224–235.
- Kempe, S., Kazmierczak, J., and Degens, E. T., 1989. The soda ocean concept and its bearing on biotic and crustal evolution. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomineralization in Plants and Animals* Proceedings of the 5th International Symposium Biomineralization, Arlington, Texas, May, 1986, New York: Plenum Press, pp. 29–43.
- Kempe, S., Kazmierczak, J., Landmann, G., Konuk, T., Reimer, A., and Lipp, A., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kretsinger, R. H., 1977. Evolution of the informational role of calcium in eukaryotes. In Wasserman, R. H., Corradino, R. A., Kretsinger, R. H., MacLennan, D. H., and Siegel, F. L. (eds.), *Calcium Binding Proteins and Calcium Function*. New York: North Holland Publishing, pp. 63–7.
- Kretsinger, R. H., 1983. A comparison of the roles of calcium in biomineralization and in cytosolic signaling. In Westbroek, P., and De Jong, E. W. (eds.), *Biomineralization and Biological Metal Accumulation*. Dordrecht: D. Reidel Publishing Co., pp. 123–131.
- López-García, P., Kazmierczak, J., Benzerara, K., Kempe, S., Guyot, F., and Moreira, D., 2005. Bacterial diversity and carbonate precipitation in the microbialites of the highly alkaline Lake Van, Turkey. *Extremophiles*, **9**, 263–274.
- Möller, P., and Bau, M., 1993. Rare-earth patterns with positive cerium anomaly in alkaline waters from Lake Van, Turkey. *Earth and Planetary Science Letters*, **117**, 671–676.
- Morse, J. W., and Mackenzie, F. T., 1998. Hadean ocean carbonate geochemistry. *Aquatic Geochemistry*, **4**, 301–319.
- Pegler, K., and Kempe, S., 1988. The carbonate system of the North Sea: Determination of alkalinity and TCO₂ and calculation of PCO₂ and SICal (Spring 1986). In Kempe, S., Liebezeit, G., Dethlefsen, V., and Harms, U. (eds.), *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications to Fisheries Biology*, Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, SCOPE/UNEP Sonderband, **65**, pp. 35–87.
- Petrychenko, O. Y., Peryt, T. M., and Chechel, E. I., 2005. Early Cambrian water chemistry from fluid inclusions in halite from Siberian evaporates. *Chemical Geology*, **219**, 149–161.

- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Ries, J. B., Anderson, M. A., and Hill, R. T., 2008. Seawater Mg/Ca controls polymorph mineralogy of microbial CaCO_3 : A potential for calcite-aragonite seas in Precambrian time. *Geobiology*, **6**, 106–119.
- Snyder, W. D., and Fox, S. W., 1975. A model for the origin of stable protocells in a primitive alkaline ocean. *Biosystems*, **7**, 222–229.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Lakes
 Tufa, Freshwater

SOILS

Erika Kothe
 Friedrich Schiller University Jena, Jena, Germany

Synonyms

Pedosphere

Definition

Soil: refers to the product of mineral weathering and secondary mineral formation, (microbial) mineralization, humus formation, and the resulting element mobilization/immobilization in the upper Earth crust in a pedogenetic process involving chemical, physical, and biological activities.

Introduction

Soil is the basis of terrestrial life, particularly for agriculture, forestry, and generally land-use by man (Driessen et al., 2001). It is also the largest terrestrial ecosystem dominated by high numbers of microorganisms and soil-living animals as well as the root systems of plants. The biota within this ecosystem, not only interact but also actively shape their environment (Fiedler et al., 2002). This can be easily seen with root systems or earthworm/mole tunnels, but the metabolic activities of microorganisms by far exceed these visible alterations. Microbes are essential for the decomposition of plant litter followed by humification. The pedogenesis differs with respect to base rock material, climatic conditions, and other factors leading to soil series of different soil types. Root exudates as well as microbiological acidification and release of

chelating agents take part in mobilization and immobilization processes of mineral elements, essential or ecotoxically relevant alike, and thus are major players in import into food chains, and input into water paths or as volatiles into the atmosphere.

Soil function

The soils of the world can be characterized from a more structural or a more functional point of view (FAO-UNESCO, 2002), and the geobiologically important functions involving metabolically active soil dwellers are characterized with reference to microbiology, botany and agriculture, and zoology.

Soil function is essential for plant nutrition. The capacity to solubilize nutrients like P and minerals including K, Na, Mg, Ca, and trace elements, as well as availability of S or N are largely the product of microbial mobilization from minerals, microbial degradation of organic matter, or fixation from air (e.g., for nitrogen). Soil mineralogy largely determines the buffering capacity for minerals adsorbed to different fractions (Jones and Bassington, 1998). The sustainability of microbial activity is connected to water availability for growth, and water storage and availability are also major functions for plant growth. Detrimental processes of soil degeneration like soil acidification, desertification, clay mineral destruction, or decrease of organic contents are global problems mankind experiences which will have a large impact on socioeconomic scales.

Agriculture and nutrition in modern systems are coupled to the use of fertilizers and plant protection chemicals which can constitute a threat to the water pathways, if management of agricultural use is not performed according to the laws and provisions on soil and water protection, good agricultural practice, or integrated farming, and monitoring has to be included to ensure best practice. Soil density increases due to the use of heavy machinery which leads to soil degradation in high-yield agricultural systems the world over.

In order to assess possible problems with land-use and soil degeneration, soil science has provided a very good reference system to classify and monitor soils based on soil mechanics, soil physics, soil genesis, soil composition, and soil functions reviewed in different contexts below (IUSS Working Group WRB, 2006). As soil can be regarded as a major sink for carbon depending on land-use, this might become more important as climate change discussions are gaining impact. Generally, soils and larger views on landscape should be regarded as eminent players in global cycles (Meir et al., 2006).

Soil texture

The soil texture can be divided in the proportion of sand (S: 63 μm –2 mm), silt (U: 2–63 μm), and clay (T: smaller than 2 μm) after removing skeleton particles (larger than 2 mm). While sand soils have more than 70 volume % of sand, clay soil is mainly composed of clay (>50%) with

- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Ries, J. B., Anderson, M. A., and Hill, R. T., 2008. Seawater Mg/Ca controls polymorph mineralogy of microbial CaCO_3 : A potential for calcite-aragonite seas in Precambrian time. *Geobiology*, **6**, 106–119.
- Snyder, W. D., and Fox, S. W., 1975. A model for the origin of stable protocells in a primitive alkaline ocean. *Biosystems*, **7**, 222–229.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Lakes
 Tufa, Freshwater

SOILS

Erika Kothe
 Friedrich Schiller University Jena, Jena, Germany

Synonyms

Pedosphere

Definition

Soil: refers to the product of mineral weathering and secondary mineral formation, (microbial) mineralization, humus formation, and the resulting element mobilization/immobilization in the upper Earth crust in a pedogenetic process involving chemical, physical, and biological activities.

Introduction

Soil is the basis of terrestrial life, particularly for agriculture, forestry, and generally land-use by man (Driessen et al., 2001). It is also the largest terrestrial ecosystem dominated by high numbers of microorganisms and soil-living animals as well as the root systems of plants. The biota within this ecosystem, not only interact but also actively shape their environment (Fiedler et al., 2002). This can be easily seen with root systems or earthworm/mole tunnels, but the metabolic activities of microorganisms by far exceed these visible alterations. Microbes are essential for the decomposition of plant litter followed by humification. The pedogenesis differs with respect to base rock material, climatic conditions, and other factors leading to soil series of different soil types. Root exudates as well as microbiological acidification and release of

chelating agents take part in mobilization and immobilization processes of mineral elements, essential or ecotoxically relevant alike, and thus are major players in import into food chains, and input into water paths or as volatiles into the atmosphere.

Soil function

The soils of the world can be characterized from a more structural or a more functional point of view (FAO-UNESCO, 2002), and the geobiologically important functions involving metabolically active soil dwellers are characterized with reference to microbiology, botany and agriculture, and zoology.

Soil function is essential for plant nutrition. The capacity to solubilize nutrients like P and minerals including K, Na, Mg, Ca, and trace elements, as well as availability of S or N are largely the product of microbial mobilization from minerals, microbial degradation of organic matter, or fixation from air (e.g., for nitrogen). Soil mineralogy largely determines the buffering capacity for minerals adsorbed to different fractions (Jones and Bassington, 1998). The sustainability of microbial activity is connected to water availability for growth, and water storage and availability are also major functions for plant growth. Detrimental processes of soil degeneration like soil acidification, desertification, clay mineral destruction, or decrease of organic contents are global problems mankind experiences which will have a large impact on socioeconomic scales.

Agriculture and nutrition in modern systems are coupled to the use of fertilizers and plant protection chemicals which can constitute a threat to the water pathways, if management of agricultural use is not performed according to the laws and provisions on soil and water protection, good agricultural practice, or integrated farming, and monitoring has to be included to ensure best practice. Soil density increases due to the use of heavy machinery which leads to soil degradation in high-yield agricultural systems the world over.

In order to assess possible problems with land-use and soil degeneration, soil science has provided a very good reference system to classify and monitor soils based on soil mechanics, soil physics, soil genesis, soil composition, and soil functions reviewed in different contexts below (IUSS Working Group WRB, 2006). As soil can be regarded as a major sink for carbon depending on land-use, this might become more important as climate change discussions are gaining impact. Generally, soils and larger views on landscape should be regarded as eminent players in global cycles (Meir et al., 2006).

Soil texture

The soil texture can be divided in the proportion of sand (S: 63 μm –2 mm), silt (U: 2–63 μm), and clay (T: smaller than 2 μm) after removing skeleton particles (larger than 2 mm). While sand soils have more than 70 volume % of sand, clay soil is mainly composed of clay (>50%) with

only small portions of sand (<20%). Most soils in humic conditions are sandy loam (with at least 1/3 sand, 1/3 silt, and up to 1/3 loam) or sandy silt soils (with at least 1/3 sand, at least 1/3 silt, and lower amounts of loam).

These differences in texture are also reflected in mineral composition of the respective soils. While sand soil contains 80% of quartz, this mineral constitutes only 25% of clay soil. In contrast, 50% clay minerals (mostly illit and vermiculite, as well as smaller portions of montmorillonite and kaolinit) are present in clay soil while sand soil contains only 5% of clay minerals. As clay minerals show the highest buffering potential for water and mineral elements, like alkali and earth alkali elements, soil types with higher clay mineral contents show high sorption capacities. Sandy loam/silt shows the highest fertility because of a harmonized ration between water storage and sorption capacities.

Water-retention potentials are of major importance for soil functioning. While sand soil has a water capacity of only 10 volume %, 2/3 of that water (7 volume %) is bio-available. Loam has 45 volume % water capacity of which, however, only a small proportion, usually less than 12 volume %, is available to plant roots. Sand soil has the highest aeration with 30 volume % of gas-filled pores. This is dependent on the pore sizes. Pores that are smaller than 10 μm retain water. Large pores of over 50 μm diameter, in contrast, are air-filled and contain only adsorption water. Capillary water in unsaturated soil is found in pores of 5–10 μm diameter. This is important for the soil function with respect to plant growth. As plant root hairs can just about enter pores of 5–10 μm , most water transport in soil is not simply taken from water-filled pores, but has to be extracted with sucking tension. The investigation of in situ soil water, however, remains a challenge (Weihermüller et al., 2007).

Soil type

Soil types are distinguished depending on the development of soil (pedogenesis) over space and time. The formation of a soil type generally takes a long time, upward of 1000 years. The soil type also is dependent on the climatic conditions under which the pedogenesis has taken place. A typical example of this is the formation of laterite soils in tropical areas. Such soil types show strong coloration, with iron-rich red soil types dominating in most areas. This is due to mobilization and wash-out of elements released from minerals and retaining of insoluble iron(hydroxy)oxides.

The function of mineralic, climatic, and biological conditions over time leads to formation of soils defined by different horizons. These can extend over cm up to 100 m and more, depending on the conditions experienced.

Horizons and soil profiles

A visual inspection of a soil profile already leads to immediate distinction of several layers in a typical soil. In an autochthonous soil formation, the lowest typical layer

above groundwater is the C horizon, the base rock on which the soil develops. It presents the basis for the elemental composition of the soil developing on its surface.

The B horizon (subsoil) is the next layer, clearly dominated by weathering products. Immigration of clay and sesquioxides (Fe and Al-oxihydroxides) may be found to dominate such B horizons which is specified in B_v (weathering dominated), B_t (loam accumulation), and B_s (sesquioxides) subclassification.

The uppermost layer is the A horizon (topsoil) which is rich in humus resulting from degradation of leaf litter and often shows a darker color due to organic contents enriched in this horizon. The uppermost layer of humus also is specifically termed A_o horizon. In the A horizon, microbial populations are largest and metabolic activities of soil microorganisms can be shown easily by soil respiration analyses. The A horizon also is the site of dominant plant root penetration which again stabilizes and supports the soil structure as well as the microorganisms present. Again, this horizon can be subdivided into layers A_h or A_1 (uppermost layer rich in humus), A_p (altered upper layer due to ploughing) and A_e or A_2 (subsequent layer influenced by wash-out processes). A layer of litter above the topsoil horizon is formed by decaying plant material. Depending on soil type, not all horizons have to be present.

Soil biology

The animals found in soil, or edaphone, are earthworms, nematodes, insects, specifically collembolids, and moles, to name the most prominent. Their function in soil is especially to mix the soil, to draw leaves under the soil surface which accelerates decay because of higher humidity and better accessibility for microbes, and to form passages for water interflow allowing rain to enter a soil more easily.

Plant roots stabilize soil and protect from wind and water erosion. Decaying plant roots leave passages also allowing for preferential water flow. And living roots contain special tissues for aeration and therefore improve oxygen availability for microorganisms in soil. At the same time, up to 30% of plant net photosynthesis products can be excreted by the roots in the root exudates. These consist mainly of organic acids, like acetate, oxalate, malate, and citrate. These are able to feed microbes in the rhizosphere and also chelate iron and supply the plant with iron. On the other hand, these organic acids, as well as sugars and amino acids form root exudates, provide nutrition for soil microorganisms which multiply in the vicinity of roots. This constitutes the rhizosphere, the specific area found around a plant root (Watt et al., 2006). More directly associated with the plant root are the rhizoplane bacteria which colonize the surface of the plant root. Symbiotic root associations include the nitrogen-fixing rhizobial symbioses with leguminaceous plants, and the mycorrhizal mutual symbioses between fungi and plant roots. It has been stated that microbial populations structure the plant biodiversity seen above-ground by such soil-derived associations (van der Heijden et al., 2007).

The soil surface, if no plants are present, is often covered by a crust consisting of a biofilm with cyanobacteria, fungi, bacteria, and lichens. This crust will lead to covering of bare soils which will then be prone to succession with mosses and plants.

Soil microbiology

Soils are the major place of element turnover and biogeochemical cycling in terrestrial ecosystems (Crawford et al., 2005). The degradation of plant litter, decay of leaves, carcasses, feces, and wood rotting are maintained by soil microorganisms with subsequent processes of humification. In addition, denitrification, nitrogen fixation, and element mobilization are performed by the metabolic activities of soil microorganisms. The formation of humus and the binding of elements with humic and fulvic acids drive soil development, and its function for the soil ecosystem cannot be overestimated.

Populations of soil microorganisms contain eukaryotes, bacteria, and archaea. Depending on the metabolic functions prevailing, the soil generated at the site will be dominated by formation of humic and fulvic acids, like in moorlands, by high mobilization of cations, and the formation of dissolved organic matter (DOC) in luvisols and podzols (in woodlands) or tchernozem (in agricultural land and grasslands). To have an idea of the scale, in 1 hectare soil it has been calculated that 8 tons of microorganisms are present, while the edaphon makes only for 2 tons of biomass. With the smaller size of microbes, this makes for very high numbers of soil microorganisms consisting of eukaryotic, bacterial, and archaeal cells.

The eukaryotic population is dominated by fungi with minor populations of ciliates, flagellates, algae including oomycetes and amoebae including myxomycetes. Of special importance are the mycorrhizal fungi forming a mutual symbiosis with plant roots thereby influencing not only plant physiology, but also soil as the hyphae extending into soil can add to soil structure and texture (Rillig and Mummey, 2006).

The archaea found in soil include especially the anaerobic methanogens which can lead to methane exhalation. However, methane oxidation performed by other groups of related archaea, already sequester a large portion of methane in soils. Methane exhalation is more often seen from swamps and flooded areas.

The major taxa of soil bacteria are the Gram-negative proteobacteria, the cytophaga, and Gram-positive bacilli and actinobacteria. Microbial interactions are a driving force for the community structure (Wardle, 2006). Per gram dry weight of soil, usually 10^7 – 10^9 bacteria are found. However, the bacterial population so far mainly had been analyzed by plating techniques, which will allow only those to be found that are growing under the applied laboratory conditions. Estimates of direct counts have indicated higher numbers with only 1–10% of bacteria being cultivable. Modern techniques relying not on cultivation but on DNA extraction from soil with subsequent

amplification and cloning of 16S rDNA sequences give a better insight in microbial soil populations. New taxa occur by these analyses and the phylogenetic associations observed from the obtained 16S rDNA sequences show that entire branches in the phylogenetic tree of bacteria can be described. It will remain a task to now find possibilities to cultivate these organisms in order to evaluate their physiological properties. Only such new investigations will allow to assess the role of new classes of bacteria in soil function and specific pathways for microbial degradation and soil formation will be uncovered as such investigations and modeling making use of the results will progress (O'Donnell et al., 2007).

Humification

Soil organic matter is the basis for humification (Kögel-Knabner et al., 1990). The characteristics of soil organic matter have been analyzed in some detail (Schaumann, 2006). Humus (Stevenson, 1994) provides essential functions in soils. The formation of humic acids and fulvic acids is essential for A horizon development, and especially the A_h horizon of tchernozem is rich in humus. Both humic acids (brown and grey) and shorter-lived fulvic acids have been described as recalcitrant matter with turnover times of thousands of years. These figures are re-evaluated now as it becomes clear that microbial metabolism uses these recalcitrant substrates and incorporates the carbon moieties into their cell mass. However, it is clear that the humic and fulvic acids constitute a major portion of soil matter and that their specific function in water retention and mineral element adsorption contributes a great deal to soil fertility.

Humus constituents also are important for (temporal) fixation of minerals and organic exudates. Their release will be controlled by the microbiota prevalent in the environment (Watteau and Berthelin, 1994).

Summary

Soil is the basis of terrestrial life and both evolves from biological activities and in turn allows and shapes terrestrial ecosystems. Soil types can be distinguished with different compositions, functionalities, and fertilities for production in agronomy and forestry, or water quality. The development of a soil type, such as tchernozem, rendzina, and podzol (and in tropic conditions, latozol) is dependent on base rock, climate, physical and chemical weathering, and formation of humic substances which takes thousands of years. The pedogenesis, in addition, is a result of root functions and plant litter degradation. The resulting soil can be characterized in vertical direction by soil horizons, which are distinguished from the A horizon with high humus contents, over a B horizon with lower humus contents, to the C horizon mainly consisting of base rock material. Soil series attempts to combine soil types and soil structure to reveal parameters for soil function.

Bibliography

- Crawford, J. W., Harris, J. A., Ritz, K., and Young, J. M., 2005. Towards an evolutionary ecology of life in soil. *Trends in Ecology and Evolution*, **20**, 81–87.
- Driessen, P., Deckers, J., Spaargaren, O., and Nachtergaele, F., 2001. *Lecture Notes on the Major Soils of the World*. World Soil Resources Reports 94, Rome: FAO.
- FAO-UNESCO, 2002. *Digital Soil Map of the World and Derived Soil Properties*. Land and Water Digital Media Series No. 1 Rev. 1. Rome: FAO.
- Fiedler, S. H., Jungkunst, P. F., Kleber, M., Sommer, M., Jahn, R., and Stahr, K., 2002. Linking soil classification and soil dynamics – pedological and ecological perspectives. *Journal of Plant Nutrition and Soil Science*, **165**, 517–529.
- IUSS Working Group WRB, 2006. *World Reference Base for Soil Resources: Atlas*. Leuven, The Netherlands: ISRIC-FAO-ISSS-Acco, p. 63.
- Jones, D. L., and Bassington, D. S., 1998. Sorption of organic acids in acid soils and its implication in the rhizosphere. *European Journal of Soil Science*, **49**, 447–455.
- Kögel-Knabner, I., Hatcher, P. G., and Zech, W., 1990. *Decomposition and Humification Processes in Forest Soils: Implications from Structural Characterization of Forest Soil Organic Matter*. Kyoto, Japan: Trans. 14th Int. Congr. Soil Sci., pp. 218–223.
- Meir, P., Cox, P., and Grace, J., 2006. The influence of terrestrial ecosystems on climate. *Trends in Ecology and Evolution*, **21**, 254–260.
- O'Donnell, A. G., Young, J. M., Rushton, S. P., Shirley, M. D., and Crawford, J. W., 2007. Visualization, modeling and prediction in soil microbiology. *Nature Reviews Microbiology*, **5**, 689–699.
- Rillig, M. C., and Mummey, D. L., 2006. Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.
- Schaumann, G. E., 2006. Soil organic matter beyond molecular structure, part I: macromolecular and supramolecular characteristics. *Journal of Plant Nutrition and Soil Science*, **169**, 145–156.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: Wiley.
- van der Heijden, M. G., Bardgett, R. D., and van Straalen, N. M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.
- Wardle, D. A., 2006. The influence of biotic interactions on soil biodiversity. *Ecology Letters*, **9**, 870–886.
- Watt, M., Silk, W. K., and Passioura, J. B., 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of Botany*, **97**, 839–855.
- Watteau, F., and Berthelin, J., 1994. Microbial dissolution of iron and aluminum from soil minerals: efficiency and specificity of hydroxamate siderophores compared to aliphatic acids. *European Journal of Soil Biology*, **30**, 1–9.
- Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., and Pütz, T., 2007. In situ soil water extraction: a review. *Journal Of Environmental Quality*, **36**, 1735–1748.

Cross-references

Bacterial Clay Autigenesis
 Biogeochemical Cycles
 Carbon Cycle
 Denitrification
 Microbial Degradation
 Microbial-Metal Binding
 Mycorrhizae
 Nitrogen Fixation
 Siderophores

SPECIES (MICROBIAL)

Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “*Microbial Communities, Structure, and Function*” for further reading.

SPELEOTHEMS

Roman Aubrecht
 Comenius University, Bratislava, Slovakia

Synonyms

Cave deposits; Cave sinters

Definition

The word “speleothem” is from Greek and means “cave deposit.” According to its origin, this term can be used for any cave deposits, but it is used mostly for secondary mineral deposits formed (precipitated) in caves. In this entry, speleothem is understood as constructively formed, new cave material. Materials originated by destructive phenomena, such as erosion, weathering, etching, biocorrosion, or bioerosion are not subjects of this entry.

Biogenic and abiogenic speleothems

Most of the speleothems originate inorganically by precipitation of various minerals from solutions. The minerals are dissolved by undersaturated fluids and precipitate when the solutions become supersaturated, e.g., by evaporation, outgassing, or pH changes. Mineralogical compositions of speleothems vary depending on the environment in which the caves are formed. The most common speleothem-forming mineral is calcite (as most of the caves are formed of limestones), forming well-known sinter forms, such as dripstones (stalactites, stalagmites) and flowstones. Locally, mostly in hydrothermally influenced cavern systems, speleothems may be formed by its rhombic modification – aragonite. Gypsum and other sulfate minerals commonly form speleothems in gypsum karst but are widespread also in other types of caves. Speleothems formed in silicate rocks (e.g., sandstones, quartzites, granites, etc., [Figure 1](#)) are relatively rare, composed mostly of various forms of silica (from opal-A to microquartz depending on its recrystallization). Other speleothems can be represented by ferroan and manganese oxides and hydroxides forming cave flowstones.

Silica Biomineralization, Sponges
Sinter
Stromatolites
Sulfate-Reducing Bacteria

SPONGES (PORIFERA) AND SPONGE MICROBES

Friederike Hoffmann¹, Marie-Lise Schläppy²

¹Sars International Centre for Marine Molecular Biology, Bergen, Norway

²Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

Sponge-associated bacteria; Sponge-associated microbes; Sponge-associated microbiota; Sponge bacteria; Sponge-microbe systems; Sponge symbionts

Definition

Sponges are sessile multicellular aquatic animals (Lat. Porifera = bearing pores) whose bodies have a typical body plan which allows water to pass through the animals. Sponges are characterized by a skeleton made of calcareous, siliceous spicules or horny fibers.

Sponge microbes: Bacteria and archaea that live in close and permanent association with sponges, usually in the mesohyl between sponge cells.

Introduction

Sponges are sedentary benthic organisms found in shallow waters on tropical coral reefs to the Arctic and the deep sea. Some species, however, are restricted to freshwater ecosystems. Sponges are characterized by a typical body plan built around a system of water canals and chambers (Figure 1a). Within the chambers, flagellated cells called choanocytes produce a water current that enters the sponge body through surface pores (ostia) and leaves through larger openings called oscula. Hence, sponges are filter feeders and incorporate particles from the water as energy and nutrient source. An external cell layer (pinacoderm) encloses the sponge mesohyl, a glycosidic matrix containing several cell types which perform a variety of functions. Sponge cells show a low degree of specialization and a high degree of independence so that the sponge body in some respect resembles a protozoan colony. However, sponges are, without any doubt, placed as true members of the Metazoan (Ax, 1995; Müller, 1998a, b).

Sponges have been grouped into three classes: Calcarea, Hexactinellida, and Demospongiae. Calcarea are characterized by the presence of calcareous spicules. The upgrade of this class to the phylum level has recently been proposed by Borchiellini et al. (2001). Hexactinellida are characterized by siliceous spicules of hexactine structure and syncytial tissue organization.

Demospongiae, the most numerous and diverse class, is a nonmonophyletic group (Boury-Esnault, 2006). They generally have a mineral skeleton made of siliceous spicules, but several lineages, like the common bath sponge, have no mineral skeleton but a network of fibers instead. Modern sponge phylogeny is now based on a combination of molecular methods and sponge morphological features, but sponge spicules are still important features for species determination and identification of sponges in the fossil record.

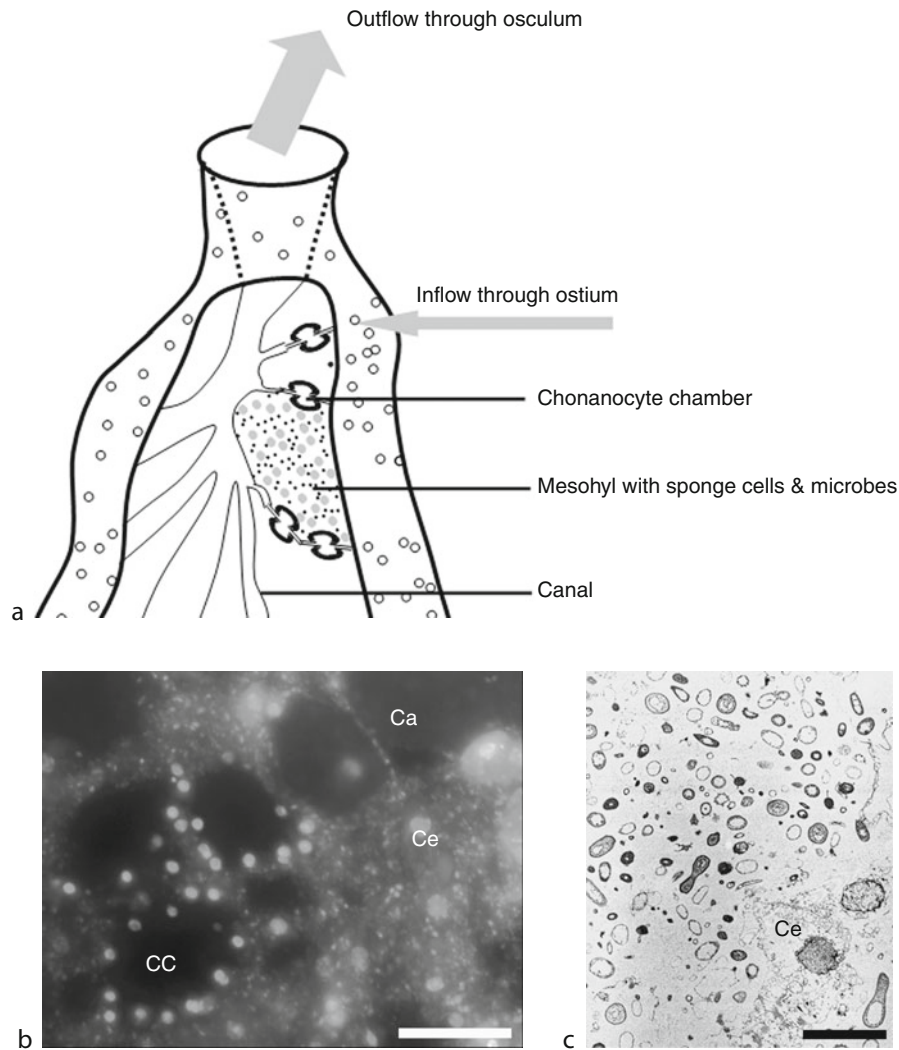
Sponges form one of the deepest radiations of the Metazoa and can be regarded as one of the oldest animal phylum still alive. The spicule record for sponges starts in the late Proterozoic (Reitner and Wörheide, 2002), while chemofossil records even indicate the presence of sponges – or their direct ancestors – already in the early Proterozoic: specific C₃₀ steranes (24-isopropylcholestanes), which are unambiguous biomarkers for demosponges, were found in 1,800 Ma old stromatolites (McCaffrey et al., 1994; Moldowan et al., 1994).

Choanoflagellates, a monophyletic group of protists which can show a colonial lifestyle, are the closest sister group of the Metazoa (Medina et al., 2001) and show a morphological resemblance to the flagellated choanocytes of sponges. It is, therefore, widely agreed that the “urmetazoa,” the last common ancestors of all Metazoa, would have many traits found in sponges (see review in Gaidos et al., 2007).

Numerous sponge species host vast amounts of microbes in their mesohyl matrix (Figure 1b and c) or sometimes in specialized cells, while others contain only few or no microorganisms at all. The former have been termed bacteriosponges (Reiswig, 1981) or high-microbial-abundance sponges (Hentschel et al., 2003) while the latter are referred to as “low-microbial-abundance sponges.” Bacterial population densities in bacteriosponges may reach 10⁸–10¹⁰ microbes per gram of sponge wet weight, while those in low-microbial-abundance sponges are within the range of natural seawater.

The first studies exploring the microbial community of sponges were based on electron microscopy and isolation of microbes and yielded invaluable information about the microorganism morphotypes and their specific location within the sponge (e.g., Wilkinson, 1978). Microbes were found to exist extra- and intra-cellularly (e.g., Vacelet and Donadey, 1977) and even inside the nucleus of sponge cells (Vacelet, 1970). More recently, the advent of molecular techniques have enabled a detailed phylogenetic description of the microorganisms associated with sponges (Hentschel et al., 2006), whereas environmental genomics have given insights into their metabolic and physiological properties (Grozdanov and Hentschel, 2007).

Phylogenetically complex, yet highly sponge-specific microbial communities were identified in numerous species over large geographical ranges (Hentschel et al., 2002). Sequences representing 16 bacterial phyla and both major archaeal lineages have been recovered to date (see



Sponges (Porifera) and Sponge Microbes, Figure 1 The sponge–microbe system (a) schematic representation of a sponge with microbes in the mesohyl (b) fluorescence micrograph of a DAPI stained tissue section of the sponge *Geodia barretti*, CC = choanocyte chamber, Ca = canal, Ce = sponge cells. Large DAPI signals represent nuclei of sponge cells, while small signals represent sponge microbes. Scale bar = 20 μ m (c) transmission electron microscope image of the sponge *G. barretti* showing sponge cells (lower right corner) and numerous, morphologically different sponge microbes. Scale bar = 5 μ m.

review by Taylor et al., 2007; Hentschel et al., 2003, 2006). One of these phyla, the Poribacteria, represents a new bacterial phylum, which is related to the Planctomycetales and has, so far, only been found in sponges (Fieseler et al., 2004, 2006). The other microbial phyla found in sponges are also found in other environments; however, many sponge microbes seem to form, within these phyla, monophyletic, sponge-specific 16S rRNA sequence clusters, which are absent in seawater (Taylor et al., 2007; Hentschel et al., 2003, 2006).

These recent review articles provide an excellent summary of our current knowledge about the diversity and phylogenetic affiliation of sponge microbes, and this topic will therefore not be addressed here in more detail. This article, in contrast, aims to focus on the geobiological

aspects of the sponge–microbe system: the question of a long-standing relationship between sponges and microbes; the consequences of fluctuating oxygen concentrations in sponge tissue for the sponge–microbe system; and the nature and possible function of microbial processes within sponges.

Sponge microbes: secret passengers through evolutionary history?

Did sponges and their associated microbes have an ancient partnership which remained unchanged since the origin of sponges (Precambrian), or is it a more recent but stable association which pertains to date? In an extensive review of this topic, Taylor et al. (2007) propose three scenarios which may explain the occurrence of sponge-specific

microbes. Firstly, it can be the product of a symbiosis of ancient origin perpetuated through the vertical (i.e., from adult to embryo) transmission of sponge-specific microorganisms. Secondly, the association can be the product of environmental acquisition through specific (immune system recognition, presence of favorable substrate, or bacterial protective capsule) or unspecific enrichment (rare sea-water microbes get trapped, accumulated and become thus detectable in the sponge). Finally, a combination of vertical and environmental transmissions is also possible.

The strongest evidence for a long-standing, close symbiotic relationship between sponges and some microorganisms comes from the demonstration of coevolution by comparative phylogenetic studies of sponges and their associated microbes. Erpenbeck et al. (2002) used a mitochondrial marker, cytochrome oxidase subunit 1 (CO1) and showed that the derived phylogenetic trees of four out of six symbionts were largely congruent with a tree containing sequences from the corresponding host sponges. This suggests that cospeciation had occurred (Erpenbeck et al., 2002). A subsequent study indicated a high degree of host specificity between the filamentous cyanobacterium *Oscillatoria spongelliae* and various dictyoceratid sponges (Thacker and Starnes, 2003). When comparing the phylogeny of 23 geographically, morphologically, and environmentally distant sponge species (based on their 28S rDNA gene sequences) and their associated archaea (based on 16S rDNA), a genus-specific association of these sponges with Crenarchaeota of the Marine Group I was apparent (Holmes and Blanch, 2007). These results suggest close coevolution and thus, a long-term symbiosis between these archaea and their sponge hosts. Coevolution requires that the host and symbiont maintain close association over evolutionary time. Vertical transmission, the mechanism through which coevolution could occur, has been shown for numerous sponge species belonging to all three classes of Porifera (Ereskovsky et al., 2005; Sharp et al., 2007; Schmitt et al., 2007; and references therein), including in species with highly varied reproductive strategies.

A pitfall for these comparative phylogenetic studies is that the phylogeny of sponges themselves is not yet fully resolved. This is particularly true for the Demosponges where phylogeny from the subclass to the family level is still in a state of flux (Boury-Esnault, 2006). Accordingly, our understanding of symbiont evolution in sponges will continue to develop only in parallel with improvement of our knowledge about host phylogeny. A recently initiated CO1 sequencing project for taxonomically diverse sponges (www.spongebarcoding.org) may provide data to achieve this goal in the future.

Further support for a possible ancient origin of the sponge–microbe system comes from biomarker studies. Various fatty acids of probable microbial origin occur in a wide range of sponges, irrespective of host phylogeny or geographic location (Thiel et al., 1999, 2002). The apparent absence of some of these biomarkers from

marine sediments and seawater led to the suggestion that the fatty acids and their microbial producers have been present in the sponges since ancient times.

The final type of evidence for an ancient and close association between sponges and microorganisms comes from the fossil record. Reef mounds constructed by siliceous sponges and cyanobacterial mats in the early Cambrian show that sponges and microbes closely coexisted hundreds of millions of years ago (Brunton and Dixon, 1994). Whether these microbes actually lived within the sponge tissue, as modern sponge microbes do, remains, at this stage of our knowledge, unclear.

In other words, Precambrian microorganism acquisition which started as a mere hypothesis is gaining support through studies of phylogeny, biomarkers, and fossil record. Thus, there is increasing evidence for a long-standing coevolution between sponges and certain microbial lineages, which may even date back to the roots of sponge evolution in the anaerobic, microbe-dominated world of the Proterozoic. There is evidence that a close association between sponges and bacteria may even have provided some of the genetic source material for Metazoan evolution (Lakshminarayan et al., 2004).

The alternative scenario, environmental acquisition, can, however, not be ruled out for certain sponge microbes or, in some cases, may be the more likely explanation. In this scenario, it is assumed that sponge-specific microbes are in fact also present in seawater but that our techniques fail to detect them because of their rarity. In the sponge, specific enrichment of those rare microorganisms could occur as a result of a selection through the sponge immune system (Müller and Müller, 2003) or because those microbes which possess a capsule are protected from sponge cells phagocytosis and would increase in number within the sponge body, thus becoming detectable. Unspecific enrichment is also possible and could happen through the ability of sponges to filter large amounts of water through their body. The likelihood of rare microbes being detected by our current techniques is higher in a sponge than in seawater as they might accumulate and be present in high enough numbers to be found.

The third scenario proposed by Taylor et al. (2007) is a combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) which seems the most likely scenario to explain microbial diversity that we find in sponges today (Hentschel et al., 2002).

An anaerobic world in sponges

Until recently, sponge metabolism was viewed as being based on aerobic respiration, similarly to all Metazoa. Oxygen is usually supplied in excess to the sponge body through the water current created by the choanocytes (flagellated cells) (Reiswig, 1974). The remarkable ability of sponges to pump large amounts of water through their bodies has led to the assumption that permanent oxygen saturation exists within the sponge body. However, the

presence of anaerobic microbes in some sponge species hinted toward the presence of spatial or temporal anoxic niches (e.g., Webster et al., 2001; Hoffmann et al., 2005a; Schläppy et al., 2010a).

Gatti et al. (2002) and Schönberg et al. (2004) were the first to use microsensors to investigate the oxygen distribution in the tissue of living sponges at a microscale. Gatti et al. (2002) used oxygen micro-optodes on sponges' primorpha (multicellular aggregates composed of previously dissociated single sponge cells) and described oxygen deficiency in *Suberites domuncula*. Clark-type oxygen microelectrodes have since been used to explore the fine-scale oxygen dynamics in several sponge species from the Mediterranean and the North Atlantic. Anoxic regions have been detected in the cold-water sponge *Geodia barretti* (Hoffmann et al., 2005a) and in its explants (Hoffmann et al., 2005b), as well as in the Mediterranean sponges *Dysidea avara* (Schläppy et al., 2007) and *Chondrosia reniformis* (Figure 2) kept in aquaria or cultivation tanks with recirculating seawater.

Similarly, fluctuating oxygen concentrations leading to anoxia lasting up to 1 h were also found in *Dysidea avara* in the field when measured with an oxygen microelectrode applied by SCUBA diving (Schläppy et al., 2010a). A positive correlation between pumping activity and oxygen content was described in the tissue and in the exhaled water of *Dysidea avara* (Schläppy et al., 2007) and *Aplysina aerophoba* (Hoffmann et al., 2008). When the sponge ventilated, its body was oxygen saturated. Figure 2a shows a typical profile of a pumping sponge, with oxygen saturated water above the sponge surface and near-saturated water within the sponge tissue (compare also Hoffmann et al., 2007; Schläppy et al., 2007).

Oxygen concentrations decreased dramatically when pumping activity ceased. After 15 min without ventilation, the entire sponge body was anoxic with the exception of a 1 mm surface layer where oxygen penetrated due to molecular diffusion over the sponge surface (Hoffmann et al., 2008).

Oxygen profiles are typically diffusive over the surface and into the tissue of a nonpumping sponge (Figure 2b) (compare also Hoffmann et al., 2005b, 2008; Schläppy et al., 2007, 2010a). In situ, many sponge species reduce or even stop their pumping activity for several hours at irregular intervals (Reiswig, 1971; Vogel, 1977; Pile et al., 1997). It remains unclear whether the interruption of ventilation is caused by external events such as, for example, a high sediment load in the water (Gerodette and Flechsig, 1979; Leys et al., 1999), decrease in salinity (Fell et al., 1989), or by an intrinsic rhythm specific to a species (see Reiswig, 1971). In nonpumping sponges, molecular diffusion across a diffusive boundary layer at the sponge surface is the only source of oxygen.

The consequences of these findings are twofold. Firstly, all sponge cells and microbes which lay as deeper as 1 mm below the sponge surface need to be able to survive without direct and regular access to oxygen. Since a lack of ventilation causes tissue anoxia, the parts of the sponge

body which are not affected by surface diffusion must be alternately exposed to oxic and anoxic conditions. Secondly, by modulating the presence or absence and the magnitude of water flow through their bodies sponges have the possibility of actively switching between aerobic and anaerobic metabolism. It follows that sponge-associated microorganisms must be able to tolerate both situations and their metabolism may be activated or inactivated depending on the oxygen concentration in the sponge. This means that anaerobic microbial processes, such as sulfate reduction, for example, may take place (Hoffmann et al., 2005a; but see Schläppy et al., 2010b).

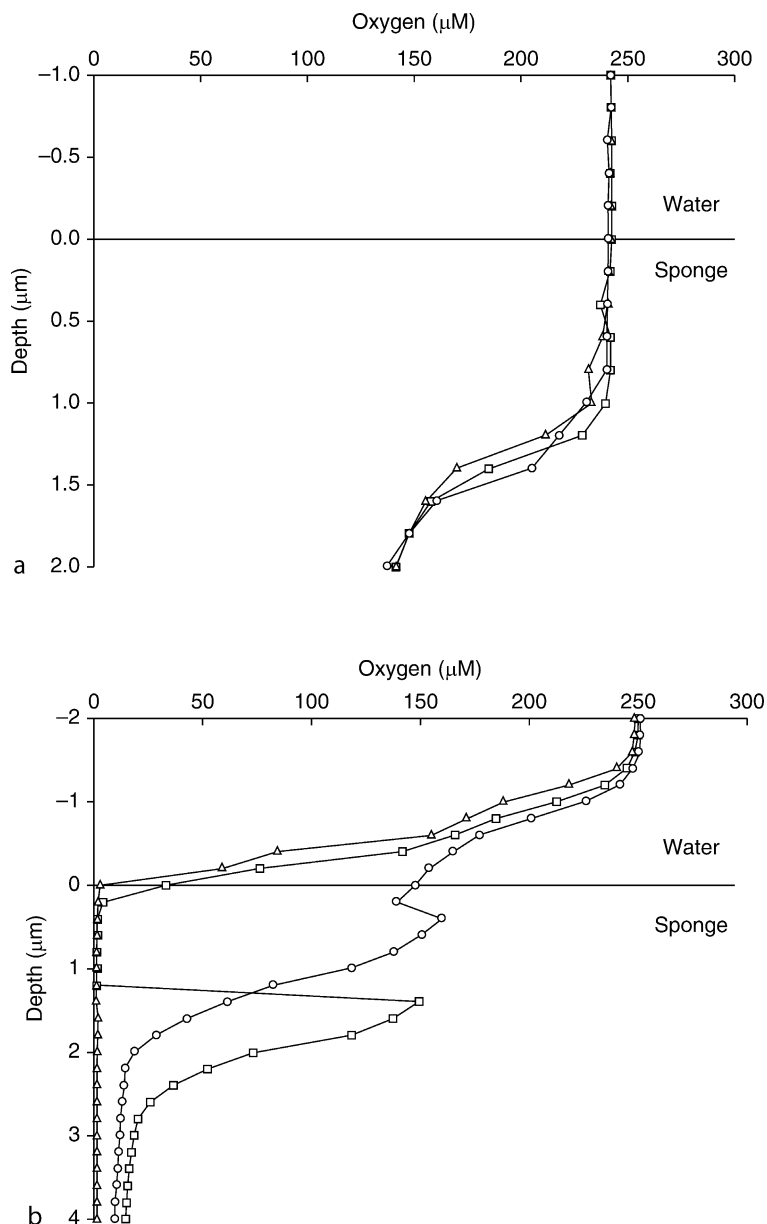
Another possible consequence of varying oxygen concentrations in sponge tissue is reported by Müller et al. (2004a, b). Using *Suberites domuncula* and its alphaproteobacterial symbiont (SB2) as a model system, they showed that the expression of genes which were essential for metabolic interaction of microbe and host was maximal under aerated conditions. Coupled with the observed loss of SB2 cells from the sponge surface under low-oxygen conditions, it was concluded that the oxygen level is responsible for regulating the bacterial fauna in *S. domuncula*. Whether this type of mechanism is equally important in other sponge-microbe systems remains to be investigated.

Tissue anoxia in sponges may act as a controlling factor toward the number of anaerobic sponge-associated microbes and act as a regulator of the nature and extent of their metabolic activity. Alternatively, it may act as a mechanism through which the sponge can kill undesirable seawater microbes. Regardless of whether anoxia in sponges is intentional or inevitable, many sponges can survive long periods of tissue anoxia and either tolerate or even foster anaerobic microbes and corresponding anaerobic microbial activity within their bodies. A close association with a diverse microbial community including (facultative) anaerobic or microaerophilic microorganisms may have been particularly advantageous at a time when fluctuating oxygen conditions were present on the Earth.

Interactions between sponges and sponge microbes

While our knowledge about phylogeny and metabolic capacities of the sponge microbes continuously increases, the role that microbes play for the sponge host is still a matter of debate. Microbes undoubtedly benefit from the nutrient-rich and protected environment of the sponge mesohyl, but the benefit to the sponge host remains unclear.

Sponge microbes may consume carbon sources which are not accessible to the sponge and may be subsequently ingested by the sponge cells. This process is commonly known as "microbial farming" and is well described (Wilkinson and Garrone, 1980; Ilan and Abelson, 1995; Vacelet et al., 1996 and own observation). Autotrophic



Sponges (Porifera) and Sponge Microbes, Figure 2 Oxygen profiles above and within *Chondrosia reniformis* showing (a) a pumping specimen with oxygen-saturated water above the sponge surface and near-saturated water within the sponge (b) a nonpumping specimen with oxygen-saturated water up to 1.5 cm above the sponge surface, a gradual decrease in the diffusive boundary layer and low oxygen concentration approaching anoxia from 2.5 cm into the sponge body. Note the different Y axes.

symbionts transform inorganic carbon (DIC) into organic carbon, which may then be transferred to the host. Energy transfer between symbiont and sponge is best described for phototrophic symbionts (e.g., cyanobacteria: Wilkinson, 1983). Chemolithotrophic processes, such as nitrification, are similar in principle. The energy which is needed for carbon fixation, however, is gained by a chemical process, the aerobic oxidation of ammonium to nitrite first and then of nitrite to nitrate. The presence of nitrifying microbes as well as the transformation of

ammonium to nitrite and nitrate have been demonstrated in many sponge species (e.g., Diaz and Ward, 1997; Diaz et al., 2004; Bayer et al., 2007; Schläppy et al., 2010b). In addition to carbon fixation, these microbes efficiently remove the sponge waste product ammonium and the toxic nitrite. Heterotrophic microbes consume simple compounds (dissolved organic carbon, DOC), while sponge cells prefer small particles such as pelagic bacteria or phytoplankton (Willenz, 1980; Pile et al., 1996; Witte et al., 1997; Ribes et al., 1999). Some sponges, however,

have been identified as important DOC sinks (Yahel et al., 2003; de Goeij et al., 2008). Interestingly, these sponges host high amount of associated bacteria. This leads to the conclusion that DOC is consumed by the sponge microbes, which in turn may be consumed by the sponge cells. A diverse community of associated microorganisms with high metabolic diversity is able to metabolize a wide array of simple molecules and to transfer them into biomass as a food resource for the sponge.

The second possibility for a mutual interaction between sponges and microbes lies within the production of secondary metabolites. Sponges produce a large variety of bioactive compounds (Blunt et al., 2003; Paul and Puglisi, 2004), which act as defenses against surface fouling, infection, or predation. Sponge microbes, which are obviously not harmed by the antimicrobial metabolites, are in fact often the actual producers of these compounds (Hildebrand et al., 2004; Piel, 2004, 2006). In addition to protecting the sponge, secondary metabolites act as a controlling factor in the host–microbe interaction.

At least some groups of sponge microbes are clearly beneficial to the sponge host, while others may be commensals.

Summary

Sponges are amongst the most ancient Metazoans on the Earth and are distributed over a wide range of aquatic ecosystems. Phylogenetically complex, yet highly sponge-specific microbial communities live in close association with numerous sponge species. A combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) seems the most likely scenario to explain microbial diversity that we find in sponges today. Tissue anoxia is a feature found in several sponges species and is related to sponge pumping activity. Sponge microbes with autotrophic, heterotrophic, aerobic, or anaerobic metabolisms may contribute to the nutrition of the host. Other interactions between sponges and sponge microbes involve the synthesis of secondary metabolites. Due to modulation of internal oxygen concentration and the production of secondary metabolites, sponges are able to control their microbial communities. While our knowledge about the phylogeny of sponge microbes is increasing rapidly, many questions concerning the activity of sponge microbes and their interaction with the host sponge still remain unanswered. Future challenges will involve linking microbial phylogeny to microbial function on a microscale within the sponge tissue, and to examine the complex interactions within the sponge–microbe system from the origin of Metazoan life until today.

Bibliography

Ax, P., 1995. *Das System der Metazoa I*. Stuttgart: Fischer.
 Bayer, K., Schmitt, S., and Hentschel, U., 2007. Microbial nitrification in Mediterranean sponges: possible involvement of ammonium-oxidizing betaproteobacteria. In Custódio, M., Lôbo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera*

Research: Biodiversity, Innovation, Sustainability: Série Livros. Museu Nacional, Rio de Janeiro, pp. 165–171.
 Blunt, J. W., Copp, B. R., Munro, H. G. M., Northcote, P. T., and Prinsep, M. R., 2003. Marine natural products. *Natural Product Report*, **20**, 1–48.
 Borchellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J., and Le Parco, Y., 2001. Sponge paraphyly and the origin of metazoa. *Journal of Evolutionary Biology*, **14**, 171–179.
 Boury-Esnault, N., 2006. Systematic and evolution of demospongiae. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **84**, 205–224.
 Brunton, F. R., and Dixon, O. A., 1994. Siliceous sponge-microbe biotic associations and their recurrence through the phanerozoic as reef mound constructors. *Palaios*, **9**, 370–387.
 de Goeij, J. M., Moodley, L., Houtekamer, M., Carballeira, N. M., and van Duyl, F. C., 2008. Tracing ¹³C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca Caerulea*: evidence for Dom-feeding. *Limnology & Oceanography*, **53**, 1376–1386.
 Diaz, M. C., and Ward, B. B., 1997. Sponge-mediated nitrification in tropical benthic communities. *Marine Ecology Progress Series*, **156**, 97–107.
 Diaz, M. C., Akob, D., and Cary, C. S., 2004. Denaturing gradient gel electrophoresis of nitrifying microbes associated with tropical sponges. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova*, **68**, 279–289.
 Ereskovsky, A. V., Gonobobleva, E., and Vishnyakov, A., 2005. Morphological evidence for vertical transmission of symbiotic bacteria in the viviparous sponge *Halisarca Dujardini* Johnston (porifera, demospongiae, halisarcida). *Marine Biology*, **146**, 869–875.
 Erpenbeck, D., Breeuwer, A. J., van der Velde, H. C., and Van Soest, R. W. M., 2002. Unravelling host and symbiont phylogenies of halichondrid sponges (Demospongiae, Porifera) using a mitochondrial marker. *Marine Biology*, **141**, 377–386.
 Fell, P. E., Knight, P.-A., and Rieders, W., 1989. Low-salinity tolerance of and salinity-induced dormancy in the estuarine sponge *Microciona Prolifera* (Ellis & Solander) under long-term laboratory culture. *Journal of Experimental Marine Biology and Ecology*, **133**, 195–211.
 Fieseler, L., Horn, M., Wagner, M., and Hentschel, U., 2004. Discovery of the novel candidate phylum “Poribacteria” in marine sponges. *Applied & Environmental Microbiology*, **70**, 3724–3732.
 Fieseler, L., Quaiser, A., Schleper, C., and Hentschel, U., 2006. Analysis of the first genome fragment from the marine sponge-associated, novel candidate phylum poribacteria by environmental genomics. *Environmental Microbiology*, **8**, 612–624.
 Gaidos, E., Dubuc, T., Dunford, M., McAndrew, P., Padilla-Gamino, J., Studer, B., Weersing, K., and Stanley, S., 2007. The precambrian emergence of animal life: a geobiological perspective. *Geobiology*, **5**, 351–373.
 Gatti, S., Brey, T., Müller, W. E. G., Heilmayer, O., and Holst, G., 2002. Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. *Marine Biology*, **140**, 1075–1085.
 Gerodette, T., and Flechsig, A. O., 1979. Sediment-induced reduction in the pumping rate of the tropical sponge *Verongia Lacunosa*. *Marine Biology*, **55**, 103–110.
 Grozdanov, L., and Hentschel, U., 2007. An environmental genomics perspective on the diversity and function of marine sponge-associated microbiota. *Current Opinion in Microbiology*, **10**, 215–220.
 Hentschel, U., Hopke, J., Horn, M., Friedrich, A. B., Wagner, M., Hacker, J., and Moore, B. S., 2002. Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied and Environmental Microbiology*, **68**, 4431–4440.

- Hentschel, U., Fieseler, L., Wehrl, M., Gernert, C., Steinert, M., Hacker, J., and Horn, M., 2003. Microbial diversity of marine sponges. In Müller, W. E. G. (ed.), *Marine Molecular Biotechnology*. Berlin: Springer, pp. 59–88.
- Hentschel, U., Usher, K. M., and Taylor, M. W., 2006. Marine sponges as microbial fermenters. *FEMS Microbiology Ecology*, **55**, 167–177.
- Hildebrand, M., Waggoner, L. E., Lim, G. E., Shar, K. H., Ridley, C. P., and Haygood, M. G., 2004. Approaches to identify, clone, and express symbiont bioactive metabolite genes. *Natural Product Reports*, **21**, 122–142.
- Hoffmann, F., Larsen, O., Thiel, V., Rapp, H. T., Pape, T., Michaelis, W., and Reitner, J., 2005a. An anaerobic world in sponges. *Geomicrobiology Journal*, **22**, 1–10.
- Hoffmann, F., Larsen, O., Rapp, H. T., and Osinga, R., 2005b. Oxygen dynamics in choanosomal sponge explants. *Marine Biology Research*, **1**, 160–163.
- Hoffmann, F., Sauter, E., Sachs, O., Røy, H., and Klages, M., 2007. Oxygen distribution in *Tentorium Semisuberites* and in its habitat in the Arctic deep sea. In Custódio, M., Lôbo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera Research: Biodiversity, Innovation, Sustainability: Série Livros*. Museu Nacional, Rio de Janeiro, pp. 379–382.
- Hoffmann, F., Røy, H., Bayer, K., Hentschel, U., Pfannkuchen, M., Brümmer, F., and de Beer, D., 2008. Oxygen dynamics and transport in the Mediterranean sponge *Aplysina Aerophoba*. *Marine Biology*, **153**, 1257–1264.
- Holmes, B., and Blanch, H., 2007. Genus-specific associations of marine sponges with Group I Crenarchaeota. *Marine Biology*, **150**, 759–772.
- Ilan, M., and Abelson, A., 1995. The life of a sponge in a Sandy Lagoon. *Biological Bulletin*, **189**, 363–369.
- Lakshminarayan, M. I., Aravind, L., Coon, S. L., Klein, D. C., and Koonin, E. V., 2004. Evolution of cell-cell signaling in animals: Did late horizontal gene transfer from bacteria have a role? *Trends in Genetics*, **20**, 292–299.
- Leys, S. P., Mackie, G. O., and Meech, R. W., 1999. Impulse conduction in a sponge. *Journal of Experimental Biology*, **202**, 1139–1150.
- McCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Paleoenvironmental implications of Novel C30 steranes in precambrian to cenozoic age petroleum and Bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Medina, M., Collins, A. G., Silberman, J. D., and Sogin, M. L., 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *PNAS*, **98**, 9707–9712.
- Moldowan, J. M., Dahl, J., Jacobsen, S. R., Huizinga, B. J., McCaffrey, M. A., and Summons, R. E., 1994. Molecular fossil evidence for late proterozoic – early paleozoic environments. *Terra Nova Abstracts*, **14**, 4.
- Müller, W. E. G., 1998a. *Molecular Evolution: Towards the Origin of Metazoa*. Berlin: Springer.
- Müller, W. E. G., 1998b. Origin of metazoa: sponges as living fossils. *Naturwissenschaften*, **85**, 11–25.
- Müller, W. E. G., and Müller, I. M., 2003. Origin of the metazoan immune system: identification of the molecules and their functions in sponges. *Integrative & Comparative Biology*, **43**, 281–292.
- Müller, W. E. G., Grebenjuk, V. A., Thakur, N. L., Thakur, A. N., Batel, R., Krasko, A., Müller, I. M., and Breter, H. J., 2004a. Oxygen-controlled bacterial growth in the sponge *Suberites Domuncula*: toward a molecular understanding of the symbiotic relationships between sponge and bacteria. *Applied and Environmental Microbiology*, **70**, 2332–2341.
- Müller, W. E. G., Perovic, S., Schroder, H. C., and Breter, H. J., 2004b. Oxygen as a morphogenic factor in sponges: expression of a tyrosinase gene in the sponge suberites *Domuncula*. *Micron*, **35**, 87–88.
- Paul, V. J., and Puglisi, M. P., 2004. Chemical mediation of interactions among marine organisms. *Natural Product Reports*, **21**, 189–209.
- Piel, J., 2004. Metabolites from symbiotic bacteria. *Natural Product Reports*, **21**, 519–538.
- Piel, J., 2006. Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. *Current Medicinal Chemistry*, **13**, 39–50.
- Pile, A. J., Patterson, M. R., and Witman, J. D., 1996. In situ grazing on plankton < 10 µM by the boreal sponge *Mycale Lingua*. *Marine Ecology Progress Series*, **141**, 95–102.
- Pile, A. J., Patterson, M. R., Savarese, M., Chernykh, V. I., and Fialkov, V. A., 1997. Trophic effects of sponge feeding within lake Baikal's littoral zone. 1. In situ pumping rate. *Limnology and Oceanography*, **42**, 171–178.
- Reiswig, H. M., 1971. In situ pumping activities of tropical demospongiae. *Marine Biology*, **9**, 38–50.
- Reiswig, H. M., 1974. Water transport, respiration and energetics of three tropical marine sponges. *Journal of Experimental Marine Biology and Ecology*, **14**, 231–249.
- Reiswig, H. M., 1981. Partial carbon and energy budgets of the bacteriosponge *Verongia Fistularis* (Porifera: Demospongiae) in Barbados. *Marine Ecology Progress Series*, **2**, 273–293.
- Reitner, J., and Wörheide, G., 2002. Non-lithistid fossil demospongiae – origins of their palaeobiodiversity and highlights in history of preservation. In Hooper, J. N. A., and Soest, R. W. M. v. (eds.), *Systema Porifera*. New York: Kluwer, pp. 52–68.
- Ribes, M., Coma, R., and Gili, J. M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series*, **176**, 179–190.
- Schläppy, M.-L., Hoffmann, F., Røy, H., Wijffels, R. H., Mendola, D., Sidri, M., and de Beer, D., 2007. Oxygen dynamics and flow patterns of *Dysidea avara* (Porifera, Demospongiae). *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1677–1682.
- Schläppy, M.-L., Schöttner, S. I., Lavik, G., Kuypers, M. M. M., de Beer, D., and Hoffmann, F., 2010a. Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Marine Biology*, **157**, 593–602, doi:10.1007/s00227-009-1344-5.
- Schläppy, M.-L., Weber, M., Mendola, D., Hoffmann, F., and de Beer, D., 2010b. Heterogeneous oxygenation resulting from active and passive flow in two Mediterranean sponges, *Dysidea avara* and *Chondrosia reniformis*. *Limnology and Oceanography*, **55**(3), 1289–1300. doi:10.4319/lo.2010.55.3.1289.
- Schmitt, S., Weisz, J., Lindquist, N., and Hentschel, U., 2007. Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. *Applied and Environmental Microbiology*, **73**, 2067–2078.
- Schönberg, C. H. L., Hoffmann, F., and Gatti, S., 2004. Using microsenors to measure sponge physiology. *Bollettino dei Musei e degli Istituti Biologici dell' Università di Genova*, **68**, 593–604.
- Sharp, K., Eam, B., Faulkner, D., and Haygood, M., 2007. Vertical transmission of diverse microbes in the tropical sponge *Corticium* Sp. *Applied and Environmental Microbiology*, **73**, 622–629.
- Taylor, M. W., Radax, R., Steger, D., and Wagner, M., 2007. Sponge-associated microorganisms: evolution, ecology, and

- biotechnological potential. *Microbiology and Molecular Biology Reviews*, **71**, 259–347.
- Thacker, R. W., and Starnes, S., 2003. Host specificity of the symbiotic cyanobacterium *Oscillatoria Spongelia* in marine sponges, *Dysidea spp.* *Marine Biology*, **142**, 643–648.
- Thiel, V., Jenisch, A., Wörheide, G., Löwenberg, A., Reitner, J., and Michaelis, W., 1999. Mid-chain branched alkanic acids from “living fossil” demosponges: a link to ancient sedimentary lipids? *Organic Geochemistry*, **30**, 1–14.
- Thiel, V., Blumenberg, M., Hefter, J., Pape, T., Pomponi, S., Reed, J., Reitner, J., Wörheide, G., and Michaelis, W., 2002. A chemical view of the most ancient metazoa – biomarker chemotaxonomy of Hexactinellid sponges. *Naturwissenschaften*, **89**, 60–66.
- Vacelet, J., 1970. Description De Cellules à Bactéries Intranucléaires Chez Des Eponges *Verongia*. *Journal de Microscopie*, **9**, 333–346.
- Vacelet, J., and Donadey, C., 1977. Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, **30**, 301–314.
- Vacelet, J., Fiala-Médioni, A., Fisher, C. R., and Boury-Esnault, N., 1996. Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous Cladorhizid sponge. *Marine Ecology Progress Series*, **145**, 77–85.
- Vogel, S., 1977. Current-induced flow through living sponges in nature. *Proceedings of the National Academy of Science USA*, **74**, 2069–2071.
- Webster, N. S., Wilson, K. J., Blackall, L. L., and Hill, R. T., 2001. Phylogenetic diversity of bacteria associated with the marine sponge *Rhopaloeides odorabile*. *Applied and Environmental Microbiology*, **67**, 434–444.
- Wilkinson, C. R., 1978. Microbial association in sponges. II. Numerical analysis of sponge and water bacterial populations. *Marine Biology*, **49**, 169–176.
- Wilkinson, C. R., 1983. Net primary productivity in coral reef sponges. *Science*, **219**, 410–412.
- Wilkinson, C. R., and Garrone, R., 1980. Nutrition of marine sponges. Involvement of symbiotic bacteria in the uptake of dissolved carbon. In Smith, D. C., and Tiffon, Y. (eds.), *Nutrition in Lower Metazoa*. Oxford: Pergamon Press, pp. 157–161.
- Willenz, P., 1980. Kinetic and morphological aspects of particle ingestion by the freshwater sponge *Ephydatia Fluviatilis* L. In Smith, D. C., and Tiffon, Y. (eds.), *Nutrition in Lower Metazoa*. Oxford: Pergamon Press, pp. 163–178.
- Witte, U., Brattegard, T., Graf, G., and Springer, B., 1997. Particle capture and deposition by deep-sea sponges from the Norwegian-Greenland Sea. *Marine Ecology Progress Series*, **154**, 241–252.
- Yahel, G., Sharp, J. H., Marie, D., Hase, C., and Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: bulk DOC is the major source for carbon. *Limnology & Oceanography*, **48**, 141–149.

Cross-references

[Aerobic Metabolism](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Early Earth](#)
[Early Precambrian Eukaryotes](#)
[Histology](#)
[Microbial Ecology of Submarine Caves](#)
[Microsensors for Sediments, Microbial Mats, and Biofilms](#)
[Origins of the Metazoa](#)
[Symbiosis](#)

STROMATACTIS

Roman Aubrecht

Comenius University, Bratislava, Slovakia

Definition

Stromatactis is defined as a mass of spar (with partial substitution of internal sediment) which has smooth base, digitate roof, occurs in swarms and has reticulate distribution (Bathurst, 1982). Neuweiler et al. (2001) distinguished the terms “stromatactis,” “inhibited stromatactis,” and “aborted stromatactis” (Figure 1). Stromatactis originated when early internal sedimentation occurred in the open space of the cavity network followed by centripetal cementation by early marine cements in residual cavity space. Aborted stromatactis resulted from internal sediment filling of the entire or near-entire original cavity network. Inhibited stromatactis refers to incipient marine cementation of the stromatactid cavity followed by entire or near-entire internal sediment filling.

Stromatactis often (but not always) occur in mud-mounds (see entry [Mud Mounds](#)). Spar bodies similar to stromatactis but not fitting to all the points of this definition are termed in literature as “stromatactoid” or “stromatactis-like” bodies. Similar to stromatactis but smaller are fenestral pores occurring in intertidal limestones. Similar to limestones with stromatactis structures are “zebra limestones” in which the spar bodies are flat, straight, elongated, and the individual spar layers are not always interconnected.

Evolution of the term stromatactis

The term stromatactis was first used by Dupont (1881, 1882). Until present, there is still no agreement in the way of its origin. There are two main groups of opinions about the origin of stromatactis: abiogenic and biogenic. Most researchers agree that stromatactis originally represented cavities which were later filled with internal sediment and sparry calcite. The suggested abiogenic origins for stromatactis included internal erosion and reworking of small cavities (e.g., Kukal, 1971; Wallace, 1987; Bridges and Chapman, 1988; Matyszkiewicz, 1993, 1997), dewatering or escape of fluids (Heckel, 1972; Desbordes and Maurin, 1974; Bernet-Rollande et al., 1981), slumps (Schwarzacher, 1961), and freshwater karstification (Dunham, 1969). Most recent ideas involve frozen clathrate hydrates in the calcareous mud, after which the stromatactis cavities remained (Krause, 2001) or the cavities are interpreted as a result of sedimentation of stirred polydisperse sediment (Hladil, 2005; Hladil et al., 2006; Hladil et al., 2007). Some researchers supposed that stromatactis did not represent cavities and originated either by neomorphism or recrystallization of the calcareous mud (Black, 1952; Orme and Brown, 1963; Ross et al., 1975) or by dynamic metamorphism (Logan and Semeniuk, 1976). Among the opinions

MICROBIALITES, MODERN

Christophe Dupraz¹, R. Pamela Reid², Pieter T. Visscher¹

¹University of Connecticut, Storrs, CT, USA

²University of Miami, Miami, FL, USA

Synonyms

“Cryptalgal sedimentary rock” (Aitken, 1967); Microbolite (Riding, 1991)

Definition

Microbialite: “Organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987), or “Benthic microbial deposits” (Riding, 1991).

Introduction

In this encyclopedia, microbialite is discussed under two chapters: (1) modern and (2) fossil. This part discusses modern microbialites, with emphasis on processes of formation. The fossil extension and classification of microbial deposits (fossil forms being more diverse) are treated in the “fossil microbialite” section of the encyclopedia (see Chapter *Microbialites, Stromatolites, and Thrombolites*). Microbialites are composed of trapped, bound, and/or precipitated sediment, and exhibit a range of mineralogies (Figure 1). This chapter focuses on carbonate microbialites, which are the most widespread and the most studied.

Microbialites are “rocks” that are produced, induced, or influenced by benthic microbial communities, primarily bacteria and sometimes also microeukaryotes, such as diatoms. These microbial communities are organized in mats or biofilms (e.g., Stolz, 2000), consisting of microscopic organisms enveloped in an organic matrix of extracellular polymeric substances (EPS; Decho, 1990; Bhaskar and Bhosle, 2005; see Chapter *Extracellular Polymeric Substances (EPS)*). Because the microbial mat is dominantly organic, it is rarely preserved in the fossil record. In some unusual cases, exceptional taphonomic conditions can preserve intact cells and even the EPS structure (e.g., silicification or preserved remnants of EPS matrices, Golubic and Hofmann, 1976; Barbieri et al., 2004; Barbieri and Cavalazzi, 2005; Altermann et al., 2006; Benzerara et al., 2006; Kumar and Pandey, 2008; Lepot et al., 2008). More commonly, the organic matter decomposes to carbon dioxide (CO₂) or methane (CH₄), although in some cases incomplete degradation can lead to organic matter enrichment of the sediment. Evidence of the original microbial mat or biofilm is generally indirect (e.g., specific sedimentary (bio)structure, micromorphology, organic biomarkers, and stable isotopic fractionation). Microbial mats can help preserve sedimentary structures in the fossil record (“microbially-induced sedimentary structures” – MISS; Noffke et al., 2003). The most

spectacular examples are carbonate microbialites. Examples of modern microbialites in the following section are followed by a discussion of processes of formation.

Types of modern microbialite

Microbialite descriptions incorporate a range of scales, from macrometers to micro/nanometers (e.g., Shapiro, 2000). Megastructure relates to the geographical extension of a microbial deposit and overall deposit morphology (e.g., ridges, biostrome, and bioherm). Macrostructure characterizes the morphology of an individual buildup (e.g., domal, branching, knobby). Mesostructure refers to structures visible with the naked eye, such as lamination. Microstructure is observed with a microscope, such as light, confocal, scanning electron (SEM), or transmission electron microscope (TEM).

Microbialite classification is based on mesostructure with four common types (Figure 2): laminated microbialites classified as stromatolites, thrombolites are clotted, leiolites are structureless, and dendrolites are dendritic (Burne and Moore, 1987; Riding, 1991, 2000; Dupraz and Strasser, 1999, 2002). All of these structures exhibit a wide range of microstructures including micropeloidal, densely micritic, or agglutinated microfabrics (Riding, 1991; Dupraz and Strasser, 1999). Three types of modern microbialites are described in the following section; dendrolites have not yet been found in modern environments. For further description of microbialite classification and microstructure see Chapter *Microbialites, Stromatolites, and Thrombolites*.

Stromatolite

The term stromatolite is derived from the Greek “stroma,” meaning mattress or stratum and “lithos,” meaning rock. The term was first introduced by Kalkowsky in 1908 to refer to “laminated benthic microbial deposits” (see also Riding, 1999; Chapter *Microbialites, Stromatolites, and Thrombolites*). The first modern stromatolites were found in the Shark Bay, Western Australia in 1954 in the hypersaline water of Hamelin Pool. Until this date, scientists believed that stromatolites were extinct, found only in the fossil record. Although the biogenicity of 3.5 Ga stromatolites has been questioned (Buick et al., 1981; Lowe, 1994; Grotzinger and Knoll, 1999; Lindsay et al., 2003), all modern microbialites called stromatolites are associated with microbial communities that induce, or serve as organic substrate for, precipitation (Riding, 2000). Modern stromatolites are found in many different locations including marine, hypersaline, freshwater, and even continental environments.

Open marine stromatolites

The Bahamas hosts the only known examples of open marine stromatolites. These structures are found at a variety of locations on the margins of Exuma Sound including Schooner Cays, Lee Stocking Island, Stocking Island Highborne Cay, and Darby Island (Dravis, 1983;

Carbonates

Calcite - CaCO_3
 Mg-Calcite - $(\text{Mg}_x\text{Ca}_{1-x})\text{CO}_3$
 Aragonite - CaCO_3
 Vaterite - CaCO_3
 Monohydrocalcite - $\text{CaCO}_3 \cdot \text{H}_2\text{O}$
 Protodolomite - $\text{MgCa}(\text{CO}_3)$
 Hydrocerussite - $\text{Pb}_3(\text{CO}_3)_2(\text{OH})_2$
 Amorphous forms - $\text{CaCO}_3 \cdot \text{H}_2\text{O}$ or CaCO_3

Phosphates

Octacalcium phosphate - $\text{Ca}_8\text{H}_2(\text{PO}_4)_6$
 Brushite - $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$
 Francolite - $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$
 Carbonated-hydroxylapatite (dahllite) - $\text{Ca}_5(\text{PO}_4, \text{CO}_3)_3(\text{OH})$
 Whitlockite - $\text{Ca}_8\text{H}_2(\text{Mg, Fe})_2 + 2(\text{PO}_4)_4\text{I}_4$
 Struvite - $\text{Mg}(\text{NH}_4)(\text{PO}_4) \cdot 6\text{H}_2\text{O}$
 Vivianite - $\text{Fe}_3 + 2(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$
 Amorphous Calcium Phosphate (at least 6 forms) variable
 Amorphous Calcium Pyrophosphate - $\text{Ca}_2\text{P}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$

Sulfates

Gypsum - $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
 Barite - BaSO_4
 Celestite - SrSO_4
 Jarosite - $\text{KFe}_3^{+3}(\text{SO}_4)_2(\text{OH})_6$

Sulfides

Pyrite - FeS_2
 Hydrotroilite - $\text{FeS} \cdot \text{nH}_2\text{O}$
 Sphalerite - ZnS
 Galena - PbS
 Hydrotroilite $\text{FeS} \cdot \text{nH}_2\text{O}$
 Sphalerite ZnS
 Wurtzite ZnS
 Greigite Fe_3S_4
 Mackinawite $(\text{Fe, Ni})_9\text{S}_8$
 Amorphous Pyrrhotite Fe_{1-x}S ($x = 0-0.17$)
 Acanthite Ag_2S

Arsenates

Orpiment - As_2S_3

Hydrated Silica

Amorphous Silica - $\text{SiO}_2 \cdot \text{nH}_2\text{O}$

Chlorides

Atacamite - $\text{Cu}_2\text{Cl}(\text{OH})_3$

Fluorides

Fluorite - CaF_2
 Hieratite - K_2SiF_6

Oxides

Magnetite - Fe_3O_4
 Amorphous Iron Oxide - Fe_2O_3
 Amorphous Mn Oxide - Mn_3O_4
 Amorphous Ilmenite $\text{Fe}^{+2}\text{TiO}_3$

Metals

Sulfur - S

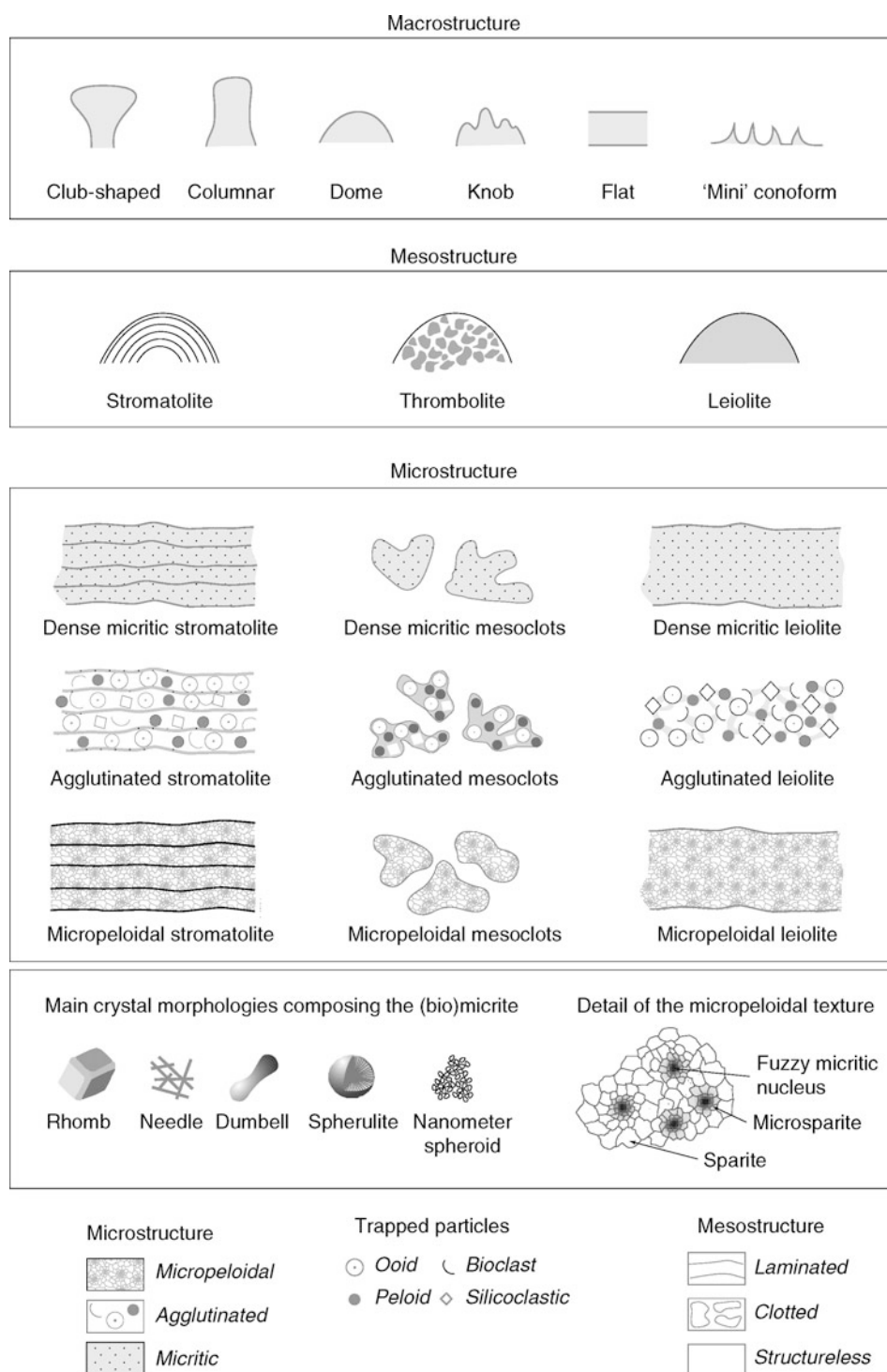
Hydroxides and Hydrous Oxides

Goethite - $\alpha\text{-FeOOH}$
 Ferrihydrite - $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
 Lepidocrocite - $\gamma\text{-FeOOH}$
 Ferrihydrite - $5\text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$
 Todorokite - $(\text{Mn}^{+2}\text{CaMg})\text{Mn}_3^{+4}\text{O}_7 \cdot \text{H}_2\text{O}$
 Birnessite - $\text{Na}_4\text{Mn}_{14}\text{O}_{27} \cdot 9\text{H}_2\text{O}$

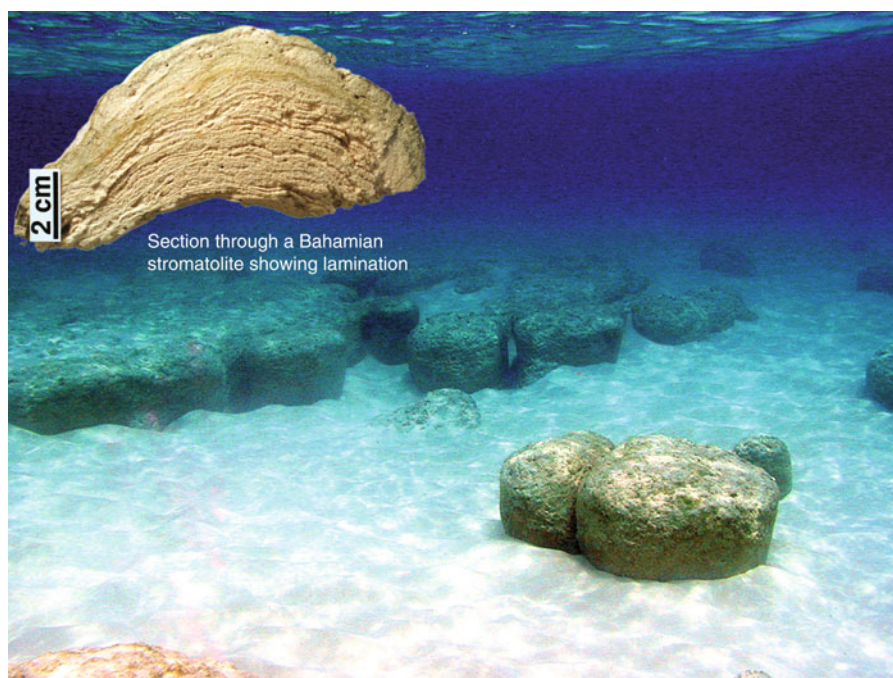
Organic Crystals following Lowenstam & Weiner (1989)

Whewellite - $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$
 Weddellite - $\text{CaC}_2\text{O}_4 \cdot (2+x)\text{H}_2\text{O}$
 Mn Oxalate - $\text{Mn}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
 Earlandite - $\text{Ca}_3(\text{C}_6\text{H}_5\text{O}_2)_2 \cdot 4\text{H}_2\text{O}$
 Glushinskite - $\text{MgC}_2\text{O}_4 \cdot 4\text{H}_2\text{O}$
 Manganese Oxalate - $\text{Mn}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$
 Sodium urate - $\text{C}_5\text{H}_3\text{N}_4\text{NaO}_3$
 Uric Acid - $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$
 Ca tartrate - $\text{C}_4\text{H}_4\text{CaO}_6$
 Ca malate - $\text{C}_4\text{H}_4\text{CaO}_5$
 Paraffin Hydrocarbon
 Guanine - $\text{C}_5\text{H}_3(\text{NH}_2)\text{N}_4\text{O}$

Microbialites, Modern, Figure 1 Nomenclature and chemical compositions of minerals produced by biologically-related mineralization processes. (Modified from Weiner and Dove, 2003; data from Lowenstam and Weiner, 1989; Simkiss and Wilbur, 1989; Mann, 2001; Weiner and Addadi, 2002.)



Microbialites, Modern, Figure 2 Descriptive terminology for microbialite macro-, meso, and microfabrics. (From Kennard and James, 1986; Riding, 1991; Schmid, 1996; Dupraz and Strasser, 1999; Shapiro, 2000.)



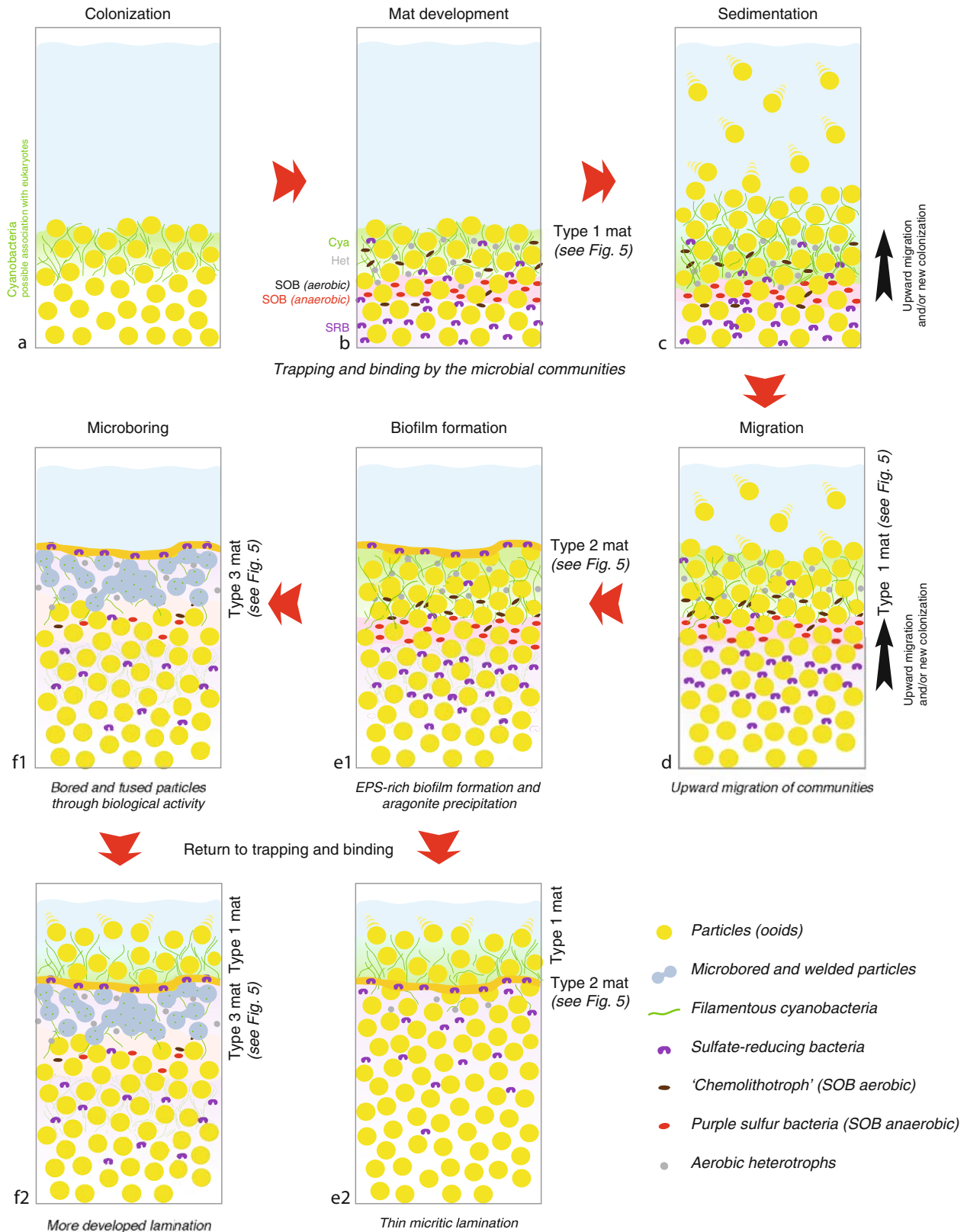
Microbialites, Modern, Figure 3 Underwater photograph showing shallow subtidal stromatolites (Darby Island, Bahamas) with a vertical section (upper left hand insert) showing well-developed lamination.

Dill et al., 1986; Reid et al., 1995). These stromatolites are coarse grained structures, composed of ooid sand; they form in intertidal and subtidal environments, exposed to high wave energy and strong tidal currents (e.g., Gebelein, 1976; Reid et al., 2000). Many of the stromatolites exhibit well-developed laminae (Figure 3; e.g., Reid et al., 2000). The lamination results from iterative growth of different types of microbial mats on the top of the build-ups (Figure 4). Each microbial mat forms a distinct mineral product, which can be recognized in thin section and SEM (Figure 5; Reid et al., 2000). The first mat type (1) is dominated by the filamentous cyanobacterium *Schizothrix*, which traps and binds ooids. The stickiness of the mat EPS results in the trapping of particles, which are subsequently bound by the upward growth of the cyanobacterial filaments. Other microorganisms (e.g., diatoms that produce copious amounts of EPS) can also participate in trapping grains. The second mat community (2) consists of a thin biofilm with abundant heterotrophic bacteria. Precipitation of aragonite within this biofilm forms a micritic lamina, which caps the underlying ooids. The third mat type (3) is characterized by an abundance of the endolithic coccoid cyanobacterium *Solentia* spp. *Solentia* tunnels through grains, leaving bore holes filled with EPS. Aragonite precipitation within the EPS results in micritization of the grains and, when tunnels cross between grains, welds the grains together. This process forms a well-cemented layer (Macintyre et al., 2000; Reid and Macintyre, 2000; Reid et al., 2000). The in situ precipitation of carbonate, which leads to early lithification of

the stromatolite-forming mats, is a result of prokaryotic activity (Visscher et al., 2000; Reid et al., 2003a).

Dominance of prokaryotic communities on the surfaces of modern marine stromatolites, is favored by the selective environmental pressure of periodic burial by sand (Andres and Reid, 2006; Kromkamp et al., 2007). In contrast to eukaryotes, which are typically killed when buried for periods of weeks to months, prokaryotes such as cyanobacteria are able to survive and recover from long burial events (Kromkamp et al., 2007). This attribute may contribute to the success of stromatolites in modern and past environments. Eukaryotic colonization (macroalgae: e.g., *Batophora*), during week- to month-long periods of exposure disrupts the lamination resulting from the cycling of prokaryotic mats, producing intervals of crude to no lamination within the stromatolite head.

It should be noted that trapping and binding alone does not form a stromatolite. The key factor in allowing development of a lithified laminated buildup is a hiatus in sediment accretion and corresponding development and lithification of a microbial biofilm. During long hiatus periods (i.e., several weeks), surfaces infested with the coccoid bacterium *Solentia* forms thicker, well-cemented lamina of fused ooid grains. Precipitation of aragonitic needles associated with the activity of sulfate-reducing bacteria is a key feature in carbonate precipitation within Bahamian stromatolites (Visscher et al., 2000). Precipitation resulting from anaerobic heterotrophic activity is supported by isotopic data (Andres et al., 2005).



Microbialites, Modern, Figure 4 (Continued)

Factors leading to the cycling of surface communities responsible for lamination in Bahamian stromatolites are currently under investigation (Andres and Reid, 2006). Both biological and environmental factors may be important (Figure 6; Seong-Joo et al., 2000; Reid et al., 2003a). Biological factors include microbial ecological interactions, and production and consumption of organic and inorganic compounds. Extrinsic factors include temperature, light, nutrients, and hydrodynamics, which could affect the stickiness of EPS and/or sediment supply (Figure 6).

Hypersaline and saline stromatolites

In contrast to open marine environments, where modern stromatolites are rare, living stromatolites are more common in hypersaline environments. This is because microbial communities are able to cope with high salinity, whereas most eukaryotes, such as macroalgae and burrowing/grazing invertebrates, which compete with or ingest cyanobacteria and disrupt lamination, are largely excluded (Fischer, 1965; Awramik, 1971, 1982, 1992; Walter and Heys, 1985; Riding, 2006).

Stromatolites in Shark Bay, Western Australia are the most famous stromatolites thriving in hypersaline water. They are found in the Hamelin Pool Marine Nature Reserve of the Shark Bay UNESCO World Heritage Site. Ranging from 55–70 ppt throughout the year (Playford, 1990), Hamelin Pool has approximately double normal marine salinity and hosts abundant and diverse stromatolites. Shark Bay stromatolites were discovered in 1954 by Johnstone, Playford, and Chase of the West Australian Petroleum Pty. Ltd (Playford and Cockbain, 1976). They were the first modern stromatolites discovered with sizes and shapes comparable to fossil counterparts (Logan, 1961; Logan et al., 1974; Playford and Cockbain, 1976; Playford, 1979; Bauld et al., 1979; Bauld, 1981; Burns et al., 2004). Like Bahamian stromatolites, Shark Bay structures are formed by both (1) microbial trapping and binding and (2) microbial precipitation. Both processes are important in the intertidal zone, forming coarse grained sandy stromatolites. Microbial precipitation is the primary accretionary mechanism in the subtidal zone of Shark Bay, forming muddy, micritic stromatolites (Reid et al., 2003b).

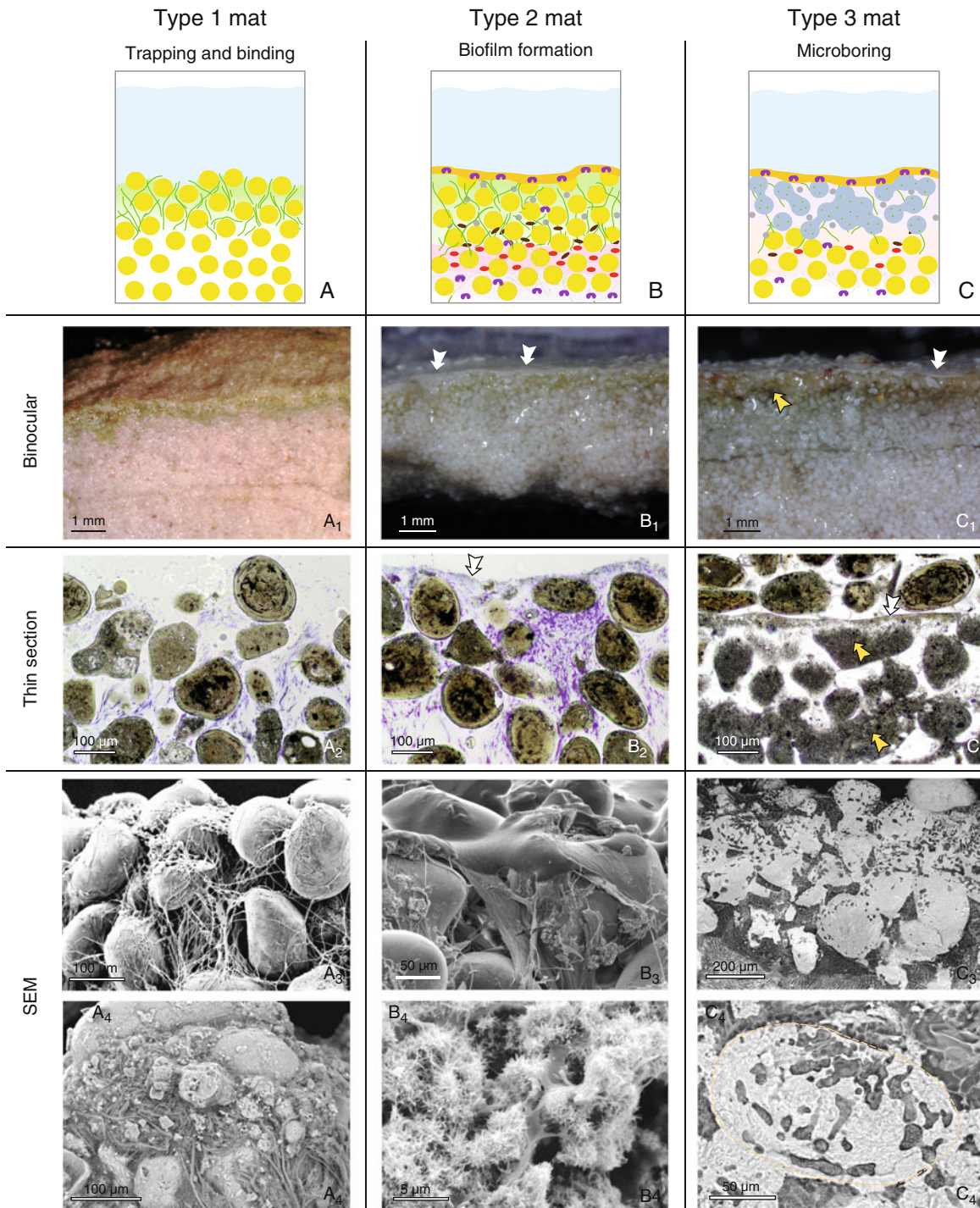
As a result of the dominant sandy textures of Shark Bay stromatolites and open marine stromatolites in the Bahamas, some authors have proposed that these stromatolites are not appropriate analogs for fossil stromatolites, which are generally display fine-grained, micritic microstructures (Awramik and Riding, 1988). Despite their overall coarse-grained texture, these modern stromatolites have micritic laminae formed as a result of microbially-induced precipitation (Reid et al., 2000; Visscher et al., 2000). Moreover, the ecological model developed for Bahamian stromatolites, in which lamination results from the cycling of prokaryotic communities on the stromatolite surface, is likely applicable to the growth of Shark Bay stromatolites. Indeed, a model of iterative accretion of laminae, which record both microbial and environmental fingerprints and progressively shape the emergent morphology, may serve as a conceptual model for the growth of fossil stromatolites.

In addition to Shark Bay, living stromatolites are also present in many other saline and hypersaline environments. Several saline lakes in Australia harbor stromatolites, including Lake Thetis, near Cervantes, lakes on Rottne Island (Reitner et al., 1996), and Lake Clifton near Mandurah (e.g., Grey et al., 1990). Cyanobacterial stromatolitic domes have been described in the intertidal zone of Bermuda (e.g., Sharp, 1969; Golubic and Focke, 1978) and small crudely laminated knobs have been found in a hypersaline lake on Bonaire Island in the Netherlands Antilles (Golubic and Focke, 1978). Various islands in the Bahamas also have hypersaline lakes with well-developed microbial mats and stromatolites, including Storr's Lake on San Salvador. Storr's lake harbors alternating stromatolitic and thrombolitic buildups (Mann and Hoffman, 1984; McNeese, 1988; Neumann et al., 1988; Mann and Nelson, 1989; Pentecost, 1989; Zabielski, 1991).

Fresh water and continental stromatolites

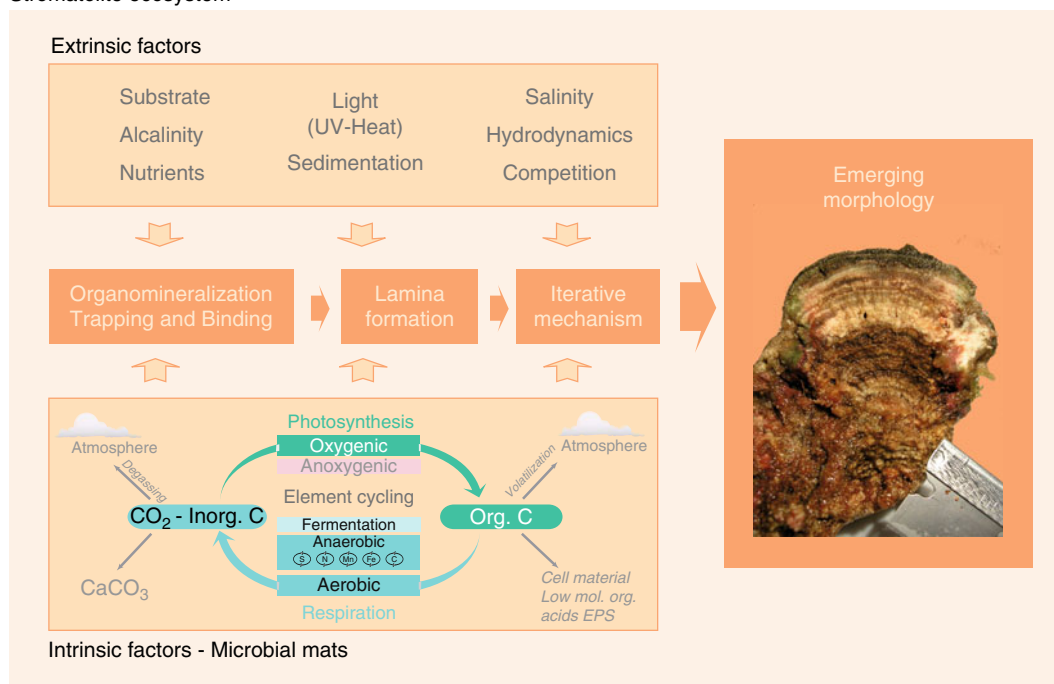
Numerous examples of freshwater laminated microbialite have been published. One of the most widespread examples of these microbialites is travertines. Tufa stromatolites (meteoene travertine following Pentecost (2005)) are freshwater fluviatile tufas, which locally exhibit thick laminated crusts formed by calcified cyanobacteria (Pentecost, 1978; Riding, 1991b, 2000). Most of the precipitation of carbonate in these travertines results from physicochemical

Microbialites, Modern, Figure 4 Model of coarse-grained open-marine stromatolite formation. (a) Cyanobacteria colonize and stabilize mobile ooid sand (trapping and binding). (b) A complex microbial mat develops in the upper few millimeters of the sediment (Cya cyanobacteria; SRB sulfate reducing bacteria; SOB sulfide oxidizing bacteria (aerobic and anaerobic); Het aerobic heterotrophs; see also Figure 8). (c–d) As a result of sediment accretion, the microbial mat migrates upward, continuing trapping and binding. Cyanobacteria (and heterotrophs) can survive massive burial. No significant in situ precipitation is observed at this stage, which corresponds to the Type 1 mat in Figure 5. (e) During a hiatus in sediment accretion, an EPS-rich biofilm develops, draping over the ooids (Type 2 mat, Figure 5). This biofilm rapidly lithifies due to the activity of anaerobic heterotrophs (i.e., SRB), forming a thin lamina of micritic aragonite (see Figure 5b4). (f) A prolonged hiatus in trapping and binding results in colonization of ooid grains under the biofilm by the endolithic cocoid cyanobacteria *Solenia* sp. (Type 3 mat, Figure 5) Precipitation of aragonite in the bore holes made by these endoliths destroys the internal microstructure of ooids, changing their color from golden brown to gray. Precipitation in bore holes crossing between grains fuses them together (Figure 5c2–4). Resumption of trapping and trapping corresponds to formation of a new Type 1 mat (e₂, f₂). The iterative succession of these three different mat types is responsible for the lamination observed in open-marine stromatolite (Figure 3).



Microbialites, Modern, Figure 5 Micrographs illustrating the main features of the surface mats responsible for the formation of modern marine stromatolites. Stromatolite lamination results from the iterative growth at the surface of the build up of three mat types: (1) Type 1 mats are dominated by filamentous cyanobacteria, which trap carbonate sand grains through EPS stickiness (A₁₋₄); (2) Type 2 mats consist of a continuous biofilm, draping the stromatolite surface (white arrows in B₁₋₃); the biofilm is composed of extracellular polymeric substances (EPS, B₃) containing numerous heterotrophic bacteria. This biofilm rapidly lithifies as a result of precipitation of aragonite needles within the biofilm (B₄); (3) Type 3 mats are characterized by endolith-infested ooid grains (yellow arrows in C₁₋₂), which appear gray and are fused together, below a surface biofilm. (A₁-B₁-C₁) binocular micrographs, (A₂-B₂-C₂) petrographic micrographs, (A₃-B₃-B₄) high vacuum scanning electron microscope images (SEM; chemical drying), and (A₄-C₃-C₄) cryo-SEM (frozen samples) images.

Stromatolite ecosystem



Microbialites, Modern, Figure 6 Interactive processes within the stromatolite ecosystem. Intrinsic (microbial mat-related) and extrinsic (environment-related) factors work together to determine the emergent stromatolite morphology and fabric, which can be described at microscale (biomineralization, trapping, and binding), mesoscale (laminae formation), and macroscale (iterative mechanism leading to morphology) levels.

degassing of CO₂ through resurgence, cascades, or waterfalls. Photosynthetic uptake of CO₂ by cyanobacteria can, however, be responsible for the precipitation of calcium carbonate in slow running and low DIC streams (Verrecchia et al., 1995; Merz-Preiss and Riding, 1999; Arp et al., 2001). Relatively low DIC and high Ca²⁺ concentration is required for photosynthesis to influence carbonate alkalinity and enable precipitation (Arp et al., 2001, 2003).

Fresh water stromatolites are also present in Antarctic lakes, which are permanently ice-covered (Parker et al., 1981). These lakes are seasonally fed by glaciers, which modify salinity from fresh-to-saline while oxygen levels vary from anoxic to supersaturated. The stromatolites are formed through sediment trapping and binding as well as carbonate precipitation in cyanobacterial-dominated (e.g., *Phormidium*, *Oscillatoria*) microbial mats. Other non-marine environments with stromatolites include alkaline lakes, such as the Caldera lakes of Niufo'ou Island (Tonga), with spectacular laminated buildups (Kazmierczak and Kempe, 2006).

Laminated deposits, referred to as terrestrial stromatolites (Verrecchia et al., 1995; Riding, 2000), are observed in "non-water-saturated" environments. These deposits have also been called lichen stromatolites (Klappa, 1979) and subaerial stromatolites (Riding, 1991b). They consist of laminar calcretes formed by microbial activity or in close association with microbial communities.

Determining between biogenic and non-biogenic calcretes is often difficult as carbonate precipitation may be caused by physicochemical evaporation (e.g., Read, 1976), and/or cyanobacterial activity (e.g., Verrecchia et al., 1995).

Thrombolite

The term thrombolite was introduced by Aitken (1967) for microbial "structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric". Since this initial definition, thrombolites and stromatolites have been recognized as two distinct microbialite structures (Kennard and James, 1986). The key microstructure of thrombolites is mesoclots, which produce the clotted fabric (Shapiro, 2000). The mesoclots consist of polymorphic (simple spheroids to polylobate), millimeter to centimeter-sized, aggregates which display a variety of forms, from simple spheroids to polylobate masses (e.g., Shapiro, 2000). Clotted mesostructures have been described for many fossil thrombolites, which are mostly composed of fine-grained carbonate. Therefore, many authors define mesoclots as mostly composed of micrite or micropeloids (Kennard, and James, 1986; Dupraz and Strasser, 1999; Shapiro, 2000). However, modern thrombolites, as well examples from the Miocene, show agglutinated, clotted fabrics (Riding, 2000). The mesoclots of modern thrombolites thus consist of micrite, micropeloids, or agglutinated particles (Figure 2).

The clotted fabric of thrombolites can have a variety of origins. The fabric can be attributed “to the in situ calcification of coccoid or coccoid-dominated microbial communities” (Kennard and James, 1986). Other mechanisms involve bioturbation and bioerosion, and thrombotic mesostructures within large Bahamian stromatolites are often due to disruption of the lamination by eukaryotic colonization (e.g., *Batophora* sp. and other algae).

In some cases, environmental conditions may trigger changes between thrombolite and stromatolitic fabrics. In Storr’s Lake (San Salvador, Bahamas), well-developed thrombolites alternate with stromatolitic layers (McNeese, 1988; Mann and Nelson, 1989). This alternation might be triggered by lake-level fluctuations and associated turbidity variability. This mechanism results in a succession of phototrophic-dominated (stromatolites) to heterotrophic-dominated communities (thrombolites) as surface communities, as modeled by Dupraz et al. (2006).

Many other modern lakes host thrombolite deposits: Lake Clifton, Lake Tethys, Lake Richmond and different lakes on Rottnest Island (Australia), Kelly and Pavillon lakes (British Columbia, Canada), lakes on Bonaire Island (Netherlands Antilles, southern Caribbean; Kobluk and Crawford, 1990), Lago Sarmiento (Patagonia, Chile), Poza Azul lake (Cuatro Ciénegas, Mexico), Yellow Stone National Park (USA), Green Lake (Fayetteville, New York, USA). In addition, travertines showing thrombotic features have been referred to as “Tufa Thrombolite” (Riding, 2000).

Leiolite

The term leiolite comes from the Greek word “leios,” meaning uniform or smooth and “lithos,” (meaning rock) and was originally applied to late Miocene (Messinian) deposits in Spain (Figure 2; Braga et al., 1995). Leiolite is characterized by relatively structureless, aphanitic, mesostructure, lacking lamination or clots, and can be a synonym for “cryptomicrobial” as used by Kennard and James (1986). Most authors tend to classify microbialites as either stromatolites or thrombolites, despite the fact that many of these deposits lack the defining mesostructures and would more appropriately be termed leiolite (see Riding, 2000). No examples of modern leiolite have been published. However, examples could include non-laminated, non-thrombotic portions of Bahamian or Shark Bay stromatolites, carbonate crust formation in hypersaline lakes (Dupraz et al., 2004), and biological stabilization of sand dune by chasmolithic microorganisms (Hillgaertner et al., 2001).

Other types of microbialites

Microbial processes or the presence of microbes are often intimately related to sedimentary processes in modern carbonate systems, although the resulting deposits may not be considered as true microbialite and classified as stromatolite, thrombolite, or leiolite. These microbial carbonate

deposits include meteogene and thermogene travertine (Pentecost, 2005), where microbial influence creates specific microfabrics. Spectacular examples of travertines can be observed in many alkaline lakes, forming carbonate towers (e.g., Pyramid Lake, Nevada (Benson, 1994)), Lake Can, Turkey (Kempe et al., 1991), and Mono Lake, California (e.g., Bischoff et al., 1993; Riding, 2000). Carbonate chimneys are also formed in hydrothermal systems, where microbial activity is important (e.g., “Lost City” Hydrothermal Field; Kelley et al., 2001; Ludwig et al., 2006).

Other deposits that are difficult to classify include microbial calcrete – a terrestrial carbonate the formation of which is strongly influenced by bacteria and fungi (Jones and Wilson, 1986; Wright et al., 1988; Verrecchia and Verrecchia, 1994; Verrecchia et al., 1995). In addition, in continental environments, the formation of needle fiber calcite (NFC) is related to fungi activity (Verrecchia and Verrecchia, 1994; Cailleau et al., 2004, 2005). Mondmilch or Moonmilch – a soft paste-like or powdery cave deposit – includes important microbial populations, which influence precipitation (Thraillkill, 1976; Canaveras et al., 2006). Microbial mats are also involved in the formation of oncoids, which are unattached spherical stromatolites (e.g., Bathurst, 1966), and microbial activity may play a role in the formation of “whittings” (Thompson, and Ferris, 1990; Robbins, and Blackwelder, 1992).

Processes of microbialite formation

Early lithification is a key process in the formation of modern carbonate microbialites and is critical for the preservation of these structures in the fossil record. Lithification processes start at the time of deposition and continue during burial (diagenesis). Lithification consists of in situ precipitation of minerals, which progressively cement trapped grains (if present), and preserve the biosedimentary structures. Two closely coupled components are fundamental in carbonate precipitation within microbial mats: (1) the alkalinity engine and (2) the organic matrix in which this mineral is forming.

The alkalinity engine

The precipitation of carbonate minerals depends on the availability of carbonate ions, specific cations (e.g., Ca^{2+} , Mg^{2+}), and suitable nucleation sites. Carbonate minerals may precipitate when a solution is saturated with respect to that mineral. The degree of saturation is calculated via the saturation index of the specific mineral:

$$\text{SI} = \log(\text{IAP}/K_{\text{ps}}) \quad (1)$$

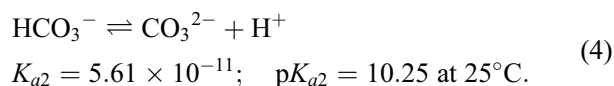
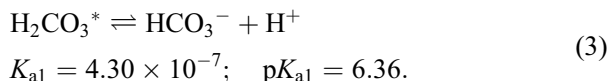
IAP is the “ion activity product” and K_{sp} is the solubility product of the corresponding mineral (solid phase). In the case of CaCO_3 , both the carbonate alkalinity and the activity of the available cations are taken into account

when calculating the saturation index (Stumm and Morgan, 1996):

$$SI_{\text{CaCO}_3} = \text{Log}(\{ \text{Ca}^{2+} \} * \{ \text{CO}_3^{2-} \} / K_{\text{sp}}). \quad (2)$$

The activity of a given chemical compound corresponds to the concentration of that compound multiplied by the activity coefficient. The solubility products for aragonite and calcite are $10^{-6.19}$ and $10^{-6.37}$, respectively, at 25°C, 1 bar atmospheric pressure and 35 PSU salinity (Zeebe and Wolf-Gladrow, 2001). A solution is supersaturated with respect to a given mineral when SI is greater than 0, meaning that K_{sp} is smaller than IAP. Experimental evidence showed that a supersaturation must be greater than 0.8 in order to have spontaneous precipitation of CaCO_3 (Kempe and Kazmierczak, 1994). Arp et al. (2001) used SI greater than 1 (i.e., a 10-fold supersaturation) as a prerequisite for carbonate precipitation in non-marine environment.

Carbonate ion activity, $\{ \text{CO}_3^{2-} \}$, depends on the carbonate equilibrium. CO_2 dissolves in water to form carbonic acid (H_2CO_3). The amount of dissolved CO_2 is proportional to the partial pressure of CO_2 ($p\text{CO}_2$) in the gas phase in contact with the liquid (i.e., Henry's Law). This value varies with pressure and temperature. Low temperature and high pressure allow for more CO_2 dissolution in water. Carbonic acid is weak acid that will only partially dissociate in an aqueous solution to produce H^+ ion and the conjugate base. It will deprotonate as follows (with pK_s value for fresh water):

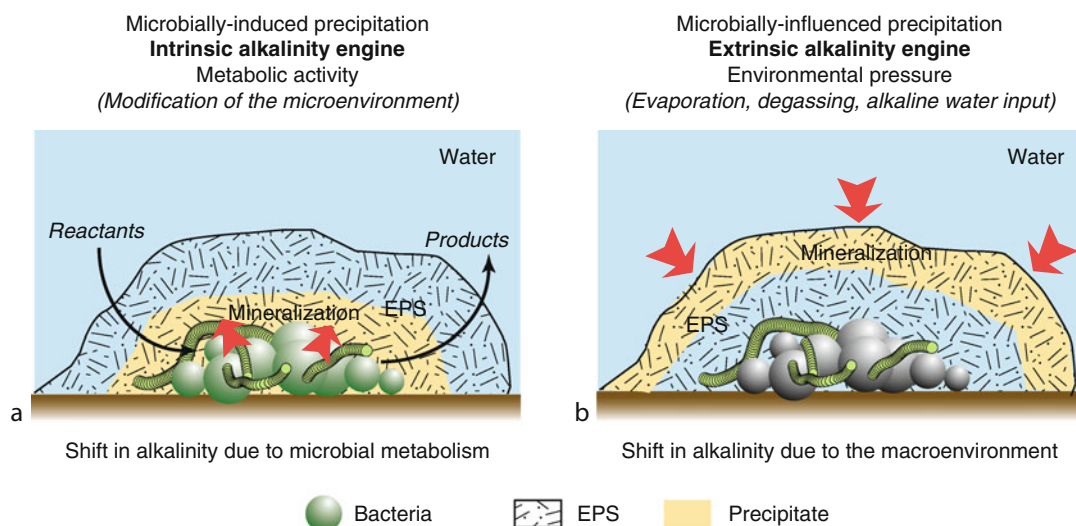


The two pK_s values vary as a function of the salinity. For seawater conditions, pK_{a1} and pK_{a2} are 5.9 and 8.9, respectively (at 25°C, 1 bar atmospheric pressure and 35 PSU salinity; Zeebe and Wolf-Gladrow, 2001).

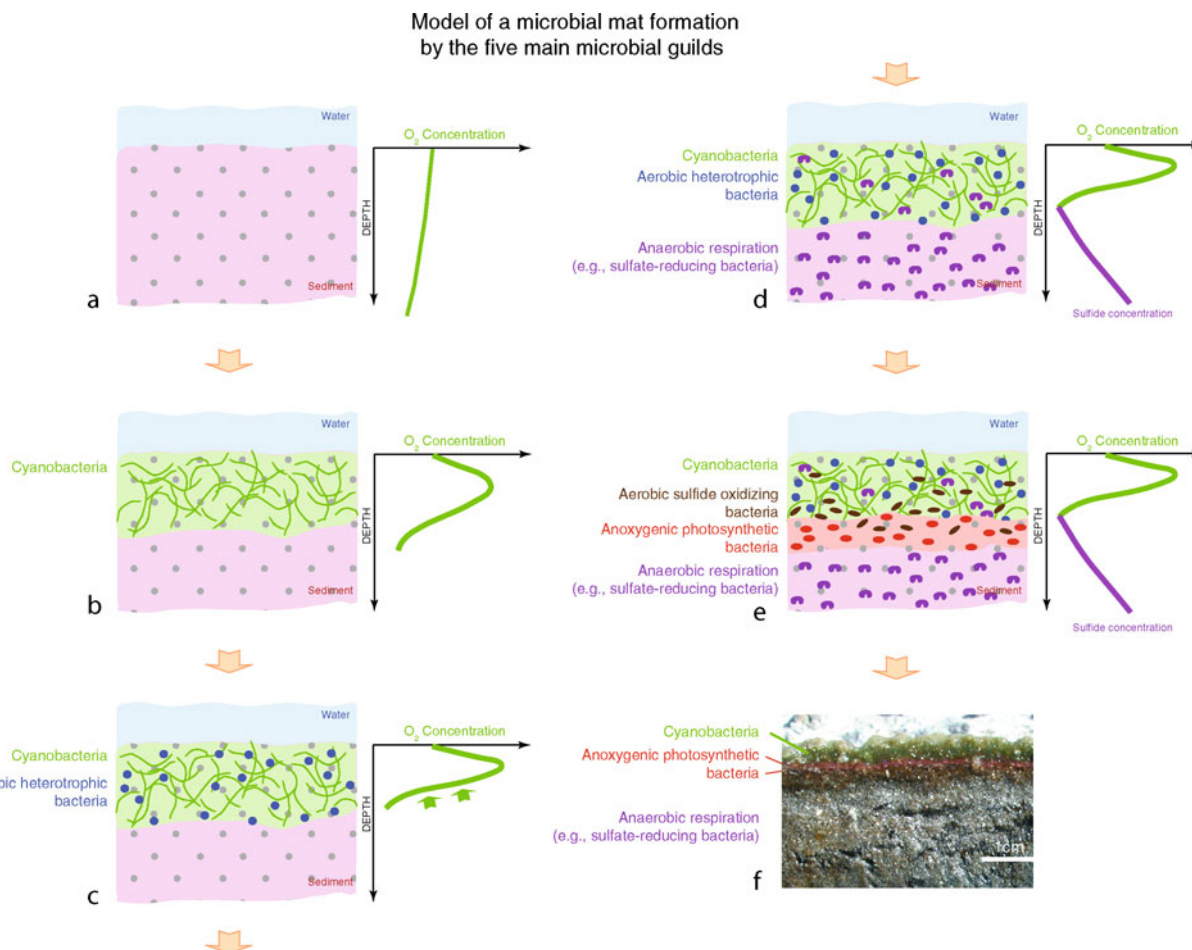
Availability of carbonate ions is a prerequisite for precipitation of carbonate minerals. The amount of bicarbonate and carbonate ions in solution denotes carbonate alkalinity, which is a part of total alkalinity, including borate, hydroxide, phosphate, and silicate. In marine and most of freshwater environments, phosphate and silicate are minor components, and the carbonate equilibrium typically drives global alkalinity. Various processes can have an impact of carbonate alkalinity, indirectly promoting precipitation or dissolution of carbonate mineral. The sum of processes that creates alkalinity represents the “alkalinity engine.” This engine is intrinsically driven when alkalinity is affected by microbial communities, or extrinsically driven when physico-chemical processes of the macro-environment cause alkalinity shifts (Figure 7).

Intrinsic alkalinity engine (metabolism)

The way microbial communities acquire their energy and their source of carbon (i.e., through metabolism) can have a strong impact on local carbonate alkalinity. In a simplified view of the microbial carbon cycle, the production of organic matter through photosynthesis (oxygenic or anoxygenic) is coupled with the oxidation of organic carbon by aerobic (oxygen as oxidant) or



Microbialites, Modern, Figure 7 Model of carbonate precipitation within biofilms. Increases in alkalinity leading to precipitation can be (a) intrinsic, resulting from microbial metabolism or (b) extrinsic, resulting from environmental factors (b). In both the cases, the EPS matrix, which embeds the microbial communities, has a pivotal role in determining mineral morphology, composition, and mineralogy.



Microbialites, Modern, Figure 8 Model of microbial mat formation by the five main guilds (van Gernerden, 1993). (a) Typical diffusion of O₂ through the water-sediment interface in the absence of microbial activity. (b) Cyanobacteria colonize the sediment, fixing (reduce) CO₂ to form biomass using solar energy (photosynthesis) and using water as electron donor. O₂ is a byproduct (visible in the upper millimeter of the depth profile). (c) The autotrophically-produced organic carbon is degraded by aerobic heterotrophs (aerobic respiration) using O₂ as final electron acceptor. The consumption of oxygen results in, or is reflected by, a sharper O₂ gradient in the sediment (arrows) and the formation of an anoxic zone below the active cyanobacterial layer. (d) Anaerobic heterotrophs then develop in the anoxic zone (mostly SRB in this example). (e) SRB produces sulfide compounds, which can then feed anoxygenic photosynthesis and aerobic sulfide oxidation. (f) Examples of a hypersaline mat from the Bahamas showing classical zonation of bacterial guilds.

anaerobic (SO₄²⁻, NO₃⁻, Fe³⁺, etc. as oxidant) respiration (Figure 8). Most microbial metabolites (products of metabolism) are efficiently recycled within the mats, completely closing the cycles of major elements. Many microbial mats function as light-driven engines, fueled only by sunlight.

The biogeochemical niche of microorganisms can be described through three overarching metabolic characteristics related to energy generation and biomass acquisition: the energy source, the electron donor used for energy generation, and the carbon source for biomass production. Each of these three properties have two possibilities: potential energy sources are light (photo-) or chemical redox reactions (chemo-), electron donors can be organic (organo-)

or inorganic (litho-), and biomass can be derived from CO₂ fixation (autotrophy) or from carbon that is already fixed (heterotrophy). For example, a cyanobacterium derives energy from light (photo) uses water as electron donor (litho) and fixes CO₂ for biomass (auto-); it is hence designated as a photolithoautotroph (trophos means “to feed”). A typical aerobic bacterium that uses organic carbon is a chemoorganoheterotroph, etc.

Different types of metabolism impact carbonate precipitation and dissolution by taking up (autotrophy) or releasing (respiration) CO₂ and through production or consumption of organic acids. Changes in organic acid concentrations modify alkalinity and pH of the mats (Ehrlich, 1996; Visscher and Stolz, 2005). Mechanisms

that can promote precipitation of carbonate minerals include the following:

1. *Oxygenic photosynthesis* (producing O_2) by cyanobacteria (photolithoautotrophy). Uptake of CO_2 during photosynthetic activity leads to precipitation of carbonate in the sheath or in the direct vicinity of the cyanobacteria (Pentecost and Riding, 1986; Frey et al. and Verrecchia, 1998, 1999; Merz-Preiss and Riding, 1999; Riding, 2000) (Figure 9). The increase in alkalinity is attributed to an exchange of HCO_3^- and OH^- through the cell membrane (Figure 9). Photosynthesis is an important mechanism for microbialite formation in freshwater environment (Arp et al., 2001), where it may help overcome the kinetic barrier for $CaCO_3$ precipitation, even in highly supersaturated freshwater settings (Shiraishi et al., 2008).
2. *Aerobic respiration* (chemoorganoheterotrophy) generally dissolves calcium carbonate through production of CO_2 . However, precipitation can occur in a well-buffered alkaline environment when CO_2 produces carbonate ions. Carbonate minerals can also be formed when aerobic respiration consumes strong organic acids and produce CO_2 that will be degassed. This process is proposed for carbonate precipitation in tropical soil associated to the oxalate-carbonate cycle (Braissant et al., 2004). In the absence of O_2 , some bacteria can generate energy through fermentation, which process uses the same compound (e.g., organic carbon, inorganic sulfur) both as electron donor and acceptor. Most fermentation of organic carbon will lead to dissolution of carbonate (Visscher and Stolz, 2005).
3. *Anaerobic respiration*: certain bacteria can oxidize organic matter using respiratory chains that do not use O_2 as final electron acceptor (also chemoorganoheterotrophy). These bacteria can use, e.g., sulfate (sulfate-reduction), nitrate (nitrate-reduction), iron (iron-reduction), manganese (manganese reduction), or even HCO_3^- . These types of metabolism can promote precipitation of carbonate through various processes (e.g., Visscher and Stolz, 2005): consumption of organic acid, production of base (e.g., NH_3), removing precipitation inhibition (removing of SO_4^{2-}), etc. Sulfate reduction is the main process in marine environment due to high concentration of SO_4^{2-} in seawater.
4. *Chemolithoautotrophy*: Some bacteria do not oxidize organic matter to generate energy. They will instead use the chemical energy (chemo) produced by the oxidation of inorganic (litho) electron donors such as H_2 , CO , Fe^{2+} , NH_4^+ and HS^- in order to fix CO_2 (autotrophy). Sulfide (in sediments) and ammonium (in the water column) oxidation are the most abundant chemolithoautotrophic electron donor in marine environment. Aerobic sulfide oxidation and ammonium oxidation is likely to induce dissolution of carbonate minerals, whereas anaerobic sulfide oxidation (e.g., using nitrate as electron acceptor) may induce

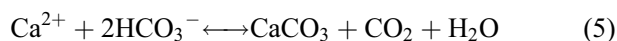
precipitation (Visscher and Stolz, 2005). However, most of these types of metabolism are all autotrophic, i.e., fixing CO_2 . The uptake of CO_2 can increase carbonate alkalinity and induce precipitation of carbonates in similar way that physicochemical degassing of CO_2 does.

Extrinsic alkalinity engine

The determination of alkalinity-driving forces that form microbialites is generally a complex task. In many cases, a combination of intrinsic and extrinsic factors is responsible for precipitation. There are however, a few examples where the macroenvironment acts as a major player in microbial mat lithification. The two important physicochemical processes that can lead to carbonate precipitation in microbial mats are water evaporation and CO_2 degassing. Even in these cases, however, the microbial communities of the mats can serve as substrates for physicochemical carbonate precipitation and affect mineral mineralogy or morphology.

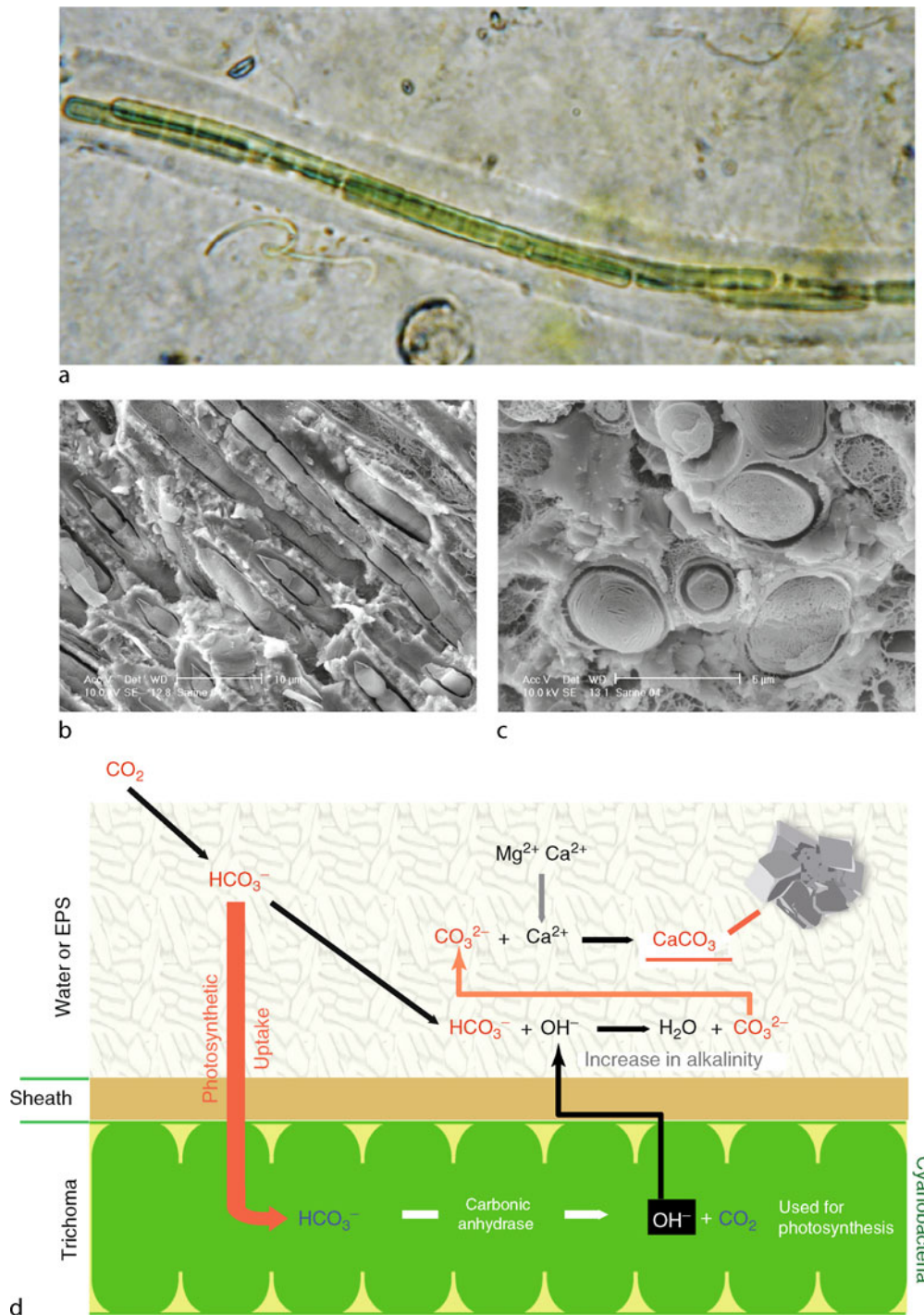
In the marine environment, solar evaporation of water may lead to the formation of evaporitic minerals (e.g., Warren, 2006). Although a variety of carbonate minerals can be produced through this mechanism (e.g., calcite, ikaite, aragonite, Mg-calcite, dolomite, and magnesite), evaporites largely consist of halite and gypsum. Examples of microbial mats associated with gypsum deposits have been described (e.g., Babel, 2004). Moreover, bacteria trapped in gypsum crystals are able to survive for millions of year (Vreeland et al., 2000, 2007). The specific role of microbes in gypsum nucleation is, however, not clear as the evaporation processes generally obscure microbial signatures. For reviews on evaporites, see Yechieli, and Wood (2002) or Warren (2006).

Precipitation of carbonate can also result from degassing of CO_2 and forms travertines. Travertines represent chemically-mediated continental carbonate deposits that form around sinter sources along streams and lakes (Pentecost, 2005). The precipitated minerals, consisting of aragonite or calcite, are mainly related to the release of CO_2 from the system, resulting in supersaturation with respect to calcium carbonate. This can be illustrated by the buffering equation of the carbonate:

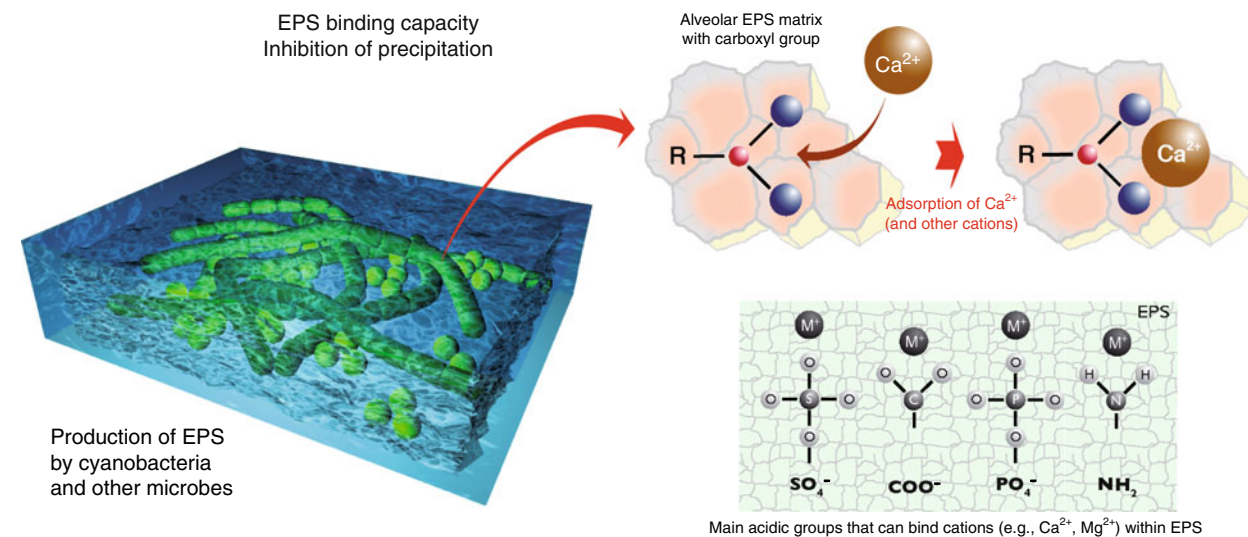


Removal of CO_2 through degassing will drive this equation to the right, promoting precipitation of $CaCO_3$. Similarly, increasing dissolved CO_2 drive the equation to the left, resulting in dissolution of calcium carbonate.

Travertines can be classified based on the source of the CO_2 that is degassed (Pentecost, 2005). "Metogene travertines" are formed by degassing of meteoric carbon dioxide, whereas "thermogene travertines" are formed by the degassing of hydrothermal CO_2 . In metogene travertines, the CO_2 can originate from the atmosphere or from the soil-zone. In thermogene travertines, the bulk of the CO_2



Microbialites, Modern, Figure 9 Role of photosynthesis in the precipitation of carbonate minerals. (a) Photomicrograph of *Microcoleus* spp., a filamentous cyanobacterium showing the trichoma (chains of cells) embedded in a sheath. (b) Longitudinal section through a freshwater travertine (Sarine River, Switzerland) showing precipitation of CaCO_3 in the sheath of the filamentous cyanobacteria *Oscillatoria* sp. and *Phormidium* sp. (c) Perpendicular section through the same travertine. (d) Model of photosynthetically-driven carbonate precipitation. CO_2 dissolve in water, forming bicarbonate ions (the most common form of DIC between pH 5 and 9). The bicarbonate is taken up by cells of the trichoma; the enzyme carbonic anhydrase inside the cells produces CO_2 (used for photosynthesis) and OH^- . The release of hydroxyl ions outside the trichoma increases the alkalinity in the surrounding area of the cyanobacteria and induces precipitation of CaCO_3 (provided Ca^{2+} ions are available).

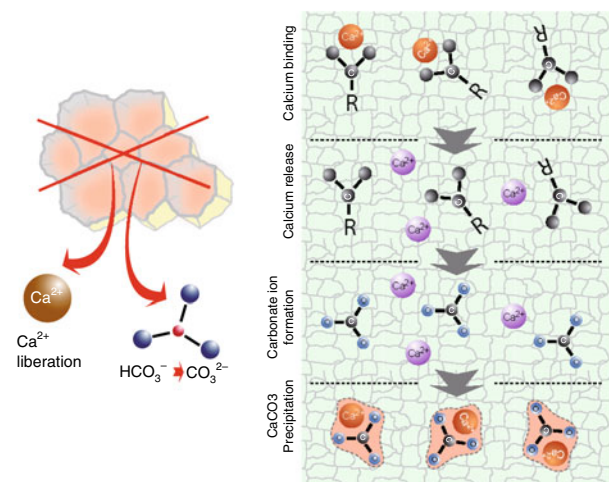


EPS alteration
leading to precipitation of carbonates

Biologically-induced mineralization

EPS Degradation

Microbial degradation of EPS liberating calcium and inorganic carbon (increasing the carbonate mineral saturation index)
Various types of mineralogy and morphology



Free calcium

Bound calcium

Acidic group

Sterically blocked acidic group

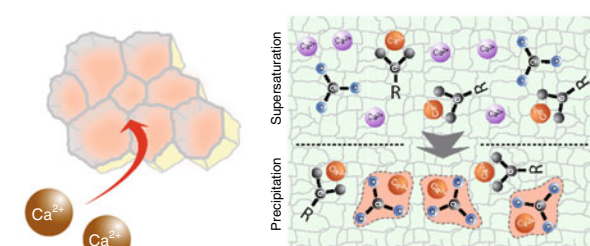
Carbonate ion

Nucleation of CaCO₃

Biologically-influenced mineralization

Extrinsic Supersaturation of EPS

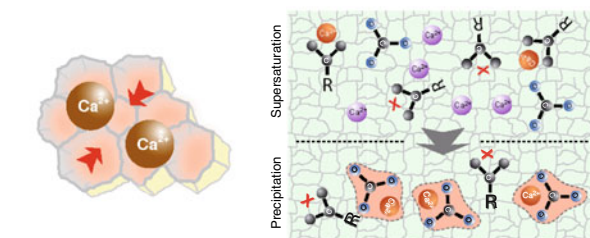
Forcing of mineralization on the EPS matrix.
Organic matrix and kinetic of precipitation can control mineral morphology.



Intrinsic Supersaturation of EPS:

Steric Hindrance

Changes in availability of functional groups (steric hindrance).
Cation binding capacity does not reflect abundance of functional groups



Microbialites, Modern, Figure 10 Mechanistic role of the organic EPS matrix in microbial mat lithification. The exopolymeric substances are produced by various groups of bacteria (especially cyanobacteria). EPS can bind large amounts of cations (i.e., Ca²⁺), reducing the calcium carbonate saturation index, inhibiting precipitation. Alteration of the EPS matrix can end this inhibition, allowing carbonate precipitation to commence. EPS alteration is achieved via two main pathways: (1) EPS degradation, and (2b) intrinsic supersaturation of the EPS binding capacity (modified from Dupraz and Visscher, 2005).

originates from thermal processes. These hydrothermal systems are commonly associated with region of recent volcanic or tectonic activities. It is important to note that the term travertine has variable usage in the literature. Many authors use travertine only for hot water deposits, using “tufa” for cold-water deposits (Pedley, 2000). Other authors use tufa for actively-forming deposits and travertine for non-active, fossil forms (see Chapter *Tufa, Freshwater*).

Although carbonate precipitation in travertines and tufas is driven by abiotic processes, precipitation often initiates on organic substrates, e.g., leaves, woods, algae, or microbial mats. In these cases, mineralogy and morphology are strongly influenced by the organic matrix (e.g., Fouke et al., 2000; Farmer, 2000; Pentecost, 2005; Turner and Jones, 2005). Microbial mats at hot springs, for example, can therefore have a profound impact on thermogene travertine by providing templates for mineralization, even though precipitation is due to vigorous CO₂ degassing (Farmer, 2000; Fouke et al., 2000). Travertine formation is thus an example of biologically-influenced mineralization, since the carbonate precipitation is not due to biological activity, but microorganisms indirectly affect the characteristics of the resulting minerals.

The organic matrix (EPS)

Microbial communities generally produce extracellular polymeric substances (EPS; see Chapter *Extracellular Polymeric Substances (EPS)*). This organic matrix is an important part of the microbial mat, preventing desiccation, retaining essential nutrients, protecting against UV radiation, and providing water channels for transport of metabolites and signaling compounds (Decho, 1990, 2000). Cyanobacteria are often considered to be the major producers of EPS. However, many other bacteria can produce EPS matrix, including aerobic and anaerobic (chemoorgano)heterotrophs, anoxygenic phototrophs, and chemolithoautotrophs (De Philippis et al., 1998, 2001; Stal, 2000, 2003; Richert et al., 2005). Anaerobic bacteria, especially sulfate-reducing bacteria, which play a key role in carbon metabolism in marine microbial mats (Thode-Andersen, and Jorgensen, 1989; Canfield and DesMarais, 1991; Visscher et al., 1991), are known to produce large amounts of EPS (Bosak and Newman, 2005; Braissant et al., 2007).

The EPS matrix is generally the location where the carbonate minerals nucleate and grow (Dupraz et al., 2004). EPS contains negatively-charged acidic groups (e.g., carboxyl, sulfhydryl, amine, hydroxyl groups), which can bind a large amount of mono- and divalent cations, notably Ca²⁺ (Braissant et al., 2007). This binding capacity may inhibit the precipitation of carbonate minerals by depleting calcium from the surrounding microenvironment (Figure 10). The saturation index of calcite depends on the carbonate alkalinity and the availability in calcium. Even if carbonate ions are in solution, the lack of free Ca²⁺ may inhibit precipitation (Dupraz and Visscher, 2005). Therefore, the physicochemical properties of the polymer

matrix, such as the acidity or composition of the functional groups associated with the EPS, are key factors in the formation of modern microbialites.

In order to precipitate calcium carbonate in microbial mats, the Ca²⁺-binding capacity of the EPS has to be reduced. Various mechanisms have been proposed (e.g., Dupraz and Visscher, 2005), which can be grouped into two main processes (Figure 10): (1) degradation of the EPS matrix and (2) intrinsic or extrinsic supersaturation of cation-binding sites (e.g., Dupraz and Visscher, 2005). As EPS is a sugar polymer, it serves as metabolic substrate for (chemoorgano) heterotrophic bacteria. Depolymerization (hydrolysis) of EPS, possibly by fungi and heterotrophs, including spirochetes (Harwood and Canale-Parola, 1984; Weaver and Hicks, 1995), must occur before sugar monomers are available. Microbial degradation of EPS liberates the calcium and produces inorganic carbon (under alkaline conditions, the dominant species is carbonate ion). Alternatively, if the amount of Ca exceeds the number of binding sites in the EPS, free calcium will be present and available for precipitation. These conditions can be achieved through extrinsic factors (the environment) through continuous input of Ca. It can also result from the steric hindrance of acidic groups within the EPS. Although EPS may indeed possess abundant functional groups capable of binding Ca²⁺ or Mg²⁺ ions, these groups may be “sterically-inhibited” (i.e., blocked) via molecular-scale interactions into a structurally complex EPS (Figure 10).

Summary

Microbialites are “organosedimentary deposits that accrete as a result of a benthic microbial community, which traps and binds sediment and/or forms the locus of mineral precipitation” (Kennard and James, 1986). Living examples of these structures can be found in many modern environments including marine, hypersaline, freshwater, and even continental settings. Microbialites are classified based on mesostructure as stromatolites (laminated), thrombolites (clotted), and leiolites (structureless). Among the most spectacular examples are modern stromatolites, which were first discovered in a hypersaline lagoon of Shark Bay in 1954. The only known examples of stromatolites presently forming in open marine environments are in the Bahamas. Lamination in these coarse-grained structures results from the iterative growth of three different types of microbial mats on the surfaces of the build-ups. These microbial communities are responsible for successive “trapping and binding” of sediment (type 1) and in situ precipitation of calcium carbonate (types 2 and 3). Both processes are essential for stromatolites formation. Modern thrombolites and leiolites are less well-studied than modern stromatolites.

Early lithification is a key process in the formation of modern microbialites. Lithification consists of in situ precipitation of minerals, which preserves the biosedimentary structures. Two key and closely coupled components

involved in carbonate precipitation within microbial mats are (1) the alkalinity engine and (2) the organic matrix. The alkalinity engine, which effectively changes the saturation index, is *intrinsically driven* when microbial metabolism is the dominant process causing carbonate precipitation. The alkalinity engine is *extrinsically driven* when the macroenvironment is responsible for microbial mat lithification. In both the cases, the organic matrix of extracellular polymeric substances (EPS), which embed the microbial communities, is the physical location where carbonate minerals nucleate and grow. Fresh EPS contains negatively-charged acidic groups, which bind large amount of cations (e.g., Ca^{2+}), inhibiting CaCO_3 precipitation. In order to precipitate carbonate, this inhibition has to be reduced by degradation of the EPS or by oversaturation of the cation-binding capacity. Microbial activity is ultimately responsible for EPS production and degradation leading mineral precipitation.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Altermann, W., Kazmierczak, J., Oren, A., and Wright, D. T., 2006. Cyanobacterial calcification and its rock-building potential during 3.5 billion years of Earth history. *Geobiology*, **4**, 147–166.
- Andres, M. S., and Reid, R. P., 2006. Growth morphologies of modern marine stromatolites: a case study from Highborne Cay, Bahamas. *Sedimentary Geology*, **185**, 319–328.
- Andres, M. S., Sumner, D. Y., Reid, R. P., and Swart, P. K., 2005. Isotopic fingerprints of microbial respiration in aragonite from Bahamas stromatolites. *Geology*, **34**, 973–976.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Arp, G., Reimer, A., and Reitner, J., 2003. Microbialite formation in seawater of increased alkalinity, Satonda Crater Lake, Indonesia. *Journal of Sedimentary Research*, **73**, 105–127.
- Awramik, S. M., 1971. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **174**, 825–827.
- Awramik, S. M., 1982. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **216**, 171–173.
- Awramik, S. M., 1992. The history and significance of stromatolites. In Schidlowski, M., Kimberley, M. M., McKirdy, D. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Energy and Mineral Resources*. Berlin: Springer, pp. 435–449.
- Awramik, S. M., and Riding, R., 1988. Role of algal eukaryotes in subtidal columnar stromatolite formation. *Proceeding of National Academy of Science, USA*, **85**, 1327–1329.
- Babel, M., 2004. Models for evaporite, selenite and gypsum microbialite deposition in ancient saline basins. *Acta Geologica Polonica*, **54**, 219–249.
- Barbieri, R., and Cavalazzi, B., 2005. Microbial fabrics from Neogene cold seep carbonates Northern Apennine, Italy. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 143–155.
- Barbieri, R., Ori, G. G., and Cavalazzi, B., 2004. A Silurian cold-seep ecosystem from the Middle Atlas, Morocco. *Palaios*, **19**, 527–542.
- Bathurst, R. G. C., 1966. Boring algae, micrite envelopes and lithification of molluscan biosparite. *Geological Journal*, **5**, 15–32.
- Bauld, J., 1981. Geobiological role of cyanobacterial mats in sedimentary environments: production and preservation of organic matter. *BMR Journal of Australian Geology and Geophysics*, **6**, 307–317.
- Bauld, J., Chambers, L. A., and Skyring, G. W., 1979. Primary productivity, sulfate reduction and sulfur isotope fractionation in algal mats and sediments of Hamelin pool, Shark Bay, W. A. Australia. *Journal of Marine and Freshwater Research*, **30**, 753–764.
- Benson, L., 1994. Carbonate deposition, Pyramid Lake Subbasin, Nevada; 1, Sequence of formation and elevational distribution of carbonate deposits (tufas). *Palaeogeography Palaeoclimatology and Palaeoecology*, **109**, 55–87.
- Benzerara, K., Menguy, N., Lopez-Garca, P., Yoon, T.-H., Kazmierczak, J., Tyliczszak, T., Guyot, F., and Brown, G. E. Jr., 2006. Nanoscale detection of organic signatures in carbonate microbialites. *PNAS*, **103**, 9440–9445.
- Bhaskar, P. V., and Bhosle, N. B., 2005. Microbial extracellular polymeric substances in marine biogeochemical processes. *Current Science*, **88**, 45–53.
- Bischoff, J. L., Stine, S., Rosenbauer, R. J., Fitzpatrick, J. A., and Stafford, T. W., 1993. Ikaite precipitation by mixing of shoreline springs and lake water, Mono Lake, California, USA. *Geochimica Cosmochimica Acta*, **57**, 3855–3865.
- Bosak, T., and Newman, D. K., 2005. Microbial kinetic controls on calcite morphology in supersaturated solutions. *Journal of Sedimentary Research*, **75**, 190–199.
- Braga, J. C., Martin, J. M., and Riding, R., 1995. Controls on microbial dome fabric development along a carbonate-siliciclastic shelf-basin transect, Miocene, SE Spain. *Palaios*, **10**, 347–361.
- Braissant, O., Cailleau, G., Aragno, M., and Verrecchia, E. P., 2004. Biologically induced mineralization in the tree *Milicia excelsa* (Moraceae): its causes and consequences to the environment. *Geobiology*, **2**, 59–66.
- Braissant, O., Decho, A. W., Dupraz, C., Glunk, C., Przekop, K. M., and Visscher, P. T., 2007. Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals. *Geobiology*, **5**, 401–411.
- Buick, R., Dunlop, J. S. R., and Groves, D. I., 1981. Stromatolite recognition in ancient rocks: an appraisal of irregularly laminated structures in an Early Archaean chert-barite unit from North Pole, Western Australia. *Alcheringa*, **5**, 161–181.
- Burne, R. V., and Moore, L. S., 1987. Microbialites: organosedimentary deposits of benthic microbial communities. *Palaios*, **2**, 241–254.
- Burns, B. P., Goh, F., Allen, M., and Nellan, B. A., 2004. Microbial diversity of extant stromatolites in the hypersaline marine environment of Shark Bay, Australia. *Environmental Microbiology*, **6**, 1096–1101.
- Cailleau, G., Braissant, O., and Verrecchia, E. P., 2004. Biomineralization in plants as a long-term carbon sink. *Naturwissenschaften*, **91**, 191–194.
- Cailleau, G., Braissant, O., Dupraz, C., and Verrecchia, E. P., 2005. Biological control on CaCO_3 accumulations in ferrallitic soils of Biga, Ivory Coast. *Catena*, **59**, 1–17.
- Canaveras, J. C., Cuezva, S., Sanchez-Moral, S., Lario, J., Laiz, L., Gonzales, J. M., and Saiz-Jimenez, C., 2006. *Naturwissenschaften*, **93**, 27–32.
- Canfield, D. E., and DesMarais, D. J., 1991. Aerobic sulfate reduction in microbial mats. *Science*, **251**, 1471–1473.
- Decho, A. W., 1990. Microbial exopolymer secretions in ocean environments: their role(s) in food webs and marine processes. *Oceanography and Marine Biology Annual Review*, **28**, 73–154.
- Decho, A. W., 2000. Exopolymer microdomains as a structuring agent for heterogeneity within microbial biofilms. In Riding,

- R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 1–9.
- De Philippis, R., Margheri, M. C., Materassi, R., and Vincenzini, M., 1998. Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. *Applied and Environmental Microbiology*, **64**, 1130–1132.
- De Philippis, R., Sili, C., Papperi, R., and Vincenzini, M., 2001. Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *Journal of Applied Phycology*, **13**, 293–299.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P., 1986. Giant subtidal stromatolites forming in normal salinity water. *Nature*, **324**, 55–58.
- Dravis, J. J., 1983. Hardened subtidal stromatolites, Bahamas. *Science*, **219**, 385–386.
- Dupraz, C., and Strasser, A., 1999. Microbialites and microencrusts in shallow coral bioherms (Middle to Late Oxfordian, Swiss Jura Mountains). *Facies*, **40**, 101–130.
- Dupraz, C., and Strasser, A., 2002. Nutritional modes in coral-microbialite reefs (Jurassic, Oxfordian, Switzerland). *Palaos*, **17**, 449–471.
- Dupraz, C., and Visscher, P. T., 2005. Microbial lithification in marine stromatolites and hypersaline mats. *Trends in Microbiology*, **13**, 429–438.
- Dupraz, C., Visscher, P. T., Baumgartner, L. K., and Reid, R. P., 2004. Microbe-mineral interactions: early CaCO₃ precipitation in a Recent hypersaline lake (Eleuthera Islands, Bahamas). *Sedimentology*, **51**, 745–765.
- Dupraz, C., Patissina, R., and Verrecchia, E. P., 2006. Simulation of stromatolite morphospace using 'DLA-CA' growth model': translation of energy in morphology. *Sedimentary Geology*, **185**, 185–203.
- Ehrlich, H. L., 1996. *Geomicrobiology*, 3rd edn. (revised and expanded). New York: Marcel Dekker, Inc.
- Farmer, J. D., 2000. Hydrothermal systems: doorways to early biosphere evolution. *GSA Today*, **10**, 1–10.
- Fischer, A. G., 1965. Fossils, early life, and atmospheric history. In *Proceeding National Academy of Science. USA, Washington*, Vol. 53, pp. 1205–1215.
- Fouke, B. W., Farmer, J. D., Des Marais, D. J., Pratt, L., Sturchio, N. C., Burns, P. C., and Discipulo, M. K., 2000. Depositional facies and aqueous-solid geochemistry of travertine-depositing hot springs (Ange Terrace, Mammoth Hot Springs, Yellowstone National Park, USA). *Journal of Sedimentary Research*, **70**, 565–585.
- Freytet, P., and Verrecchia, E. P., 1998. Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. *Sedimentology*, **45**, 535–563.
- Freytet, P., and Verrecchia, E. P., 1999. Calcitic radial palisadic fabric in freshwater stromatolites: diagenetic and recrystallized feature or physicochemical sinter crust. *Sedimentary Geology*, **126**, 97–102.
- Gebelein, C. D., 1976. The effects of the physical, chemical and biological evolution of the earth. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 499–515.
- Golubic, S., and Focke, J. W., 1978. Phormidium hendersonii Howe: identify and significance of a modern stromatolites building microorganism. *Journal of Sedimentary Petrology*, **48**, 751–764.
- Golubic, S., and Hofmann, H. J., 1976. Comparison of Holocene and mid-Precambrian Entophysalidaceae (Cyanophyta) in stromatolitic algal mats: cell division and degradation. *Journal of Paleontology*, **50**, 1074–1082.
- Grey, K., Moore, L. S., Burne, R. V., Pierson, B. K., and Bauld, J., 1990. Lake Thetis, Western Australia: an example of saline lake sedimentation dominated by benthic microbial processes. *Australian Journal of Marine and Freshwater Research*, **41**, 275–300.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Review of Earth and Planetary Science*, **27**, 313–358.
- Harwood, C. S., and Canale-Parola, E., 1984. Ecology of spirochetes. *Annual Review of Microbiology*, **38**, 161–192.
- Hillgaertner, H., Dupraz, C., and Hug, W. A., 2001. Microbially induced stabilization of carbonate sands in marine phreatic environments or are micritic meniscus cements good indicators for vadose diagenesis? *Sedimentology*, **48**, 117–131.
- Jones, D., and Wilson, M. J., 1986. Biomineralization in crustose lichens. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals*. The Systematics Association, Special Volume 30. New York: Oxford University Press, pp. 91–105.
- Kalkowsky, E., 1908. Oolith und Stromatolith im nord-deutschen Buntsandstein. *Zeitschrift der deutschen geologischen Gesellschaft*, **60**, 68–125.
- Kazmierczak, J., and Kempe, S., 2006. Genuine modern analogues of Precambrian stromatolites from caldera lakes of Niuafu'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kelley, D. S., Karson, J. A., Blackman, D. K., Frueh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Shrenk, M. O., Roe, K. K., Lebon, G. T., and Rivizzigno, P., and the AT3–60 Shipboard Party, 2001. An off-axis hydrothermal vent field near the Mid-Atlantic ridge at 30° N. *Nature*, **412**, 145–149.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems, and biocalcification processes. *Bulletin de l'Institut oceanographique, Monaco*, **13**, 61–117.
- Kempe, S., Reimer, A., Lipp, A., Kazmierczak, J., Landmann, G., and Konuk, T., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites: two distinct types of microbial structures. *Palaos*, **1**, 492–503.
- Klappa, C. F., 1979. Lichen stromatolites; criterion for subaerial exposure and a mechanism for the formation of laminar calcretes (caliche). *Journal of Sedimentary Petrology*, **49**, 387–400.
- Kobluk, D. R., and Crawford, D. R., 1990. A modern hypersaline organic mud- and gypsum dominated basin and associated microbialites. *Palaos*, **5**, 134–148.
- Kromkamp, J. C., Perkins, R., Dijkman, N., Consalvey, M., Andres, M., and Reid, R. P., 2007. Resistance to burial of cyanobacteria in stromatolites. *Aquatic Microbial Ecology*, **48**, 123–130.
- Kumar, S., and Pandey, S. K., 2008. Discovery of organic-walled microbiota from the black-bedded chert, Balman Limestone, the Bhandar Group, Lakheri area, Rajasthan. *Current Science*, **94**, 797–800.
- Lepot, K., Benzerara, K., Brown, G. E. Jr., and Philippot, P., 2008. Microbially influenced formation of 2.724-million-year-old stromatolites. *Nature Geoscience*, **1**, 118–121.
- Lindsay, J. F., Brasier, M. D., McLoughlin, N., Green, O. R., Fogel, M., McNamara, K., Steele, A., and Mertzman, S. A., 2003. Abiotic Earth – establishing a baseline for earliest life, data from the Archaean of Western Australia. *Lunar and Planetary Institute, Annual Meeting. Lunar, Planetary Institute Contribution*, **1156**, 1137.
- Logan, B. W., 1961. Cryptozoon and associate stromatolites from the Recent, Shark Bay, Western Australia. *The Journal of Geology*, **69**, 517–533.
- Logan, B. W., Hoffman, P., and Gebelein, C. D., 1974. Algal mats, cryptalgal fabrics and structures, Hamelin Pool, Western Australia. *AAPG Memoir*, **22**, 140–194.

- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 387–390.
- Lowenstam, H. A., and Weiner, S., 1989. *On Biomineralization*. New York: Oxford University Press.
- Ludwig, K. A., Kelley, D. S., Butterfield, D. A., Nelson, B., and Früh-Green, G., 2006. Formation and evolution of carbonate chimneys at the Lost City Hydrothermal Field. *Geochemica Cosmochimica Acta*, **70**, 3625–3645.
- Macintyre, I. G., Prufert-Bebout, L., and Reid, R. P., 2000. The role of endolithic cyanobacteria in the formation of lithified laminae in Bahamian stromatolites. *Sedimentology*, **47**, 915–921.
- Mann, S., 2001. *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. New York: Oxford University Press.
- Mann, C. J., and Hoffman, L. R., 1984. Algal mounds in Storr's Lake, San Salvador, Bahamas. In *Proceeding of the 2nd Symposium on Geology of the Bahamas*, CCFL Bahamian Field Station, pp. 41–51.
- Mann, C. J., and Nelson, W. M., 1989. Microbialitic structures in Storr's Lake, San Salvador Island, Bahamas Islands. *Palaos*, **4**, 287–293.
- McNeese, L. R., 1988. *The stromatolites of Storr's Lake, San Salvador, Bahamas*. MSc thesis, University of North Carolina at Chapel Hill, Department of Geology.
- Merz-Preiss, M., and Riding, R., 1999. Cyanobacterial tufa calcification in two freshwater streams; ambient environment, chemical thresholds biological processes. *Sedimentary Geology*, **126**, 103–124.
- Neumann, C. A., Bebout, B. M., McNeese, L. R., Paul, C. K., and Paerl, H. W., 1988. Modern stromatolites and associated mats: San Salvador, Bahamas. In *Proceedings of the 4th Symposium on the geology of the Bahamas*. Bahamas Field Station, San Salvador, pp. 235–251.
- Noffke, N., Gerdes, G., and Klenke, T., 2003. Benthic cyanobacteria and their influence on the sedimentary dynamics of peritidal depositional systems (siliciclastic, evaporitic salty, and evaporitic carbonatic). *Earth-Science Reviews*, **62**, 163–176.
- Parker, B. C., Simmons, G. M., Love, F. G. Jr., Wharton, R. A., and Seaburg, K. G. Jr., 1981. Modern stromatolites in Antarctic Dry Valley Lakes. *BioScience*, **31**, 656–661.
- Pedley, M., 2000. Ambient temperature freshwater microbial tufas. In Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 179–186.
- Pentecost, A., 1978. Blue-green algae and freshwater carbonate deposits. *Proceedings of the Royal Society of London*, **200**, 43–61.
- Pentecost, A., 1995. The quaternary travertine deposits of Europe and Asia Minor. *Quaternary Science Reviews*, **14**, 1005–1028.
- Pentecost, A., 2005. *Travertine*. Berlin: Springer.
- Pentecost, A., and Riding, R., 1986. Calcification in cyanobacteria. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomineralization in Lower Plants and Animals*. The Systematic Association, Special Volume 30, pp. 73–90.
- Perry, R. S., McLoughlin, N., Lynne, B. Y., Sephton, M. A., Oliver, J. D., Perry, C. C., Campbell, K., Engel, M. H., Farmer, J. D., Brasier, M. D., and Staley, J. T., 2007. Defining biominerals and organominerals: Direct and indirect indicators of life. *Sedimentary Geology*, **201**, 157–179.
- Playford, P. E., 1979. Stromatolite research in Western Australia. *Journal of the Royal Society of Western Australia*, **62**, 13–20.
- Playford, P. E., 1990. Geology of the Shark Bay area, Western Australia. In Berry, P. F., Bradshaw, S. D., and Wilson, B. R. (eds.), *Research In Shark Bay*. West Australian Museum, pp. 13–31.
- Playford, P. E., and Cockbain, A. E., 1976. Modern algal stromatolites at Hamelin Pool, a hypersaline barred basin in Shark Bay, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 389–411.
- Read, J. F., 1976. Calcretes and their distinction from stromatolites. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 55–71.
- Reid, R. P., and Macintyre, I. G., 2000. Microboring versus recrystallization: further insight into the micritization process. *Journal of Sedimentary Research*, **70**, 24–28.
- Reid, R. P., Macintyre, I. G., Browne, K. M., Steneck, R. S., and Miller, T., 1995. Modern marine stromatolites in the Exuma Cays, Bahamas: uncommonly common. *Facies*, **33**, 1–18.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. K., Bebout, B. M., Dupraz, C., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and Des Marais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reid, R. P., Dupraz, C., Visscher, P. T., Decho, A. W., and Sumner, D. Y., 2003a. Microbial processes forming modern marine stromatolites: microbe-mineral interactions with a three-billion-year rock record. In Krumbein, W. E., Paterson, D. M., and Zavarzin, G. A. (eds.), *Fossil and Recent Biofilms - A Natural History of Life on Earth*. Dordrecht: Kluwer, pp. 103–118.
- Reid, R. P., James, N. P., Macintyre, I. G., Dupraz, C. P., and Burne, R. V., 2003b. Shark Bay Stromatolites: microfibrils and reinterpretations of origins. *Facies*, **49**, 243–270.
- Reitner, J., Paul, J., Arp, G., and Hause-Reitner, D., 1996. Lake Thetis Domal Microbialites – a complex framework of calcified biofilms and organomicrites (Cervantes, Western Australia). In Reitner, J., Neuweiler, F., and Gunkel, F. (eds.), *Global and Regional Controls on Biogenetic Sedimentation. I. Reef Evolution*. Research Reports. Göttingen: Göttinger Arb. Geol. Paläont., Sonderband, Vol. 2, pp. 85–89.
- Richert, L., Golubic, S., Le Guedes, R., Ratiskol, J., Payri, C., and Guesennec, J., 2005. Characterization of exopolysaccharides produced by cyanobacteria isolated from Polynesian microbial mats. *Current Microbiology*, **51**, 379–384.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous Algae and Stromatolites*. Berlin: Springer, pp. 21–51.
- Riding, R., 1999. The term stromatolite: towards an essential definition. *Lethaia*, **32**, 321–330.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Riding, R., 2006. Microbial carbonate abundance compared with fluctuations in metazoan diversity over geological time. *Sedimentary Geology*, **185**, 229–238.
- Robbins, L. L., and Blackwelder, P. L., 1992. Biochemical and ultrastructural evidence for the origin of whittings; a biologically induced calcium carbonate precipitation mechanism. *Geology*, **20**, 464–468.
- Schmid, D. U., 1996. Marine microbolithe und mikroinkrustierer aus dem Oberjura. *Profil*, **9**, 101–251.
- Seong-Joo, L., Browne, K. M., and Golubic, S., 2000. On stromatolites lamination. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 16–24.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaos*, **15**, 166–169.
- Sharp, J. H., 1969. Blue-green algae and carbonates – Schizothrix calcicola and algal stromatolites from Bermuda. *Limnology and Oceanography*, **14**, 568–578.
- Shiraishi, F., Bissett, A., de Beer, D., Reimer, A., and Arp, G., 2008. Photosynthesis, respiration and exopolymer calcium-binding in biofilm calcification (Westerhoefer and Deinschwanger Creek, Germany). *Geomicrobiology Journal*, **25**, 83–94.

- Simkiss, K., and Wilbur, K., 1989. *Biomining. Cell Biology and Mineral Deposition*. San Diego: Academic.
- Stal, L. J., 2000. Microbial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Dordrecht: Kluwer.
- Stal, L. J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal*, **20**, 463–478.
- Stolz, J. F., 2000. Structure of microbial mats and biofilms. In Riding, R. E., Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 1–9.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry*. New York: Wiley, 1022 pp.
- Thode-Andersen, S., and Jorgensen, B. B., 1989. Sulfate reduction and the formation of ^{35}S -labeled FeS, FeS₂, and S(0) (elemental sulfur) in coastal marine sediments. *Limnology and Oceanography*, **34**, 793–806.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Thrallkill, J., 1976. Speleothems. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 73–86.
- Turner, E. C., and Jones, B., 2005. Microscopic calcite dendrites in cold-water tufa: implications for nucleation of micrite and cement. *Sedimentology*, **52**, 1043–1066.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine geology*, **113**, 3–25.
- Verrecchia, E. P., and Verrecchia, K. E., 1994. Needle-fiber calcite: a critical review and a proposed classification. *Journal of Sedimentary Research*, **64**, 650–664.
- Verrecchia, E. P., Freytag, P., Verrecchia, K. E., and Dumont, J. L., 1995. Spherulites in calcareous laminar crusts: biogenic CaCO₃ precipitation as a major contributor to crust formation. *Journal of Sedimentary Research*, **A65**, 690–700.
- Visscher, P. T., and Stolz, J. F., 2005. Microbial mats as bioreactors: populations, processes and products. *Palaeogeography Palaeoclimatology, Paleogeography*, **219**, 87–100.
- Visscher, P. T., Beukema, J., and van Gemerden, H., 1991. *In situ* characterization of sediments: measurements of oxygen and sulfide profiles. *Limnology and Oceanography*, **36**, 1476–1480.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Vreeland, R. H., Rosenzweig, W. D., and Powers, D. W., 2000. Isolation of a 250 million year old halotolerant bacterium from a primary salt crystal. *Nature*, **407**, 897–900.
- Vreeland, R. H., Lowenstein, T., Timofeeff, M., Satterfield, C., DiFerdinando, J., Jones, J., Monson, A., Rosenzweig, W. D., Cho, B. C., Park, J. S., Wallace, A., and Grant, W. D., 2007. The isolation of live cretaceous (121–112 million years old) halophilic *Archaea* from primary salt crystals. *Geomicrobiology Journal*, **24**, 275–282.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Warren, J. K., 2006. *Evaporites: Sediments, Resources and Hydrocarbons*. New York: Springer.
- Weaver, D. T., and Hicks, R. E., 1995. Biodegradation of *Azotobacter vinelandii* exopolymer by Lake Superior microbes. *Limnology and Oceanography*, **40**, 1035–1041.
- Weiner, S., and Addadi, L., 2002. At the cutting edge. Perspectives. *Science*, **298**, 375–376.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization and the problem of the vital effect. In Dove, P. M., Weiner, S., and De Yoreo, J. J. (eds.), *Biomining. Cell Biology and Mineral Deposition*. Washington: Mineralogical Society of America, Vol. 54, pp. 1–31.
- Wright, V. P., Platt, N. H., and Wimbeldon, W. A., 1988. Biogenic laminar calcareous: evidence of calcified root-mat horizons in paleosols. *Sedimentology*, **35**, 603–620.
- Yechieli, Y., and Wood, W. W., 2002. Hydrogeologic processes in saline systems: playas, sabkhas, and saline lakes. *Earth-Science Reviews*, **58**, 343–365.
- Zabielski, V. P., 1991. The depositional history of Storr's Lake San Salvador, Bahamas. Unpublished PhD thesis, University of North Carolina.
- Zeebe, R. E., and Wolf-Gladrow, D. (eds.), 2001. *CO₂ in Seawater: Equilibrium, Kinetics and Isotopes*. Amsterdam: Elsevier, 346 pp.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomining](#)
[Microbial Mats](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Organomineralization](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Tidal Flats](#)
[Tufa, Freshwater](#)

MICROBIALITES, STROMATOLITES, AND THROMBOLITES

Robert Riding
 University of Tennessee, Knoxville, TN, USA

Microbialite Definition

Microbialites are “organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987, pp. 241–242).

Introduction

Microbial carbonates are produced by the interaction of microbial growth and metabolism, cell surface properties, and extracellular polymeric substances (EPS) with mineral precipitation and grain trapping. The early lithification that is essential for the accretion and preservation of benthic microbial carbonates is both biologically mediated and environmentally dependent. Consequently, microbialite history reflects not only microbial mat evolution, but also long-term changes in seawater and atmospheric chemistry that have influenced microbial metabolism and seawater carbonate saturation state.

Microbialites are in place benthic sediments produced by microbial processes. The term “microbialite” has been most widely used to describe carbonate stromatolites, thrombolites, and similar structures that occur as domes and columns in the shallow waters of lakes and seas, but it can also apply to many additional authigenic accumulations in which microbes are locally conspicuous, such as

- Simkiss, K., and Wilbur, K., 1989. *Biomining. Cell Biology and Mineral Deposition*. San Diego: Academic.
- Stal, L. J., 2000. Microbial mats and stromatolites. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria. Their Diversity in Time and Space*. Dordrecht: Kluwer.
- Stal, L. J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal*, **20**, 463–478.
- Stolz, J. F., 2000. Structure of microbial mats and biofilms. In Riding, R. E., Awramik, S. M. (eds.), *Microbial Sediments*. New York: Springer, pp. 1–9.
- Stumm, W., and Morgan, J. J., 1996. *Aquatic Chemistry*. New York: Wiley, 1022 pp.
- Thode-Andersen, S., and Jorgensen, B. B., 1989. Sulfate reduction and the formation of ^{35}S -labeled FeS, FeS₂, and S(0) (elemental sulfur) in coastal marine sediments. *Limnology and Oceanography*, **34**, 793–806.
- Thompson, J. B., and Ferris, F. G., 1990. Cyanobacterial precipitation of gypsum, calcite, and magnesite from natural alkaline lake water. *Geology*, **18**, 995–998.
- Thrallkill, J., 1976. Speleothems. In Walter, M. R. (ed.), *Stromatolites*. Developments in Sedimentology. Amsterdam: Elsevier, Vol. 20, pp. 73–86.
- Turner, E. C., and Jones, B., 2005. Microscopic calcite dendrites in cold-water tufa: implications for nucleation of micrite and cement. *Sedimentology*, **52**, 1043–1066.
- Van Gemerden, H., 1993. Microbial mats: a joint venture. *Marine geology*, **113**, 3–25.
- Verrecchia, E. P., and Verrecchia, K. E., 1994. Needle-fiber calcite: a critical review and a proposed classification. *Journal of Sedimentary Research*, **64**, 650–664.
- Verrecchia, E. P., Freytag, P., Verrecchia, K. E., and Dumont, J. L., 1995. Spherulites in calcareous laminar crusts: biogenic CaCO₃ precipitation as a major contributor to crust formation. *Journal of Sedimentary Research*, **A65**, 690–700.
- Visscher, P. T., and Stolz, J. F., 2005. Microbial mats as bioreactors: populations, processes and products. *Paleogeography Paleoclimatology, Paleogeography*, **219**, 87–100.
- Visscher, P. T., Beukema, J., and van Gemerden, H., 1991. *In situ* characterization of sediments: measurements of oxygen and sulfide profiles. *Limnology and Oceanography*, **36**, 1476–1480.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Vreeland, R. H., Rosenzweig, W. D., and Powers, D. W., 2000. Isolation of a 250 million year old halotolerant bacterium from a primary salt crystal. *Nature*, **407**, 897–900.
- Vreeland, R. H., Lowenstein, T., Timofeeff, M., Satterfield, C., DiFerdinando, J., Jones, J., Monson, A., Rosenzweig, W. D., Cho, B. C., Park, J. S., Wallace, A., and Grant, W. D., 2007. The isolation of live cretaceous (121–112 million years old) halophilic *Archaea* from primary salt crystals. *Geomicrobiology Journal*, **24**, 275–282.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Warren, J. K., 2006. *Evaporites: Sediments, Resources and Hydrocarbons*. New York: Springer.
- Weaver, D. T., and Hicks, R. E., 1995. Biodegradation of *Azotobacter vinelandii* exopolymer by Lake Superior microbes. *Limnology and Oceanography*, **40**, 1035–1041.
- Weiner, S., and Addadi, L., 2002. At the cutting edge. Perspectives. *Science*, **298**, 375–376.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization and the problem of the vital effect. In Dove, P. M., Weiner, S., and De Yoreo, J. J. (eds.), *Biomining. Cell Biology and Mineral Deposition*. Washington: Mineralogical Society of America, Vol. 54, pp. 1–31.
- Wright, V. P., Platt, N. H., and Wimbeldon, W. A., 1988. Biogenic laminar calcareous: evidence of calcified root-mat horizons in paleosols. *Sedimentology*, **35**, 603–620.
- Yechieli, Y., and Wood, W. W., 2002. Hydrogeologic processes in saline systems: playas, sabkhas, and saline lakes. *Earth-Science Reviews*, **58**, 343–365.
- Zabielski, V. P., 1991. The depositional history of Storr's Lake San Salvador, Bahamas. Unpublished PhD thesis, University of North Carolina.
- Zeebe, R. E., and Wolf-Gladrow, D. (eds.), 2001. *CO₂ in Seawater: Equilibrium, Kinetics and Isotopes*. Amsterdam: Elsevier, 346 pp.

Cross-references

[Biofilms](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Biomineralization](#)
[Microbial Mats](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Organomineralization](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Tidal Flats](#)
[Tufa, Freshwater](#)

MICROBIALITES, STROMATOLITES, AND THROMBOLITES

Robert Riding
 University of Tennessee, Knoxville, TN, USA

Microbialite Definition

Microbialites are “organosedimentary deposits that have accreted as a result of a benthic microbial community trapping and binding detrital sediment and/or forming the locus of mineral precipitation” (Burne and Moore, 1987, pp. 241–242).

Introduction

Microbial carbonates are produced by the interaction of microbial growth and metabolism, cell surface properties, and extracellular polymeric substances (EPS) with mineral precipitation and grain trapping. The early lithification that is essential for the accretion and preservation of benthic microbial carbonates is both biologically mediated and environmentally dependent. Consequently, microbialite history reflects not only microbial mat evolution, but also long-term changes in seawater and atmospheric chemistry that have influenced microbial metabolism and seawater carbonate saturation state.

Microbialites are in place benthic sediments produced by microbial processes. The term “microbialite” has been most widely used to describe carbonate stromatolites, thrombolites, and similar structures that occur as domes and columns in the shallow waters of lakes and seas, but it can also apply to many additional authigenic accumulations in which microbes are locally conspicuous, such as

some tufa, travertine, speleothem and spring, seep, and vent deposits. A series of terms and definitions were proposed between 1967 and 1987 in attempts to distinguish benthic sediments formed by microbial sediment trapping and/or precipitation. The first term, *cryptalgal*, was proposed by Aitken (1967, p. 1163) for rocks or rock structures “believed to originate through the sediment-binding and/or carbonate precipitating activities of non-skeletal algae.” The second was redefinition of *stromatolite* by Awramik and Margulis’ (1974) as “organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” Both Aitken (1967, p. 1163) and Awramik and Margulis (1974) used blue-green algae to indicate cyanobacteria, and therefore Kennard and James (1986, p. 496) replaced *cryptalgal* by *cryptomicrobial*. The third term was *microbialite* (Burne and Moore, 1987), which effectively repeated Awramik and Margulis’ (1974) definition of *stromatolite*, thereby broadening its scope. The primary focus of all these definitions was stromatolites and thrombolites.

Research during the 1900s revealed many details concerning the nature and history of stromatolites, but it also led to nomenclatural uncertainty as it became necessary to accommodate newly recognized deposits, such as thrombolites. Aitken (1967, p. 1164) sought to clarify use of “algal” in carbonate rock descriptions by distinguishing “rocks composed of the remains of skeletal calcareous algae . . . from those formed by noncalcareous blue-green . . . and green . . . algae.” He termed the latter *cryptalgal*, defined as rocks or rock structures “believed to originate through the sediment-binding and/or carbonate-precipitating activities of nonskeletal algae” (Aitken, 1967, p. 1163). Aitken (1967, Fig. 1) regarded both thrombolites and stromatolites as *cryptalgal* deposits, distinguishing by their respective clotted and laminated macrofabrics. However, Awramik and Margulis’ (1974) proposed a broader definition of *stromatolite* as “megascopic organosedimentary structures produced by sediment trapping, binding and/or precipitation as a result of growth and metabolic activity of organisms, primarily blue-green algae.” This therefore subsumed thrombolite as a category within *stromatolite*.

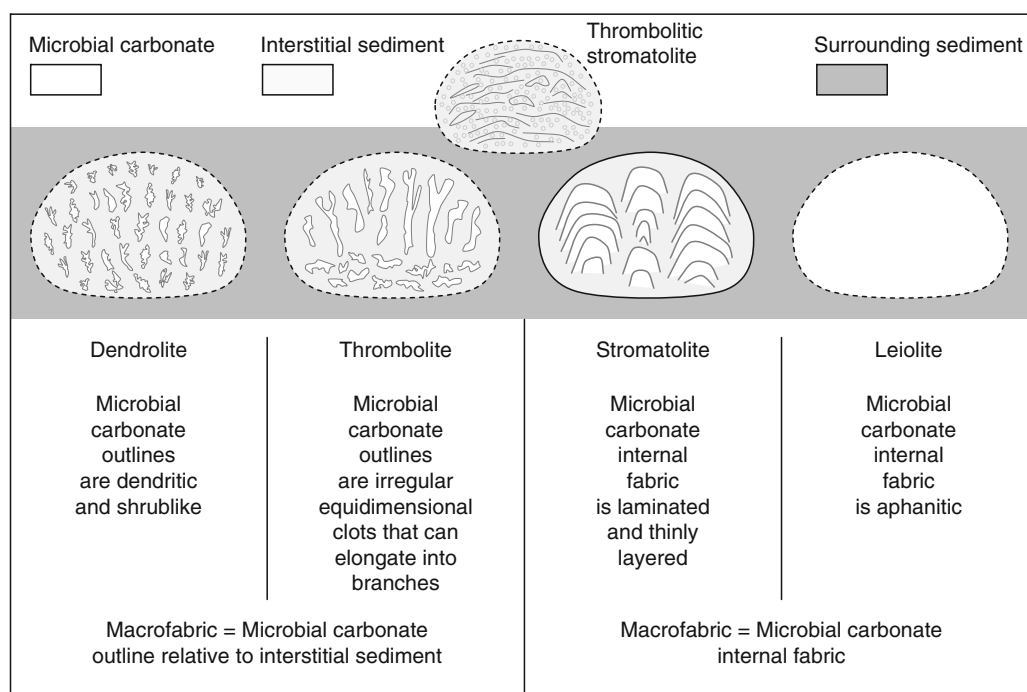
As a result of these and other developments, by the 1980s stromatolites could variously be regarded as (1) microbial and laminated (e.g., Kalkowsky, 1908; Hofmann, 1969; Krumbein, 1983), (2) microbial but not necessarily laminated (Awramik and Margulis, 1974), and (3) laminated but not necessarily organic (Semikhatov et al., 1979) (see Riding, 1999, p. 324). Since the wording of Burne and Moore’s (1987, pp. 241–242) definition of *microbialite* closely followed that of Awramik and Margulis’ (1974) definition of *stromatolite*, it therefore required return to a narrower definition of *stromatolite*, as layered or laminated structures. This relatively narrow definition was intended by Kalkowsky (1908) and is implied by his term *stromatolith*; *stromat*, Greek for “to spread out,” suggests a layer. Burne and Moore’s

(1987) new term, *microbialite*, encompassed more diverse benthic microbial deposits. The outcome of these developments was that *stromatolite* and *thrombolite* could be considered as equal categories within *microbialite*, mirroring Aitken’s (1967, Fig. 1) classification of *stromatolites* and *thrombolites* within *cryptalgal* carbonates. Subsequently, these and other varieties of *microbialite* have commonly been distinguished by their dominant internal macrofabric: *stromatolite* (laminated), *thrombolite* (clotted), *dendrolite* (dendritic), and *leiolite* (aphanitic) (Riding, 2000, pp. 189–195) (Figure 1).

Given the importance of stromatolites in the development of these concepts, this orderly arrangement of terms under the *microbialite* umbrella relied heavily on stromatolites being microbial structures. However, as soon as Kalkowsky’s (1908) article was published, researchers began to point out similarities between stromatolites and abiogenic precipitates (Reis, 1908; Bucher, 1918), and this chorus has continued ever since. Logan et al. (1964, p. 69) suggested recognition of “inorganic stromatolites”; Hofmann (1973, Fig. 5) showed the difficulty of distinguishing stromatolites from other laminites; and Semikhatov et al. (1979, p. 994) proposed a descriptive rather than purely genetic definition of *stromatolite*. Reports of essentially abiogenic stromatolites have persisted, especially in the Proterozoic (Grotzinger and Read, 1983; Grotzinger, 1989a; Pope et al., 2000). Attempts to separate abiogenic and biogenic stromatolites are obstructed by *hybrid stromatolites* (see below, *Stromatolites*) consisting of millimetric alternations of abiogenic crust and lithified microbial mat (Riding, 2008). Consequently, although microbial mat models seem to apply well to all or most thrombolites, dendrolites and leiolites, they do not account for all stromatolite-like deposits, particularly in the Precambrian. The implications of this complication for stromatolite interpretation as well terminology have still to be worked out.

Microbialites in space and time

Microbes occupy a very broad range of environments, including waters of widely differing chemistry and composition, and their involvement in sedimentation is equally varied. Most microbialites are carbonate (e.g., aragonite, calcite, dolomite) in composition, but siliceous, phosphatic, iron, manganese, and sulfate examples also occur. The microbes in microbialites are dominantly bacteria, including cyanobacteria, together with small algae. From the perspective of biocalcification, *microbialite* carbonates are bioinduced. Bacteria in general exert relatively weak control over the organic site and mineralogy of calcification, and present-day microbial carbonates appear to be most widespread in environments where precipitation is inorganically favored. This environmental dependence means that where rapid cooling and degassing strongly favor mineral precipitation irrespective of organic intervention, as in hot water springs and vents, the overall sedimentary contribution of microbes may be



Microbialites, Stromatolites, and Thrombolites, Figure 1 Microbial carbonates defined by macrofabric: leiolite (aphanitic), stromatolite (laminated), thrombolite (clotted), dendrolite (dendritic). Examples show domes and associated sediment. Not to scale. All categories are integrational. In addition to domes/mounds, overall shape can include columns, layers, and irregular masses. A complication is that leiolite and stromatolite macrofabrics are defined by features within (i.e., internal to) the microbial carbonate, such as lamination. In contrast, thrombolite and dendrolite macrofabrics are defined by the external shape of individual masses of microbial carbonate, such as clots or small shrub-like masses. Thrombolitic stromatolite (Aitken, 1967), typified by some Shark Bay columns, is internally weakly clotted and crudely laminated. It is essentially agglutinated, in contrast to Neoproterozoic and early Palaeozoic thrombolites with calcified microbial microfabrics. In the latter, the clots may be prostrate and irregular, and also vertically extended into amalgamated elongate branches (e.g., *Favosamacteria*).

relatively minor. In such cases, they influence the fabric more than the process of deposition. In contrast, where waters are less saturated for minerals, microbial activity can play a major role in sediment precipitation. These differing roles are reflected in the form and fabrics of the resulting deposits.

Sensitivity to environmental influence is also reflected in the secular distribution of microbialites. Without ruling out the influence of other factors – such as competition with other organisms – marine microbial carbonate abundance through time significantly reflects fluctuation in carbonate saturation state (Fischer, 1965; Grotzinger, 1990; Riding and Liang, 2005). Microbial carbonates can therefore be relatively sensitive proxies for seawater carbonate chemistry, and for atmospheric composition, since cyanobacterial sheath calcification is promoted by increased bicarbonate uptake (Merz, 1992) when CO_2 levels decline (Riding, 2006).

Scope

The term microbialite, with its emphasis on benthic microbial deposits, encompasses a wide range of sediments; what does it not cover? “Benthic” would seem to exclude both allochthonous and interstitial microbial sediments.

If so, then biogenic “whiting” deposits produced by photo-synthetically induced nucleation of small CaCO_3 crystals in the water column are not microbialites, and neither are diagenetic bacterially induced precipitates such as cements and concretions. However, these somewhat artificial distinctions could be blurred, as in some soil crust and beachrock deposits. On the other hand, “microbial” must exclude abiogenic stromatolites, identified in both Precambrian and Phanerozoic sediments (Grotzinger and Read, 1983; Pope et al., 2000). This is a complication, especially since microbialite (Burne and Moore, 1987) is based on a preexisting definition of stromatolite proposed by Awramik and Margulis (1974), and arose from a need for a general term to encompass stromatolite, thrombolite, and related deposits. Such difficulties of definition should be resolved by increased understanding of both present-day and ancient examples of these important and geologically widespread deposits.

Stromatolites

Definition

Stromatolites are laminated benthic microbial deposits (Riding, 1991).

Introduction

Kalkowsky (1908) proposed the term stromatolite (*Stromatolith*) for columns and domes in early Triassic playa lake oolites, but similar structures were already long known from examples such as those in the late Cambrian of New York State that Steele (1825, pp. 17–18, pl. 2) described as “calcareous concretions” and Hall (1883) named *Cryptozoon* (Figure 2). The search for present-day analogs of these ancient stromatolites led from freshwater tufa (Walcott, 1914; Roddy, 1915) and marsh deposits (Black, 1933) to marginal marine domes (Ginsburg et al., 1954) and columns (Logan, 1961). These discoveries strongly supported Kalkowsky’s (1908) inference that stromatolites are essentially microbial deposits. But optimism that they could provide appropriate analogs for all ancient marine stromatolites was tempered by studies of Precambrian examples that include significant abiogenic precipitated components (e.g., Serebryakov, 1976, p. 633; Grotzinger and Knoll, 1999, p. 314). Some definitions of stromatolite have therefore encompassed both microbial and abiogenic layered/laminated authigenic crusts that characteristically form domical and columnar morphologies.

Definitions of stromatolite range from that of Kalkowsky (1908) to that of Semikhatov et al. (1979, p. 993): “a stromatolite is . . . an attached, laminated, lithified, sedimentary growth structure, accretionary away from a point or limited surface of initiation. Although characteristically of microbial origin and calcareous composition, it may be of any origin, composition, shape, size, or age.” Understanding of Kalkowsky’s view has been hindered by a definition incorrectly attributed to him by Krumbein (1983, p. 499): “stromatolites are organogenic, laminated, calcareous rock structures, the origin of which is clearly related to microscopic life, which in itself must not be fossilised.” Kalkowsky (1908) did not write this (see Riding, 1999, p. 323), but it has been repeated as if it were a literal translation from his paper (e.g., Ginsburg, 1991, p. 25; Feldmann and McKenzie, 1998, p. 201; Grotzinger and Knoll, 1999, p. 316; McLoughlin et al.,

2008, p. 96). Compounding this mistake, the somewhat awkward wording (use of “must not” rather than “need not”) has been cited as “paradoxical,” and “confusion” to be avoided, and also as an example of the deficiencies of genetic definitions (Grotzinger and Knoll, 1999, p. 316; McLoughlin et al., 2008, p. 96). In his 1908 paper, Ernst Kalkowsky did not provide a specific definition of stromatolite, but he did repeatedly emphasize that it is a laminated organic structure. He thought that the life forms involved were “*niedrig organisierte pflanzliche Organismen*” (simple plantlike organisms, Kalkowsky, 1908, p. 125). It is therefore reasonable to conclude that Kalkowsky essentially regarded stromatolites as laminated microbial deposits (Riding, 1999).

However, at the same time that support was growing for Kalkowsky’s (1908) biogenic interpretation of stromatolites, the contrary view was also being expressed. Reis (1908) interpreted stromatolites as inorganic precipitates, Bucher (1918) compared them with hot spring sinter. Semikhatov et al. (1979, p. 994) were also concerned by the similarities between microbial stromatolites and “morphologically similar structures of nonmicrobial origin” such as those in caves and hot springs, and therefore suggested their descriptive definition. Subsequent research has shown that some stromatolite-like deposits are essentially abiogenic (e.g., Grotzinger and Read, 1983; Grotzinger and James, 2000, p. 9). Pope et al. (2000, p. 1139) contrasted microbial and abiogenic stromatolites, and considered “isopachous stromatolites to have been dominated by chemogenic precipitation in the absence of microbial mats, and the growth of peloidal stromatolites to have been controlled by sedimentation in the presence of microbial mats.”

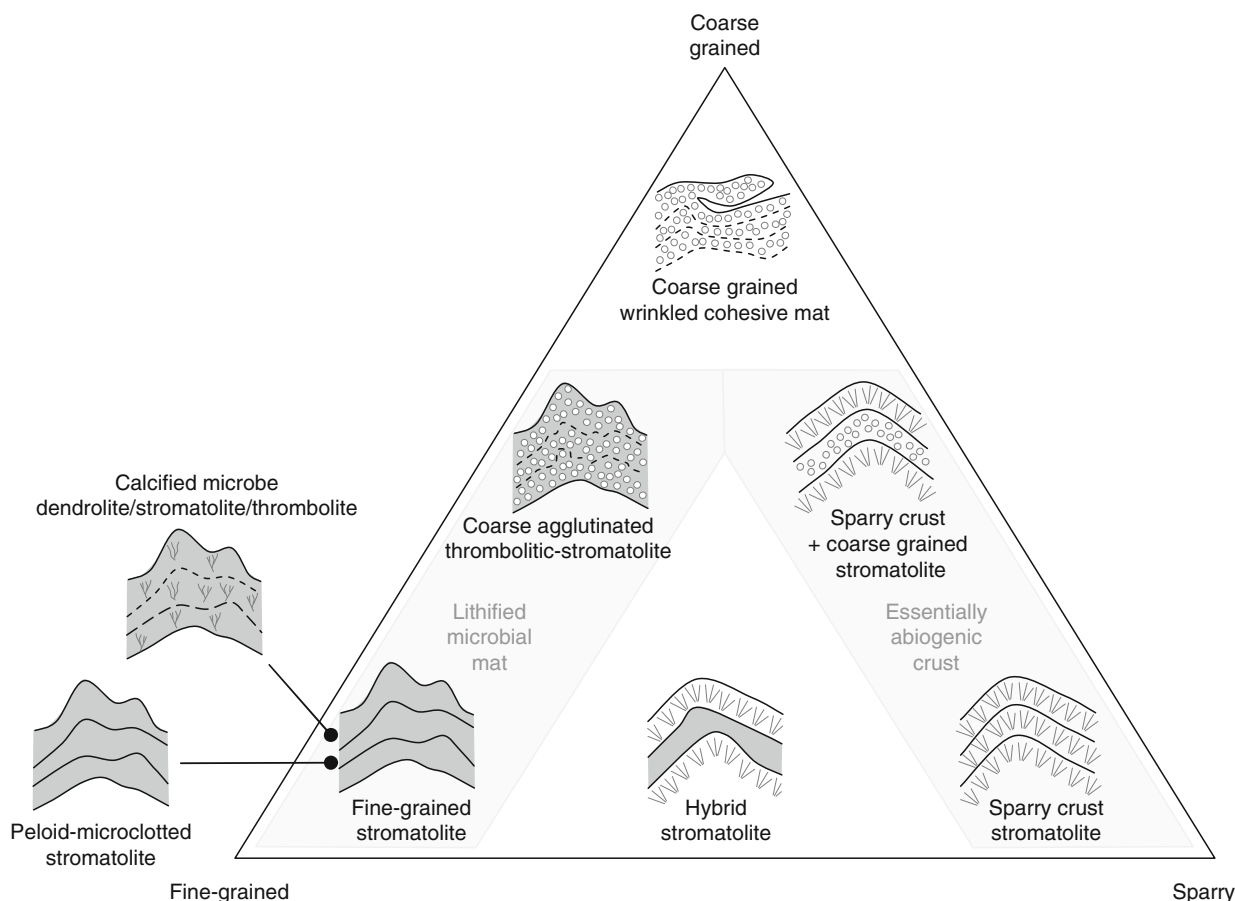
Fabric criteria can be used to distinguish abiogenic evenly layered sparry crust from fine-grained lithified microbial mats with uneven layering. In well-preserved examples, it should therefore be possible to tell abiogenic and biogenic stromatolites apart. However, as noted under microbialite, the millimetric interlayering of these two fabrics that can occur in hybrid stromatolites (Riding, 2008) makes such straightforward separation impossible. Consequently, stromatolites are regarded here, essentially, as laminated benthic microbial deposits, but they can contain abiogenic precipitates and be intimately inter-layered with them. In the marine realm, there is a strong secular perspective to this. Many Phanerozoic and Neoproterozoic stromatolites are probably essentially lithified microbial mats, whereas many older examples probably contain at least some precipitated abiogenic crust.



Microbialites, Stromatolites, and Thrombolites, Figure 2 Late Cambrian (late Franconian–early Trempealeauan) stromatolite (*Cryptozoon*), Hoyt Limestone, Petrified Gardens, Saratoga Springs, New York, USA. Coin diameter ~3 cm.

Stromatolite types

Stromatolite micro- and macrofabrics commonly intergrade with those of dendrolites and thrombolites (Figure 3). Their formation can involve up to three main processes: microbial precipitation, inorganic precipitation, and grain trapping (Figure 4). The main types represent



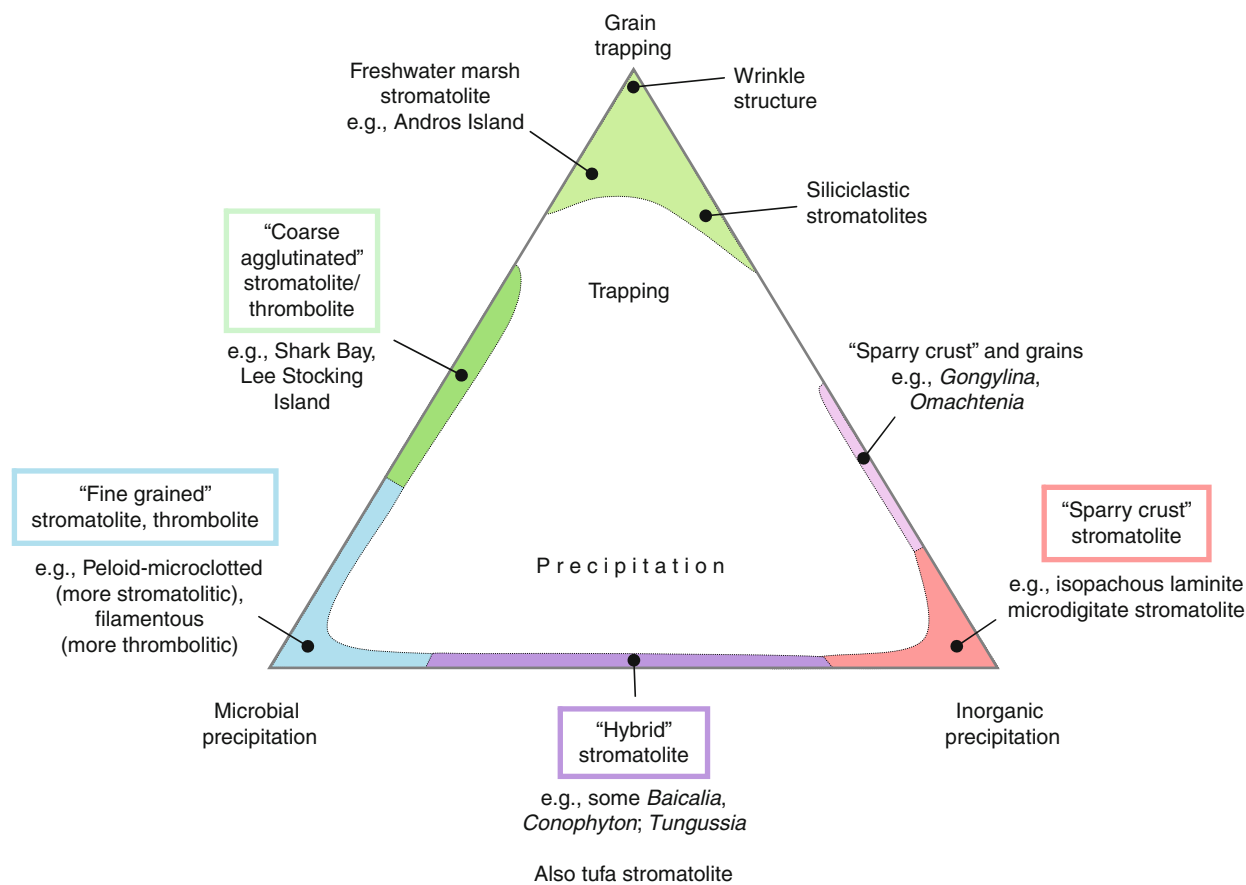
Microbialites, Stromatolites, and Thrombolites, Figure 3 Stromatolites and thrombolites based on micro/macrob fabric. All except wrinkled cohesive mats are syngedimentarily lithified by microbial and/or abiogenic processes. Fine-grained varieties include two distinctive types: (i) stromatolites with peloidal-microclotted microfabrics and (ii) dendrolites and thrombolites with abundant filamentous and other calcified microbial microfossils.

lithified microbial mat, sparry crust, and combinations of these (“hybrid stromatolite”).

Lithified microbial mat stromatolites exhibit a variety of intergradational fabrics. Two main types are fine-grained and coarse agglutinated. They form diverse stratiform, domical and columnar structures and, overall, tend to show relatively uneven to discontinuous layers with relatively poor inheritance and can include abundant fenestrae. Fine-grained stromatolites appear mainly to be products of syngedimentary microbial precipitation and are dominated by fine-grained (micrite, microspar) and filamentous fabrics. Fine-grained microfabrics are typically clotted and peloidal and are probably largely produced by heterotrophic bacterial calcification (e.g., dissimilatory sulfate reduction) of EPS and other cell products. Filamentous microfabrics are dominated by tubiform microfossils such as *Girvanella* that reflect photosynthetic cyanobacterial sheath calcification, but they too have fine-grained matrices. On their own, peloid-microclotted fabrics tend to be stromatolitic, and have been termed spongiostrome (Gürich, 1906; Pia, 1927, p. 36).

Filamentous fabrics tend to be more crudely layered and grade to thrombolitic. They have been termed porostromate (Pia, 1927, pp. 36–40), skeletal (Riding, 1977), and calcimicrobial (James and Gravestock, 1990). Coarse agglutinated stromatolites/thrombolites are produced by trapping sandy sediment by uncalcified EPS and erect filaments that can include microalgae. Present-day examples include some Shark Bay and Lee Stocking Island columns. They often have crudely layered macrofabrics (Logan, 1961) and have been termed thrombolitic stromatolites (Aitken, 1967).

Sparry crust (Riding, 2008) can form stromatolite-like abiogenic precipitates (Grotzinger and Rothman, 1996) characterized by even, often isopachous, laterally persistent layers with good inheritance (Pope et al., 2000). They have been most widely recognized in the Palaeoproterozoic and Mesoproterozoic (Grotzinger and Knoll, 1999, Fig. 6a, b), with microdigitate forms occupying peritidal environments (Grotzinger and Read, 1983) and isopachous laminite (Jackson, 1989) relatively deeper water facies.



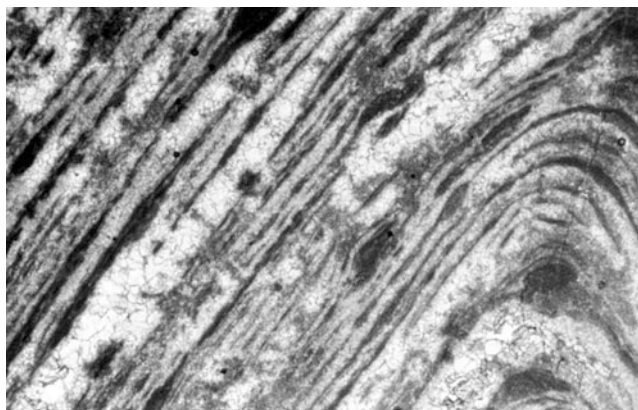
Microbialites, Stromatolites, and Thrombolites, Figure 4 Stromatolitic and thrombolitic carbonates related to grain trapping, and syngenic microbial and inorganic precipitation. Developed from Riding (2008, Fig. 12). Principal categories are coarse agglutinated, fine-grained, hybrid and sparry crust. Those dominated by microbial precipitation and/or grain trapping are microbial mats. Sparry crusts are essentially abiogenic precipitates. Hybrid stromatolites are combinations of lithified microbial mat and sparry crust. Fine-grained varieties include filamentous (also termed porostromate, skeletal, and calcimicrobial) which is more thrombolitic and peloidal-microclotted (also termed spongostromate) which is more stromatolitic. Microbial mats dominated by trapping, but with relatively minor early lithification include freshwater marsh stromatolites (Black, 1933) and wrinkle marks (Runzelmarken, Häntzschel and Reineck, 1968) mainly in siliciclastic sands and silts. For secular distributions see Fig. 11.

Hybrid crust stromatolites (Riding, 2008) typically consist of light-dark, often millimetric, alternations of sparry and fine-grained crust. These build stromatolites with well-developed even, although not usually isopachous, layering that is laterally quite persistent with generally good inheritance, as in some *Conophyton* (e.g., Walter, 1972, p. 86) (Figure 5). This layering is therefore intermediate in regularity between that of stromatolite-like sparry crust and fine-grained crust stromatolites. Hybrid crust appears to be a major component of Palaeoproterozoic (e.g., Sami and James, 1996) and Mesoproterozoic stromatolites (e.g., Petrov and Semikhatov, 2001), which can include very large domical and conical examples. Laminated freshwater cyanobacterial mats in shallow lakes of Andros Island show similarities to hybrid stromatolite, with fine-grained incipiently lithified cyanobacterial mats alternating with laminar fenestrae and elongate open voids (Monty, 1976, Fig. 4). If this structure were early lithified,

it could resemble some *Conophyton* and *Baicalia* fabrics, as Bertrand-Sarfati (1976) suggested.

Intertidal mats and wrinkle structures

At the present day, intertidal and supratidal sediments are commonly colonized by cyanobacteria dominated mats in siliciclastic (e.g., Cameron et al., 1985; Stal et al., 1985), evaporitic (e.g., Gerdes et al., 2000), and carbonate (Black, 1933; Logan et al., 1974) environments (Figure 6). Where these mats are cohesive, but insufficiently early lithified, they do not show significant accretion but nonetheless stabilize layers of sediment. These microbialites that were unlithified during their formation can be preserved in place, and also imprinted and disrupted, e.g., by desiccation and water movement. They and their incorporated sediment may be cracked, curled, and folded, and this syngenic deformation can be preserved after burial. Such patterned surfaces in



Microbialites, Stromatolites, and Thrombolites,
Figure 5 *Conophyton garganicum*. The fabric is interpreted as Hybrid stromatolite composed of alternating submillimetric layers of fine-grained lithified microbial mat and light-colored essentially abiogenic sparry layers. Middle Riphean, Russia, stratigraphic unit and locality not known. Specimen donated to Geological Survey of Canada by M.A. Semikhatov; photograph courtesy of Hans Hofmann. Width of view 8 mm.



Microbialites, Stromatolites, and Thrombolites,
Figure 6 Wrinkled microbial mat with carbonate sediment; supratidal flat, Crane Key, Florida Bay, USA. Red pen cap ~5 cm long.

Mesoproterozoic limestones were named *Kinneyia* by Walcott (1914, p. 107, pl. 11, Fig. 3) and subsequently linked to “Runzelmarken” (wrinkle marks, Häntzschel and Reineck, 1968) and other mat-related structures (Hagadorn and Bottjer, 1997). They have also been termed microbially induced sedimentary structures (MISS) (Noffke et al., 1996). The exact origins of these bedding plane structures are probably complex and varied (Porada et al., 2008) but they have been widely linked to deformation of microbial mats and have been used to infer the presence of mats in the Proterozoic (Horodyski, 1982) and Archaean (Noffke et al., 2006).

Siliciclastic stromatolites

In contrast to wrinkle structures, siliciclastic stromatolites can possess large domical morphologies and considerable synoptic relief. They are much scarcer than carbonate stromatolites, and are only known from mixed siliciclastic-carbonate environments that provide both siliciclastic grains and the early lithification required to maintain their support (Martín et al., 1993). Most examples are Palaeozoic, e.g., Ordovician (Davis, 1968), Devonian (Draganits and Noffke, 2004), Carboniferous (Bertrand-Sarfati, 1994), and Permian (Harwood, 1990), but diverse examples also occur in the late Miocene of South-east Spain (Martín et al., 1993; Braga and Martín, 2000). Based on the proportion of siliciclastic grains they contained, Martín et al. (1993) recognized three compositional types: “carbonate,” <10%; “siliciclastic-carbonate,” 10–50%; and “sandstone,” >50%. They are typically metric, locally decametric, domes with both stromatolitic and thrombolitic macrofabrics, associated with marginal marine beach (Braga and Martín, 2000), fan-delta and conglomeratic debris-flow deposits (Martín et al., 1993), and also with oolitic stromatolites–thrombolites (Braga et al., 1995). Sediment within the more siliciclastic domes includes abundant quartz, mica, and lithic fragment sand, and occasional quartz and metamorphic rock granules and pebbles.

Thrombolites

Definition

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Introduction

Aitken’s (1967) seemingly straightforward definition of thrombolite contained the seeds of more confusion than might have been anticipated. Since stromatolites are generally regarded as internally laminated; it could be expected that thrombolites are internally clotted. However, stromatolitic laminae are internal features of microbial carbonate, whereas “clot” could be used to describe both the external shape and internal structure of microbial carbonate (Figure 1). Aitken’s (1967) descriptions of Cambro-Ordovician examples, and his emphasis that thrombolite clotted fabric is macroscopic, have directed most researchers to regard “clots” as centimetric patches of microbial carbonate within interstitial sediment. In this view, a thrombolite dome consists of numerous such clots within lighter colored interstitial detrital matrix. Consequently, the dome has an overall macroclotted fabric, but the individual clots themselves are not necessarily internally composed of smaller clots, although they can be. This contrasts with stromatolitic domes in which each individual stromatolite is internally laminated and its shape is described separately, e.g., as domical, columnar, digitate, etc.

Aitken's (1967) concept of clots as patches of microbial carbonate within matrix is appropriate where they are irregularly rounded, but in digitate thrombolites the patches elongate into decimetric branches (e.g., Armella, 1994, Fig. 9) and use of "clot" to describe these is awkward. If some of Aitken's (1967) thrombolite domes contain dendritic fabrics, as seems likely, then why did he stress their clotted macrofabric? A likely explanation is that, in domes composed of radial branches, any section other than a vertical one through the dome center (e.g., Armella, 1994, Fig. 9) tends to show clot-like rounded outlines of oblique sections of branches. If this is correct, then to be consistent the branches should also be regarded as clots. This approach was followed by Armella (1994) and Kennard (1994) (although they used *thromboid* as an equivalent term to clot) to describe rounded lobate patches and elongate columns alike. There seems little doubt that this is what Aitken (1967) intended, and Shapiro (2000, p. 166) recognized that he used clot to describe the thrombolite columns themselves. Nonetheless, in describing similar branched Cambro-Ordovician thrombolites (*Favosamaceria*), Shapiro (2000) and Shapiro and Awramik (2006) broadened "clot" to refer to both millimetric clots within the branches and centimetric clots embedded in interstitial sediment. And there is a further complication. Aitken (1967) suggested that poorly laminated columns at Shark Bay are "thrombolitic stromatolites." As a result, in addition to describing Cambro-Ordovician domes with well-defined fine-grained clots, thrombolite has been applied to agglutinated present-day microbial columns in which the clots are much less distinct patchy fabrics.

These complications of usage still require clarification. At present, it is safe to say that "clot" (and the equivalent terms mesoclot and thromboid) has not been used consistently in thrombolite studies. It has been applied to millimetric patches within microbial carbonate, to centimetric lobate patches and also extended columns of microbial carbonate surrounded by detrital carbonate sediment, to transverse sections of these columns (here termed pseudoclots), and to diffuse patches of trapped sand, as well as to secondarily enhanced clots. Given these complications, it is understandable that earlier workers often referred to thrombolites as "unlaminated stromatolites" (e.g., Aitken, 1967, p. 1166; Pratt, 1982a; Schopf and Klein, 1992, p. 1202). Nonetheless, thrombolites can generally be regarded as benthic microbial carbonates with macroclotted fabric.

Thrombolite types

Two main types of thrombolite are calcified microbe and coarse agglutinated (Riding, 2000, pp. 192–193). Both intergrade with microbial mat stromatolites and primarily reflect the presence of major components that form irregular aggregates rather than thin layers. Nonetheless, the two types have quite distinct origins:

- (i) *Calcified microbe thrombolites* include the classic Cambro-Ordovician examples described by Aitken (1967) and also by Pratt and James (1982) and Kennard and James (1986). Aitken (1967) noted that burrows and trilobite fragments are common in these thrombolites, and examples of similar age often show mottled interiors and stromatolitic outer coatings (Figure 7). They consist of clots composed of framework whose most recognizable components are calcified microfossils, such as *Girvanella* filaments and *Renalcis* botryoids. The clots may be irregular centimetric amoeboid forms (Figure 8) or extend vertically into elongate (Armella, 1994, Fig. 9) meandriform and laterally amalgamated (Pratt and



Microbialites, Stromatolites, and Thrombolites, Figure 7 Late Cambrian (Trempealeauan) thrombolite column with mottled interior (within dotted line) and dark stromatolitic coating (between white arrows). Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA. Pen ~15 cm long.

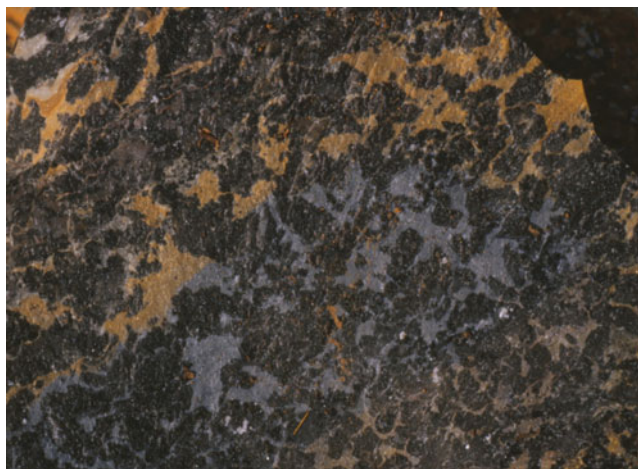


Microbialites, Stromatolites, and Thrombolites, Figure 8 Late Cambrian (Trempealeauan) thrombolite, Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA.

James, 1982, Figs. 9, 12) branches named *Favosamaceria* by Shapiro and Awramik (2006), (Figure 1). The clots are surrounded by detrital sediment infill. Progression from irregular clots to elongate branches has confused descriptive terminology. Shapiro (2000) suggested that in branched forms the clots typical of thrombolites are to be found within the branches, although Aitken (1967) seems to have regarded the columns as clots. James and Gravestock (1990) used “calcimicrobe” (calcified microbial microfossil) to refer to filamentous and botryoidal fossils that are common in Cambrian reefs. These typically form dendrolite (Figure 9) and thrombolite (Figure 10) fabrics and include calcified cyanobacteria such as *Angusticellularia*, *Botomaella*, and *Girvanella*, and also *Epiphyton* and *Renalcis*, whose affinities are less certain. Calcified microbe thrombolites are widespread in shallow marine



Microbialites, Stromatolites, and Thrombolites,
Figure 9 Dendrolite, Middle Cambrian, Zhangxia Formation,
near Jinan, Shandong, China



Microbialites, Stromatolites, and Thrombolites,
Figure 10 Thrombolite, early Cambrian, near Tiout, Anti-Atlas
Mountains, Morocco. Width of view 8 cm.

environments during the Neoproterozoic and early Palaeozoic.

- (ii) *Coarse agglutinated thrombolites* are largely composed of fenestral microbially trapped sandy sediment within finer-grained microbially lithified matrix. They are closely associated with coarse agglutinated stromatolites (Figures 3 and 4) that Aitken (1967, p. 1171) described as “thrombolitic-stromatolites.” They are only known in the late Neogene and their development appears to be linked to the rise of algal-cyanobacterial mats able to trap coarse sediment.

Coarse-grained thrombolites in the late Miocene of South-east Spain are closely associated with stromatolite fabrics in composite domes and columns up to 1.5 m high and 4 m across (Martín et al., 1993; Braga et al., 1995; Feldmann and McKenzie, 1997). They include oolitic examples in which clotted fabric is produced by irregular fenestrae up to 15 mm in size (Riding et al., 1991a, p. 123). Braga et al. (1995, Fig. 8, pp. 358–359) attributed the formation of these thrombolite fabrics to “a complex of irregular agglutination, microbial calcification, skeletal encrustation, and erosional processes.”

Logan (1961) regarded Shark Bay columns as stromatolites but recognized that the lamination is often poor, describing some as “crudely laminated with laminae of 1–10 mm. in thickness” (p. 526, pl. 1, Fig. 4). Playford and Cockbain (1976, p. 403) observed that “Hamelin Pool stromatolites range from unlaminated (thrombolites) to finely laminated; most show only crudely developed lamination.” Walter (1972, p. 64) attributed “the crude lamination of many Shark Bay stromatolites” to the thick irregular mats and coarse sediment. Logan et al. (1974, p. 154) related carbonate fabric to mat type, with thick irregular pustular mat dominated by *Entophysalis* in particular producing “unlaminated to poorly laminated” fenestral fabric, and Gebelein (1974) suggested that clotted fabric could reflect degradation of organic material. Similar Bahamian subtidal columns (Dravis, 1983) are coarse-grained and crudely laminated (Dill et al., 1986) and it has been suggested that abundant algae, including diatoms in these mats contribute to the coarse texture and poor lamination typical of both Shark Bay (Awramik and Riding, 1988) and Bahamian (Riding et al., 1991b) columns.

Mesozoic-Cenozoic thrombolites: Thrombolites are also common in the mid-late Jurassic (e.g., Leinfelder et al., 1993; Parcell, 2002; Kopaska-Merkel, 2003; Mancini et al., 2004), often in association with sponges and stromatolites in deeper water (e.g., Jansa et al., 1988; Leinfelder et al., 1994, p. 37; Dromart et al., 1994) and with corals in shallow-water (e.g., Bertling and Insalaco, 1998; Dupraz and Strasser, 1999; Olivier et al., 2003; Olivier et al., 2006; Helm and Schülke, 1998, 2006). They exhibit a variety of forms, including

arborescent and pendant that commonly occur as thick crusts on frame-building invertebrates, and are also closely associated with a variety of problematic encrusting organisms such as *Bacinella*, *Lithocodium*, and *Shamovella/Tubiphytes*, as well as annelids and foraminifers (Leinfelder et al., 1993, Fig. 6; Olivier et al., 2003, Fig. 4). Similar – but more often stromatolitic – crusts occur in late Neogene, including present-day, scleractinian reefs (Riding et al., 1991c; Montaggioni and Camoin, 1993; Leinfelder et al., 1993, pp. 222–224), and provide important clues to the formation of microbial carbonate microfabrics (Reitner, 1993; Reitner et al., 2000). In addition, the macrofabrics of these Mesozoic-Cenozoic examples broadly resemble those of early Palaeozoic calcified microbe thrombolites, but with significantly different skeletal components. Their microfabrics are fine-grained, with peloids and microclotted micrite (Olivier et al., 2006, Fig. 9), similar to those of many lithified microbial mat stromatolites.

Tufa thrombolite and stromatolite (Riding, 2000, pp. 191, 194) forms in calcifying lakes and streams as a result of cyanobacterial calcification that includes sheath impregnation and encrustation, together with calcification of associated microbes. It was comparisons with freshwater tufa that first led to recognition of links between stromatolites and cyanobacteria (Walcott, 1914; Roddy, 1915). These complex fabrics show considerable variety due to local differences in organisms and the extent of biocalcification and external encrustation. There are similarities between tufa stromatolites and hybrid crust stromatolites (Riding, 2008, p. 88) (Figure 4).

Kennard and James (1986, p. 498) noted that, although “poorly laminated and partially clotted microbial structures” occur on some present-day hypersaline marine shorelines (Laguna Mormona, Shark Bay) and in some lakes of various salinities (Great Salt Lake, Lake Clifton, Green Lake), in these examples “individual microbial clots are generally poorly defined” and “form an irregular, botryoidal-like, mesoscopic fabric that is unlike any of the fabrics observed by us in Lower Paleozoic thrombolites.” However, Moore and Burne (1994, pp. 23) considered that thrombolites in brackish to normal marine Lake Clifton, Western Australia, do closely resemble some early Palaeozoic examples in mesoclot shape and “the interframework of infilled fenestrae” and they pointed out that in Lake Clifton filamentous cyanobacteria (*Scytonema*) are important in thrombolite formation. Moore and Burne (1994, p. 21) noted that fine laminae, initially present in the Lake Clifton structures, are syndimentarily destroyed during mesoclot formation as carbonate precipitation continues, and also that the interframework fenestrae are intrinsic features “primarily related to the topography of the surface of the developing microbialite rather than to excavation of the structure by metazoan activity.” Great Salt Lake “algal mounds,” that consist of precipitated aragonitic framework and internal sediment (Halley, 1976, p. 439, Fig. 3), have a sub-centimetric thrombolitic fabric. Ferris et al. (1997)

suggested that thrombolitic fabrics in Kelly Lake, British Columbia, reflect a greater degree of calcification than in stromatolites.

Post-depositional thrombolites Aitken (1967, p. 1171) noted that thrombolite macroclots are prone to accentuation by recrystallization. This emphasizes a fundamental difference from stromatolites, whose laminae are essentially primary and not likely to be significantly enhanced by diagenesis. In contrast, clots could be significantly enhanced, e.g., by selective dolomitization, and in some cases secondarily produced, e.g., by bioturbation.

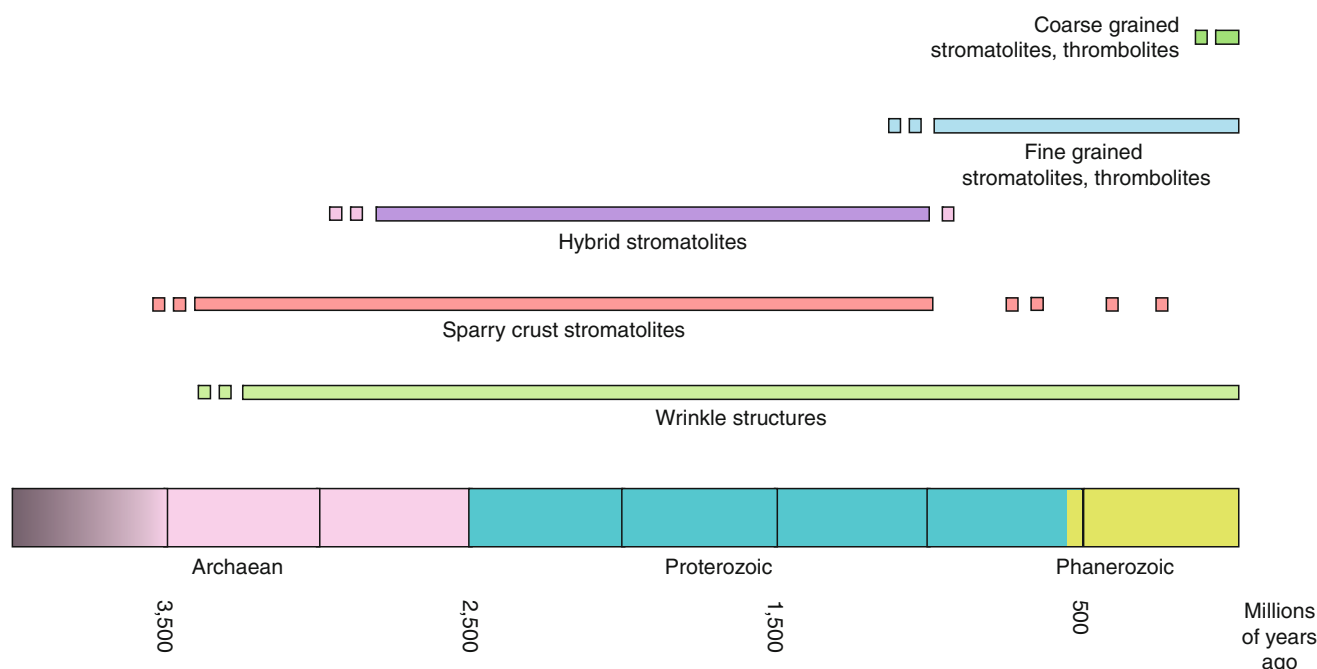
Stromatolites and thrombolites through time

Microbialite types are generally long ranging. Nonetheless, they show some distinctive differences in time distribution (Figure 11). Marine sparry crusts and hybrid stromatolites are typically Precambrian, and fine-grained stromatolites and thrombolites mainly range Neoproterozoic to the present day. Wrinkle structures show the longest range, whereas lithified coarse-grained carbonate thrombolites and stromatolites are only known from the past 10 Ma or less.

Archaean and Proterozoic

Stromatolites are relatively scarce in Archaean rocks until nearly the end of the eon. Their history begins with ~3.45 Ga coniform examples in the Pilbara region of Western Australia. These show fine continuous laminae (Lowe, 1980, 1983) and sparry microfabrics (Hofmann et al., 1999, Fig. 3). Their origins, and those of other Pilbara stromatolites, have been debated (e.g., Lowe, 1994, 1995; Buick et al., 1995), with recent studies supporting a biogenic origin (Hofmann et al., 1999, p. 1260–1261; Allwood et al., 2006, p. 717). Wrinkle and associated structures also suggest the presence of microbial mats in ~2,900 Ma siliclastic sediments of South Africa (Noffke et al., 2008), but these too are generally scarce. However, stromatolites are abundant in the ~2.55 Ga Campbellrand-Malmani carbonate platform of South Africa, locally forming elongate domes 10 m across and 40 m or more in length (Beukes, 1987, p. 9). In addition, these late Archaean carbonates contain distinctive “fenestrate microbialites” (Sumner and Grotzinger, 2004) consisting of millimetric to centimetric areas of light colored cement outlined by thin net-like layers (Figure 12). These have been interpreted as wispy convoluted microbial mats that were encrusted by calcite as they formed (Sumner, 1997a, b).

Large, often decametric, stromatolites are also conspicuous components of Proterozoic carbonate platforms (e.g., Grotzinger, 1986, p. 833; Petrov and Semikhatov, 2001, Fig. 6, p. 269). Where they are well preserved, they often show interlamination of sparry and micritic layers (e.g., Sami and James, 1996, p. 217). These dark-light layers that appear to represent alternations of lithified mat and abiogenic crust, are typical of “hybrid stromatolites” and are well seen in some *Conophyton* and *Baicalia* (Riding,



Microbialites, Stromatolites, and Thrombolites, Figure 11 Approximate time distributions of major categories of marine microbialites. Coarse agglutinated mats are mainly Neogene. Fine-grained mats and thrombolites are mainly Neoproterozoic and Phanerozoic. Marine “sparry” and “hybrid” crusts are mainly Archaean and Proterozoic. Wrinkle structures range Archaean to present-day. For some possible controls, see [Figure 14](#).

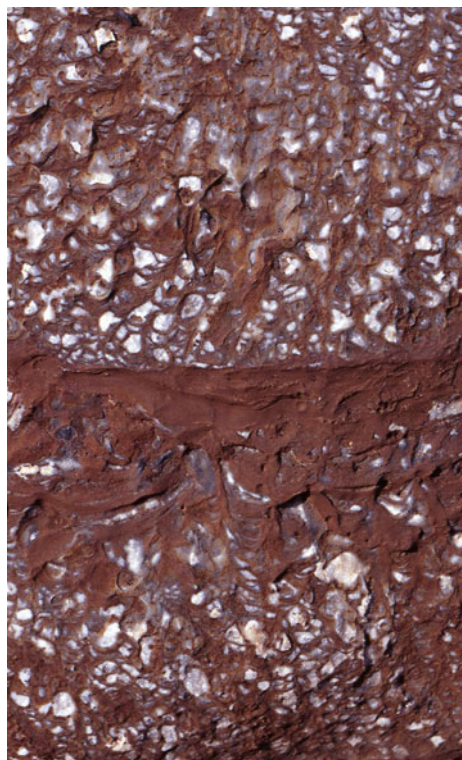
2008). Such incorporation of thin abiogenic sparry crusts appears to have contributed significantly to the size and relief of stromatolites throughout the Palaeo- and Mesoproterozoic. During the same period, extensive sheets of very small “microdigitate” stromatolites, typically <5 mm wide and <20 mm high (Hoffman, 1975, p. 262) were common in shallow peritidal environments (Figure 13). These “tufas” have been interpreted as essentially abiogenic (Grotzinger and Read, 1983).

An important control on Archaean-Proterozoic stromatolite formation was gradual decline in seawater carbonate saturation state (Grotzinger, 1989b; Grotzinger and Kasting, 1993). This progressively reduced stromatolite abundance (Grotzinger, 1990) and mediated a long-term trend from sparry crust to micritic carbonate sediments (Grotzinger and Kasting, 1993; Kah and Knoll, 1996). Transition to carbonate mud-dominated platforms ~1,400–1,300 Ma ago (Sherman et al., 2000) preceded the appearance of sheath-calcified cyanobacteria ~1,200 Ma ago (Kah and Riding, 2007). This significant transition, which led to Neoproterozoic development of calcimicrobial thrombolites (Aitken and Narbonne, 1989; Turner et al., 1993, 2000a, b), could reflect induction of CO₂-concentrating mechanisms (CCM) in cyanobacteria in response to fall in CO₂ levels below a threshold near ten times present-day levels (Riding, 2006) (Figure 14). CCMs are responses to reduced availability of inorganic carbon for photosynthesis, and in cyanobacteria include active bicarbonate uptake that

locally increases sheath pH, promoting calcification (Merz, 1992). Calcified filaments, such as *Girvanella*, altered microbial carbonate fabrics, disrupting stromatolite layering and promoting thrombolitic macro-clotted fabric. In subtidal environments, Neoproterozoic stromatolites are commonly interlayered with thrombolites with filamentous, clotted, and spongy “cellular” fabrics (Aitken and Narbonne, 1989) comparable with those of Cambro-Ordovician thrombolitic bioherms (Turner et al., 1993; 1997, p. 441, 449; 2000a, Figs. 6e, 8h and i).

Stromatolite decline

Stromatolites show long-term decline in abundance that may have commenced as early as the Palaeoproterozoic (Grotzinger, 1990) and is still observed in the Phanerozoic. Fischer (1965) suggested that decline since the Ordovician could reflect both reduction in carbonate saturation and competition by eukaryotes. Competition was subsequently emphasized when it appeared that marked late Proterozoic fall in stromatolite morphotypic diversity coincided with metazoan evolution (Awramik, 1971), but inception of decline prior to the appearance of metazoans implicates reduction in saturation state as the major influence (Grotzinger, 1990). It was also suggested that Cambrian thrombolites reflected disruption of stromatolites by burrowing organisms (Walter and Heys, 1985, pp. 150–151). However, Cambrian-Ordovician thrombolite fabrics are dominated by calcimicrobes that resist disruption (Kennard and James, 1986, p. 494) and



Microbialites, Stromatolites, and Thrombolites,
Figure 12 Fenestrate microbialite. Meshwork of large fenestrae (white) outlined by thin curved dark layers. Late Archaean Campbellrand-Malmani platform, South Africa. Width of field ~ 16 cm.



Microbialites, Stromatolites, and Thrombolites,
Figure 13 Microdigitate stromatolite, silicified after carbonate. Wumishan Formation, Mesoproterozoic, ~ 25 km north of Beijing, China. Width of view ~ 25 cm.

it has since been recognized that thrombolites appeared in the Neoproterozoic (Aitken and Narbonne, 1989), and possibly ~ 1.9 Ga in the Palaeoproterozoic (Kah and Grotzinger, 1992).

This leaves the question of the significance of trends in stromatolite diversity. Stromatolite shape reflects original

synoptic relief, determined by accretion rate relative to adjacent sediment (Figure 15). Low relative accretion rate results in low relief that makes stromatolites more prone to lateral incursion by sediment, thereby fostering complex shapes such as digitate forms (Figure 16). In contrast, high relative accretion rate results in high relief and simple shapes, such as domes and cones. Consequently, although mid-Proterozoic increase in morphotypic diversity, e.g., in branched stromatolites, has been regarded as a proxy for abundance, it more likely reflects low synoptic relief due to reduced relative accretion rate. Paradoxically, therefore, increased diversity could be sign that stromatolite growth was in decline due to reduced microbial growth and/or reduction in syndimentary lithification.

Snowball Earth

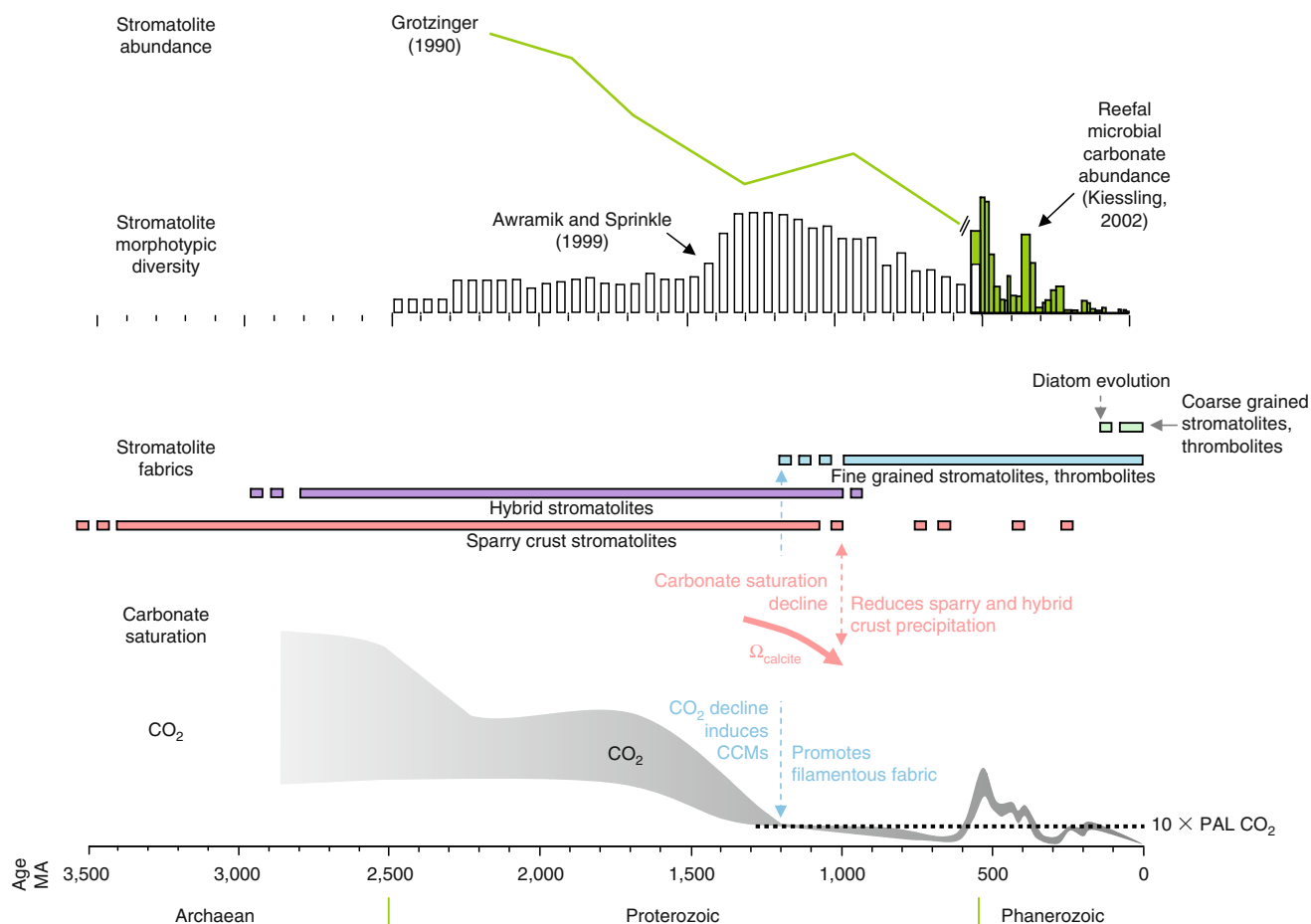
The prolonged warm Mesoproterozoic interval (Jenkins, 2003) was followed by Neoproterozoic (Sturtian, ~ 0.7 Ga Ma, Walter et al., 2000; Marinoan, ~ 0.635 Ga, Bodiselitsch et al., 2005) glaciations. Lower temperature and $p\text{CO}_2$ levels would have decreased seawater saturation state, hindering microbial calcification generally. Cooling would also have favored diffusive entry of CO_2 into cells and therefore may have slowed CCM development, further reducing cyanobacterial calcification. Nonetheless, microbialites are locally conspicuous in Cap Carbonates that immediately follow glacial deposits (e.g., Hoffman and Schrag, 2002; Corsetti and Grotzinger, 2005). Cap Carbonates have been suggested to reflect precipitation from seawater highly saturated for carbonate minerals as a result of alkaline upwelling and enhanced terrestrial weathering (Grotzinger and Knoll, 1995; Hoffman and Schrag, 2002). In the ~ 600 – 700 Ma Noonday Dolomite of California, narrow laterally amalgamated stromatolites define tubes of intervening detrital sediment fill (Figure 17). The overall organization of some Cambro-Ordovician *Favosamaceria* thrombolites (Shapiro and Awramik, 2006) resembles these “tubestones” (Corsetti and Grotzinger, 2005, p. 360).

Following Neoproterozoic “snowball” glaciations, global warming and O_2 rise could have reactivated CCM development, and rising temperature, calcium (Brennan et al., 2004), and $p\text{CO}_2$ (Berner and Kothavala, 2001) levels would have increased seawater saturation state, stimulating microbial calcification (Riding, 2006). This favored microbialite resurgence, and dendrolites, thrombolites, and stromatolites all became widespread in the early Cambrian (Rowland and Shapiro, 2002) (Figure 18).

Phanerozoic

Secular distribution

Both reefal microbial carbonates (Kiessling, 2002, Fig. 16) and calcified cyanobacteria (Arp et al., 2001) decline in abundance during the Phanerozoic, but this trend shows marked fluctuations. They are common in the late Cambrian-early Ordovician and late Devonian-early Mississippian, and scarce during the Cenozoic.

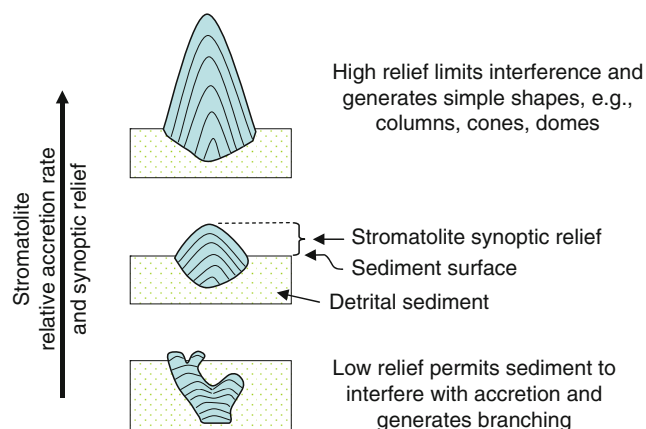


Microbialites, Stromatolites, and Thrombolites, Figure 14 Some secular trends and possible controls on microbial carbonate developments. Proterozoic stromatolite abundance (Grotzinger, 1990) and morphotypic diversity (Awramik and Sprinkle, 1999), together with Phanerozoic reefal microbial carbonate abundance (KieSSLing, 2002). These suggest overall long-term decline for at least the past 1,000 Ma. Reduction in both Sparry Crust and Hybrid stromatolites ~1,000 Ma ago may reflect decline in seawater carbonate saturation (Grotzinger, 1990). Stromatolite-like sparry crust occurs sporadically in the Phanerozoic, particularly in evaporite basins (Pope et al., 2000). Increase in thrombolites ~1,200–1,000 Ma ago could reflect development of cyanobacterial sheath calcification, reflecting inception of CO_2 -concentrating mechanisms (CCMs) as CO_2 levels declined to ~10 times present atmospheric level (PAL) (Riding, 2006). Inferred Proterozoic CO_2 trend based on Sheldon (2006), Kah and Riding (2007), Hyde et al. (2000) and Ridgwell et al. (2003). Phanerozoic CO_2 trend from Berner and Kothavala (2001, Fig. 13). Threshold for CCMs ($10 \times \text{PAL } \text{CO}_2$) based on Badger et al. (2002). Late Neogene inception of Coarse agglutinated stromatolites and thrombolite could in part reflect incorporation of diatoms into microbial mats and also generally low values of seawater carbonate saturation.

Fischer (1965) suggested that eukaryote competition and reduction in carbonate saturation state contributed to stromatolite decline from the Ordovician onward.

Carbonate saturation: Comparison with seawater saturation state for CaCO_3 minerals calculated from modeled seawater and CO_2 values shows broad positive correspondence with peaks of microbial/cyanobacterial carbonate abundance during much of the Palaeozoic and Mesozoic (Riding and Liang, 2005), supporting Fischer (1965). Lack of correspondence during the interval ~120–80 Ma ago (when calculated saturation ratio is high but microbial carbonate abundance was low) could reflect removal of carbonate deposition by pelagic plankton that significantly reduced actual saturation state.

Competition: The role of metazoan competition in late Proterozoic and early Palaeozoic stromatolite history is uncertain, and it is debatable whether metazoan grazing significantly affected stromatolite development (Pratt, 1982b) so long as carbonate saturation was high enough to ensure extensive early lithification of microbial mats. Nonetheless, it seems likely that from at least the mid-Ordovician onwards, overgrowth by skeletonized algae and invertebrates inhibited the formation of domical stromatolites and thrombolites. Subsumed within complex reef structures, microbial carbonates would instead have formed patchy and irregular crusts on and around skeletal organisms. Nonetheless, they were often important reef components (KieSSLing, 2002, Fig. 16).



Microbialites, Stromatolites, and Thrombolites,

Figure 15 Hypothetical interpretation of the significance of stromatolite shape. Shape is determined by synoptic relief that in turn reflects accretion rate of the stromatolite surface relative to adjacent sediment. In this view, enhanced stromatolite accretion generates high relief and simple shapes, such as columns, cones, and domes. In contrast, relatively low stromatolite accretion results in low relief. As a result, the stromatolite surface is prone to overlap by adjacent sediment, generating branching and irregular margins.



Microbialites, Stromatolites, and Thrombolites,

Figure 16 *Gymnosolen*, digitate stromatolite, Dalian, China, ~1.0 Ga.

Disaster biotas: The concept of stromatolite decline resulting from algal-metazoan diversification (Fischer, 1965; Garrett, 1970; Awramik, 1971) has also given rise to that of stromatolite resurgence in the aftermaths of mass extinctions (Schubert and Bottjer, 1992, p. 885). In this view, if metazoans can competitively exclude microbial carbonates then temporary reduction in metazoan abundance and diversity in the immediate aftermaths of mass



Microbialites, Stromatolites, and Thrombolites,

Figure 17 Laterally amalgamated columnar stromatolites (light color) separated by slightly darker tubular fills of detrital sediment. Noonday Dolomite, late Neoproterozoic, Mesquite Mountains, California, USA.

extinctions should permit temporary increase in microbial carbonates. Schubert and Bottjer (1992, 1995) interpreted early Triassic stromatolites as “post-mass extinction disaster forms.” However, whereas microbial carbonate reefal abundance also increased noticeably in the aftermath of late Devonian extinction, it did not increase following end-Ordovician, end-Triassic, and end-Cretaceous mass extinctions (Riding, 2006). Nonetheless, it is likely that in these situations, unconstrained by algal and invertebrate reef organisms, microbial mats were able to develop distinctive morphologies, and large domes, columns, and digitate structures have been reported, e.g., at the Permian-Triassic boundary in Sichuan, China (Kershaw et al., 1999).

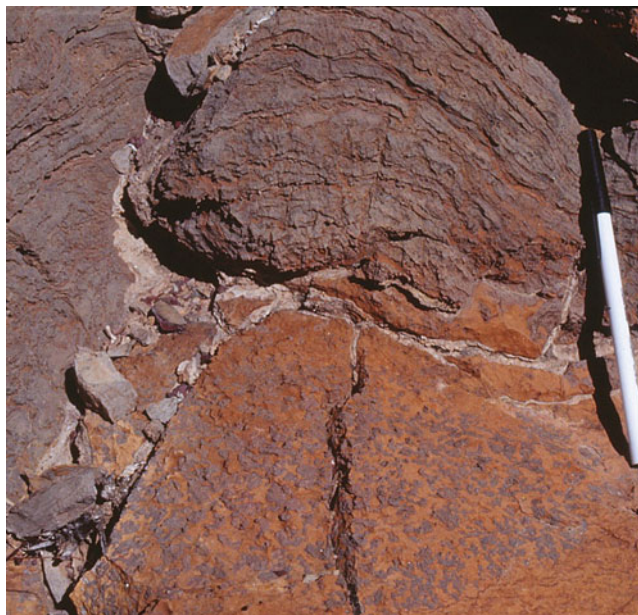
Thrombolites: Flügel (2004, p. 378) suggested that thrombolite abundance also declined after the Cambrian, although they were still locally conspicuous, e.g., in the Silurian (Kahle, 2001), Devonian (Shapiro, 2000, p. 166), Mississippian (Webb, 1987, 2005), and near the Permian-Triassic transition (Kershaw et al., 1999; Ezaki et al., 2008). Thrombolites have been widely reported in the mid-late Jurassic (see Mesozoic-Cenozoic thrombolites, above), broadly coincident with the last major peak of abundance of calcified marine cyanobacteria (Arp et al., 2001, Fig. 3d).

Evaporite stromatolites: In addition to metazoan extinction, localized increase in carbonate saturation in evaporite basins will favor microbialite development. Pope et al. (2000, p. 1139) noted that isopachously laminated stromatolites, which they considered to be dominantly abiogenic, are well developed in association with major evaporite successions and cited examples in the Proterozoic and Phanerozoic, including the Silurian Michigan Basin of North America and the late Permian Zechstein Basin of northern Europe (Pope et al., 2000,

Figs. 7, 9). These conditions therefore marked temporary returns to conditions that promoted formation of the sparry and hybrid crusts typical of the Archaean and early Proterozoic.

Neogene coarse-grained thrombolitic stromatolites

Well-known present-day examples of columns and domes occur in wave- and current-swept environments at Shark Bay (Logan, 1961) and the Bahamas (Dravis, 1983; Dill



Microbialites, Stromatolites, and Thrombolites, Figure 18 Late Cambrian (Trempealeauan) thrombolite overlain by stromatolite, Smoky Member, Nopah Fm, Dry Mountain, California, north-western Death Valley National Park, USA. Pen ~15 cm long.

et al., 1986; Riding et al., 1991b; Reid et al., 2000). They accrete bioclastic and ooid sand, and have crudely layered (Logan, 1961) and thrombolitic (Aitken, 1967, p. 1171) fabrics. Several factors conspire in their formation: water movement, mat community, and seawater chemistry. High-energy grainy conditions facilitate trapping by lifting sand to the accreting mat surface, and simultaneously deter overgrowth by reefal encrusters (Dill et al., 1989, p. 10). At Shark Bay, seasonal hypersalinity also limits competitors. Together with rapid accretion this allows decimetric, and locally metric, columns to develop. Accretion is high because the mats are thick and soft, with abundant EPS (Decho et al., 2005). In addition to cyanobacteria, they contain diatoms and filamentous green algae that enhance trapping ability (Awramik and Riding, 1988; Riding et al., 1991b). The upper mat remains soft and sticky because it is largely uncalcified, and the early lithification necessary to support these large columns mainly occurs in, or below, the lower part of the mat. Microbial lithification by sulfate reduction (Visscher et al., 2000) is limited to very thin micritic crusts (Reid et al., 2000), cyanobacterial sheaths are uncalcified (Reid et al., 2000, p. 992), and calcification of algal filaments mainly occurs in cavities (Dravis, 1983; Whittle et al., 1993, p. 224). Formation of these coarse-grained thrombolitic stromatolites is therefore largely due to their thick, soft, EPS-rich mats. These in turn reflect a combination of Cenozoic circumstances: (i) seawater saturation state that is too low for cyanobacterial sheath impregnation, but permits early lithification and (ii) the presence of fast-growing microalgae, such as diatoms, which – from a geological standpoint – are relative newcomers to mat communities (Figure 14). Coarse-grained thrombolitic stromatolite domes and columns are well developed in the late Miocene of South-east Spain (Riding et al., 1991a; Braga et al., 1995; Feldmann and McKenzie, 1997) (Figure 19) but are not known in older rocks.



Microbialites, Stromatolites, and Thrombolites, Figure 19 Coarse-grained, oolitic, composite leiolite-stromatolite-thrombolite domes, late Miocene (Messinian), Joyazo, Almería, South-east Spain. Red pen, lower left, ~15 cm long.

Summary

During the Archaean and much of the Proterozoic, both microbial mat growth and abiogenic precipitation were involved in stromatolite development that, locally, was particularly abundant from the latest Archaean to early Neoproterozoic (Grotzinger and Knoll, 1999). The combination of these biogenic and abiogenic factors was responsible for the rapid growth that enabled stromatolites, ranging from extensive small microdigitate sheets (Grotzinger and Read, 1983) to decametric domes (e.g., Hofmann, 1998, pl. 8a), to dominate carbonate platforms for 1,500 million years. Well-preserved stromatolites with dark-light lamination (Sami and James, 1996; Petrov and Semikhov, 2001) suggest that lithified mat growth alternated with abiogenic sparry crust precipitation on a millimetric scale to form inter-layered hybrid crust stromatolites (Riding, 2008).

Decline in sparry crusts and hybrid stromatolites ~1,000 Ma ago probably largely reflects reduction in seawater saturation (Grotzinger, 1990). This change also broadly coincided with the inception of cyanobacterial sheath-calcification in the Mesoproterozoic. This important development may reflect induction of CO₂-concentrating mechanisms (CCM) to assist photosynthetic carbon uptake as CO₂ levels declined (Riding, 2006). It transformed microbialite fabrics, and calcified microbe thrombolites and stromatolites with filamentous fabrics were conspicuous in shallow subtidal environments until the early Ordovician. Subsequently, stromatolites and thrombolites were widely subsumed within algal-invertebrate reefs. In these closely packed habitats, they lacked space to develop classic dome and column morphologies, and instead mainly formed reefal crusts and irregular masses. Only where competitors were environmentally excluded, or absent (as in mass extinction aftermaths), did microbial domes briefly develop extensively.

Both competition and declining carbonate saturation limited the abundance of marine microbialites from the Ordovician onwards (Fischer, 1965), and they are much less widespread and abundant in the Cenozoic than in the Palaeozoic. But microbialite communities have the ability to reinvent themselves. Diatoms together with other microalgae have significantly enhanced mat trapping ability. In environments where reefal overgrowth is limited, and coarse grains abundant, these soft mats can create large coarse-grained columns, as at Shark Bay and Lee Stocking Island. Internally these have distinctive crudely layered thrombolitic stromatolite fabrics (Aitken, 1967); in external shape, they closely resemble some Palaeoproterozoic stromatolites.

Microbialites have changed significantly during their extraordinarily long history in shape, size, fabrics, and abundance. They have responded to microbial evolution, to environmental changes that have affected both carbonate sedimentation and microbial metabolism, and to the evolution of other organisms. To add to this complexity, whereas stromatolites are essentially lithified microbial mats, they may be intimately associated with abiogenic crusts.

Microbialites archive important geobiological changes in atmospheric composition, seawater chemistry, mat evolution, and biotic interaction. Their study continues to offer many challenges and opportunities.

Bibliography

- Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.
- Aitken, J. D., and Narbonne, G. M., 1989. Two occurrences of Precambrian thrombolites from the Mackenzie Mountains, northwestern Canada. *Palaios*, **4**, 384–388.
- Allwood, A. C., Walter, M. R., Kamber, B. S., Marshall, C. P., and Burch, I. W., 2006. Stromatolite reef from the Early Archaean era of Australia. *Nature*, **441**, 714–718.
- Armella, C., 1994. Thrombolitic-stromatolitic cycles of the Cambro-Ordovician boundary sequence, Precordillera Oriental Basin, western Argentina. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 421–441.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Awramik, S. M., 1971. Precambrian columnar stromatolite diversity: reflection of metazoan appearance. *Science*, **174**, 825–827.
- Awramik, S. M., and Margulis, L., 1974. *Stromatolite Newsletter*, **2**, 5.
- Awramik, S. M., and Riding, R., 1988. Role of algal eukaryotes in subtidal columnar stromatolite formation. *Proceedings National Academy of Science USA*, **85**, 1327–1329.
- Awramik, S. M., and Sprinkle, J., 1999. Proterozoic stromatolites: the first marine evolutionary biota. *Historical Biology*, **13**, 241–253.
- Badger, M. R., Hanson, D., and Price, G. D., 2002. Evolution and diversity of CO₂ concentrating mechanisms in cyanobacteria. *Functional Plant Biology*, **29**, 161–173.
- Berner, R. A., and Kothavala, Z., 2001. GEOCARB III. A revised model of atmospheric CO₂ over Phanerozoic time. *American Journal of Science*, **301**, 182–204.
- Bertling, M., and Insalaco, E., 1998. Late Jurassic coral/microbial reefs from the northern Paris Basin - facies, palaeoecology and palaeobiogeography. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **139**, 139–175.
- Bertrand-Sarfati, J., 1976. An attempt to classify Late Precambrian stromatolite microstructure. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 251–259.
- Bertrand-Sarfati, J., 1994. *Siliciclastic-carbonate stromatolite domes in the Early Carboniferous of the Ajers Basin (eastern Sahara, Algeria)*. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic stromatolites II*. Dordrecht: Kluwer, pp. 395–419.
- Beukes, N. J., 1987. Facies relations, depositional environments and diagenesis in a major early Proterozoic stromatolitic carbonate platform to basinal sequence, Campbellrand Subgroup, Transvaal Supergroup, Southern Africa. *Sedimentary Geology*, **54**, 1–46.
- Black, M., 1933. The algal sedimentation of Andros Island Bahamas. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, **222**, 165–192.
- Bodiseltich, B., Koeberl, C., Master, S., and Reimold, W. U., 2005. Estimating duration of Neoproterozoic snowball glaciations from Ir anomalies. *Science*, **308**, 239–242.
- Braga, J., and Martín, J. M., 2000. Subaqueous siliciclastic stromatolites a case history from Late Miocene beach deposits in the Sorbas Basin, SE Spain. In Riding, R., and Awramik, S. M. (eds), *Microbial sediments*, Berlin: Springer, pp. 226–232.

- Braga, J. C., Martín, J. M., and Riding, R., 1995. Controls on microbial dome fabric development along a carbonate-siliciclastic shelf-basin transect, Miocene, S.E. Spain. *Palaaios*, **10**, 347–361.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Bucher, W., 1918. On oölites and spherulites. *Journal of Geology*, **26**, 593–609.
- Buick, R., Groves, D. I., and Dunlop, J. S. R., 1995. Abiological origin of described stromatolites older than 3.2 Ga: comment and reply. *Geology*, **23**, 191.
- Burne, R. V., and Moore, L., 1987. Microbialites; organosedimentary deposits of benthic microbial communities. *Palaaios*, **2**, 241–254.
- Cameron, B., Cameron, D., and Jones, J. R., 1985. Modern algal mats in intertidal and supratidal quartz sands, northeastern Massachusetts, USA. In Curren, H. A. (ed.), *Biogenic Structures: Their Use in Interpreting Depositional Environments*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 35, pp. 211–235.
- Corsetti, F. A., and Grotzinger, J. P., 2005. Origin and significance of tube structures in Neoproterozoic post-glacial cap carbonates: Example from Noonday Dolomite, Death Valley, United States. *Palaaios*, **20**, 348–362.
- Davis, R. A., 1968. Algal stromatolites composed of quartz sandstone. *Journal of Sedimentary Petrology*, **38**, 953–955.
- Decho, A. W., Visscher, P. T., and Reid, R. P., 2005. Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 71–86.
- Dill, R. F., Kendall, C. G. St. C., and Shinn, E. A., 1989. Giant subtidal stromatolites and related sedimentary features. Field Trip Guidebook T373, 28th International Geological Congress, Washington, DC: American Geophysical Union, 33 pp.
- Dill, R. F., Shinn, E. A., Jones, A. T., Kelly, K., and Steinen, R. P., 1986. Giant subtidal stromatolites forming in normal salinity waters. *Nature*, **324**, 55–58.
- Draganits, E., and Noffke, N., 2004. Siliciclastic stromatolites and other microbially induced sedimentary structures in an Early Devonian barrier-island environment (Muth Formation, NW Himalayas). *Journal of Sedimentary Research*, **74**, 191–202.
- Dravis, J. L., 1983. Hardened subtidal stromatolites, Bahamas. *Science*, **219**, 385–386.
- Dromart, G., Gaillard, C., and Jansa, L. F., 1994. Deep-marine microbial structures in the Upper Jurassic of western Tethys. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 295–318.
- Dupraz, C., and Strasser, A., 1999. Microbialites and microencrusts in shallow coral bioherms (Middle to Late Oxfordian, Swiss Jura mountains). *Facies*, **4**, 101–129.
- Ezaki, Y., Liu, J., Nagano, T., and Adachi, N., 2008. Geobiological aspects of the earliest Triassic microbialites along the southern periphery of the tropical Yangtze Platform: initiation and cessation of a microbial regime. *Palaaios*, **23**, 356–369.
- Feldmann, M., and McKenzie, J. A., 1997. Messinian stromatolite-thrombolite associations, Santa Pola, SE Spain: an analogue for the Palaeozoic? *Sedimentology*, **44**, 893–914.
- Feldmann, M., and McKenzie, J. A., 1998. Stromatolite-thrombolite associations in a modern environment, Lee Stocking Island, Bahamas. *Palaaios*, **13**, 201–212.
- Ferris, F. G., Thompson, J. B., and Beveridge, T. J., 1997. Modern freshwater microbialites from Kelly Lake, British Columbia, Canada. *Palaaios*, **12**, 213–219.
- Fischer, A. G., 1965. Fossils, early life, and atmospheric history. *Proceedings of the National Academy of Sciences*, **53**, 1205–1215.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks. Analysis, Interpretation and Application*. Berlin: Springer, xx + 976 pp.
- Garrett, P., 1970. Phanerozoic stromatolites: noncompetitive ecological restriction by grazing and burrowing animals. *Science*, **169**, 171–173.
- Gebelein, C. D., 1974. Biological control of stromatolite microstructure: implications for Precambrian time stratigraphy. *American Journal of Science*, **274**, 575–598.
- Gerdes, G., Krumbein, W. E., and Noffke, N., 2000. Evaporite microbial sediments. In R. Riding, R., and Awramik, S. M. (eds.), *Microbial Sediments*. Berlin: Springer, pp. 196–208.
- Ginsburg, R. N., 1991. Controversies about stromatolites: vices and virtues. In Muller, D. W., McKenzie, J. A., and Weissert, H. (eds.), *Controversies in Modern Geology; Evolution of Geological Theories in Sedimentology, Earth History and Tectonics*. London: Academic Press, pp. 25–36.
- Ginsburg, R. N., Isham, L. B., Bein, S. J., and Kuperberg, J., 1954. Laminated algal sediments of South Florida and their recognition in the fossil record. *Marine Laboratory, University of Miami, Coral Gables, Florida*, Unpublished Report, 54–20, 33 pp.
- Grotzinger, J. P., 1986. Cyclicity and paleoenvironmental dynamics, Rocknest platform, northwest Canada. *Geological Society of America Bulletin*, **97**, 1208–1231.
- Grotzinger, J. P., 1989a. Introduction to Precambrian reefs. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Areas*. Canadian Society of Petroleum Geologists Memoir 13, pp. 9–12.
- Grotzinger, J. P., 1989b. Facies and evolution of Precambrian carbonate depositional systems: emergence of the modern platform archetype. In Crevello, P. D., Wilson, J. L., Sarg, J. F., and Read, J. F. (eds.), *Controls on Carbonate Platform and Basin Development*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication 44, pp. 79–106.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. *American Journal of Science*, **290-A**, 80–103.
- Grotzinger, J. P., and James, N. P., 2000. Precambrian carbonates: evolution of understanding. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 3–20.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Grotzinger, J. P., and Knoll, A. H., 1995. Anomalous carbonate precipitates: is the Precambrian the key to the Permian? *Palaaios*, **10**, 578–596.
- Grotzinger, J. P., and Knoll, A. H., 1999. Stromatolites in Precambrian carbonates: evolutionary mileposts or environmental dipsticks? *Annual Reviews of Earth and Planetary Sciences*, **27**, 313–358.
- Grotzinger, J. P., and Read, J. F., 1983. Evidence for primary aragonite precipitation, lower Proterozoic (1.9-Ga) Rocknest Dolomite, Wopmay Orogen, Northwest Canada. *Geology*, **11**, 710–713.
- Grotzinger, J. P., and Rothman, D. R., 1996. An abiotic model for stromatolite morphogenesis. *Nature*, **383**, 423–425.
- Gürich, G., 1906. Les spongiostromides du Viséen de la Province de Namur. Musée Royal d'Histoire Naturelle de Belgique, *Mémoires*, **3**(4), 1–55, 13 pls.
- Hagadorn, J. W., and Bottjer, D. J., 1997. Wrinkle structures: microbially mediated sedimentary structures common in subtidal siliciclastic settings at the Proterozoic-Phanerozoic transition. *Geology*, **25**, 1047–1050.
- Hall, J., 1883. *Cryptozoön*, n.g.; *Cryptozoön proliferum*, nsp. New York State Museum of Natural History, 36th Annual Report of the Trustees, plate 6.

- Halley, R. B., 1976. Textural variation within Great Salt Lake algal mounds. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology*, 20, Amsterdam: Elsevier, pp. 435–445.
- Häntzschel, W., and Reineck, H.-E., 1968. Fazies-Untersuchungen im Hettangium von Helmstedt (Niedersachsen). *Mitteilungen aus dem Geologischen Staatsinstitut in Hamburg*, **37**, 5–39.
- Harwood, G., 1990. 'Sandstone stromatolites' – an example of algal-trapping of sand grains from the Permian Yates Formation, New Mexico, U.S.A. Nottingham, England: 13th International Sedimentological Congress, Abstracts-Posters, p. 97.
- Helm, C., and Schülke, I., 1998. A coral-microbialite patch reef from the late Jurassic (*florigemma*-Bank, Oxfordian) of NW Germany (Süntel Mountains). *Facies*, **39**, 75–104.
- Helm, C., and Schülke, I., 2006. Patch reef development in the *florigemma*-Bank Member (Oxfordian) from the Deister Mts (NW Germany): a type example for Late Jurassic coral thrombolite thickets. *Facies*, **52**, 441–467.
- Hoffman, P. F., 1975. Shoaling-upward shale-to-dolomite cycles in the Rocknest Formation (lower Proterozoic), Northwest Territories, Canada. In Ginsburg, R. N. (ed.), *Tidal Deposits*. New York: Springer, pp. 257–265.
- Hoffman, P. F., and Schrag, D. P., 2002. The snowball Earth hypothesis: testing the limits of global change. *Terra Nova*, **14**, 129–155.
- Hofmann, H. J., 1969. Attributes of stromatolites. *Geological Survey of Canada Paper* 69–39, 58 pp.
- Hofmann, H. J., 1973. Stromatolites: characteristics and utility. *Earth Science Reviews*, **9**, 339–373.
- Hofmann, H. A., 1998. Synopsis of Precambrian fossil occurrences in North America. In Lucas, S. B., and St-Onge, M. R. (co-ords), *Geology of Canada*, no. 7, pp. 271–376.
- Hofmann, H. J., Grey, K., Hickman, A. H., and Thorpe, R. I., 1999. Origin of 3.45 Ga coniform stromatolites in Warrawoona Group, Western Australia. *Geological Society of America Bulletin*, **111**, 1256–1262.
- Horodyski, R. J., 1982. Impressions of algal mats from the Middle Proterozoic Belt Supergroup, northwestern Montana, USA. *Sedimentology*, **29**, 285–289.
- Hyde, W. T., Crowley, T. J., Baum, S. K., and Peltier, W. R., 2000. Neoproterozoic 'snowball Earth' simulations with a coupled climate/ice-sheet model. *Nature*, **405**, 425–429.
- Jackson, M. J., 1989. Lower Proterozoic Cowles Lake foredeep reef, N.W.T., Canada. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and Adjacent Area*. Calgary: Canadian Society of Petroleum Geologists, Memoir 13, 64–71.
- James, N. P., and Gravestock, D. I., 1990. Lower Cambrian shelf and shelf margin build-ups, Flinders Ranges, South Australia. *Sedimentology*, **37**, 455–480.
- Jansa L. F., Pratt, B. R., and Dromart, G., 1988. Deep water thrombolite mounds from the Upper Jurassic of offshore Nova Scotia. In Geldsetzer, H. H. J., James, N. P., and Tebbutt, G. E. (eds.), *Reefs, Canada and adjacent areas*. Calgary: Canadian Society of Petroleum Geologists Memoir 13, 725–735.
- Jenkins, G. S., 2003. GCM greenhouse and high-obliquity solutions for early Proterozoic glaciation and middle Proterozoic warmth. *Journal of Geophysical Research*, **108**, D3, 4118, doi:10.1029/2001JD001582, 2003.
- Kah, L. C., and Grotzinger, J. P., 1992. Early Proterozoic (1.9 Ga) thrombolites of the Rocknest Formation, Northwest Territories, Canada. *Palaios*, **7**, 305–315.
- Kah, L. C., and Knoll, A. H., 1996. Microbenthic distribution of Proterozoic tidal flats: environmental and taphonomic considerations. *Geology*, **24**, 79–82.
- Kah, L. C., and Riding, R., 2007. Mesoproterozoic carbon dioxide levels inferred from calcified cyanobacteria. *Geology*, **35**, 799–802.
- Kahle, C. F., 2001. Biosedimentology of a Silurian thrombolite reef with meter-scale growth framework cavities. *Journal of Sedimentary Research*, **71**, 410–422.
- Kalkowsky, E., 1908. Oolith und Stromatolith im norddeutschen Buntsandstein. *Zeitschrift Deutschen geol. Gesellschaft*, **60**, 68–125, pls 4–11.
- Kennard, J. M., 1994. Thrombolites and stromatolites within shale-carbonate cycles, Middle–Late Cambrian Shannon Formation, Amadeus Basin, central Australia. In Bertrand-Sarfati, J., and Monty, C. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer, pp. 443–471.
- Kennard, J. M., and James, N. P., 1986. Thrombolites and stromatolites; two distinct types of microbial structures. *Palaios*, **1**, 492–503.
- Kershaw, S., Zhang, T., and Lan, G., 1999. A microbialite crust at the Permian-Triassic boundary in south China, and its palaeoenvironmental significance. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **146**, 1–18.
- Kiessling, W., 2002. Secular variations in the Phanerozoic reef ecosystem. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 72, pp. 625–690.
- Kopaska-Merkel, D. C., 2003. "Reefs" as exploration targets in the Smackover Formation. *Gulf Coast Association of Geological Sciences Transactions*, **53**, 411–421.
- Krumbein, W. E., 1983. Stromatolites - the challenge of a term in space and time. *Precambrian Research*, **20**, 493–531.
- Leinfelder, R. R., Nose, M., Schmid, D. U., and Werner, W., 1993. Microbial crusts of the Late Jurassic: composition, palaeoecological significance and importance in reef construction. *Facies*, **29**, 195–229.
- Leinfelder, R. R., Krautter, M., Laternser, R., Nose, M., Schmid, D. U., Schweigert, G., Werner, W., Keupp, H., Brugger, H., Herrmann, R., Rehfeld-Kiefer, U., Schroeder, J. H., Reinhold, C., Koch, R., Zeiss, A., Schweizer, V., Christmann, H., Menges, G., and Luterbacher, H., 1994. The origin of Jurassic reefs: current research developments and results. *Facies*, **31**, 1–56.
- Logan, B. W., 1961. *Cryptozoon* and associated stromatolites from the Recent, Shark Bay, Western Australia. *Journal of Geology*, **69**, 517–533.
- Logan, B. W., Rezak, R., and Ginsburg, R. N., 1964. Classification and environmental significance of algal stromatolites. *Journal of Geology*, **72**, 68–83.
- Logan, B. W., Hoffman, P., and Gebelein, C. D., 1974. Algal mats, cryptalgal fabrics, and structures, Hamelin Pool, Western Australia. *American Association of Petroleum Geologists, Memoir*, **22**, 140–194.
- Lowe, D. R., 1980. Stromatolites 3,400–3,500 Myr old from the Archean of Western Australia. *Nature*, **284**, 441–443.
- Lowe, D. R., 1983. Restricted shallow-water sedimentation of early Archean stromatolitic and avaporitic strata of the Strelley Pool Chert, Pilbara Block, Western Australia. *Precambrian Research*, **19**, 239–283.
- Lowe, D. R., 1994. Abiological origin of described stromatolites older than 3.2 Ga. *Geology*, **22**, 387–390.
- Lowe, D. R., 1995. Abiological origin of described stromatolites older than 3.2 Ga: comment and reply. *Geology*, **23**, 191–192.
- Mancini, E. A., Llinas, J. C., Parcell, W. C., Aurell, M., Badenas, B., Leinfelder, R. R., and Benson, D. J., 2004. Upper Jurassic thrombolite reservoir play, northern Gulf of Mexico. *AAPG Bulletin*, **88**, 1573–1602.
- Martin, J. M., Braga, J. C., and Riding, R., 1993. Siliciclastic stromatolites and thrombolites, late Miocene, S.E. Spain. *Journal of Sedimentary Petrology*, **63**, 131–139.
- McLoughlin, N., Wilson, L. A., and Brasier, M. D., 2008. Growth of synthetic stromatolites and wrinkle structures in the absence of

- microbes – implications for the early fossil record. *Geobiology*, **6**, 95–105.
- Merz, M. U. E., 1992. The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**, 81–102.
- Montaggioni, L. F., and Camoin, G. F., 1993. Stromatolites associated with coralgal communities in Holocene high-energy reefs. *Geology*, **21**, 149–152.
- Monty, C. L. V., 1976. The origin and development of cryptalgal fabrics. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology* 20, Amsterdam: Elsevier, pp. 193–249.
- Moore, L. S., and Burne, R. V., 1994. The modern thrombolites of Lake Clifton, western Australia. In Bertrand Sarfati, J., and Monty, C. L. (eds.), *Phanerozoic Stromatolites II*. Dordrecht: Kluwer Academic Publishers, pp. 3–29.
- Noffke, N., Beukes, N., and Hazen, R., 2006. Microbially induced sedimentary structures in the 2.9 Ga old Brixton Formation, Witwatersrand Supergroup, South Africa. *Precambrian Research*, **146**, 35–44.
- Noffke, N., Beukes, N., Bower, D., Hazen, R. M., and Swift, D. J. P., 2008. An actualistic perspective into Archean worlds - (cyano-) bacterially induced sedimentary structures in the siliciclastic Nhlazatse Section, 2.9 Ga Pongola Supergroup, South Africa. *Geobiology*, **6**, 5–20.
- Noffke, N., Gerdes, G., Klenke, T., and Krumbein, W. E., 1996. Microbially induced sedimentary structures – examples from modern sediments of siliciclastic tidal flats. *Zentralblatt für Geologie und Paläontologie, Teil 1, 1995, Heft 1/2*, 307–316.
- Olivier, N., Hantzpergue, P., Gaillard, C., Pittet, B., Leinfelder, R. R., Schmid, D. U., and Werner, W., 2003. Microbialite morphology, structure and growth: a model of the Upper Jurassic reefs of the Chay Peninsula (Western France). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **193**, 383–404.
- Olivier, N., Lathuilière, B., and Thiry-Bastien, P., 2006. Growth models of Bajocian coral-microbialite reefs of Chargey-lès-Port (eastern France): palaeoenvironmental considerations. *Facies*, **52**, 113–127.
- Parcell, W. C., 2002. Sequence stratigraphic controls on the development of microbial fabrics and growth forms - implication for reservoir quality distribution in the Upper Jurassic (Oxfordian) Smackover Formation, Eastern Gulf Coast, USA. *Carbonates and Evaporites*, **17**, 166–181.
- Petrov, P. Yu., and Semikhatov, M. A., 2001. Sequence organization and growth patterns of late Mesoproterozoic stromatolite reefs: an example from the Burovaya Formation, Turukhansk Uplift, Siberia. *Precambrian Research*, **111**, 257–281.
- Pia, J., 1927. Thallophyta. In Hirmer, M. (ed.), *Handbuch der Paläobotanik* 1, Munich: Oldenbourg, pp. 31–136.
- Playford, P. E., and Cockbain, A. E., 1976. Modern algal stromatolites at Hamelin Pool, a hypersaline barred basin in Shark Bay, Western Australia. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 389–411.
- Pope, M. C., Grotzinger, J. P., and Schreiber, B. C., 2000. Evaporitic subtidal stromatolites produced by in situ precipitation: textures, facies associations, and temporal significance. *Journal of Sedimentary Research*, **70**, 1139–1151.
- Porada, H., Ghergut, J., and Bouougri, E. H., 2008. Kinneyia-type wrinkle structures – critical review and model of formation. *Palaos*, **23**, 65–77.
- Pratt, B. R., 1982a. Stromatolitic framework of carbonate mudmounds. *Journal of Sedimentary Research*, **52**, 1203–1227.
- Pratt, B. R., 1982b. Stromatolite decline – a reconsideration. *Geology*, **10**, 512–515.
- Pratt, B. R., and James, N. P., 1982. Cryptalgal-metazoan bioherms of early Ordovician age in the St. George Group, western Newfoundland. *Sedimentology*, **29**, 543–569.
- Reid, R. P., Visscher, P. T., Decho, A. W., Stolz, J. F., Bebout, B. M., Dupraz, C., Macintyre, I. G., Paerl, H. W., Pinckney, J. L., Prufert-Bebout, L., Steppe, T. F., and DesMarais, D. J., 2000. The role of microbes in accretion, lamination and early lithification of modern marine stromatolites. *Nature*, **406**, 989–992.
- Reis, O. M., 1908. Kalkowsky: Ueber Oolith und Stromatolith im norddeutschen Buntsandstein. *Neues Jahrbuch für Mineralogie, Geologie und Paläontologie*, **2**, 114–138.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia); formation and concepts. *Facies*, **29**, 3–39.
- Reitner, J., Thiel, V., Zankl, H., Michaelis, W., Wörheide, G., and Gautret, P., 2000. Organic and biogeochemical patterns in cryptic microbialites. In Riding, R. E., and Awramik, S. M. (eds.), *Microbial Sediments*, Berlin: Springer, pp. 149–160.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Riding, R., 1977. Skeletal stromatolites. In Flügel, E. (ed.), *Fossil Algae, Recent Results and Developments*, Berlin: Springer-Verlag, pp. 57–60.
- Riding, R., 1991. Classification of microbial carbonates. In Riding, R., (ed.), *Calcareous algae and stromatolites*. Berlin: Springer-Verlag, pp. 21–51.
- Riding, R., 1999. The term stromatolite: towards an essential definition. *Lethaia*, **32**, 321–330.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47** (Suppl. 1), 179–214.
- Riding, R., 2006. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic-Cambrian changes in atmospheric composition. *Geobiology*, **4**, 299–316.
- Riding, R., 2008. Abiogenic, microbial and hybrid authigenic carbonate crusts: components of Precambrian stromatolites. *Geologia Croatica*, **61**(2–3), 73–103.
- Riding, R., and Liang, L., 2005. Geobiology of microbial carbonates: metazoan and seawater saturation state influences on secular trends during the Phanerozoic. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 101–115.
- Riding, R., Braga, J. C., and Martín, J. M., 1991a. Oolite stromatolites and thrombolites, Miocene, Spain: analogues of Recent giant Bahamian examples. *Sedimentary Geology*, **71**, 121–127.
- Riding, R., Awramik, S. M., Winsborough, B. M., Griffin, K. M., and Dill, R. F., 1991b. Bahamian giant stromatolites: microbial composition of surface mats. *Geological Magazine*, **128**, 227–234.
- Riding, R., Martín, J. M., and Braga, J. C., 1991c. Coral stromatolite reef framework, Upper Miocene, Almería, Spain. *Sedimentology*, **38**, 799–818.
- Roddy, H. J., 1915. Concretions in streams formed by the agency of blue-green algae and related plants. *Proceedings American Philosophical Society*, **54**, 246–258.
- Rowland, S. M., and Shapiro, R. S., 2002. Reef patterns and environmental influences in the Cambrian and earliest Ordovician. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, **72**, pp. 95–128.
- Sami, T. T., and James, N. P., 1996. Synsedimentary cements as Paleoproterozoic platform building blocks, Pethei Group, northwestern Canada. *Journal of Sedimentary Research*, **66**, 209–222.
- Schopf, J. W., and Klein, C., 1992. Glossary of technical terms. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere: A Multidisciplinary Study*. Cambridge, UK: Cambridge University Press, pp. 1189–1204.
- Schubert, J. K., and Bottjer, D. J., 1992. Early Triassic stromatolites as post-mass extinction disaster forms. *Geology*, **20**, 883–886.

- Semikhatov, M. A., Gebelein, C. D., Cloud, P., Awramik, S. M., and Benmore, W. C., 1979. Stromatolite morphogenesis - progress and problems. *Canadian Journal of Earth Science*, **16**, 992–1015.
- Serebryakov, S. N., (1976) Biotic and abiotic factors controlling the morphology of Riphean stromatolites. In Walter, M. R. (ed.), *Stromatolites, Developments in Sedimentology* 20, Amsterdam: Elsevier, pp. 321–336.
- Shapiro, R. S., 2000. A comment on the systematic confusion of thrombolites. *Palaaios*, **15**, 166–169.
- Shapiro, R. S., and Awramik, S. M., 2006. *Favosamaceria cooperi* new group and form: a widely dispersed, time-restricted thrombolite. *Journal of Paleontology*, **80**, 411–422.
- Sheldon, N. D., 2006. Precambrian paleosols and atmospheric CO₂ levels. *Precambrian Research*, **147**, 148–155.
- Sherman, A. G., James, N. P., and Narbonne, G. M., 2000. Sedimentology of a late Mesoproterozoic muddy carbonate ramp, northern Baffin Island, Arctic Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 275–294.
- Stal, L. J., van Gernerden, H., and Krumbein, W. E., 1985. Structure and development of a benthic marine microbial mat. *FEMS Microbiology Ecology*, **31**, 111–125.
- Steele, J. H., 1825. A description of the Oolitic Formation lately discovered in the county of Saratoga, and state of New-York. *American Journal of Science*, **9**, 16–19, part of pl. 2.
- Sumner, D. Y., 1997a. Carbonate precipitation and oxygen stratification in late Archean seawater as deduced from facies and stratigraphy of the Gamohaan and Frisco formations, Transvaal Supergroup, South Africa. *American Journal of Science*, **297**, 455–487.
- Sumner, D. Y., 1997b. Late Archean calcite-microbe interactions: two morphologically distinct microbial communities that affected calcite nucleation differently. *Palaaios*, **12**, 302–318.
- Sumner, D. Y., and Grotzinger, J. P., 2004. Implications for Neoproterozoic ocean chemistry from primary carbonate mineralogy of the Campbellrand-Malmani platform, South Africa. *Sedimentology*, **51**, 1–27.
- Turner, E. C., Narbonne, G. M., and James, N. P., 1993. Neoproterozoic reef microstructures from the Little Dal Group, northwestern Canada. *Geology*, **21**, 259–262.
- Turner, E. C., James, N. P., and Narbonne, G. M., 1997. Growth dynamics of Neoproterozoic calcimicrobial reefs, Mackenzie mountains, northwest Canada. *Journal of Sedimentary Research*, **67**, 437–450.
- Turner, E. C., Narbonne, G. M., and James, N. P., 2000a. Framework composition of early Neoproterozoic calcimicrobial reefs and associated microbialites, Mackenzie Mountains, N.W.T., Canada. In Grotzinger, J. P., and James, N. P. (eds.), *Carbonate Sedimentation and Diagenesis in the Evolving Precambrian World*. Tulsa, OK: Society of Economic Paleontologists and Mineralogists. SEPM special publication, 67, pp. 179–205.
- Turner, E. C., James, N. P., and Narbonne, G. M., 2000b. Taphonomic control on microstructure in early Neoproterozoic reefal stromatolites and thrombolites. *Palaaios*, **15**, 87–111.
- Visscher, P. T., Reid, R. P., and Bebout, B. M., 2000. Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites. *Geology*, **28**, 919–922.
- Walcott, C. D., 1914. Cambrian geology and paleontology III: Precambrian Algonkian algal flora. *Smithsonian Miscellaneous Collection*, **64**, 77–156.
- Walter, M. R., 1972. Stromatolites and the biostratigraphy of the Australian Precambrian and Cambrian. *Special Papers in Palaeontology*, **11**, 190 pp, 33 pls.
- Walter, M. R., and Heys, G. R., 1985. Links between the rise of the Metazoa and the decline of stromatolites. *Precambrian Research*, **29**, 149–174.
- Walter, M. R., Veevers, J. J., Calver, C. R., Gorjan, P., and Hill, A. C., 2000. Dating the 840–544 Ma Neoproterozoic interval by isotopes of strontium, carbon, and sulfur in seawater, and some interpretative models. *Precambrian Research*, **100**, 371–433.
- Webb, G. E., 1987. Late Mississippian thrombolite bioherms from the Pitkin Formation of northern Arkansas. *Geological Society of America, Bulletin*, **99**, 686–698.
- Webb, G. E., 2005. Quantitative analysis and paleoecology of earliest Mississippian microbial reefs, lowermost Gudman formation, Queensland, Australia: not just post-disaster phenomena. *Journal of Sedimentary Research*, **75**, 875–894.
- Whittle, G. L., Kendall, C. G. St. C., Dill, R. F., and Rouch, L., 1993. Carbonate cement fabrics displayed: a traverse across the margin of the Bahama Platform near Lee Stocking Island in the Exuma Cays. *Marine Geology*, **110**, 213–243.

Cross-references

Biofilms
 Calcified Cyanobacteria
 Cap Carbonates
 Microbial Biomineralization
 Microbial Communities, Structure, and Function
 Microbial Mats
 Microbialites, Modern
 Organomineralization
 Snowball Earth

MICROBIAL-METAL BINDING

Kurt O. Konhauser, David A. Fowle
 University of Alberta, Edmonton, Alberta, Canada

Definition

The accumulation of metal cations to microbes, largely through adsorptive processes to the outer cellular surfaces.

Overview

All bacteria have low isoelectric points (below pH 2 in most cases), and consequently, they interact with soluble metal cations and have them intimately associated with their surfaces (Harden and Harris, 1953). Considering their ubiquity in the near-surface environment and their characteristically large surface area-to-volume ratios, bacteria can have a significant influence on metal mobility and speciation in these settings.

Some bound metals (e.g., Ca and Mg) serve the purpose of stabilizing the negative charges of the anionic functional groups, and thus are relatively “fixed” into place, while other metals are much more exchangeable and provide a temporary positive charge to counter the negative charge induced by the deprotonation of the cell’s surface functional groups (Carstensen and Marquis, 1968). The strength of the metal–microbial bond is quantified by the surface complexation/binding constant (K_M), where M refers to the specific metal of interest. The greater its surface complex formation constant, the less likely

(e) association of SFF with or occurrence within SFF of other morphological bioforms such as fossil *Gallionella* or *Metallogenium*-like structures; (f) presence of vertically oriented elements (may resemble stalactites) resulting from mineral encrustation of filaments, filament strands, or mats while they are still flexible; and (g) inferred environmental conditions compatible with microbial growth, such as an abundant energy source and temperatures between ambient and $<120^{\circ}\text{C}$.

Types of organisms/substrates involved: The micro-morphology of SFF indicates that only filamentous micro-organisms are involved in their formation. Filamentous substrates of microbiological origin may include archaea, bacteria, and fungi. Some fabrics formed by encrustation of mineral fibers (these may be dissolved later) may show similar morphologies, but can usually be discriminated by evidence for characteristics typical of mineral fibers: Radial growth, variable fiber thickness, straight development, brittle behavior, and remnants of mineral fibers.

Conclusions

SFF represent a common, but often overlooked, type of biosignature from subsurface environments. SFF often display macroscopically visible fabrics that are easily recognizable and may be regarded as subsurface microbialites and the most conspicuous expression of the deep biosphere, representing in some ways an analog to stromatolites from surface environments. The very common occurrence in a wide variety of different rock types demonstrates that SFF are a common expression of subsurface life, wherever filamentous forms generate a large amount of biomass in relatively short time, allowing mineralization before organic filaments are destroyed. Organisms involved were chemolithotrophs (based on oxidation of Fe, S), but may also include heterotrophs living on chemolithotrophic primary producers. The formation in subsurface environments implies that no photosynthesizing organisms are involved. SFF-like features represent a target in the search for past life on Mars (Hofmann, 2007), as they might potentially be found in nearly all rock types.

Bibliography

- Baele, J. M., 1998. Reliques silicifiées et minéralisées de paléokarsts post-varisques sur le Dévonien en Belgique méridionale (Entre-Sambre-et-Meuse). *Annales de la Société Géologique du Nord*, **6**(2), 127–133.
- Bowerbank, J. S., 1842. On the spongy origin of moss agates and other siliceous bodies. *AMNH*, **10**, 9–18, 84–91.
- Daubenton, L. J. M., 1782. Sur les causes qui produisent trois sortes d'herborisations dans les pierres. *Mémoires de l'Académie Royale des Sciences*, 667–673.
- Feldmann, M., Neher, J., Jung, W., and Graf, F., 1997. Fungal quartz weathering and iron crystallite formation in an Alpine environment, Piz Alv, Switzerland. *Eclogae Geologicae Helvetiae*, **90**, 541–556.
- Hill, C., and Forti, P., 1997. *Cave Minerals of the World*. Huntsville: National Speleological Society.
- Hofmann, B., 1989. Genese, Alteration und rezentess Fließ-System der Uranlagerstätte Krunkelbach (Menzenschwand, Südschwarzwald). *Nagra Technical Report* 88–30, Nagra, Baden.
- Hofmann, B. A., 2007. Morphological biosignatures from subsurface environments: recognition on planetary missions. *Space Science Reviews*, DOI 10.1007/s11214-007-9147-9.
- Hofmann, B. A., and Farmer, J. D., 2000. Filamentous fabrics in low-temperature mineral assemblages: are they fossil biomarkers? implications for the search for a subsurface fossil record on the early Earth and Mars. *Planetary and Space Science*, **48**, 1077–1086.
- Hofmann, B. A., Farmer, J. D., von Blanckenburg, F., and Fallick, A. E., 2008. Subsurface filamentous fabrics: an evaluation of possible modes of origins based on morphological and geochemical criteria, with implications for exoplaeontology. *Astrobiology*, **8**, 87–117.
- Kretzschmar, M., 1982. Fossile Pilze in Eisen-Stromatolithen von Warstein (Rheinisches Schiefergebirge). *Facies*, **7**, 237–260.
- Razumovsky, G., 1835. Les agates mousseuses. *Bulletin de la société géologique de France*, **6**, 165–168.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie*. Göttingen: Universitätsdruckerei Göttingen.
- Trewin, N. H., and Knoll, A. H., 1999. Preservation of Devonian chemotrophic filamentous bacteria in calcite veins. *Palaos*, **14**, 288–294.
- Weitschat, W., 1986. Phosphatisierte Ammonoideen aus der Mittleren Trias von Central-Spitzbergen. *Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg*, **61**, 249–279.

Cross-references

[Biofilms and Fossilization](#)
[Biosignatures in Rocks](#)
[Chemolithotrophy](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Gallionella](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Karst Ecosystems](#)
[Microbialites, Stromatolites, and Thrombolites](#)

SULFATE-REDUCING BACTERIA

Heribert Cypionka
 University of Oldenburg, Oldenburg, Germany

Synonyms

Dissimilatory sulfate reducers; Sulfate-reducing prokaryotes

Definition

Sulfate reducers are prokaryotic microorganisms (Archaea or Bacteria) that use sulfate as electron acceptor of an anaerobic respiration process.

Introduction

Sulfur is one of the essential elements for life. It makes about 1% of the dry mass of living organisms and is present in two amino acids, iron sulfur clusters, and some coenzymes. Several organisms can use sulfate as sulfur source and carry out assimilatory sulfate reduction, which yields sulfide groups of amino acids. In contrast, dissimilatory sulfate reducers release large amounts of hydrogen sulfide (H_2S and HS^-) as product of their energy metabolism and cause the characteristic smell of rotten eggs. The process is carried out by a heterogeneous group of bacteria and archaea that occur in anoxic environments with temperatures up to 105°C (Widdel, 1988; Castro et al., 2000). Due to the high sulfate concentration of seawater (28 mM), sulfate reduction is especially important in marine sediments but does also occur in many other anoxic environments, e.g., lake sediments, animal guts, etc.

Evolution

The capacity of dissimilatory sulfate reduction appears to be very ancient. Its antiquity can be derived from phylogenetic analyses as well as from the geologic record (Canfield and Raiswell, 1999; Shen and Buick, 2004). Near the root of the phylogenetic tree are numerous Archaea or Bacteria metabolizing sulfur species including prokaryotes living from dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, and anoxygenic photosynthesis. As in a mixture of different isotopes of the same element lighter species are (mass-dependent) preferably metabolized by enzymes, isotope analysis of sediments can give strong evidence for their biogenic origin. The sulfur isotope distribution (^{32}S , ^{34}S , and ^{36}S) in microscopic pyrites in the ~ 3.47 billion years (Ga) old North Pole barite deposit of northwestern Australia provides the oldest evidence of microbial sulfate reduction and the earliest indication of a specific microbial metabolism. Furthermore, the observation of non-mass-dependent sulfur isotope ratios in sedimentary rocks more than 2.45 Ga old and the disappearance of this signal in younger sediments is taken as one of the strongest lines of evidence for the transition from an anoxic to an oxic atmosphere around 2.45 Ga ago (Farquhar et al., 2007).

Phylogeny

More than 120 species of sulfate-reducing prokaryotes (SRP), belonging to four bacterial and two archaeal phyla are known today (Thauer et al., 2007). The largest group are (1) mesophilic delta-proteobacteria (e.g., *Desulfovibrio*, *Desulfobacter*, *Desulfobulbus*), the other groups are (2) thermophilic Gram-negative bacteria (*Thermodesulfovibrio*), (3) *Desulfobium*, a recently established thermophilic genus not closely related to other bacteria, (4) Gram-positive bacteria with the spore-forming genus *Desulfotomaculum*, (5) Euryarcheota with the hyperthermophile *Archaeoglobus*, and (6) Crenarcheota with the hyperthermophile *Caldivirga* (Itoh et al., 1999).

Physiological groups

The most important substrates utilized by SRP are fermentation products as H_2 , alcohols, and organic acids like acetate, propionate, and butyrate. While most sulfate reducers have a limited spectrum of substrates that support growth, new isolates have been obtained that metabolize a wide variety of compounds including hexadecane, toluene, and several types of substituted aromatics (Hansen, 1994). Physiologically, sulfate reducers can be separated in two groups: Representatives of the first group generally grow fast and oxidize organic substrates incompletely to acetate, while members of the second group, grow more slowly, but oxidize their substrates completely to CO_2 . An important electron donor oxidized with sulfate as terminal electron acceptor is methane. However, so far, it has not been clarified whether sulfate reducers can oxidize methane directly or if they receive reducing equivalents in another form from an archaeal partner.

Pathway of sulfate reduction

Sulfate reduction is a rather complicated process (Figure 1). First, the double-charged anion has to be taken up by specific transport systems. Then ATP is hydrolyzed to activate sulfate to adenosine phosphosulfate (APS). This is now reduced to (bi-)sulfite, which is reduced to sulfide by sulfite reductase. APS reductase and sulfite reductase are the key enzymes. The genes coding for them (*apr* and *dsr*) are often used in molecular studies to analyze phylogeny or community structures of sulfate-reducing SRP.

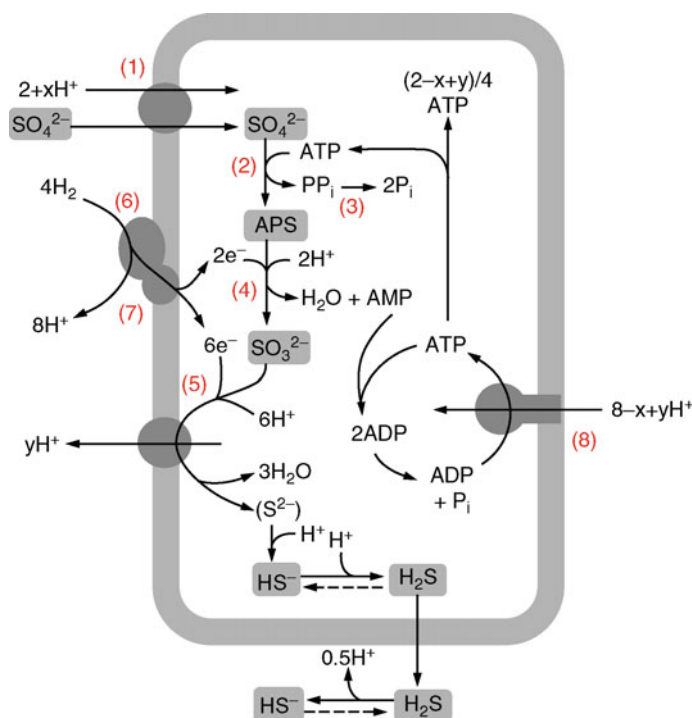
Reduction of sulfate with H_2 has a ΔG_0 of -151 kJ/mol of sulfate, which is about 1/6 of the value possible with oxygen as electron acceptor. Accordingly, there is not more than 1 ATP conserved per sulfate reduced.

Alternative electron acceptors

Most sulfate reducers can utilize other sulfur compounds than sulfate, often even with a better growth yield. Sulfite is an intermediate of sulfate reduction, but does not require an energy-dependent activation. Thiosulfate can be transformed to sulfide and sulfite in a single-step catalyzed thiosulfate reductase. Some sulfate reducers can also reduce elemental sulfur. Several sulfate reducers can utilize partially reduced compounds in the absence of an electron donor: Thiosulfate, sulfite, and elemental sulfur can be disproportionated to sulfate and sulfide, and the resulting (low amount of) free energy can be used for energy conservation (Bak and Cypionka, 1987).

Furthermore, some SRP can reduce nitrate or nitrite to ammonia, some can reduce metal ions as Fe^{3+} , Mn^{4+} or even U^{6+} (Lovley, 1993), some can reduce CO_2 to acetate (homoacetogenesis), and many can grow by fermentation of simple organic molecules like pyruvate.

Several SRB have been found to reduce molecular oxygen at high rates. The process is coupled to ATP conservation. However, it appears to be a defense mechanism. Sustainable growth of sulfate reducers with O_2 as electron acceptor has never been observed (Cypionka, 2000).



Sulfate-Reducing Bacteria, Figure 1 Steps of dissimilatory sulfate reduction and chemiosmotic energy conservation with H_2 as electron donor. (1) Uptake by symport with protons, (2) ATP sulfurylase, (3) pyrophosphatase, (4) APS reductase, (5) sulfite reductase, (6) hydrogenase, (7) cytochrome c, (8) ATP synthase (Cypionka, 2006).

Summary

Sulfate-reducing prokaryotes reduce sulfate to sulfide in an anaerobic respiration process. Sulfate reduction is ancient and found in Bacteria and Archaea. The main electron donors used by sulfate reducers are fermentation products. Sulfate reduction yields little energy and proceeds via several steps. Sulfate reducers are anaerobes, although some of them are able to reduce oxygen not coupled to growth. Alternatively, other sulfur compounds, nitrate or nitrite, and some oxidized metal ions can be used by some strains.

Bibliography

- Bak, F., and Cypionka, H., 1987. A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. *Nature*, **326**, 891–892.
- Canfield, D. E., and Raiswell, R., 1999. The Evolution of the Sulfur Cycle. *American Journal of Science*, **299**, 697–723.
- Castro, H. F., Williams, N. H., and Ogram, A., 2000. Phylogeny of sulfate-reducing bacteria. *FEMS Microbiology Ecology*, **31**, 1–9.
- Cypionka, H., 2000. Oxygen respiration by *Desulfovibrio* species. *Annual Review of Microbiology*, **54**, 827–848.
- Cypionka, H., 2006. *Grundlagen der Microbiologie*. Heidelberg: Springer-Verlag.
- Farquhar, J., Peters, M., Johnston, D. T., Strauss, H., Masterson, A., Wiechert, U., and Kaufman, A. J., 2007. Isotopic evidence for Mesoarchaeal anoxia and changing atmospheric sulphur chemistry. *Nature*, **449**, 706–710.
- Hansen, T. A., 1994. Metabolism of sulfate-reducing prokaryotes. *Antonie van Leeuwenhoek*, **66**, 165–185.

- Itoh, T., Suzuki, K., Sanchez, P. C., and Nakase, T., 1999. *Caldivirga maquilensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *International Journal of Systematic Bacteriology*, **49**, 1157–1163.
- Lovley, D. R., 1993. Dissimilatory metal reduction. *Annual Review of Microbiology*, **47**, 263–290.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth-Science Reviews*, **64**, 243–272.
- Thauer, R. K., Stackebrandt, E., and Hamilton, W. A., 2007. Energy metabolism and phylogenetic diversity of sulphate-reducing bacteria. In Barton, L. L., and Hamilton, W. A. (eds.), *Sulphate-Reducing Bacteria*. Environmental and Engineered Systems. Cambridge: Cambridge University Press, pp. 1–37.
- Widdel, F., 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In Zehnder, A. J. B. (ed.), *Biology of Anaerobic Microorganisms*. New York: John Wiley & Sons, pp. 469–585.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Deep Biosphere of Sediments](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Sulfur Cycle](#)
[Sulfur Isotopes](#)

SULFIDE MINERAL OXIDATION

D. Kirk Nordstrom

U.S. Geological Survey, Boulder, CO, USA

Synonyms

Sulfide-mineral weathering; Sulfide-ore oxidation

Definition

Sulfide mineral. A metal-sulfide compound, such as pyrite (FeS_2), which forms at high temperature ($>50^\circ\text{C}$) in well-crystallized veins or masses and at low temperatures ($<50^\circ\text{C}$) in poorly crystalline and fine-grained particles.

Oxidation. The chemical process of reacting with oxygen. More generally, the chemical process of removing electrons from an atom or group of atoms.

Introduction

Metal-sulfide minerals are valuable as ores for metals that have a wide variety of uses from jewelry to components in vehicles and electronic equipment. They are found primarily in hydrothermal mineral deposits that occur in numerous geologic environments. The most common sulfide mineral is pyrite; other important sulfide ore minerals include chalcopyrite (copper ore), molybdenite (molybdenum ore), sphalerite (zinc ore), galena (lead ore), and cinnabar (mercury ore). When these minerals are exposed to weathering at the Earth's surface, either through natural processes or as a result of mining activities, they react with oxygen in air in the presence of water to form drainage that is frequently acidic and detrimental to the environment because of the high metal concentrations. The general term for this water is acid-rock drainage. When it originates from mining activities, it is called acid-mine drainage (Nordstrom and Alpers, 1999). Acid-rock drainage is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. Extensive research has been employed to understand these processes, so that mine-site remediation can be more cost-effective and long-lasting. Weathering of sulfide minerals leads to natural enrichment in copper, gold, and silver in a process known as supergene enrichment. Copper dissolves in the infiltrating water and precipitates when it reaches unweathered sulfides downgradient, enriching the original sulfides. Gold and silver are highly insoluble and are enriched in the residual iron oxide cap or gossan that forms above the unweathered ore deposit. Sulfide-mineral oxidation from both mining and natural weathering is a major source of both dissolved sulfate and dissolved metals in natural waters.

Metal extraction of primary ores and of waste piles from previous mining activities is also accomplished by hydrometallurgical techniques that have optimized sulfide-mineral oxidation by increasing the temperature of leaching, adding catalytic reagents, and bioleaching by

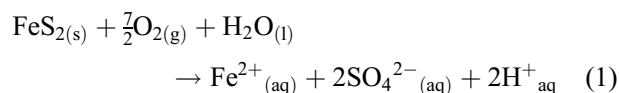
adding microorganisms that are specially suited for increasing the rate of sulfide-mineral oxidation.

Chemolithotrophy

Acid-mine drainage has been known since the early days of mining by ancient civilizations and was documented by early Greek and Roman writers (Nordstrom, 2009). Oxidation of sulfide minerals by microorganisms, however, has a more recent history. Winogradsky (1888) recognized that microbes could actually derive metabolic energy from the oxidation of inorganic compounds. These microbes gain energy from the oxidation (or reduction) of inorganic compounds and acquire their carbon needs from the carbon dioxide in the air (autotrophy). Hence, many of them are chemolithoautotrophs or chemoautotrophs for short. During the first quarter of the twentieth century, it was suspected that microbes affected the oxidation of sulfide minerals, but it was not until Rudolfs and Helbronner (1922) demonstrated that microbes could oxidize zinc sulfide, and Colmer and Hinkle (1947) isolated *Thiobacillus ferrooxidans*, now *Acidithiobacillus ferrooxidans*, from acid-mine drainage that microbes were recognized to be important catalysts in the production of acid-mine drainage from sulfide-mineral oxidation.

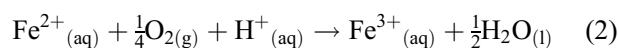
Biogeochemistry

The overall reaction of a metal sulfide like pyrite with air and water can be represented by the equation:

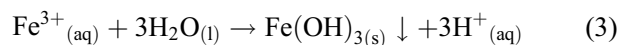


Pyrite + oxygen + water $\rightarrow$ acid ferrous sulfate solution.

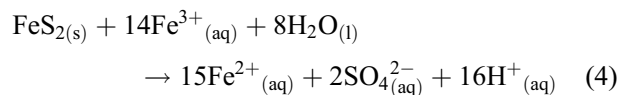
The acidity originates from the protons needed to balance out some of the loss of electrons from iron and sulfur oxidation. The dissolved ferrous iron further oxidizes to ferric iron:



Dissolved ferric iron easily hydrolyzes and precipitates as a colloidal phase, usually a mixture of fine-grained minerals, represented by either ferric hydroxide or commonly called hydrous ferric oxide (HFO):



Dissolved ferric iron is the oxidant that directly attacks the pyrite surface so that the more correct way to write the reaction would be:



Because dissolved ferrous iron oxidizes (Equation 2) much more slowly than the oxidation of pyrite by dissolved ferric iron (Equation 4), the oxidation of pyrite

would become negligible were it not for chemolithoautotrophic microorganisms. Many types of bacteria and archaea have been found to gain energy from the oxidation of ferrous iron and reduced sulfur in acidic environments (Norris, 1990; Nordstrom and Southam, 1997; Bond et al., 2000; Ehrlich, 2002). Some bacteria, such as *Acidithiobacillus ferrooxidans*, are capable of oxidizing both ferrous iron and reduced sulfur compounds (hydrogen sulfide, elemental sulfur, thiosulfate, and tetrathionate). Other bacteria, such as *Leptospirillum ferrooxidans*, are only able to oxidize ferrous iron and some bacteria, such as *Acidithiobacillus thiooxidans*, are only capable of oxidizing reduced sulfur compounds. Iron-oxidizing archaea include *Acidianus brierleyi*, *Sulfolobus acidocaldarius* (Nordstrom and Southam, 1997), *Ferroplasma acidiphilum* (Golyshina et al., 2000), and *Ferroplasma acidarmanus* (Edwards et al., 2000). Although *Acidithiobacillus ferrooxidans* has been the subject of more studies, *Leptospirillum ferrooxidans* seems to be the more common species in acid-mine drainage (Rawlings et al., 1999; Bond et al., 2000).

The geochemistry of sulfide-mineral oxidation and its effect on surface-water quality can vary substantially depending on the composition and grain size of the sulfide minerals. Common sulfide minerals are usually either disulfides like pyrite (FeS_2) and molybdenite (MoS_2) or monosulfides like sphalerite (ZnS) and galena (PbS). Molybdenite is hydrophobic and rather insoluble so that it oxidizes and weathers very slowly whereas sphalerite is more soluble and readily reacts with acid water to produce

hydrogen-sulfide gas. The grain size of these minerals can greatly affect their oxidation and dissolution rate. The smaller the grain size, the faster they react. Hence, fine-grained crystals of pyrite can react more quickly than large crystals of sphalerite even though sphalerite would dissolve more quickly if it were the same grain size as pyrite. Marcasite has the same composition as pyrite but it reacts more rapidly because it has a different crystal structure that is more amenable to oxidative weathering.

As acid-mine drainage mixes with surface waters, the dissolved ferrous iron oxidizes and precipitates to hydrous ferric oxide (HFO) which is an excellent sorbent for trace metals. Consequently, some of the dissolved copper, lead, zinc, arsenic, and other metals will be sorbed onto colloidal particles of HFO and decrease the potential toxicity, one of several processes known as natural attenuation. This process is enhanced by the growth of masses of bacteria and algae which also sorb some of the metals, removing them from solution and decreasing the concentration transported further downstream.

Environmental consequences of sulfide mineral oxidation

Acid-mine drainage from sulfide-mineral oxidation usually discharges into streams, rivers, and lakes (Figure 1). The pH values of acid-mine drainage range from 1 to 4 although values as low as -3.5 have been measured (Nordstrom et al., 2000). The high concentrations of metals such as copper, chromium, zinc, lead, cadmium,



Sulfide Mineral Oxidation, Figure 1 Effluent from the Tinto and Santa Rosa mines flowing into the Villar River, Spain. (Photograph courtesy of Manuel Caraballo Monge, Barcelona, Spain.)

mercury, manganese, iron, and aluminum found in acid-mine drainage is too toxic for invertebrates, amphibians, fish, and other forms of aquatic or terrestrial life. Only bacteria, algae, and fungi seem to survive when the pH is about 3 or less and the concentration of metals is a few hundred parts per billion or higher. Occasionally cyanide is also released into surface waters from gold processing with harmful effects. Releases of acid-mine drainage have caused the loss of enormous quantities of fish as well as harming livestock, mammalian wildlife, and crops. Two of the largest reported spills destroyed temporarily the biological habitat of two separate river systems. One spill was from the Aznalcollar impoundment in southern Spain in April, 1998, discharging 6 million cubic meters of acid water and pyritic fines into the Guadiamar River and ruining thousands of hectares of farmland. The other spill occurred in January, 2000, releasing a hundred thousand cubic meters of cyanide and heavy metals from a 4 km impoundment at the Aurul gold extraction plant near Baia Mare in northwestern Romania. The cyanide-rich water moved down to the Lapus River, then to the Tisza River, and finally to the Danube River all the way to the Black Sea. Smaller spills from the same region occurred again over the next 2 months. Approximately, 1,200 t of fish were killed from this spill, bird life was affected, thousands of fishermen were out of work, and water supplies for several towns and rural communities were badly contaminated.

Treatment technology

Innumerable ideas have been suggested for alleviating the harmful effects of sulfide-mineral oxidation and acid-mine drainage production. These suggestions include passive wetlands treatment, bioreactors, electrochemical treatment, exchange resins, reverse osmosis, and the use of various biosolids. However, the most commonly used, safe, and cost-effective treatments today are lime–lime-stone neutralization and precipitation of the metal-rich sludge in settling ponds combined with water-management techniques. An important engineering aspect is the construction of containment structures for hazardous materials that can withstand intense rainstorms and floods and that will prevent releases into surface-water and groundwater supplies.

Conclusion

Sulfide-mineral oxidation is a complex hydrobiogeochemical process involving the oxidation of metal-sulfide minerals, catalyzed by numerous bacterial and archaeal microbial species, and producing acid-rock drainage. Mining activities enhance the rate of this process, causing the production of acidic metal-rich drainage that make receiving streams, lakes, and rivers unfit for general usage and injurious to aquatic and terrestrial biota. Major spills of mine wastes into receiving waters have severely damaged valuable aquatic resources,

farmland, community water supplies, and the economic stability of those who depend on farming and fishing. Much effort has gone into the remediation of old inactive mines and current mining activities to improve water quality for beneficial uses but the scale and complexity of the problem continues to challenge engineers and regulators.

Bibliography

- Bond, P. L., Druschel, G. K., and Banfield, J. F., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Colmer, A. R., and Hinkle, M. E., 1947. The role of microorganisms in acid mine drainage. *Science*, **106**, 253–256.
- Edwards, K. J., Bond, P. L., Gihring, T. M., and Banfield, J. F., 2000. An archaeal Fe-oxidizing extreme acidophile important in acid mine drainage. *Science*, **287**, 1796–1799.
- Ehrlich, H. L., 2002. *Geomicrobiology*, 4th edn. New York: Marcel Dekker.
- Golyshina, O. V., Pivovarova, T. A., Karavaiko, G. I., Kondrat'eva, T. F., Moore, R. B., Abraham, W. R., Lunsdorf, H., Timmis, K. N., Yakimov, M. M., and Golyshin, P. N., 2000. *Ferroplasma acidiphilum*, gen. nov., sp. Nov., an acidophilic, autotrophic, ferrous-Fe-oxidizing, cell-wall-lacking, mesophilic member of the Ferroplasmaceae fam., comprising a distinct lineage of the Archaea. *International Journal of Systematic and Evolutionary Biology*, **50**, 997–1006.
- Nordstrom, D. K., 2009. Acid rock drainage and climate change. *Journal of Geochemical Exploration*, **100**, 97–104.
- Nordstrom, D. K., and Alpers, C. N., 1999. Geochemistry of acid mine waters. In Plumlee, G. S., and Logsdon, M. J. (eds.), *The Environmental Geochemistry of Mineral Deposits. Part A. Processes, Methods and Health Issues*, Reviews in Economic Geology, Littleton, CO: Society of Economic Geology, Vol. 6A, pp. 133–160.
- Nordstrom, D. K., and Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals*, Reviews in Mineralogy 35. Washington, DC: Mineralogical Society of America, pp. 361–390.
- Nordstrom, D. K., Alpers, C. N., Ptacek, C. J., and Blowes, D. W., 2000. Negative pH and extremely acid waters from Iron Mountain, California. *Environmental Science and Technology*, **34**, 254–258.
- Norris, P. R., 1990. Acidophilic bacteria and their activity in sulfide mineral oxidation. In Ehrlich, H. L., and Brierley, C. L. (eds.), *Microbial Mineral Recovery*. New York: McGraw-Hill, pp. 3–27.
- Rawlings, D. E., Tributsch, H., and Hansford, G. S., 1999. Reasons why 'Leptospirillum'-like species rather than *Thiobacillus ferrooxidans* are the dominant Fe-oxidizing bacteria in many commercial processes for biooxidation of pyrite and related ores. *Microbiology*, **145**, 5–13.
- Rudolfs, A., and Helbronner, A., 1922. Oxidation of zinc sulfide by microorganisms. *Soil Science*, **14**, 459–464.
- Winogradsky, S. N., 1888. Über Eisenbakterien. *Botanische Zeitung*, **46**, 261–276.

Cross-references

[Acid Rock Drainage](#)
[Heavy Metals](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Pyrite Oxidation](#)

SULFUR CYCLE

Michael E. Böttcher

Leibniz Institute for Baltic Sea Research, Warnemünde, Germany

Synonyms

Biogeochemical sulfur cycle; Sulfate reduction and sulfide oxidation; Transformation of sulfur compounds

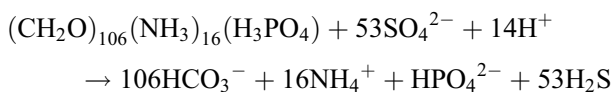
Definition

Sulfur. Chemical element that is one of the constituents of the Earth's compartments and living organisms. While the average sulfur content of the whole Earth is about 2%, the crust contains only about 0.07% sulfur (Brimblecombe, 2003).

Sulfur cycle. Biogeochemical system of biotic and abiotic transformations of inorganic and organic sulfur-bearing components, in and between, the lithosphere, hydrosphere, atmosphere, and biosphere. Sulfur initially enters the biogeochemical cycle via volcanic activity and continental erosion. Today, the modern sulfur cycle is influenced by human activity. The Earth sulfur reservoir is assumed to have been essentially constant through time (Garrels and Lerman, 1984).

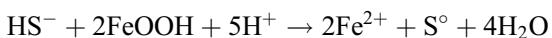
Assimilative sulfate reduction. Sulfate (SO_4^{2-}) is reduced to organic R-SH groups by plants, algae, and fungi.

Dissimilative sulfur and sulfate reduction. Hydrogen sulfide (H_2S) is produced from elemental sulfur (S^0) or dissolved sulfate by sulfur- and sulfate-reducing microorganisms, respectively. For sulfate reduction coupled to the oxidation of marine organic matter or methane, the overall reactions are:

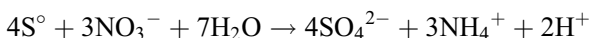


Desulfuration. Hydrogen sulfide is produced from sulfur-containing organic molecules.

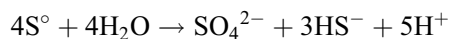
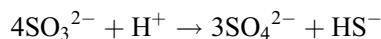
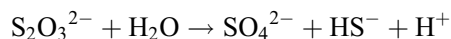
Oxidation of hydrogen sulfide. Production of sulfur intermediates, elemental sulfur, thiosulfate ($\text{S}_2\text{O}_3^{2-}$), sulfite (SO_3^{2-}), and/or sulfate by bacterial or abiotic oxidation (Jørgensen and Nelson, 2004). Possible reactions are:



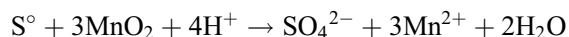
Oxidation of sulfur. Sulfate is generated by the oxidation of elemental sulfur. A possible reaction is (gradient bacterium *Thioploca*):



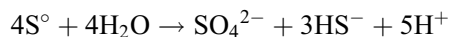
Disproportionation of sulfur intermediates. Sulfate and hydrogen sulfide are produced by the low-temperature bacterial metabolism of sulfur intermediates. The stoichiometries of the reactions are (Finster, 2008):



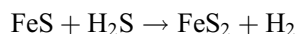
In the presence of oxy(hydroxi)des of iron(III) or manganese (IV) the following stoichiometries are obtained (Böttcher and Thamdrup, 2001; Finster, 2008):



Hydrolysis of elemental sulfur. Sulfate and hydrogen sulfide can be produced by the abiotic hydrolysis of elemental sulfur at high temperatures (Smith, 2000), according to:



Formation of iron disulfide (pyrite). The low-temperature formation of iron disulfide typically takes place via the polysulfide ($\text{S}^{\circ*}$) or hydrogen sulfide pathway by the reaction with the precursor iron monosulfide (Rickard and Luther III, 2007):



The degree of pyritization (DOP) indicates the relative amount of reactive sedimentary iron that has already been reacted to pyrite.

The global sulfur cycle

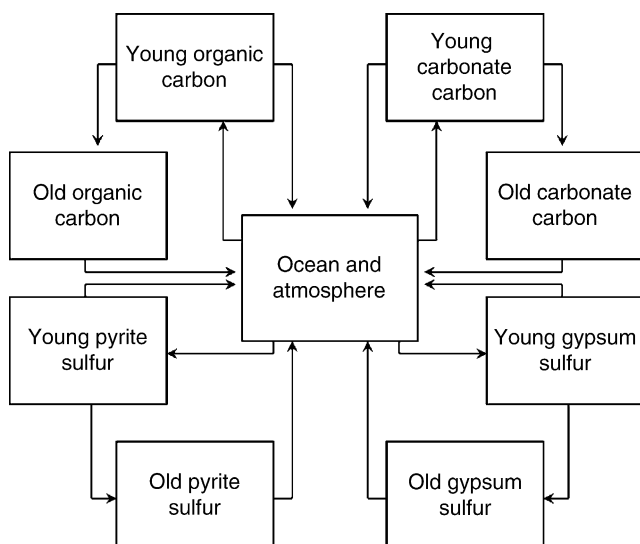
The global reduced and oxidized reservoirs of sulfur, carbon, oxygen, and selected metals are coupled on different scales via redox-driven chemical, biological, and geological processes (Figures 1–4). Parts of this cycling, as the reaction between sulfuric acid (that later became popular in industrialized times as “acid rain”) and carbonate rocks, were already described in the nineteenth century by Johann Wolfgang von Goethe (Figure 1) in his famous novel “Elective affinities” (German: “Wahlverwandschaften”; published in 1809).

On Earth, pure sulfur occurs in elemental form as for example in volcanic regions, and the quantitative use of sulfur-bearing compounds by man has been used as a proxy for economic wealth (Brimblecombe, 2003). Iron sulfide sulfur occurs in almost all types of rocks and sediments and is even found in extraterrestrial meteorites. In sediments that are not under the influence of hydrothermal activity, the occurrence of sulfide sulfur is indicative for the activity of sulfate-reducing bacteria

"... Thus what we call limestone is a more or less pure calcareous earth in combination with a delicate acid, which is familiar to us in the form of a gas. Now, if we place a piece of this stone in diluted sulphuric acid, this will place a possession of the lime, and appear with it in the form of gypsum, the gaseous acid at the same time going off in vapour. Here is a case of separation; a combination arises, and we believe ourselves now justified in applying to it the words 'Elective Affinity;' it really looks as if one relation had been deliberately chosen in preference to another...."

Johann Wolfgang von Goethe 'Elective Affinity'
(German: *Wahlverwandtschaften*)

Sulfur Cycle, Figure 1 Coupling of the sulfur and carbon cycles according to Johann Wolfgang von Goethe. (Translation from Niles, 1872.)



Sulfur Cycle, Figure 2 Mass balance model of coupling between the young and old global carbon and sulfur reservoirs. (Redrawn from Berner, 1987, as a modification of the model by Garrels and Lerman, 1984.)

(e.g., Jørgensen and Kasten, 2006). Whereas sulfur is assimilated by living organisms where it is bound to, for instance, amino acids, most of the organic sulfur found in sediments is derived from early diagenetic reaction of organic matter with reduced and intermediate sulfur species (Sinninghe Damsté and de Leeuw, 1990). The decomposition of organic matter in the presence of sulfate is catalyzed by microorganisms that gain energy from the dissimilatory reduction of sulfate (e.g., *Desulfovibrio* and *Desulfobacter*; Rabus et al., 2001). Microbial oxidation of sulfide (e.g., by green and purple sulfur bacteria; gradient bacteria as *Beggiatoa* or *Thioploca*; Jørgensen

and Nelson, 2004), disproportionation (e.g., *Desulfovibrio*, *Desulfocapsa*, Finster, 2008), or further oxidation of sulfur intermediates (e.g., *Thiobacillus*) can take place aerobically or anaerobically. In the aquatic low-temperature environment, dissolved and metal-bound sulfide may also be abiotically oxidized, e.g., by manganese dioxide (Jørgensen and Nelson, 2004). The lowest oxidation state of sulfur is $-II$ (e.g., in hydrogen sulfide). In its highest oxidation state $+VI$ (e.g., in sulfate), sulfur is bound to oxygen and found as (biogenic or abiotic) barite or evaporitic sulfate minerals (e.g., gypsum or anhydrite).

Sulfur in the Atmosphere

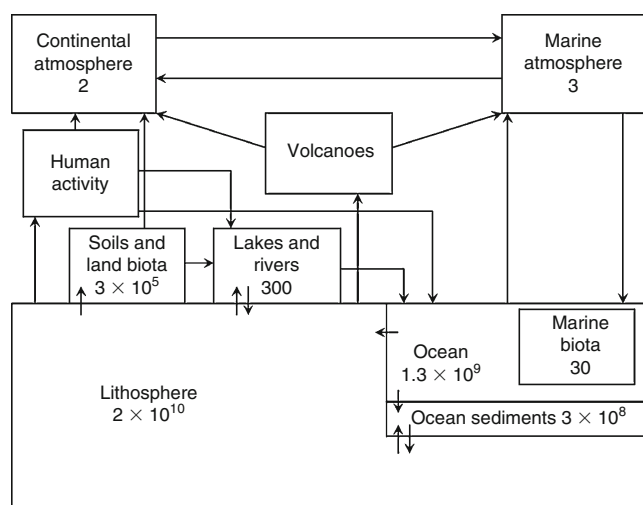
The first atmospheric sulfur components on Earth (mainly SO_2 and H_2S) were derived from volcanic activity and may have led to the first acid rain on Earth (Farquhar et al., 2000). Volcanoes still contribute to the atmospheric sulfur budget, besides emissions from the oceans (e.g., dimethyl sulfide (DMS), carbon disulfide), coastal and wetland areas. Most of the different components temporarily end as sulfate. The anthropogenic combustion of sulfur-bearing fossil fuels in the past century not only produced CO_2 , but also led to the formation of sulfuric acid ("acid rain"), which partly led to increased rock weathering that again linked the carbon and sulfur cycles (Figure 1). In the ongoing discussion of dealing with climate change, the observations from the eruption of Mount Pinatubo are taken as an event-type base for geo-engineering concepts to decrease the global surface temperature by the artificial release of sulfur components into the stratosphere (Crutzen, 2006).

Sulfur in surface and groundwaters, and freshwater sediments

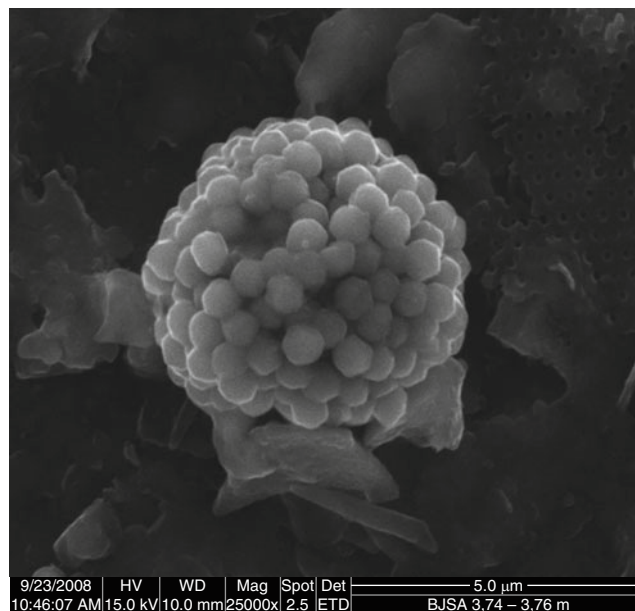
Rainwater sulfate, sulfur dioxide, and organic sulfur enter the water-unsaturated soil zone, where further

transformations take place. Geological contributions from soil and aquifer minerals vary strongly and may include gypsum dissolution and metal sulfide oxidation. Near-surface oxidation of pyrite from coal-mining activities leads today to the anthropogenically induced phenomenon of “acid mine drainage” (Holmer and Storkholm, 2001). Sulfur cycling in freshwater lake sediments associated with organic matter diagenesis is controlled by a number of factors, including temperature, light conditions, the availability of sulfate, organic matter, electron acceptors and nutrients, and macro faunal abundance (Holmer and Storkholm, 2001). Groundwater may play an important role in influencing sulfur cycling, for instance, via the

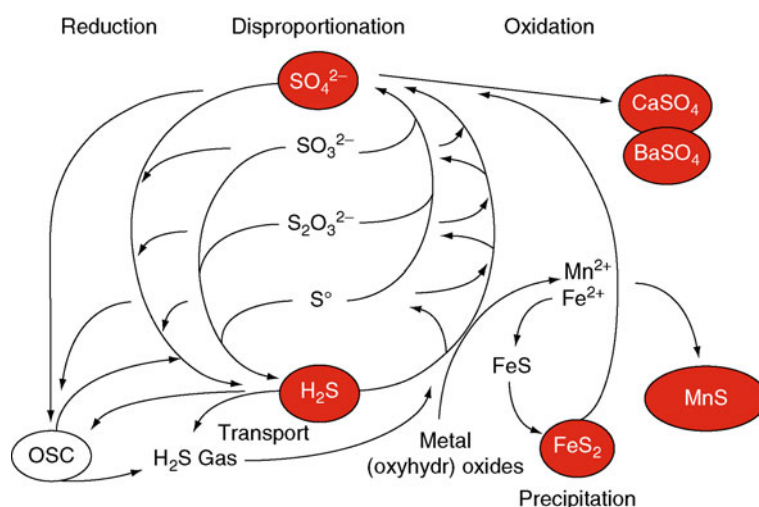
supply of evaporite-derived sulfate (Swiss Lago di Cadagno, as an example) and rock weathering (Böttcher, 1999). Sulfur cycling via organic sulfur compounds (Figure 4) plays an important role in freshwater sediments and soils (Mitchell et al., 1992; Holmer and Storkholm, 2001). Finally, sulfate may reach the ocean where further processing takes place (Figure 4).



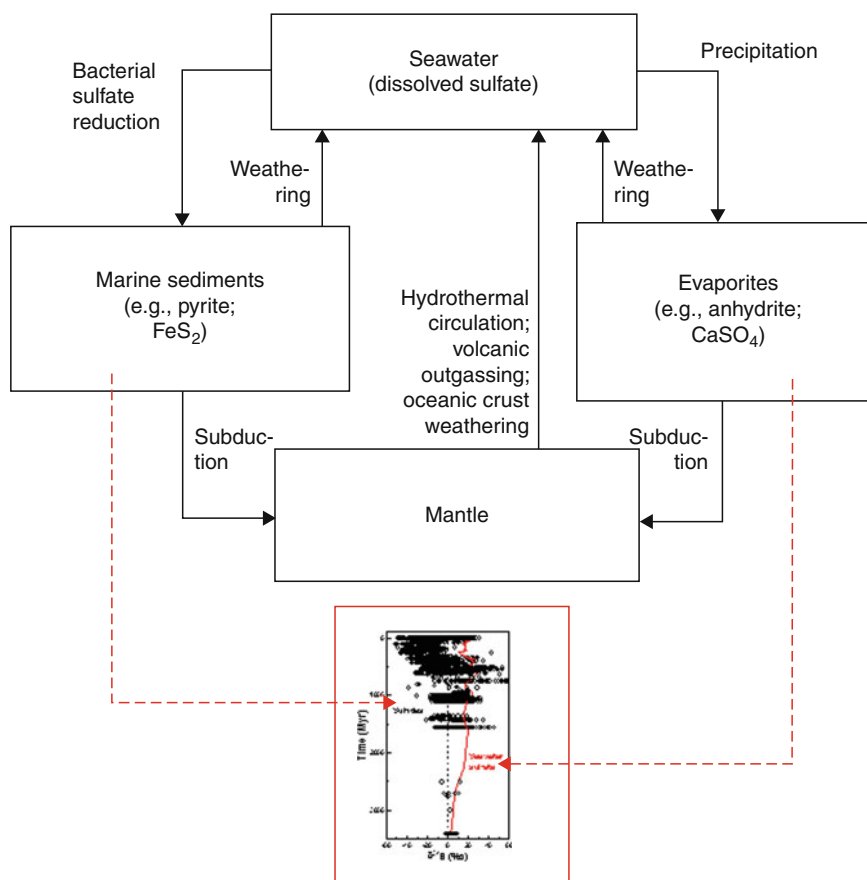
Sulfur Cycle, Figure 3 Major reservoirs and burdens of sulfur in Tg. (Modified after Brimblecombe, 2003.)



Sulfur Cycle, Figure 5 Framboidal iron disulfide (pyrite) as indicator for the activity of sulfate-reducing bacteria in sediments. (Example from marine intertidal North Sea sediments; Böttcher and Bahlo, unpublished scanning electron microscope picture, 2008.)



Sulfur Cycle, Figure 4 Simplified sedimentary sulfur cycle and its link to selected metals (neglecting, e.g., transformation of polysulfides, thionates, thiols, and methane). OSC: Organic sulfur compounds.



Sulfur Cycle, Figure 6 *Upper part:* Box model for the global sulfur cycle (after: Canfield, 2004). *Lower part:* Variation of the stable sulfur isotope composition of sedimentary sulfides and coexisting seawater sulfate (from evaporites and barite). (After data from Canfield and Teske, 1996, with additions by Böttcher, unpublished data, 1998–2000.)

Sulfur in seawater and marine sediments

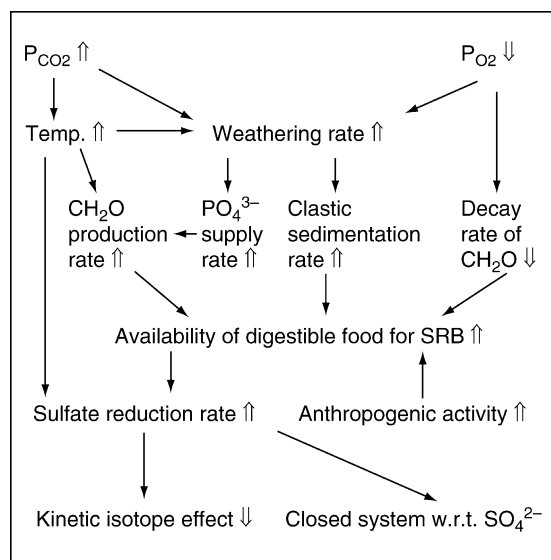
With a residence time of at least 8 million years, dissolved sulfate in seawater is well-mixed and reflects changes in the fluxes between and sizes of the major reservoirs (Figures 2 and 6). Whereas at mid-oceanic ridges, chemical sulfate reduction occurs in heated seawater that circulates through the Earth's crust, the low temperature dissimilatory sulfate reduction is linked to the enzymatic activity of sulfate-reducing bacteria which requires the abundance of metabolizable dissolved organic carbon compounds or methane (Jørgensen and Kasten, 2006). The anaerobic oxidation of methane associated with microbial reduction of sulfate is an important trap to prevent methane from escaping into the water column (Jørgensen and Kasten, 2006). In continental margin sediments, microbial sulfate reduction accounts for up to more than 50% of the organic matter oxidation (Skyring, 1987; Jørgensen and Kasten, 2006). Sulfate reduction may also take place in anoxic water columns (e.g., Black Sea). Most of the biogenic sulfide is reoxidized at the benthic or pelagic redoxclines by microbial or chemical processes (Jørgensen and Nelson, 2004) leading to the

metastable formation of intermediate sulfur species, or finally sulfate. The sulfur intermediates can be further oxidized, reduced, or disproportionated (Figure 4). Only a small portion of sulfide is finally buried as iron sulfides (essentially pyrite; Figure 5) or organic sulfur (Figures 2 and 4).

A number of external factors may directly or indirectly influence the rates of microbial sulfate reduction and sulfide burial efficiency, on different scales that can be reflected by the contents of sedimentary sulfur and the “reservoir” effect reflected in overall isotope discrimination (Figure 7).

Geochemical tracers for sulfur cycling: Stable sulfur and oxygen isotopes

Fractionation of stable sulfur (in dissolved and solid phase sulfur) and oxygen isotopes (in sulfate and sulfate-bearing minerals) takes place via biological and abiotic transformations and leads to isotope signatures that can be used to identify sources and biological, chemical, and physical processes involved (Canfield and Raiswell, 1999; Böttcher, 2010). Oxygen isotope fractionation can be used



Sulfur Cycle, Figure 7 Compilation of potential factors influencing availability of oxidizable organic matter available to sulfate-reducing bacteria and consequences for sulfate reduction rates and associated kinetic and reservoir size-induced sulfur isotope effect. (Modified after Ohmoto, 1992.)

to trace sulfate sources in aerobic environments (Clark and Fritz, 1997). The composition of dissolved sulfate and sedimentary diagenetic barite after modification by microbial sulfate, on the other hand, is finally governed by intracellular oxygen isotope exchange toward equilibration with surrounding water (Wortmann et al., 2007). The fossil sedimentary isotope record of pyrite (Figure 6) and anhydrite/barite/carbonate-bound sulfur, reflects the coupled changes in the atmospheric composition, the biogeochemistry of the sedimentary sulfur cycle and the fluxes between the different sulfur-bearing reservoirs (Figures 1–4 and 6) (e.g., Berner, 1987; Canfield and Teske, 1996; Canfield and Raiswell, 1999; Canfield, 2004).

Conclusion

The sulfur cycle is of general importance due to its involvement in the anaerobic mineralization of organic matter, and therefore, tight coupling to the carbon cycle. Its geochemical signature in sediments provides hints for identification of key processes associated with the evolution of life and the atmosphere on Earth. Some of the many open questions pertaining to the (bio)geochemistry and geomicrobiology of sulfur are (1) the importance of microbial versus chemical sulfide oxidation, (2) the quantitative importance of metastable intermediate and reduced sulfur species in the low-temperature sulfur cycle (Rickard and Morse, 2005; Finster, 2008), (3) the quantitative role of sulfur cycling for the deep biosphere (Jørgensen and D'Hondt, 2006), and (4) the role of metal

sulfides in extreme environments on the pre-biotic Earth for the later development of the precursors of life (Rickard and Luther III, 2007).

Bibliography

- Berner, R. A., 1987. Models for carbon and sulfur cycles and atmospheric oxygen: application to Paleozoic geologic history. *American Journal of Sciences*, **287**, 177.
- Böttcher, M. E., 1999. The stable isotope geochemistry of the sulfur and carbon cycles in a modern karst environment. *Isotopes in Environment and Health Studies*, **35**, 39.
- Böttcher, M. E., 2010. Isotopes (sulfur). In Reiter, J., and Thiel, V. (eds.), *Encyclopaedia of Geobiology*. Berlin: Springer, in press.
- Böttcher, M. E., and Thamdrup, B., 2001. Anaerobic oxidation and stable isotope fractionation associated with bacterial sulfur disproportionation in the presence of MnO₂. *Geochimica et Cosmochimica Acta*, **65**, 1573.
- Brimblecombe, P., 2003. The global sulfur cycle. In Schlesinger, W. (ed.), *Treatise on Geochemistry, Biogeochemistry*. Amsterdam: Elsevier, Vol. 8, pp. 645.
- Canfield, D. E., 2004. The evolution of the Earth surface sulfur cycle. *American Journal of Sciences*, **304**, 839.
- Canfield, D. E., and Raiswell, R., 1999. The evolution of the sulfur cycle. *American Journal of Science*, **290**, 697.
- Canfield, D. E., and Teske, A., 1996. Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulfur isotope studies. *Nature*, **328**, 127.
- Clark, I. D., and Fritz, P., 1997. *Environmental Isotopes in Hydrogeology*. Boca Raton: Lewis Publishers.
- Crutzen, P. J., 2006. Albedo enhancement by stratospheric sulfur injections: a contribution to resolve a policy dilemma? *Climate Change*, **77**, 211.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulphur cycle. *Science*, **4**, 756.
- Finster, K., 2008. Microbiological disproportionation of inorganic sulfur compounds. *Journal of Sulfur Chemistry*, **29**, 281.
- Garrels, R. M., and Lerman, A., 1984. Coupling of the sedimentary sulfur and carbon cycles – an improved model. *American Journal of Sciences*, **284**, 989.
- Holmer, M., and Storkholm, P., 2001. Sulfate reduction and sulphur cycling in lake sediments: a review. *Freshwater Biology*, **46**, 431.
- Jørgensen, B. B., and D'Hondt, S., 2006. A starving majority deep beneath the seafloor. *Science*, **314**, 932.
- Jørgensen, B. B., and Kasten, S., 2006. Sulfur cycling and methane oxidation. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*. Berlin: Springer, p. 271.
- Jørgensen, B. B., and Nelson, D. C., 2004. Sulfide oxidation in marine sediments: geochemistry meets microbiology. In Ahmend, J. P., Edwards, K. J., and Lyons, T. W. (eds.), *Sulfur Biogeochemistry – Past and Present*. Geological Society of America Special Paper, p. 379.
- Mitchell, M. J., David, M. B., and Harrison, R. B., 1992. Sulphur dynamics of forest ecosystems. In Howarth, R. W., Stewart, J. W. B., and Ivanov, M. V. (eds.), *Sulphur Cycling on the Continents: Wetlands, Terrestrial Ecosystems and Associated Water Bodies*. John Wiley & Sons, New York, p. 215.
- Niles, D. W., 1872. *Goethe's Elective Affinities (with an introduction by Victoria C. Woodhall)*. Boston: D. W. Niles, p. 41.
- Ohmoto, H., 1992. Biogeochemistry of sulfur and the mechanisms of sulfide-sulfate mineralization in Archean oceans. In Schidlowski, M., Golubic, S., Kimberley, M. M., McKirdy, D. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Mineral & Energy Resources*, Berlin: Springer, p. 378.

- Rabus, R., Hansen, T., and Widdel, F., 2001. Dissimilatory sulfate- and sulfur-reducing prokaryotes. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.) *The Prokaryotes: An evolving electronic resource for the microbiological community*. Heidelberg, Springer Science Online. Available from: www.prokaryotes.com.
- Rickard, D., and Luther III, G. W., 2007. Chemistry of iron sulfides. *Chemical Reviews*, **107**, 514.
- Rickard, D., and Morse, J. W., 2005. Acid volatile sulfide (AVS). *Marine Chemistry*, **97**, 141.
- Sinninghe Damsté, J. S., and de Leeuw, J. W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. *Organic Geochemistry*, **16**, 1077.
- Skyring, G. W., 1987. Sulfate reduction in coastal ecosystems. *Geomicrobiology Journal*, **5**, 295.
- Smith, J. W., 2000. Isotopic fractionations accompanying sulfur hydrolysis. *Geochemical Journal*, **34**, 95.
- Wortmann, U. G., Chernyavsky, B., Bernasconi, S. M., Brunner, B., Böttcher, M. E., and Swart, P. K., 2007. Oxygen isotope biogeochemistry of pore water sulfate in the deep biosphere: dominance of isotope exchange reactions with ambient water during microbial sulfate reduction (ODP Site 1130). *Geochimica et Cosmochimica Acta*, **71**, 4221.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Biogeochemical Cycles](#)
[Sulfur Isotopes](#)

SULFUR ISOTOPES

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Radioactive sulfur isotopes; Stable sulfur isotopes

Definition

Sulfur. A chemical element that is one of the constituents of living organisms and of the Earth.

Isotopes. Isotopes are different nuclear forms of the same element. For a given element, a constant number of protons but different numbers of neutrons in the nucleus correspond to different isotopes. Sulfur of standard atomic mass 32.065 u has 18 isotopes, most of which are not stable and undergo radioactive decay.

Stable sulfur isotopes. Stable isotopes are isotopes that do not decay within an experimentally observable time frame. Stable isotopes have essentially the same chemical characteristics and, therefore, their behavior is chemically almost identical. The different masses lead to isotope fractionation in chemical, physical, and especially biological reactions. Four stable isotopes occur in nature: ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S (4.21%), and ^{36}S (0.02%) (Hoefs, 2008). Typically, the ratio of the two most

abundant sulfur isotopes is measured and presented in the conventional δ notation as opposed to the Vienna-Canyon-Diablo-Troilite (V-CDT) standard ($\delta^{34}\text{S}$ values per mil versus the V-CDT standard). In aerobic surface environments, the sulfur isotopic composition of sulfate is often used for source identification (e.g., anthropogenic versus geogenic sulfate in rain, river, and ground waters (e.g., Clark and Fritz, 1997). Only recently has the fractionation of the minor isotopes ^{33}S and ^{36}S also been introduced into sulfur isotope biogeochemistry (e.g., Farquhar et al., 2003).

Radioactive sulfur isotopes. Sulfur has 14 unstable isotopes. Radioactive sulfur with mass 35 is formed in the atmosphere from cosmic ray spallation of argon with mass 40 and has a half-life of 87 days. Therefore, it is used in hydrological studies of biologically less active surface environments. ^{35}S -labeled sulfur compounds are used in experiments to determine microbial and chemical sulfur turnover, upon microbial dissimilatory sulfate reduction, for instance (e.g., Kallmeyer et al., 2004). The other radioactive sulfur isotopes are all short-lived.

Fractionation of stable sulfur isotopes

Sulfur isotopes are fractionated upon microbial and abiotic sulfur transformations and the isotope signal may be preserved in sedimentary sulfide- and sulfate-bearing solids, organic matter, and to a minor extent, elemental sulfur (e.g., Canfield, 2001; Amend et al., 2004). The majority of geochemical and microbial studies on sulfur isotopes have so far focused on the fractionation of ^{32}S to ^{34}S . The most important isotope discrimination step occurs during dissimilatory reduction of dissolved sulfate within the bacterial cell. The overall change in the valence state of sulfur by pure cultures leads to hydrogen sulfide that is enriched in the lighter isotope by up to about 50 per mil when compared to sulfate (Figure 1). The magnitude of isotope discrimination depends on conditions such as cellular sulfate reduction rate, substrate and sulfate concentrations, and the organisms (e.g., Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Bolliger et al., 2001; Canfield, 2001; Amend et al., 2004). A maximum fractionation upon sulfate reduction of up to about 70 per mil is obtained from pore water modeling in natural environments (e.g., Wortmann et al., 2001), which is close to the value predicted for thermodynamic equilibrium conditions. Further partial or complete oxidation of hydrogen sulfide is associated with only small isotope discrimination, but may lead to the formation of (metastable) sulfur intermediates. The bacterial disproportionation of these compounds (e.g., elemental sulfur, thiosulfate, sulfite) yields hydrogen sulfide that is further enriched in the lighter isotope compared with the original sulfide (Figure 1).

This may contribute to the magnitude of isotope partitioning found in shallow natural environments and the fossil record (Canfield, 2001).

shortly after the extreme glaciations during the late Neoproterozoic (Hoffman et al., 1998) and was nearly synchronous with the rapid evolutionary pulse that coincides with the Ediacaran and Cambrian radiations. Final assembly of Gondwanaland and a circum-Gondwanaland mountain chain is thought to have provided nutrients to the oceanic realm and also resulted in a large $^{87}\text{Sr}/^{86}\text{Sr}$ spike due to the erosion of the mountains (Squire et al., 2006). Therefore, links between the assembly of the Gondwanaland supercontinent and biological evolution are forwarded by numerous studies (Meert and Lieberman, 2008).

Bibliography

- Collins, A. S., and Pisarevsky, S. A., 2005. Amalgamating eastern Gondwana: the evolution of the circum-Indian orogens. *Earth Science Reviews*, **71**, 229–270.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Fitzsimons, I. C. W., 2000. A review of tectonic events in the East Antarctic Shield, and their implications for three separate collisional orogens. *Journal of African Earth Sciences*, **31**, 3–23.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside out? *Science*, **252**, 1409–1412.
- Hoffman, P. F., Kaufman, A. J., Halverson, G. P., and Schrag, D. P., 1998. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Kelsey, D. E., Wade, D. P., Collins, A. S., Hand, M., Sealing, C. R., and Netting, A., 2008. Discovery of a Neoproterozoic basin in the Prydz Belt in East Antarctica and its implications for Gondwana assembly and ultrahigh temperature metamorphism. *Precambrian Research*, **161**, 355–388.
- Meert, J. G., 2003. A synopsis of events related to the assembly of eastern Gondwana. *Tectonophysics*, **362**, 1–40.
- Meert, J. G., and Lieberman, B. S., 2008. The Neoproterozoic assembly of Gondwana and its relationship to the Ediacaran–Cambrian radiation. *Gondwana Research*, in press.
- Meert, J. G., and Torsvik, T. H., 2003. The making and unmaking of a supercontinent: Rodinia revisited. *Tectonophysics*, **375**, 261–288.
- Meert, J. G., Van der Voo, R., and Ayub, S., 1995. Paleomagnetic investigation of the neoproterozoic Gagwe lavas and Mbozi Complex, Tanzania and the assembly of Gondwana. *Precambrian Research*, **74**, 225–244.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Squire, R. J., Campbell, I. H., Allen, C. M., and Wilson, C. J. L., 2006. Did the Transgondwanan supermountain trigger the explosive radiation of animals on Earth? *Earth and Planetary Science Letters*, **250**, 116–133.
- Stern, R. J., 1994. Arc assembly and continental collision in the Neoproterozoic East Africa Orogen: implications for the consolidation of Gondwanaland. *Annual Reviews of Earth and Planetary Science*, **22**, 319–351.
- Yoshida, M., 2007. Geochronological data evaluation: implications for the Proterozoic tectonics of East Gondwana. *Gondwana Research*, **12**, 228–241.

Cross-references

[Breakup of Rodinia](#)
[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Origins of the Metazoa](#)
[Snowball Earth](#)
[Trace Fossils: Neoproterozoic](#)

GREAT OXYGENATION EVENT (GOE)

The term “Great Oxygenation Event” (GOE, also known as “Great Oxidation Event,” “oxygen catastrophe,” or “oxygen crisis”) describes a critical environmental change in Earth history that resulted from the appearance of diatomic oxygen (O_2), a waste product of oxygenic photosynthesis, in the atmosphere. The GOE occurred around 2.4 billion years (Ga) before present, close to the boundary between the Archean and the Proterozoic (2.5 Ga). For details, please refer to “[Critical Intervals in Earth History](#).”

GREEN ALGAE

Green algae are photosynthetic eukaryotes with simple plastids (derived from primary endosymbiosis) that are mostly microscopic and rarely more than a meter in greatest dimension. The approximate 6,000 extant species show an enormous diversity of growth habit and fine details of their cellular architecture. Green algae occur as unicellular or colonial, microscopic or macroscopic, motile planktonic, as well as benthic attached forms. Some (e.g., Dasycladales) are important carbonate producers in aquatic environments, others live in symbiotic associations with fungi to form lichens in terrestrial settings. Green algae living as endosymbionts inside heterotrophic organisms are a common phenomenon. The order Charales, in which full tissue differentiation occurs, is considered to include the closest relative of higher plants. For details, please refer to entry “[Algae \(Eukaryotic\)](#).”

GUILD

Metabolically related populations, e.g., sulfate-reducing microorganisms. See entry “[Microbial Communities, Structure, and Function](#)” for further reading.

- Sass, H., and Cypionka, H., 2004. Isolation of sulfate-reducing bacteria from the terrestrial deep subsurface and description of *Desulfovibrio cavernae* sp. nov. *Systematic and Applied Microbiology*, **27**, 541–548.
- Seiler, K.-P., and Lindner, W., 1995. Near-surface and deep groundwaters. *Journal of Hydrology*, **165**, 33–44.
- Shimizu, S., Akiyama, M., Naganuma, T., Fujioka, M., Nako, M., and Ishijima, Y., 2007. Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. *Geobiology*, **5**, 423–433.
- Simpkins, W. W., and Bradbury, K. R., 1992. Groundwater flow, velocity, and age in a thick, fine-grained till unit in southeastern Wisconsin. *Journal of Hydrogeology*, **132**, 283–319.
- Stevens, T. O., 2002. The deep subsurface biosphere. In Staley, J. T., and Reysenbach, A.-L. (eds.), *Biodiversity of Microbial Life. Foundation of the Earth's Biosphere*. New York: Wiley, 552 p.
- Stevens, T. O., 1997a. Subsurface microbiology and the evolution of the biosphere. In Amy, P., and Haldeman, D., (eds.), *The Microbiology of the Terrestrial Subsurface*. Boca Raton: CRC Press, pp. 203–221.
- Stevens, T. O., 1997b. Lithoautotrophy in the subsurface. *FEMS Microbiology Reviews*, **20**, 327–337.
- Suzina, N. E., Mulyukin, A. L., Kozlova, A. N., Shorokova, A. P., Dmitriev, V. V., Barinova, E. S., Mokhova, O. N., El'-Registan, G. I., and Duda, V. I., 2004. Ultrastructure of resting cells of some non-spore-forming bacteria. *Microbiology*, **73**, 516–529.
- Szewzyk, U., Szewzyk, R., and Stenström, T.-A., 1994. Thermophilic, anaerobic bacteria isolated from a deep borehole in granite, Sweden. *Proceedings of the National Academy of Sciences, USA*, **91**, 1810–1813.
- Ventura, G. T., Kenig, F., Reddy, C. M., Schieber, J., Frysinger, G. S., Nelson, R. K., Dinel, E., Gaines, R. B., and Schaeffer, P., 2007. Molecular evidence of late Archaean archaea and the presence of a subsurface hydrothermal biosphere. *PNAS*, **104**, 14260–14265.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkma, E., and Vishnivetskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Vreeland, R. H., Piselli, A. F., McDonnough, S., and Meyers, S. S., 1998. Distribution and diversity of halophilic bacteria in a subsurface salt formation. *Extremophiles*, **2**, 321–331.
- Wanger, G., Onstott, T. C., and Southam, G., 2008. Stars of the terrestrial deep subsurface: a novel 'star-shaped' bacterial morphotype from a South African platinum mine. *Geobiology*, **6**, 325–330.
- Zavarzina, D., Sokolova, T. G., Tourova, T. P., Chernykh, N. A., Kostrikina, N. A., and Bonch-Osmolovskaya, E. A., 2007. *Thermincola ferriacetica* sp. nov., a new anaerobic, thermophilic facultatively chemolithoautotrophic bacterium capable of dissimilatory Fe(III) reduction. *Extremophiles*, **11**, 1–7.
- Zhang, G., Dong, H., Jiang, H., Xu, Z., and Eberl, D. D., 2006. Unique microbial community in drilling fluids from Chinese Continental Scientific Drilling. *Geomicrobiology Journal*, **23**, 499–514.
- Zobell, C. E., 1945. The role of bacteria in the formation and transformation of petroleum hydrocarbons. *Science*, **102**, 364–369.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Biofilms and Fossilization](#)
[Biosignatures in Rocks](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)

[Fe\(II\)-Oxidizing Prokaryotes](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Halobacteria – Halophiles](#)
[Hydrothermal Environments, Terrestrial](#)
[Origin of Life](#)
[Permafrost Microbiology](#)
[Snowball Earth](#)
[Sulfate-Reducing Bacteria](#)

THIOESTER WORLD

Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition

Christian de Duve (1991, 1994, 1995) proposed a Hadean “Thioester World” which should have preceded the succeeding RNA world. Thioester molecules were probably very common in the proposed “primordial organic soup”. Thioesters are formed when a ‘thiol’ joins a carboxylic acid (R'–COOH) under acidic and high temperature environmental conditions. The general form is written as an organic group (R) bonded with sulfur and hydrogen, hence R-SH. H₂O is released in this process, and forms finally a thioester: R–S–CO–R'. The “Thioester World” represents a hypothetical very early stage in the origin of life that could have provided the energetic and catalytic framework of a protometabolic set of primitive chemical reactions, like in the transaction of molecular groups, and in Redox-reactions. Thioesters were probably involved in the formation of energy-rich phosphates via acylphosphates and pyrophosphates as precursor compounds. The thioester bond is a high-energy bond and probably thioesters had the same function as ATP as an energy supplier. With the help of thioesters amino acids (AS) could also spontaneously polymerise and form first simple proteins. The “primordial organic soup” was enriched with AS may be provided by carbonaceous chondrites and comets which collided with the early earth and the early earth was also characterized by an intense volcanic activity. This volcanic activity generated H₂S and thiols are expected to arise readily in these H₂S-rich fluids. In laboratory experiments, imitating the Hadean conditions on Earth, it was possible to obtain cysteamine, b-alanine and pantonic acid known as ‘pantetheine’ which is a precursor of coenzyme A (Keefe et al., 1995). The thioester world is an important hypothesis in the understanding of early life processes which may explain essential reactions expected in the prebiotic world.

Bibliography

- De Duve, C., 1991. *Blueprint for a Cell: The Nature and Origin of Life*. Burlington, NC: Carolina Biological Supply Company (Neil Patterson Publisher).

De Duve, C., 1994. *Ursprung des Lebens. Präbiotische Evolution und die Entstehung des Lebens*. Heidelberg: Spektrum (Akademischer Verlag).

De Duve, C., 1995. The beginning of life on Earth. *American Scientist*, **83**(Sept–Oct), 428–430.

Keefe, A. D., Newton, G. L., and Miller, S. L., (1995). A possible prebiotic synthesis of pantetheine, a precursor of coenzyme A. *Nature*, **373**, 683–685.

Cross-references

[Asteroid and Comet Impacts](#)

[Chondrites](#)

[Comets](#)

[Meteoritics](#)

[Origin of Life](#)

[RNA-World](#)

THIOMARGARITA

Heide N. Schulz-Vogt

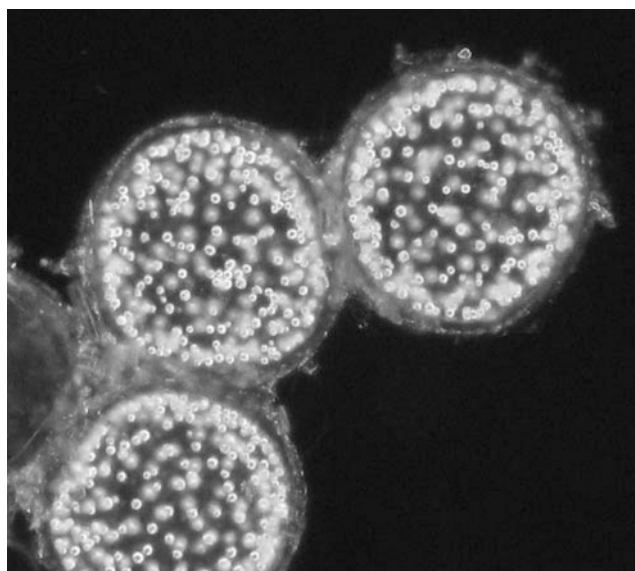
Max Planck Institute for Marine Microbiology, Bremen, Germany

Definition

Colorless, sulfur and nitrate storing bacterium belonging to the gammaproteobacteria, spherical, vacuolated.

Thiomargarita namibiensis, the largest known prokaryote, was first discovered in 1997 off the coast of Namibia and called the Namibian sulfur pearl. Together with the larger species of the genera *Beggiatoa* and *Thioploca*, they belong to a group of benthic bacteria that live by the oxidation of sulfide to sulfate with internally stored nitrate. Sulfide is first oxidized to sulfur, which can be stored by the bacteria as an energy reservoir. Nitrate is accumulated to very high concentrations (up to 800 mM) in a central vacuole, which comprises most of the cell volume. The single spherical cells are 100–300 µm in diameter and are held together in a chain by a mucus sheath ([Figure 1](#)). A new species of *Thiomargarita*, which was discovered in the Gulf of Mexico, appears as single cells without a slime sheath and can perform reductive divisions in up to three planes. These *Thiomargarita* cells resemble some of the fossils from the Doushantuo Formation in China, which have been classified as the oldest metazoan eggs and embryos.

Thiomargarita namibiensis inhabits the upper centimeters of fluid, sulfidic diatom ooze, which forms the sediment off the Namibian coast. Because of their large size, they can store enough nitrate to survive many years within the sulfidic sediment without getting into contact with nitrate from the open water. As *Thiomargarita* cells are not motile, they have to get suspended with their sediment, in order to refill the vacuole with nitrate. Off Namibia sediment resuspension may occur via frequent methane eruptions. Apart from sulfur and nitrate *Thiomargarita* cells



Thiomargarita, Figure 1 A chain of *Thiomargarita* cells (three cells visible) seen with a stereo microscope on a black background and illuminated from the side. The cells with a diameter of about 0.2 mm are held together by a mucus sheath. To the left there is part of an empty slime pocket visible. The large sulfur inclusions can be seen as shining white globules.

can also store polyphosphate, which they may release periodically resulting in high phosphate concentrations around the cells and spontaneous precipitation of phosphorus rich minerals.

Bibliography

Schulz, H. N., 2006. The genus *Thiomargarita*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, Vol. 6, pp. 1156–1163.

Cross-references

[Cold Seeps](#)

[Phosphorus, Phosphorites](#)

[Sulfur Cycle](#)

[Symbiosis](#)

[Thiotrophic Bacteria](#)

THIOTROPHIC BACTERIA

Heide N. Schulz-Vogt

Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

[Sulfur bacteria](#)

De Duve, C., 1994. *Ursprung des Lebens. Präbiotische Evolution und die Entstehung des Lebens*. Heidelberg: Spektrum (Akademischer Verlag).

De Duve, C., 1995. The beginning of life on Earth. *American Scientist*, **83**(Sept–Oct), 428–430.

Keefe, A. D., Newton, G. L., and Miller, S. L., (1995). A possible prebiotic synthesis of pantetheine, a precursor of coenzyme A. *Nature*, **373**, 683–685.

Cross-references

[Asteroid and Comet Impacts](#)

[Chondrites](#)

[Comets](#)

[Meteoritics](#)

[Origin of Life](#)

[RNA-World](#)

THIOMARGARITA

Heide N. Schulz-Vogt

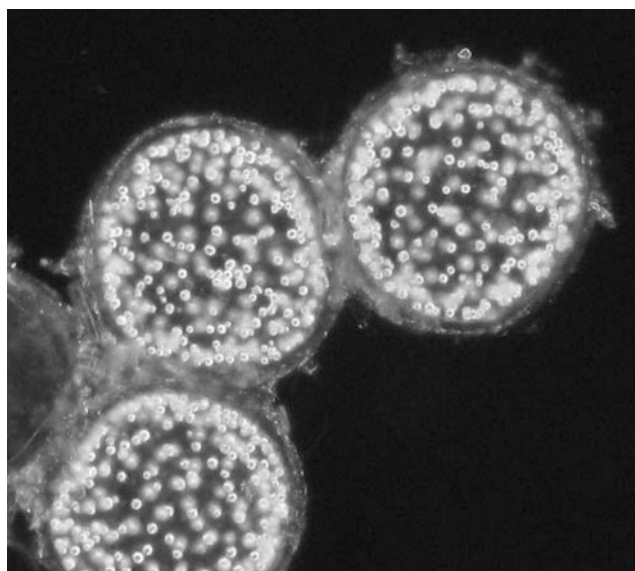
Max Planck Institute for Marine Microbiology, Bremen, Germany

Definition

Colorless, sulfur and nitrate storing bacterium belonging to the gammaproteobacteria, spherical, vacuolated.

Thiomargarita namibiensis, the largest known prokaryote, was first discovered in 1997 off the coast of Namibia and called the Namibian sulfur pearl. Together with the larger species of the genera *Beggiatoa* and *Thioploca*, they belong to a group of benthic bacteria that live by the oxidation of sulfide to sulfate with internally stored nitrate. Sulfide is first oxidized to sulfur, which can be stored by the bacteria as an energy reservoir. Nitrate is accumulated to very high concentrations (up to 800 mM) in a central vacuole, which comprises most of the cell volume. The single spherical cells are 100–300 µm in diameter and are held together in a chain by a mucus sheath ([Figure 1](#)). A new species of *Thiomargarita*, which was discovered in the Gulf of Mexico, appears as single cells without a slime sheath and can perform reductive divisions in up to three planes. These *Thiomargarita* cells resemble some of the fossils from the Doushantuo Formation in China, which have been classified as the oldest metazoan eggs and embryos.

Thiomargarita namibiensis inhabits the upper centimeters of fluid, sulfidic diatom ooze, which forms the sediment off the Namibian coast. Because of their large size, they can store enough nitrate to survive many years within the sulfidic sediment without getting into contact with nitrate from the open water. As *Thiomargarita* cells are not motile, they have to get suspended with their sediment, in order to refill the vacuole with nitrate. Off Namibia sediment resuspension may occur via frequent methane eruptions. Apart from sulfur and nitrate *Thiomargarita* cells



Thiomargarita, Figure 1 A chain of *Thiomargarita* cells (three cells visible) seen with a stereo microscope on a black background and illuminated from the side. The cells with a diameter of about 0.2 mm are held together by a mucus sheath. To the left there is part of an empty slime pocket visible. The large sulfur inclusions can be seen as shining white globules.

can also store polyphosphate, which they may release periodically resulting in high phosphate concentrations around the cells and spontaneous precipitation of phosphorus rich minerals.

Bibliography

Schulz, H. N., 2006. The genus *Thiomargarita*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, Vol. 6, pp. 1156–1163.

Cross-references

[Cold Seeps](#)

[Phosphorus, Phosphorites](#)

[Sulfur Cycle](#)

[Symbiosis](#)

[Thiotrophic Bacteria](#)

THIOTROPHIC BACTERIA

Heide N. Schulz-Vogt

Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

[Sulfur bacteria](#)

De Duve, C., 1994. *Ursprung des Lebens. Präbiotische Evolution und die Entstehung des Lebens*. Heidelberg: Spektrum (Akademischer Verlag).

De Duve, C., 1995. The beginning of life on Earth. *American Scientist*, **83**(Sept–Oct), 428–430.

Keefe, A. D., Newton, G. L., and Miller, S. L., (1995). A possible prebiotic synthesis of pantetheine, a precursor of coenzyme A. *Nature*, **373**, 683–685.

Cross-references

[Asteroid and Comet Impacts](#)

[Chondrites](#)

[Comets](#)

[Meteoritics](#)

[Origin of Life](#)

[RNA-World](#)

THIOMARGARITA

Heide N. Schulz-Vogt

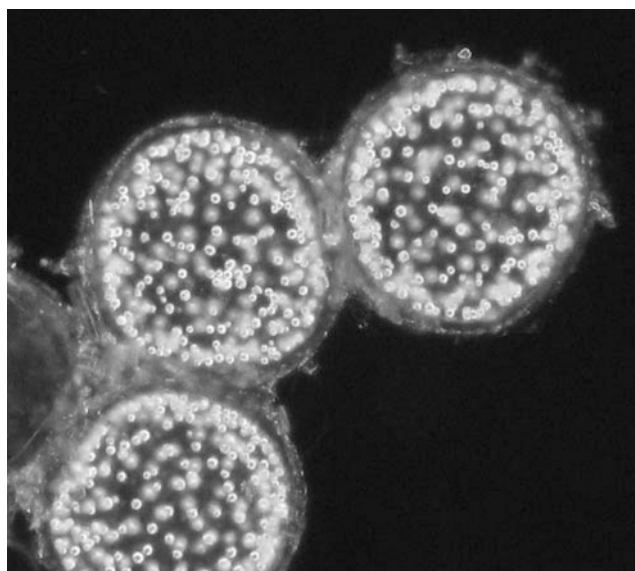
Max Planck Institute for Marine Microbiology, Bremen, Germany

Definition

Colorless, sulfur and nitrate storing bacterium belonging to the gammaproteobacteria, spherical, vacuolated.

Thiomargarita namibiensis, the largest known prokaryote, was first discovered in 1997 off the coast of Namibia and called the Namibian sulfur pearl. Together with the larger species of the genera *Beggiatoa* and *Thioploca*, they belong to a group of benthic bacteria that live by the oxidation of sulfide to sulfate with internally stored nitrate. Sulfide is first oxidized to sulfur, which can be stored by the bacteria as an energy reservoir. Nitrate is accumulated to very high concentrations (up to 800 mM) in a central vacuole, which comprises most of the cell volume. The single spherical cells are 100–300 µm in diameter and are held together in a chain by a mucus sheath ([Figure 1](#)). A new species of *Thiomargarita*, which was discovered in the Gulf of Mexico, appears as single cells without a slime sheath and can perform reductive divisions in up to three planes. These *Thiomargarita* cells resemble some of the fossils from the Doushantuo Formation in China, which have been classified as the oldest metazoan eggs and embryos.

Thiomargarita namibiensis inhabits the upper centimeters of fluid, sulfidic diatom ooze, which forms the sediment off the Namibian coast. Because of their large size, they can store enough nitrate to survive many years within the sulfidic sediment without getting into contact with nitrate from the open water. As *Thiomargarita* cells are not motile, they have to get suspended with their sediment, in order to refill the vacuole with nitrate. Off Namibia sediment resuspension may occur via frequent methane eruptions. Apart from sulfur and nitrate *Thiomargarita* cells



Thiomargarita, Figure 1 A chain of *Thiomargarita* cells (three cells visible) seen with a stereo microscope on a black background and illuminated from the side. The cells with a diameter of about 0.2 mm are held together by a mucus sheath. To the left there is part of an empty slime pocket visible. The large sulfur inclusions can be seen as shining white globules.

can also store polyphosphate, which they may release periodically resulting in high phosphate concentrations around the cells and spontaneous precipitation of phosphorus rich minerals.

Bibliography

Schulz, H. N., 2006. The genus *Thiomargarita*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, Vol. 6, pp. 1156–1163.

Cross-references

[Cold Seeps](#)

[Phosphorus, Phosphorites](#)

[Sulfur Cycle](#)

[Symbiosis](#)

[Thiotrophic Bacteria](#)

THIOTROPHIC BACTERIA

Heide N. Schulz-Vogt

Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

[Sulfur bacteria](#)

Definition

Bacteria living of sulfur compounds.

Introduction

The term thiotrophic is derived from Greek and translates to sulfur nourishment, meaning that thiotrophic bacteria live on sulfide or other reduced sulfur compounds. Traditionally, these bacteria are often called sulfur bacteria because some of them can be recognized as sulfide oxidizers in the microscope, as they store elemental sulfur within the cells or excrete elemental sulfur into the medium. Microbiologists distinguish between colorless sulfur bacteria and green and purple sulfur bacteria, the latter two being phototrophs, which use sulfide as electron donor for anoxygenic photosynthesis (Robertson and Kuenen, 2006). The purple and green sulfur bacteria are generally not called thiotrophic because they gain energy by photosynthesis and are thus classified as phototrophic, even though sulfide is required for this process. Free-living, sulfide oxidizing bacteria are, out of tradition, still called sulfur bacteria rather than thiotrophic bacteria. A large group of sulfide oxidizing bacteria lives in symbiotic association with higher organisms (Cavanaugh et al., 2006). These symbionts are generally called thiotrophic in order to distinguish them from the methanotrophic symbionts, which gain energy by the oxidation of methane. In general, the ability to live by the oxidation of reduced sulfur components is widespread among eubacteria and even archaea, but a large number of thiotrophic bacteria are found within the gammaproteobacteria (Sievert et al., 2007).

Sulfide (the sum of H_2S , HS^- , and S^{2-}) is a difficult energy source because it is toxic for respiratory processes, as it tends to bind iron. Therefore, even thiotrophic bacteria have to prevent very high sulfide concentrations. Microbiologists often avoid this problem by offering thiosulfate ($\text{S}_2\text{O}_3^{2-}$) instead of sulfide for cultivating thiotrophic bacteria. Most thiotrophs oxidize sulfide with oxygen. This reaction also occurs abiotically, but at much lower rates, so thiotrophic bacteria usually compete successfully with the chemical oxidation of sulfide to sulfur in the presence of oxygen. Thiotrophs mostly oxidize sulfide completely to sulfate, to gain the maximum of energy, but some thiotrophs excrete or internally accumulate the intermediate elemental sulfur. Other major intermediates of sulfide oxidation are thiosulfate, tetrathionate, and sulfite.

The oxidation of sulfide with nitrate instead of oxygen is an energy releasing reaction, which is used by some thiotrophic bacteria. One group of free-living sulfur bacteria is even a specialist on nitrate respiration, by possessing a large vacuole in which nitrate is stored in almost molar concentrations. Apart from the oxidation of reduced sulfur compounds bacteria can also gain energy by disproportionating thiosulfate or sulfur to sulfide and sulfate. This inorganic fermentation of thiosulfate or sulfur seems to

be a widespread process both in marine and in freshwater sediments (Jørgensen and Bak, 1991).

Sulfidic environments

A major source of sulfide in the marine environment is the anaerobic degradation of organic materials by sulfate-reducing bacteria. Consequently, high sulfide concentrations and dense populations of thiotrophic bacteria occur in areas where a lot of organic materials settle at the sea floor, as for example in upwelling areas, in eutrophic coastal environments or at whale falls. The main source of sulfide is the sediment; only in very enclosed eutrophic basins sulfide may be produced in the open water (e.g., the Black Sea). Another source of sulfide is highly reduced fluids, which are transported to the sediment surface by advection (e.g., hydrothermal vents, seeps). These are point sources of sulfide in the ocean, which can be densely populated by free-living or symbiotic, thiotrophic bacteria.

Free-living thiotrophs

Many heterotrophic bacteria are capable of also oxidizing reduced sulfur components for energy generation and many thiotrophic bacteria can also use organic carbon as substrate for growth (Robertson and Kuenen, 2006). The classical lithoautotrophic sulfide-oxidizing bacteria are the thiobacilli, which have recently been reclassified and are now distributed to several different genera (e.g., *Acidithiobacillus*, *Halothiobacillus*). Another classical and well-studied group of thiotrophic bacteria are the members of the genus *Thiomicrospira*. Both groups harbor strains which can use nitrate as alternative electron acceptor with nitrogen as the end product (*Thiobacillus denitrificans* and *Thiomicrospira denitrificans*, now *Sulfurimonas denitrificans*). It is long known that many thiotrophic bacteria can create and survive very low pH values. A well-studied example is *Thiobacillus ferrooxidans* (now *Acidithiobacillus ferrooxidans*), which causes problems in acid mine drainage but can also be used for ore leaching. Only recently a number of alkaliphilic thiotrophic bacteria have been isolated (Sorokin and Kuenen, 2005).

Among the free-living thiotrophic bacteria, there is a morphological conspicuous group of large or even gigantic cells with inclusions of elemental sulfur. These large sulfur bacteria are widespread in nature, but are difficult to cultivate. *Beggiatoa* is the longest known, best studied, and probably also the most abundant genus among the morphologically conspicuous sulfur bacteria. They form multicellular, highly motile filaments of several millimeters to centimeter length which are 1–200 μm in diameter. Other genera of morphologically conspicuous sulfur bacteria are *Thioploca*, *Thiomargarita*, *Thiothrix*, *Achromatium*, and *Thiovulum* (Schulz and Jørgensen, 2001). Among the genera *Beggiatoa*, *Thioploca*, and *Thiomargarita*, there are giant forms with vacuoles used for the internal accumulation of nitrate,

which serve as an alternative electron acceptor for sulfide oxidation. With the exception of *Thiovulum* all morphologically conspicuous sulfur bacteria are within the gammaproteobacteria. *Thiovulum* is a member of the epsilonproteobacteria and is closely related to smaller free-living thiotrophic bacteria, which only recently have been recognized to be widespread sulfide oxidizers at hydrothermal vents (Campbell et al., 2006). Habitats with very high sulfide fluxes such as hydrothermal vents often favor the growth of a vibroid epsilonbacterium called *Candidatus Arcobacter sulfidicus*, which oxidizes sulfide rapidly to elemental sulfur. The resulting filaments of elemental sulfur are left behind (Wirsén et al., 2002).

Endosymbionts

Since the discovery of abundant life at hydrothermal vent systems in 1977 it took several years before it was recognized that the gutless tube worm *Riftia* found in high numbers at vents lives on energy conserved by endosymbiotic thiotrophic bacteria (Cavanaugh et al., 1981). The bacteria live on sulfide and oxygen provided by the host and use this energy source to reduce CO₂ to organic carbon compounds, which they share with their host. Many such tight associations between invertebrate animals and thiotrophic bacteria have been reported since the first discovery not only from vent systems but also from other sulfidic marine environments. Endosymbiosis with thiotrophic bacteria seems to be especially common among Mollusca (e.g., clams and mussels) and Annelida (e.g., tube worms) (Cavanaugh et al., 2006). None of the endosymbiotic bacteria could yet be grown outside of its host, but an abundance of information was obtained by using culture-independent methods. Most endosymbiotic thiotrophs are members of the gammaproteobacteria but a few symbionts belong to the epsilonproteobacteria. Usually host animals of one species are found to contain the same or closely related bacteria, indicating a close symbiotic association. For the clam *Calyplogena* there are indications that the symbiont is transferred directly from mother to offspring (Cary and Giovannoni, 1993), whereas the tube worm *Riftia* seems to acquire symbionts de novo from the environment (Laue and Nelson, 1997). For many host animals the mode of infection with the symbiont is not clarified yet (Cavanaugh et al., 2006).

Ectosymbionts

Virtually every larger organism is to some degree colonized by bacteria living on their surface. These so-called epibionts may have harmful, beneficial, or neutral effects on the host. In sulfidic environments thiotrophic bacteria frequently populate organisms such as ciliates, shrimps, or gastropods (Ott et al., 2004). In some cases, it could be shown that the host is feeding on these bacteria or may even provide conditions favoring the growth of thiotrophic bacteria. In other cases, the benefit for the host seems to lie in the detoxification of sulfide by the

epibionts or no obvious benefit could be observed (Ott et al., 2004).

Summary

Thiotrophic bacteria (or sulfur bacteria) live by the oxidation of sulfide with oxygen or nitrate as electron acceptor. This lithotrophic metabolism is widespread among bacteria and also found in some archaea. Among the gammaproteobacteria, a group of giant bacteria is specialized on oxidizing sulfide with nitrate, which is accumulated in a vacuole. Many thiotrophic bacteria live in symbiosis with higher organisms either as endo- or as ectosymbionts.

Bibliography

- Campbell, B. J., Engel, A. S., Porter, M. L., and Takai, K., 2006. The versatile epsilon-proteobacteria: key players in sulphidic habitats. *Nature Reviews Microbiology*, **4**, 458–468.
- Cary, S. C., and Giovannoni, S. J., 1993. Transovarial inheritance of endosymbiotic bacteria in clams inhabiting deep-sea hydrothermal vents and cold seeps. *Proceedings of the National Academy of Sciences of the United States of America*, **90**, 5695–5699.
- Cavanaugh, C. M., Gardiner, S. L., Jones, M. L., Jannasch, H. W., and Waterbury, J. B., 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: possible chemoautotrophic symbionts. *Science*, **213**, 340–342.
- Cavanaugh, C. M., McKiness, Z. P., Newton, L. G., and Stewart, F. J., 2006. Marine chemosynthetic symbioses. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (ed.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, pp. 985–1011.
- Jørgensen, B. B., and Bak, F., 1991. Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat Denmark). *Applied and Environmental Microbiology*, **57**, 847–856.
- Laue, B. E., and Nelson, D. C., 1997. Sulfur-oxidizing symbionts have not co-evolved with their hydrothermal vent tube worm hosts: an RFLP analysis. *Molecular Marine Biology and Biotechnology*, **6**, 180–188.
- Ott, J., Bright, M., and Bulgheresi, S., 2004. Marine microbial thiotrophic ectosymbioses. *Oceanography and Marine Biology: An Annual Review*, **42**, 95–118.
- Robertson, L. A., and Kuenen, J. G., 2006. The colorless sulfur bacteria. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.), *The Prokaryotes. A Handbook on the Biology of Bacteria*. New York: Springer, pp. 985–1011.
- Schulz, H. N., and Jørgensen, B. B., 2001. Big bacteria. *Annual Review of Microbiology*, **55**, 105–137.
- Sievert, S. M., Keine, R. P., and Schulz-Vogt, H. N., 2007. Microbes and major elemental cycles – the sulfur cycle. In Karl, D. M., and Proctor, L. M. (eds.), *A Sea of Microbes, Oceanography*, **20**, 117–123.
- Sorokin, D. Y., and Kuenen, J. G., 2005. Haloalkaliphilic sulfur-oxidizing bacteria in soda lakes. *FEMS Microbiology Reviews*, **29**, 685–702.
- Wirsén, C. O., Sievert, S. M., Cavanaugh, C. M., Molyneux, S. J., Ahmad, A., Taylor, L. T., DeLong, E. F., and Taylor, C. D., 2002. Characterization of an autotrophic sulfide-oxidizing marine *Arcobacter* sp that produces filamentous sulfur. *Applied and Environmental Microbiology*, **68**, 316–325.

Cross-references

Beggiatoa
Chemolithotrophy
Cold Seeps
Hydrothermal Environments, Marine
Pyrite Oxidation
Sulfate-Reducing Bacteria
Sulfur Cycle
Thiomargarita

THROMBOLITES

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Bibliography

Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.

Cross-references

Microbialites, Stromatolites, and Thrombolites

TIDAL FLATS

Meinhard Simon
University of Oldenburg, Oldenburg, Germany

Synonyms

Wadden Sea: name of the world’s largest tidal flat system in Central Europe

Definition

Tidal Flat. A wide and almost flat coastal area between the mean upper and lower tidal seawater levels (eulitoral) exposed to the rhythmic tidal water movements. It consists of unconsolidated sediments and is bordered toward the land by permanently emerged areas and toward the sea by the permanently submerged sublitoral zone.

Introduction

Tidal flats occur worldwide in shallow coastal regions: in the tropics, subtropics, temperate zones, and subpolar, and polar regions. In the tropics and subtropics, such as at various regions of the east coast of South America, in Africa, Bangladesh, Indonesia, and Australia, tidal flats are closely associated with mangrove ecosystems. In other places, such as Mauretania, the Persian Gulf, and the coast of Texas, USA, tidal flats are either wide and open coastal stretches or protected by barrier islands. Large tidal flat

areas adjacent to marshlands exist on the Atlantic coast of the USA (North/South Carolina, Georgia) and Canada (Nova Scotia, Bay of Fundy), Korea, and in Europe along the Atlantic coast (Portugal, Spain, France, and Great Britain), and the North Sea. At the southern coast of the North Sea, the worldwide largest tidal flat system, the Wadden Sea ranges from The Netherlands (den Helder) through Germany to Denmark (Esbjerg). Subpolar and polar tidal flat systems are found in southern Chile, Alaska, and Siberia. Tidal flats may also develop in shallow estuaries, and thus their sediments may partially exhibit riverine properties.

A special type of tidal flats is wind-induced; these wind flats form along shallow coastlines with very little tidal water movements but occasionally persistently strong winds. The winds lead to the emergence or submergence of shallow areas for irregular periods, and the flats are characterized by microbial mats and sediments similar to those of regular tidal flats.

Formation and shaping forces

Along shallow coastlines with little wave action, the rhythmic incoming and outgoing tidal currents are the main driving forces for establishing and structuring tidal flats. A critical tidal range of at least 1 m is a prerequisite for the formation of a tidal flat. The incoming tide imports dissolved nutrients and particulate inorganic and organic matter, which settle out during reduced water movement at slack tide. This hydrographic energy gradient leads to a fractionation of the suspended material according to its settling properties. The finest material, the mud fraction (>50% of the material with a particle size <63 µm and the highest content of organic carbon up to 10% of dry weight) settles at the lowest current velocity and thus in the shallowest and least energetic zones. The other fractions consist of sandy (<5% mud) and mixed sediments (5–50% mud) and form the bulk of the sediment in highly and intermediate energetic areas (Chang et al., 2006). As a consequence, material and nutrients accumulate in tidal flats and provide the basis for highly productive ecosystems (Postma, 1981). Additional input of particulate organic and inorganic matter and nutrients may come from the adjacent coastal areas permanently emerged and/or by pore water (Kasten, this volume) and groundwater via tidal pumping (only inorganic nutrients and dissolved organic matter). Export of matter occurs mainly in the dissolved form and net export of particulates occurs only when re-suspension exceeds sedimentation for more than a tidal cycle, during storm events. The interplay of coastal and tidal currents, settling, re-suspension, and fractionation of suspended matter and sediment, results in the formation of typical depositional features, such as banks, islands, barrier island systems with distinct sediment structures, and drainage channels of different sizes and depths (Figure 1). Because of the ample nutrient supply, tidal flats are hot spots of marine productivity and biodiversity.

Cross-references

Beggiatoa
Chemolithotrophy
Cold Seeps
Hydrothermal Environments, Marine
Pyrite Oxidation
Sulfate-Reducing Bacteria
Sulfur Cycle
Thiomargarita

THROMBOLITES

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Bibliography

Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.

Cross-references

Microbialites, Stromatolites, and Thrombolites

TIDAL FLATS

Meinhard Simon
University of Oldenburg, Oldenburg, Germany

Synonyms

Wadden Sea: name of the world’s largest tidal flat system in Central Europe

Definition

Tidal Flat. A wide and almost flat coastal area between the mean upper and lower tidal seawater levels (eulitoral) exposed to the rhythmic tidal water movements. It consists of unconsolidated sediments and is bordered toward the land by permanently emerged areas and toward the sea by the permanently submerged sublitoral zone.

Introduction

Tidal flats occur worldwide in shallow coastal regions: in the tropics, subtropics, temperate zones, and subpolar, and polar regions. In the tropics and subtropics, such as at various regions of the east coast of South America, in Africa, Bangladesh, Indonesia, and Australia, tidal flats are closely associated with mangrove ecosystems. In other places, such as Mauretania, the Persian Gulf, and the coast of Texas, USA, tidal flats are either wide and open coastal stretches or protected by barrier islands. Large tidal flat

areas adjacent to marshlands exist on the Atlantic coast of the USA (North/South Carolina, Georgia) and Canada (Nova Scotia, Bay of Fundy), Korea, and in Europe along the Atlantic coast (Portugal, Spain, France, and Great Britain), and the North Sea. At the southern coast of the North Sea, the worldwide largest tidal flat system, the Wadden Sea ranges from The Netherlands (den Helder) through Germany to Denmark (Esbjerg). Subpolar and polar tidal flat systems are found in southern Chile, Alaska, and Siberia. Tidal flats may also develop in shallow estuaries, and thus their sediments may partially exhibit riverine properties.

A special type of tidal flats is wind-induced; these wind flats form along shallow coastlines with very little tidal water movements but occasionally persistently strong winds. The winds lead to the emergence or submergence of shallow areas for irregular periods, and the flats are characterized by microbial mats and sediments similar to those of regular tidal flats.

Formation and shaping forces

Along shallow coastlines with little wave action, the rhythmic incoming and outgoing tidal currents are the main driving forces for establishing and structuring tidal flats. A critical tidal range of at least 1 m is a prerequisite for the formation of a tidal flat. The incoming tide imports dissolved nutrients and particulate inorganic and organic matter, which settle out during reduced water movement at slack tide. This hydrographic energy gradient leads to a fractionation of the suspended material according to its settling properties. The finest material, the mud fraction (>50% of the material with a particle size <63 µm and the highest content of organic carbon up to 10% of dry weight) settles at the lowest current velocity and thus in the shallowest and least energetic zones. The other fractions consist of sandy (<5% mud) and mixed sediments (5–50% mud) and form the bulk of the sediment in highly and intermediate energetic areas (Chang et al., 2006). As a consequence, material and nutrients accumulate in tidal flats and provide the basis for highly productive ecosystems (Postma, 1981). Additional input of particulate organic and inorganic matter and nutrients may come from the adjacent coastal areas permanently emerged and/or by pore water (Kasten, this volume) and groundwater via tidal pumping (only inorganic nutrients and dissolved organic matter). Export of matter occurs mainly in the dissolved form and net export of particulates occurs only when re-suspension exceeds sedimentation for more than a tidal cycle, during storm events. The interplay of coastal and tidal currents, settling, re-suspension, and fractionation of suspended matter and sediment, results in the formation of typical depositional features, such as banks, islands, barrier island systems with distinct sediment structures, and drainage channels of different sizes and depths (Figure 1). Because of the ample nutrient supply, tidal flats are hot spots of marine productivity and biodiversity.



Tidal Flats, Figure 1 A tidal flat with sand banks and tidal channels of the East Frisian Wadden Sea, Germany. (Photo: S. Kotzur.)

Recent studies have shown that settling of suspended material on tidal flats is not purely hydrodynamically and physico-chemically driven. Instead bacteria associated with the suspended material actively enhance the aggregation of suspended particles, followed by the sinking of the aggregated material at slack water (Chang et al., 2006; Lunau et al., 2006). During periods of low microbial activity, such as during winter in temperate regions, aggregation is low and suspended particles that persist in the water column are more abundant and smaller in size than during the warmer, growing season. The consolidation of sediment is enhanced by biofilms/microbial mats (Neu, Reitner, this volume) at the sediment-water interface and the associated production of exopolymetric substances (EPS, see Decho, entry *Extracellular Polymeric Substances (EPS)*, this volume), consisting mainly of polysaccharides and proteins, which glue together the fine primary sediment particles (Stal, 2003). As these biofilms are more biologically active during the enhanced temperatures of the spring and summer growing seasons, the sediments are more subject to re-suspension and erosion during periods of low temperature in fall and winter (Chang et al., 2006).

Under conditions when ice may cover large areas of tidal flats, such as during particularly cold winters in the temperate zone or during regular winters in subpolar and polar regions, tidal movement of ice flows can cause considerable erosion of the unconsolidated surface sediments.

Geobiology of tidal flats

The high productivity of tidal flats is reflected in their use as resting and overwintering areas for myriads of

migrating birds, which feed on the infauna such as molluscs, crustaceans, and worms. The food bases for these animals that rework and often bioturbate the tidal flats are autotrophic and heterotrophic microbes thriving in the water column, at the sediment-water interface and within the sediment (Reise, 2002). Primary producers in the water column are dominated by pennate diatoms (Bacillariales) in the temperate and polar zones, and diatoms, dinoflagellates, and coccolithophorids in subtropical and tropical regions. The suspended matter in the water column is heavily colonized by heterotrophic bacteria which decompose a substantial part of the available organic matter and also affect the aggregation and thus deposition of the settling material. In the oxic layer of the biofilms/microbial mats at the sediment-water interface, the dominant primary producers are filamentous cyanobacteria (Friedl, this volume), and diatoms (Stal, 2003). Because of the high primary production rates and the import of organic matter, mineralization rates of organic matter and thus oxygen consumption in the sediment is very high, resulting in anoxic conditions. Hence, anoxic microbial processes largely dominate within sediments, greatly affecting the formation and diagenesis of these sediments (Mackenzie, this volume).

The composition of the tidal flat-associated microbial communities is a function of the available light, pore water advection, and redox conditions, i.e., the availability of organic carbon, electron acceptors, and donors. The shapes of the redox profiles within the sediments, i.e., oxygen and sulfide concentration (Böttcher, this volume), are a function of sediment structure, grain size, pore water advection, and the metabolic activities of the microbes. Anoxygenic phototrophic bacteria contribute



Tidal Flats, Figure 2 Farbstreifensandwatt, layers from top to bottom – brown surface: Diatoms; red: Cyanobacteria associated with purple sulfur bacteria (*Chromatiaceae*); olive: Green sulfur bacteria (*Chlorobiaceae*); white/gray: Sulfidic precipitates. A black sulfidic zone of sulfate reduction is missing in this example because of lack of iron. Scale bar: 1 cm. (Photo: H. Cypionka, www.microbiological-garden.net.)

to CO₂-fixation by oxidizing sulfide. When the sediment remains undisturbed, a vertical zonation of the autotrophic communities may form distinctly colored layers, the so-called *Farbstreifensandwatt* (Stal et al., 1985; Figure 2), with a brownish-green layer at the top (diatoms), followed by a blue-green (cyanobacteria), a pink (purple sulfur bacteria), a green (purple non-sulfur bacteria), and a black layer at the bottom (sulfate reducers). Even though sulfate reduction (Cypionka, this volume) is commonly the most important process in forming the black, sulfide-dominated anoxic sediments in tidal flats, other anoxic processes contribute to the decomposition of organic matter: fermentation, denitrification, reduction of manganese and iron, and anaerobic methane oxidation (Boetius, this volume). These processes lead to the deposition of various reduced inorganic compounds and leftover of refractory organic matter, thus structuring the sediments on a long-term range and to a depth of several meters. Even though the general microbial processes in the oxic and anoxic sediments are well-characterized, the *Bacteria* and *Archaea* involved are far from being well-described. There is some information on the partitioning of some of the major

phylogenetic prokaryotic groups in near-surface sediments (Llobet-Brossa et al., 1998; Musat et al., 2006; Wilms et al., 2006). Some players in the major processes are available as isolated strains, but many more are identified only by genetic fingerprinting, i.e., sequencing of the 16S rRNA gene or of functional genes, and the microbes are not yet isolated. Some of the tidal flat microbes appear to be closely related to prokaryotes of the deep biosphere.

Mud flats versus sand flats

The composition of the inorganic sediment in the tidal flats may vary considerably but has little influence on the general grain size distribution. Depending on the geologic setting and the coastal environment, quartz, volcanic minerals, or biogenic carbonates can dominate or constitute varying proportions of the inorganic components of the sediments. Grain size and organic carbon content differ markedly in mud flats and sand flats. The smaller grain size and higher organic content of the mud flats result in a much more compact structure and a lower pore water volume and permeability. These characteristics have led to the traditional view that decomposition rates of organic carbon are much higher in mud flats than in sand flats. Recent studies, however, have shown that mineralization rates of organic carbon in sand flats are at least as high as and often higher than in mud flats (De Beer et al., 2005). In sand flats with a high pore water volume, oxygen supply is much higher than in mud flats, supporting high aerobic mineralization rates and leading to a lower content of organic carbon. Hence, the low content of organic carbon in sand flats appears not to be a result of the lower input of organic matter or reduced microbial activities as compared to mixed or mud flats but instead reflect more favorable conditions for the microbial decomposition of organic carbon.

Geologic age

Recent tidal flats are young geobiological systems on a geologic time scale and originated in the Holocene. They typically developed in the course of the sea water level rise in shallow and flat coastal regions during the last 10,000 years after the Pleistocene, when large amounts of sediments were deposited.

Related term

Sabkha: Almost flat, vegetation-free coastal areas above the mean and upper tidal seawater level in arid subtropical regions like at the Persian and Arabian Gulf. They are covered by salt deposits of the evaporating seawater and other crystalline deposits of halite, anhydrite, and gypsum, and by microbial mats of filamentous cyanobacteria.

Summary

Tidal flats are almost flat coastal areas between the mean upper and lower tidal sea water levels and a tidal range of at least 1 m and occur in all climatic regions. They are

structured by tidal channels and banks, and accumulate particulate matter which settles out at sites of reduced water movement. As a consequence, sediment layers are formed which rapidly become anoxic below the surface because of the intense microbial decomposition processes. Due to the high input of organic matter, tidal flats are one of the most productive ecosystems in the world; this productivity is fueled by microbial processes in the water column and within the sediments of the tidal flat regions.

Bibliography

- Chang, T. S., Bartholomä, A., and Flemming, B. W., 2006. Seasonal dynamics of fine-grained sediments in a back-barrier tidal basin of the German Wadden Sea (Southern North Sea). *Journal of Coastal Research*, **22**, 328–338.
- De Beer, D., Wenzhöfer, F., Ferdelman, T. G., Boehme, S. E., Huettel, M., van Beusekom, J. E. E., Boettcher, M. E., Musat, N., and Dubilier, N., 2005. Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Romø Basin, Wadden Sea. *Limnology and Oceanography*, **50**, 113–127.
- Llobet-Brossa, E., Rossello-Mora, R., and Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence-in-situ-hybridization. *Applied and Environmental Microbiology*, **64**, 2691–2696.
- Lunau, M., Lemke, A., Dellwig, O., and Simon, M., 2006. Physical and biogeochemical controls of microaggregate dynamics in a tidally affected coastal ecosystem. *Limnology and Oceanography*, **51**, 847–859.
- Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J. E. E., de Beer, D., Dubilier, N., and Amann, R., 2006. Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Romø Basin, Wadden Sea. *Systematic and Applied Microbiology*, **29**, 333–348.
- Postma, H., 1981. Exchange of materials between the North-Sea and the Wadden Sea. *Marine Geology*, **40**, 199–213.
- Reise, K., 2002. Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, **48**, 127–141.
- Stal, L. J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiological Journal*, **20**, 463–478.
- Stal, L. J. H., van Gemerden, H., and Krumbein, W. E., 1985. Structure and development of a benthic marine microbial mat. *FEMS Microbiology Ecology*, **31**, 111–125.
- Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., and Engelen, B., 2006. Specific bacterial, archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. *Applied and Environmental Microbiology*, **72**, 2756–2764.

Cross-references

[Anaerobic Methane Oxidation with Sulfate Biofilms](#)
[Cyanobacteria](#)
[Diatoms](#)
[Evaporites](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Mats](#)
[Pore Water](#)
[Sediment Diagenesis – Biologically Controlled Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

ToF-SIMS

Peter Sjövall, Jukka Lausmaa
 SP Technical Research Institute of Sweden, Chemistry and Materials Technology, Borås, Sweden

Definition

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a method for chemical microanalysis of solid surfaces (Benninghoven, 1994; Belu, 2003; Vickerman and Briggs, 2001). The method can be used to obtain detailed information of the organic and inorganic composition of samples at a spatial resolution down to <1 µm. It is a highly surface-sensitive method, providing chemical information about the uppermost molecular layers only. The analysis is based on mass spectrometry and requires no preselection or labeling of the substances to be analyzed.

In a ToF-SIMS measurement, the sample is bombarded by a pulsed, focused, high-energy ion beam (primary ions) that causes atoms, molecular fragments, and intact molecules to be ejected from the sample surface. A small fraction of the ejected particles will be ionized (secondary ions). The secondary ions are extracted and directed into a time-of-flight (ToF) mass analyzer, where they are separated with respect to mass to charge ratio. The resulting mass spectrum represents a chemical fingerprint from the sample surface at the point of impact of the primary ion beam (Figure 1).

Characterization of the chemical substances present on the sample surface is done by identification of the ions corresponding to the peaks in the mass spectrum, in a similar way as in other mass spectrometric techniques, such as GC/MS and LC/MS. The high mass resolution of the ToF analyzer (typically $m/\Delta m \sim 5,000\text{--}10,000$) allows for detailed identification of the secondary ions.

The spatial information, i.e., the lateral distribution of the different chemical components on the sample surface, is obtained by scanning the primary ion beam over the sample surface and recording separate mass spectra from a large number of points (pixels) within the analysis area. The acquired data can be presented in different ways: (a) as mass spectra of the total analysis area or selected regions of interest, (b) as ion images, in which the signal intensity distribution from ions representing specific elements or compounds are displayed as variations in brightness over the analysis area (see Figures 1 and 2), or (c) as time evolutions of specific ion signal intensities during ion etching using a separate ion beam (depth profiling).

An important advantage of the ToF-SIMS technique, in relation to other SIMS methods, is that the ToF mass analyzer allows for so-called static SIMS analysis, i.e., analysis of the undamaged surface before the mass spectrum has been significantly altered by the molecular damage caused by the primary ions. In dynamic SIMS, the sample surface is subjected to considerably higher primary ion dose

structured by tidal channels and banks, and accumulate particulate matter which settles out at sites of reduced water movement. As a consequence, sediment layers are formed which rapidly become anoxic below the surface because of the intense microbial decomposition processes. Due to the high input of organic matter, tidal flats are one of the most productive ecosystems in the world; this productivity is fueled by microbial processes in the water column and within the sediments of the tidal flat regions.

Bibliography

- Chang, T. S., Bartholomä, A., and Flemming, B. W., 2006. Seasonal dynamics of fine-grained sediments in a back-barrier tidal basin of the German Wadden Sea (Southern North Sea). *Journal of Coastal Research*, **22**, 328–338.
- De Beer, D., Wenzhöfer, F., Ferdelman, T. G., Boehme, S. E., Huettel, M., van Beusekom, J. E. E., Boettcher, M. E., Musat, N., and Dubilier, N., 2005. Transport and mineralization rates in North Sea sandy intertidal sediments, Sylt-Romø Basin, Wadden Sea. *Limnology and Oceanography*, **50**, 113–127.
- Llobet-Brossa, E., Rossello-Mora, R., and Amann, R., 1998. Microbial community composition of Wadden Sea sediments as revealed by fluorescence-in-situ-hybridization. *Applied and Environmental Microbiology*, **64**, 2691–2696.
- Lunau, M., Lemke, A., Dellwig, O., and Simon, M., 2006. Physical and biogeochemical controls of microaggregate dynamics in a tidally affected coastal ecosystem. *Limnology and Oceanography*, **51**, 847–859.
- Musat, N., Werner, U., Knittel, K., Kolb, S., Dodenhof, T., van Beusekom, J. E. E., de Beer, D., Dubilier, N., and Amann, R., 2006. Microbial community structure of sandy intertidal sediments in the North Sea, Sylt-Romø Basin, Wadden Sea. *Systematic and Applied Microbiology*, **29**, 333–348.
- Postma, H., 1981. Exchange of materials between the North-Sea and the Wadden Sea. *Marine Geology*, **40**, 199–213.
- Reise, K., 2002. Sediment mediated species interactions in coastal waters. *Journal of Sea Research*, **48**, 127–141.
- Stal, L. J., 2003. Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiological Journal*, **20**, 463–478.
- Stal, L. J. H., van Gemerden, H., and Krumbein, W. E., 1985. Structure and development of a benthic marine microbial mat. *FEMS Microbiology Ecology*, **31**, 111–125.
- Wilms, R., Sass, H., Köpke, B., Köster, J., Cypionka, H., and Engelen, B., 2006. Specific bacterial, archaeal, and eukaryotic communities in tidal-flat sediment along a vertical profile of several meters. *Applied and Environmental Microbiology*, **72**, 2756–2764.

Cross-references

[Anaerobic Methane Oxidation with Sulfate Biofilms](#)
[Cyanobacteria](#)
[Diatoms](#)
[Evaporites](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Mats](#)
[Pore Water](#)
[Sediment Diagenesis – Biologically Controlled Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

ToF-SIMS

Peter Sjövall, Jukka Lausmaa
 SP Technical Research Institute of Sweden, Chemistry and Materials Technology, Borås, Sweden

Definition

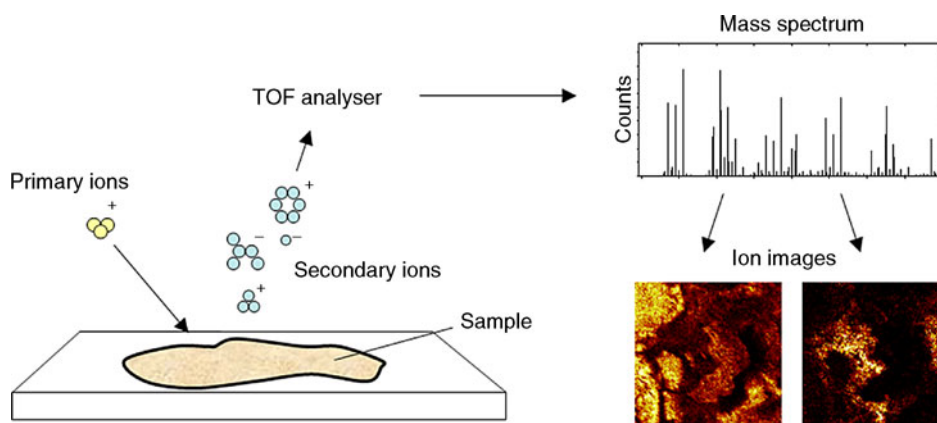
Time-of-flight secondary ion mass spectrometry (ToF-SIMS) is a method for chemical microanalysis of solid surfaces (Benninghoven, 1994; Belu, 2003; Vickerman and Briggs, 2001). The method can be used to obtain detailed information of the organic and inorganic composition of samples at a spatial resolution down to <1 µm. It is a highly surface-sensitive method, providing chemical information about the uppermost molecular layers only. The analysis is based on mass spectrometry and requires no preselection or labeling of the substances to be analyzed.

In a ToF-SIMS measurement, the sample is bombarded by a pulsed, focused, high-energy ion beam (primary ions) that causes atoms, molecular fragments, and intact molecules to be ejected from the sample surface. A small fraction of the ejected particles will be ionized (secondary ions). The secondary ions are extracted and directed into a time-of-flight (ToF) mass analyzer, where they are separated with respect to mass to charge ratio. The resulting mass spectrum represents a chemical fingerprint from the sample surface at the point of impact of the primary ion beam (Figure 1).

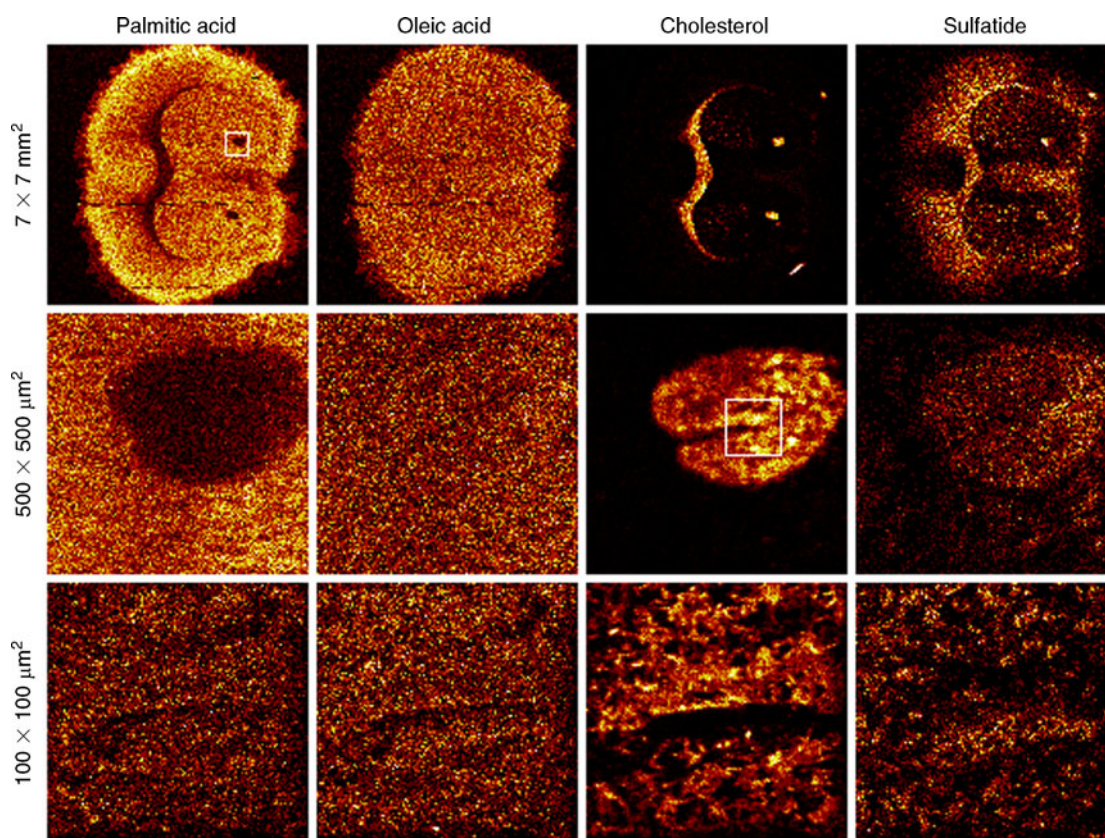
Characterization of the chemical substances present on the sample surface is done by identification of the ions corresponding to the peaks in the mass spectrum, in a similar way as in other mass spectrometric techniques, such as GC/MS and LC/MS. The high mass resolution of the ToF analyzer (typically $m/\Delta m \sim 5,000\text{--}10,000$) allows for detailed identification of the secondary ions.

The spatial information, i.e., the lateral distribution of the different chemical components on the sample surface, is obtained by scanning the primary ion beam over the sample surface and recording separate mass spectra from a large number of points (pixels) within the analysis area. The acquired data can be presented in different ways: (a) as mass spectra of the total analysis area or selected regions of interest, (b) as ion images, in which the signal intensity distribution from ions representing specific elements or compounds are displayed as variations in brightness over the analysis area (see Figures 1 and 2), or (c) as time evolutions of specific ion signal intensities during ion etching using a separate ion beam (depth profiling).

An important advantage of the ToF-SIMS technique, in relation to other SIMS methods, is that the ToF mass analyzer allows for so-called static SIMS analysis, i.e., analysis of the undamaged surface before the mass spectrum has been significantly altered by the molecular damage caused by the primary ions. In dynamic SIMS, the sample surface is subjected to considerably higher primary ion dose



ToF-SIMS, Figure 1 Schematic illustration of the ToF-SIMS analysis technique.



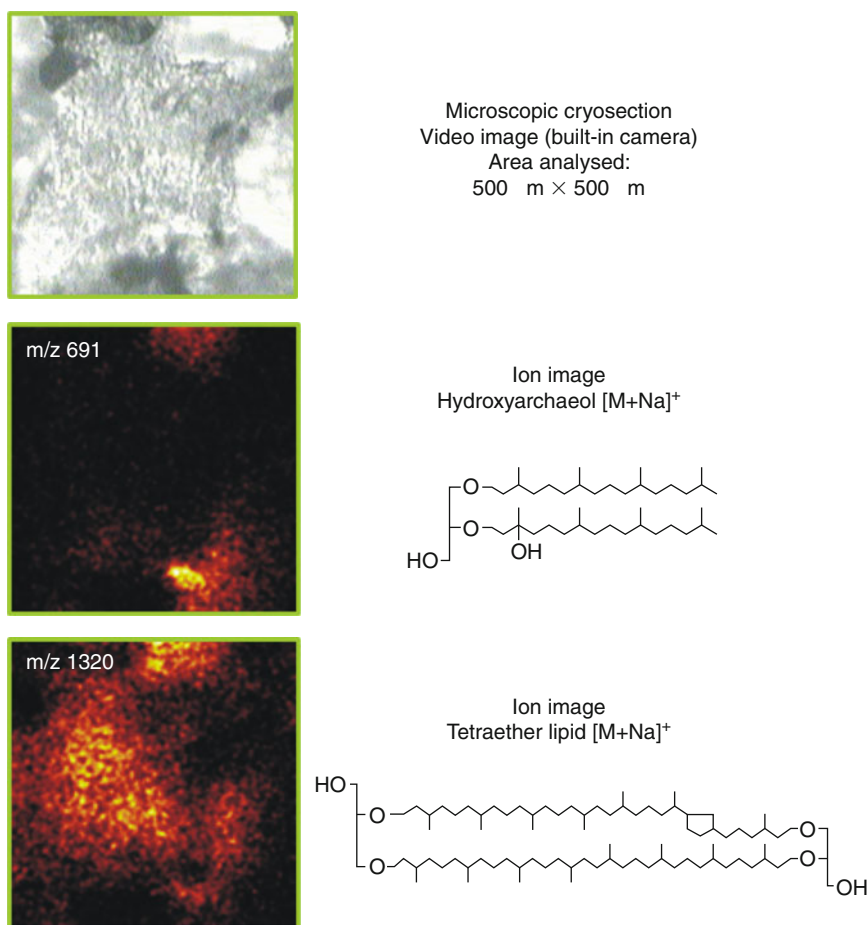
ToF-SIMS, Figure 2 Examples of ion images from ToF-SIMS analysis of a freeze-dried mouse brain tissue section. The images show the signal intensity distributions of secondary ions specific to four different lipids at three different magnification levels. The inserted squares indicate the analysis area at the next magnification level.

densities, which means that the analysis is done on a molecularly damaged and continuously eroding surface. As a consequence, ToF-SIMS is capable of providing detailed molecular information of, e.g., organic substances (up to around 5,000 Da), while dynamic SIMS is limited to

information on elements, isotopes, and small fragments on the surface.

For organic microanalysis using ToF-SIMS, the use of cluster ions, such as Bi_n^+ , $n = 3-7$, Au_3^+ , and C_{60}^+ , as primary ions has proven advantageous because

ToF-SIMS analysis of a microbial mat (microscopic cryosection)



ToF-SIMS, Figure 3 Ion images from ToF-SIMS analysis of a microbial mat cryosection retrieved from a methane seep in the Black Sea. The images show the signal intensity distributions of secondary ions of two different lipid biomarkers from methanotrophic archaea thriving in separate colonies within the microbial mat (ANME-1, tetraether lipids; ANME-2, hydroxyarchaeol, see chapters *Anaerobic Oxidation of Methane with Sulfate* and *Biomarkers (Organic, Compound-Specific Isotopes)*). Modified after Thiel et al. (2007a).

these ions produce high secondary ion yields for large (characteristic) organic ions in organic materials (Kollmer, 2004). In addition, it has been shown that some molecular information is retained in organic materials also after extensive bombardment by C_{60}^+ ions, making this ion suitable for ion etching (depth profiling) of organic materials (Wucher et al., 2007).

These capabilities make ToF-SIMS a promising tool for the investigation of organic biomarkers in different types of geobiological materials, particularly if only small sample amounts are available, or a high lateral resolution is desired. Examples are mineral phases in microbialites or microscopic samples (i.e., sections) of microbial mats (Figure 3; Thiel et al., 2007a, b; Sjövall et al., 2008). Furthermore, after hopanes and steranes have been successfully detected in different types of crude oils using ToF-SIMS (Sjövall et al., 2008; Siljeström

et al., 2009), the identification of authentic biomarkers in single oil inclusions of ancient rocks now appears to be within reach (Siljeström et al., 2010).

Bibliography

- Belu, A., Graham, D. J., and Castner, D. G., 2003. Time-of-flight secondary ion mass spectrometry: techniques and applications for the characterization of biomaterial surfaces. *Biomaterials*, **24**, 3635–3653.
- Benninghoven, A., 1994. Chemical analysis of inorganic and organic surfaces and thin films by static time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Angewandte Chemie International Edition in English*, **33**, 1023–1043.
- Kollmer, F., 2004. Cluster primary ion bombardment of organic materials. *Applied Surface Science*, **231–232**, 153–158.
- Siljeström, S., Hode, T., Lausmaa, J., Sjövall, P., Toporski, J., and Thiel, V., 2009. Detection of organic biomarkers in crude oils using ToF-SIMS. *Organic Geochemistry*, **40**, 135–143.

- Siljeström, S., Lausmaa, J., Sjövall, P., Broman, C., Thiel, V., and Hode, T., 2010. Analysis of hopanes and steranes in single oil-bearing fluid inclusions using time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Geobiology*, **8**, 37–44.
- Sjövall, P., Thiel, V., Siljeström, S., Heim, C., Hode, T., and Lausmaa, J., 2008. Organic geochemical microanalysis by time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Geostandards and Geoanalytical Research*, **32**, 267–277.
- Thiel, V., Heim, C., Arp, G., Hahmann, U., Sjövall, P., and Lausmaa, J., 2007a. Biomarkers at the microscopic range: ToF-SIMS molecular imaging of Archaea-derived lipids in a microbial mat. *Geobiology*, **5**, 413–421.
- Thiel, V., Toporski, J., Schumann, G., Sjövall, P., and Lausmaa, J., 2007b. Analysis of archaeal core ether lipids using Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS): exploring a new prospect for the study of biomarkers in geobiology. *Geobiology*, **5**, 75–83.
- Vickerman, J. C., and Briggs, D. (eds.), 2001. *ToF-SIMS: Surface Analysis by Mass Spectrometry*, Charlton, Chichester, West Sussex, UK: IM Publications and SurfaceSpectra Limited.
- Wucher, A., Cheng, J., and Winograd, N., 2007. Protocols for three-dimensional molecular imaging using mass spectrometry. *Analytical Chemistry*, **79**, 5529–5539.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Cold Seeps](#)
[Microbial Communities, Structure, and Function](#)

TRACE FOSSILS: NEOPROTEROZOIC

Sören Jensen¹, James G. Gehling², Mary L. Droser³

¹Universidad de Extremadura, Badajoz, Spain

²South Australian Museum, Adelaide, SA, Australia

³University of California, Riverside, CA, USA

Synonyms

Ichnofossils; Neoproterozoic

Definition

Neoproterozoic trace fossils give us the earliest signs of animals moving on or just below the sea floor typically in the form of small horizontal unbranched trails and furrows. Despite their modest appearances, these trace fossils are important in that they provide an undisputable minimum age for the appearance of bilaterian animals. Remarkable are the recent discoveries of the Ediacaran organisms, *Kimberella* and *Dickinsonia*, in association with their trace fossils. These associations suggest exploitation of microbial mats, and a similar behavior probably explains also the simple horizontal Neoproterozoic trace fossils.

Introduction

Much of the Neoproterozoic sedimentary record is without the sign of burrows, trails, or other types of animal

trace fossils. A continuous record of widely accepted trace fossil appear only after about 560 Ma ago (Figure 1), and by comparison to the Phanerozoic the diversity and complexity is low and the size and depth of bioturbation are modest and generally considered in terms of a few millimeters. Indeed, though the transition between Ediacaran and Phanerozoic ichnofossil assemblages is somewhat gradual, the difference is nevertheless striking, notably in what is missing in the former. The Ediacaran lacks any evidence of trace fossils produced by animals with arthropod-type appendages, and traces showing adjustment to sedimentation (spreiten). There is little evidence for permanently maintained open burrows, and there are essentially no complex burrow systems. The Ediacaran trace fossil record is almost exclusively known from shallow-water marine deposits. Temporal trends in Ediacaran trace fossils are as yet tentative, but it can be noted that no trace fossils are currently known from the oldest associations of Ediacara-type fossils and that the first three-dimensional burrow systems, appearing shortly before the Ediacaran–Cambrian boundary, overlapping the range of some of the youngest Ediacara-type fossils (Figure 1). Among early studies, that of Seilacher (1956) was one of the first papers in which the contrasting nature of Proterozoic and Phanerozoic trace fossils was clearly stated, and the broad synthesis of Crimes (1987) provided a summary of the literature to date. The paper by Narbonne et al. (1987) outlined the Ediacaran–Cambrian sequence of trace fossils in sections in Newfoundland and set the stage for the subsequent definition of the base of the Cambrian on the basis of trace fossils in this region. Recent overviews and discussions of Ediacaran trace fossils, where the reader can find additional references to earlier papers, can be found in Jensen (2003), Droser et al. (2005), Seilacher et al. (2005), Jensen et al. (2006), Weber et al. (2007), Seilacher (2007), and Fedonkin and Vickers-Rich (2007). Currently, one of the major areas of study of Ediacaran trace fossils is, perhaps somewhat surprisingly, in deciding what is and what is not a trace fossil. In this brief overview, we will consider Ediacaran trace fossils under three headings.

Ediacaran trace fossils: true and false

Traditional trace fossils: Meaning trace fossils that can reasonably be assigned to ichnotaxa also described from the Phanerozoic. The most common Ediacaran trace fossils are essentially horizontal, unbranched, and with few exceptions small (a few millimeters wide), grooves or ridges on the upper or lower surface of sandstone beds (Figure 2c and d). These trace fossils, which are generally referred to as *Helminthoidichnites*, represent transitional movement through the sediment, close to the sediment–water interface, and there may be lateral raised ridges where sediment was pressed to the sides (e.g., Droser et al., 2005). They have been interpreted as mining of decaying microbial mats (Seilacher, 1999). Other possible Ediacaran trace fossils include

- Siljeström, S., Lausmaa, J., Sjövall, P., Broman, C., Thiel, V., and Hode, T., 2010. Analysis of hopanes and steranes in single oil-bearing fluid inclusions using time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Geobiology*, **8**, 37–44.
- Sjövall, P., Thiel, V., Siljeström, S., Heim, C., Hode, T., and Lausmaa, J., 2008. Organic geochemical microanalysis by time-of-flight secondary ion mass spectrometry (ToF-SIMS). *Geostandards and Geoanalytical Research*, **32**, 267–277.
- Thiel, V., Heim, C., Arp, G., Hahmann, U., Sjövall, P., and Lausmaa, J., 2007a. Biomarkers at the microscopic range: ToF-SIMS molecular imaging of Archaea-derived lipids in a microbial mat. *Geobiology*, **5**, 413–421.
- Thiel, V., Toporski, J., Schumann, G., Sjövall, P., and Lausmaa, J., 2007b. Analysis of archaeal core ether lipids using Time of Flight - Secondary Ion Mass Spectrometry (ToF-SIMS): exploring a new prospect for the study of biomarkers in geobiology. *Geobiology*, **5**, 75–83.
- Vickerman, J. C., and Briggs, D. (eds.), 2001. *ToF-SIMS: Surface Analysis by Mass Spectrometry*, Charlton, Chichester, West Sussex, UK: IM Publications and SurfaceSpectra Limited.
- Wucher, A., Cheng, J., and Winograd, N., 2007. Protocols for three-dimensional molecular imaging using mass spectrometry. *Analytical Chemistry*, **79**, 5529–5539.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Cold Seeps](#)
[Microbial Communities, Structure, and Function](#)

TRACE FOSSILS: NEOPROTEROZOIC

Sören Jensen¹, James G. Gehling², Mary L. Droser³

¹Universidad de Extremadura, Badajoz, Spain

²South Australian Museum, Adelaide, SA, Australia

³University of California, Riverside, CA, USA

Synonyms

Ichnofossils; Neoproterozoic

Definition

Neoproterozoic trace fossils give us the earliest signs of animals moving on or just below the sea floor typically in the form of small horizontal unbranched trails and furrows. Despite their modest appearances, these trace fossils are important in that they provide an undisputable minimum age for the appearance of bilaterian animals. Remarkable are the recent discoveries of the Ediacaran organisms, *Kimberella* and *Dickinsonia*, in association with their trace fossils. These associations suggest exploitation of microbial mats, and a similar behavior probably explains also the simple horizontal Neoproterozoic trace fossils.

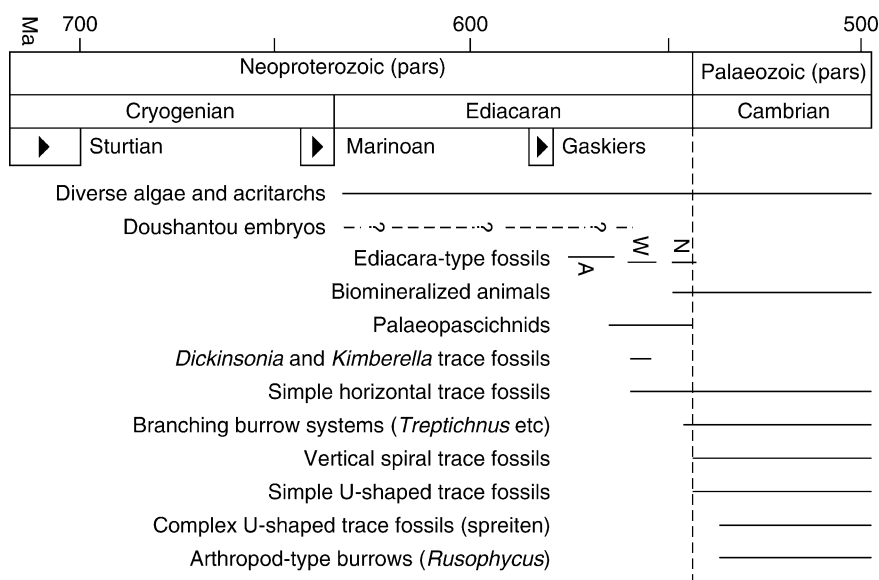
Introduction

Much of the Neoproterozoic sedimentary record is without the sign of burrows, trails, or other types of animal

trace fossils. A continuous record of widely accepted trace fossil appear only after about 560 Ma ago (Figure 1), and by comparison to the Phanerozoic the diversity and complexity is low and the size and depth of bioturbation are modest and generally considered in terms of a few millimeters. Indeed, though the transition between Ediacaran and Phanerozoic ichnofossil assemblages is somewhat gradual, the difference is nevertheless striking, notably in what is missing in the former. The Ediacaran lacks any evidence of trace fossils produced by animals with arthropod-type appendages, and traces showing adjustment to sedimentation (spreiten). There is little evidence for permanently maintained open burrows, and there are essentially no complex burrow systems. The Ediacaran trace fossil record is almost exclusively known from shallow-water marine deposits. Temporal trends in Ediacaran trace fossils are as yet tentative, but it can be noted that no trace fossils are currently known from the oldest associations of Ediacara-type fossils and that the first three-dimensional burrow systems, appearing shortly before the Ediacaran–Cambrian boundary, overlapping the range of some of the youngest Ediacara-type fossils (Figure 1). Among early studies, that of Seilacher (1956) was one of the first papers in which the contrasting nature of Proterozoic and Phanerozoic trace fossils was clearly stated, and the broad synthesis of Crimes (1987) provided a summary of the literature to date. The paper by Narbonne et al. (1987) outlined the Ediacaran–Cambrian sequence of trace fossils in sections in Newfoundland and set the stage for the subsequent definition of the base of the Cambrian on the basis of trace fossils in this region. Recent overviews and discussions of Ediacaran trace fossils, where the reader can find additional references to earlier papers, can be found in Jensen (2003), Droser et al. (2005), Seilacher et al. (2005), Jensen et al. (2006), Weber et al. (2007), Seilacher (2007), and Fedonkin and Vickers-Rich (2007). Currently, one of the major areas of study of Ediacaran trace fossils is, perhaps somewhat surprisingly, in deciding what is and what is not a trace fossil. In this brief overview, we will consider Ediacaran trace fossils under three headings.

Ediacaran trace fossils: true and false

Traditional trace fossils: Meaning trace fossils that can reasonably be assigned to ichnotaxa also described from the Phanerozoic. The most common Ediacaran trace fossils are essentially horizontal, unbranched, and with few exceptions small (a few millimeters wide), grooves or ridges on the upper or lower surface of sandstone beds (Figure 2c and d). These trace fossils, which are generally referred to as *Helminthoidichnites*, represent transitional movement through the sediment, close to the sediment–water interface, and there may be lateral raised ridges where sediment was pressed to the sides (e.g., Droser et al., 2005). They have been interpreted as mining of decaying microbial mats (Seilacher, 1999). Other possible Ediacaran trace fossils include



Trace Fossils: Neoproterozoic, Figure 1 Schematic chart to show the approximate temporal distribution of trace fossils and selected other groups of fossils during the Ediacaran and early Cambrian. Only a selection of Cambrian trace fossil types is depicted. Note that trace fossils are only known from the latest part of the Neoproterozoic, and postdate the Gaskiers glaciation. The age of Doushantuo embryos, currently the oldest direct evidence for probable animals, is uncertain. Approximate position of glaciations is indicated by boxes with filled triangle. Abbreviations: A, Avalon association; N, Nama association; W, White Sea association.

small star-shaped forms and simple vertical tubes, but these are rare.

Ediacara-type organisms associated with their trace fossils: These remarkable fossils are known from northern Russia and South Australia. The possibly mollusc-grade *Kimberella* has been found associated with radial fan-shaped structures (Figure 2e and f) apparently representing raspings as well as possible traces of movement (Fedonkin et al., 2007). *Dickinsonia* has been found associated with disjunct rows of imprints (Figure 2g), perhaps representing pulses of degradation of a microbial mat (Gehling et al., 2005). These associations are all the more remarkable because associations of trace fossils with their producers are exceptionally rare in the Phanerozoic in normal marine settings.

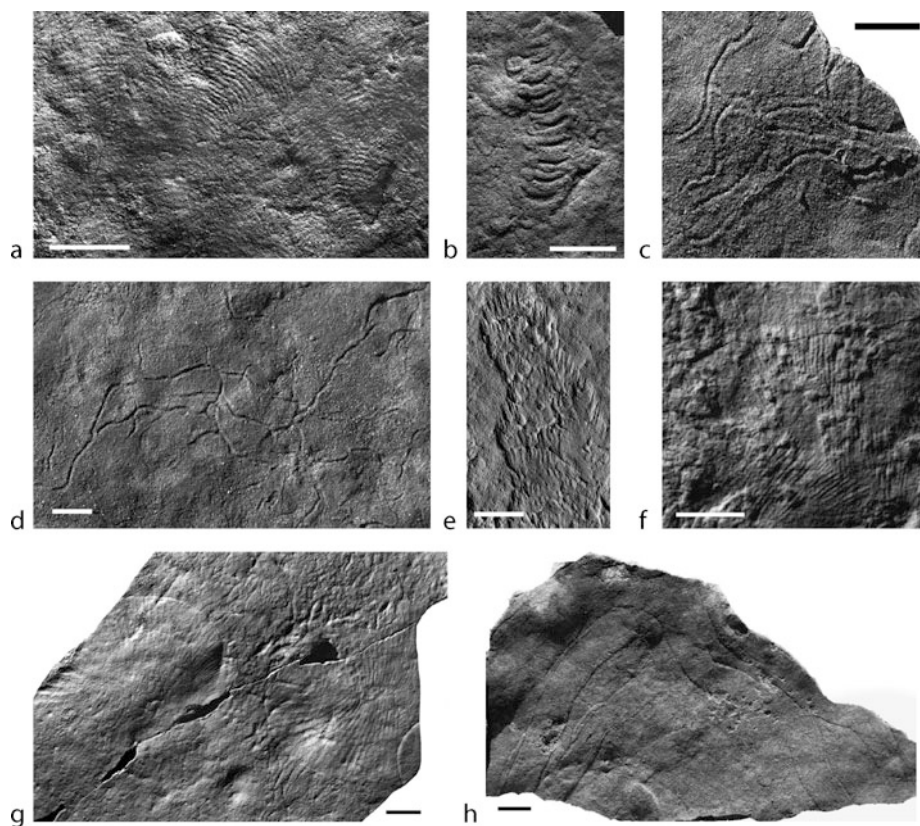
False and doubtful Neoproterozoic trace fossils: The list of purported Ediacaran trace fossils is in fact quite extensive but many of these are now believed to be better explained as body fossils (Seilacher et al., 2005; Droser et al., 2005; Jensen et al., 2006). Common examples are *Yelovichnus* and *Palaeopasichnus*, which consist of short series of closely spaced kidney- or sausage-shaped objects (Figure 2a and b). Rather than trace fossils, these long-ranging forms, may have been algal or xenophyophore protists (Seilacher et al., 2005). Other supposed trace fossils represent the three-dimensional preservation of tubular organisms (Figure 2h) (Droser and Gehling, 2008). The evaluation of some of these purported Ediacaran trace fossil remains problematic. For example, *Nenoxites* might be an early example of a back-filled burrow (Seilacher et al., 2005). It is, however, clear that a considerable

non-trace fossil Ediacaran organismal diversity is hidden here.

Broader significance of Neoproterozoic trace fossils

Sediment properties and paleoecology: The rise of bioturbation had dramatic effects on sediment properties as well as on seafloor ecology, as entailed in the agronomic revolution or Cambrian substrate revolution (Seilacher, 1999; Bottjer et al., 2000). The gradual appearance of deeper and more intense bioturbation and by larger animals that took place during the late Ediacaran and early Paleozoic led to a shift from a seafloor largely covered with microbial mats and with a sharp sediment–water interface to a biogeochemically more active and deeper zone of surface sediments (McIlroy and Logan, 1999; Droser et al., 2002; Meysman et al., 2006). Many late Ediacaran and early Cambrian organisms may have had a lifestyle adapted to mat grounds (including the early trace fossil-makers) and a relatively firm muddy sea floor and these would have been negatively affected by the rise of bioturbation (Seilacher, 1999; Bottjer et al., 2000; Dornbos, 2006).

Evolution of metazoans: The Ediacaran trace fossil record is important in the discussion of the appearance of metazoans and in particular that of mobile animals. An obvious but important point is that the preservation potential of a trace fossil is decoupled from that of its producer. Thus, one line of argument goes that the appearance of trace fossils is unlikely to substantially postdate the appearance of mobile benthic animals and that the



Trace Fossils: Neoproterozoic, Figure 2 Ediacaran trace fossils (c–g) and trace fossil-like organisms (a, b, and h) (a) *Yelovichnus*, branching sets of collapsed, crescentic-tubes (b) *Palaeopascichnus*, a serial set of lozenge-shaped tubes with collapsed centers (c and d) *Heminthoidichnites*, groove trails with levees; junctions represent level changes by one trail (e and f) Casts of fan-shaped sets of bifid rasping traces in a microbial mat (g) Low-relief casts of two serial resting traces made by a single specimen of *Dickinsonia costata* (d) Composite casts of large, partly filled, tubular fossils. All from the Ediacara Member of South Australia. Scale bars correspond to 2 cm. Except for (c) and (d), all fossils are on bed soles.

radiation of bilaterian animals would have occurred in animals of a size that would readily leave a trace fossil record (Budd and Jensen, 2000; Jensen et al., 2005). The late Ediacaran appearance of trace fossils and the subsequent radiation at the Ediacaran–Cambrian boundary fits in with evidence from diverse fossil groups (and at least some molecular clock studies) of the appearance of increasingly diverse ecosystems following the Marinoan glaciation (Peterson and Butterfield, 2005; Butterfield, 2007) (Figure 1). Organisms other than metazoans are potential trace fossil makers (Matz et al., 2008) but the Ediacaran to Cambrian trace fossil diversification can only be explained as being part of a metazoan radiation. It is not known to what extent the types of trace fossils found in the Ediacaran was the result of limited neurological sophistication, limited capability to burrow deeply and/or withstand anoxic sediments or if the type of trace fossil largely reflect a particular type of feeding adapted to microbial mats in an environment with little competition. It has been suggested that the appearance of deeper burrows were a response to increased predation (e.g., Dzik, 2005).

Summary

Animal trace fossils first appear close to the end of the Neoproterozoic era typically in the form of simple horizontal burrows or trails, a few millimeters wide; although, the exact diversity of Ediacaran trace fossils is currently a hot topic. Particularly noteworthy is the occurrence of trace fossils made by the Ediacara-type organisms *Dickinsonia* and *Kimberella*. The appearance of Neoproterozoic trace fossils and their subsequent radiation at the Ediacaran–Cambrian transition most likely correspond to the diversification of animals and ecosystems at this time.

The rise of bioturbation probably played an important role in structuring early benthic ecosystems.

Bibliography

(The list of reference is heavily biased toward the most recent literature, including recent reviews. Important earlier studies can be found by consulting these papers.)
 Bottjer, D. J., Hagadorn, J. W., and Dornbos, S. Q., 2000. The Cambrian substrate revolution. *GSA Today*, **10**, 1–7.

- Budd, G. E., and Jensen, S., 2000. A critical reappraisal of the fossil record of the bilaterian phyla. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 253–295.
- Butterfield, N. J., 2007. Macroevolution and macroecology through deep time. *Palaeontology*, **20**, 41–55.
- Crimes, T. P., 1987. Trace fossils and correlation of late Precambrian and early Cambrian strata. *Geological Magazine*, **124**, 97–119.
- Dornbos, S. Q., 2006. Evolutionary palaeoecology of early epifaunal echinoderms: response to increasing bioturbation levels during the Cambrian radiation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 225–239.
- Droser, M. L., Jensen, S., and Gehling, J. G., 2002. Trace fossils and substrates of the terminal Proterozoic–Cambrian transition: implications for the record of early bilaterians and sediment mixing. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 12572–12576.
- Droser, M. L., Gehling, J. G., and Jensen, S., 2005. Ediacaran trace fossils: true and false. In Briggs, D. E. G. (ed.), *Evolving Form and Function: Fossils and Development*. New Haven: Yale Peabody Museum, pp. 125–138.
- Droser, M. L., and Gehling, J. G., 2008. Synchronous aggregate growth in an abundant new Ediacaran tubular organism. *Science*, **319**, 1660–1662.
- Dzik, J., 2005. Behavioral and anatomical unity of the earliest burrowing animals and the cause of the “Cambrian explosion”. *Paleobiology*, **31**, 503–521.
- Fedonkin, M. A., and Vickers-Rich, P., 2007. First traces of motion. In *The Rise of Animals: Evolution and Diversification of the Kingdom Animalia*. Baltimore: John Hopkins University Press, pp. 205–217.
- Fedonkin, M. A., Simonetta, A., and Ivantsov, A. Y., 2007. New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. In Vickers-Rich, P., and Komarow, P. (eds.), *The Rise and Fall of the Ediacaran Biota*. London: Geological Society Special Publications, 286, pp. 157–179.
- Gehling, J. G., Droser, M. L., Jensen, S., and Runnegar, B. N., 2005. Ediacara organisms: relating form to function. In Briggs, D. E. G. (ed.), *Evolving Form and Function: Fossils and Development*. New Haven: Yale Peabody Museum, pp. 43–66.
- Jensen, S., 2003. The Proterozoic and Earliest Cambrian trace fossil record: patterns, problems and perspectives. *Integrative and Comparative Biology*, **43**, 219–228.
- Jensen, S., Droser, M. L., and Gehling, J. G., 2005. Trace fossil preservation and the early evolution of animals. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **220**, 19–29.
- Jensen, S., Droser, M. L., and Gehling, J. G., 2006. A critical look at the Ediacaran trace fossil record. In Xiao, S., and Kaufman, J. K. (eds.), *Neoproterozoic Geobiology and Paleobiology*. Dordrecht: Springer, pp. 115–157.
- Matz, M. V., Frank, T. M., Marshall, N. J., Widder, E. A., and Johnsen, S., 2008. Giant deep-sea protist produce bilaterian-like traces. *Current Biology*, **18**, 1–6.
- McIlroy, D., and Logan, G. A., 1999. The impact of bioturbation on infaunal ecology and evolution during the Proterozoic–Cambrian transition. *Palaaios*, **14**, 58–72.
- Meysman, F. J. R., Middelburg, J. J., and Heip, C. H. R., 2006. Bioturbation: a fresh look at Darwin’s last idea. *Trends in Ecology and Evolution*, **21**, 688–695.
- Narbonne, G. M., Myrow, P., Landing, E., and Anderson, M. A., 1987. A candidate stratotype for the Precambrian–Cambrian boundary, Fortune Head, Burin Peninsula, southeastern Newfoundland. *Canadian Journal of Earth Sciences*, **24**, 1277–1293.
- Peterson, K. J., and Butterfield, N. J., 2005. Origin of the eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 9547–9552.
- Seilacher, A., 1956. Der Beginn des Kambriums als biologische Wende. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **103**, 155–180.
- Seilacher, A., 1999. Biomat-related lifestyle in the Precambrian. *Palaaios*, **14**, 86–93.
- Seilacher, A., 2007. *Trace Fossil Analysis*. Berlin: Springer.
- Seilacher, A., Buatois, L. A., and Mangano, M. G., 2005. Trace fossils in the Ediacaran–Cambrian transition: behavioral diversification, ecological turnover and environmental shift. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 323–356.
- Weber, B., Steiner, M., and Zhu, M. Y., 2007. Precambrian–Cambrian trace fossils from the Yangtze Platform (South China) and the early evolution of bilaterian lifestyles. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 328–349.

Cross-references

[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Microbial Mats](#)
[Origins of the Metazoa](#)

TUFA, FRESHWATER

Akihiro Kano
 Kyushu University, Fukuoka, Japan

Synonyms

Travertines (in definition of Pentecost, 2005)

Definition

Tufas are calcareous deposits formed by inorganic or microbiologically induced precipitation of calcium carbonate in a wide range of freshwater environments, including streams, rivers, and lakes. Calcium carbonate precipitates principally on the tufa surface, and the term excludes lacustrine marl in which carbonate particles are formed in water column. Tufas are commonly developed in limestone areas from tropical to temperate climatic zones (Pentecost, 1995) showing a variety of geometry from a huge riverine barrage to a small mound developed just beside water springs (Pedley, 1990). Following the definitions of Ford and Pedley (1996) that tufas are carbonate deposited in cool or near ambient temperature freshwater and in open-air conditions, the term excludes carbonate deposits in hydrothermal water (travertines) and in a limestone cave (speleothems). Some workers prefer the definition of the term carrying textural implication because the term tufa was originally derived from a Greek word *tophos* which refers porous deposits, such as carbonate tufa and volcanic tuff.

- Budd, G. E., and Jensen, S., 2000. A critical reappraisal of the fossil record of the bilaterian phyla. *Biological Reviews of the Cambridge Philosophical Society*, **75**, 253–295.
- Butterfield, N. J., 2007. Macroevolution and macroecology through deep time. *Palaeontology*, **20**, 41–55.
- Crimes, T. P., 1987. Trace fossils and correlation of late Precambrian and early Cambrian strata. *Geological Magazine*, **124**, 97–119.
- Dornbos, S. Q., 2006. Evolutionary palaeoecology of early epifaunal echinoderms: response to increasing bioturbation levels during the Cambrian radiation. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **237**, 225–239.
- Droser, M. L., Jensen, S., and Gehling, J. G., 2002. Trace fossils and substrates of the terminal Proterozoic–Cambrian transition: implications for the record of early bilaterians and sediment mixing. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 12572–12576.
- Droser, M. L., Gehling, J. G., and Jensen, S., 2005. Ediacaran trace fossils: true and false. In Briggs, D. E. G. (ed.), *Evolving Form and Function: Fossils and Development*. New Haven: Yale Peabody Museum, pp. 125–138.
- Droser, M. L., and Gehling, J. G., 2008. Synchronous aggregate growth in an abundant new Ediacaran tubular organism. *Science*, **319**, 1660–1662.
- Dzik, J., 2005. Behavioral and anatomical unity of the earliest burrowing animals and the cause of the “Cambrian explosion”. *Paleobiology*, **31**, 503–521.
- Fedonkin, M. A., and Vickers-Rich, P., 2007. First traces of motion. In *The Rise of Animals: Evolution and Diversification of the Kingdom Animalia*. Baltimore: John Hopkins University Press, pp. 205–217.
- Fedonkin, M. A., Simonetta, A., and Ivantsov, A. Y., 2007. New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. In Vickers-Rich, P., and Komarow, P. (eds.), *The Rise and Fall of the Ediacaran Biota*. London: Geological Society Special Publications, 286, pp. 157–179.
- Gehling, J. G., Droser, M. L., Jensen, S., and Runnegar, B. N., 2005. Ediacara organisms: relating form to function. In Briggs, D. E. G. (ed.), *Evolving Form and Function: Fossils and Development*. New Haven: Yale Peabody Museum, pp. 43–66.
- Jensen, S., 2003. The Proterozoic and Earliest Cambrian trace fossil record: patterns, problems and perspectives. *Integrative and Comparative Biology*, **43**, 219–228.
- Jensen, S., Droser, M. L., and Gehling, J. G., 2005. Trace fossil preservation and the early evolution of animals. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **220**, 19–29.
- Jensen, S., Droser, M. L., and Gehling, J. G., 2006. A critical look at the Ediacaran trace fossil record. In Xiao, S., and Kaufman, J. K. (eds.), *Neoproterozoic Geobiology and Paleobiology*. Dordrecht: Springer, pp. 115–157.
- Matz, M. V., Frank, T. M., Marshall, N. J., Widder, E. A., and Johnsen, S., 2008. Giant deep-sea protist produce bilaterian-like traces. *Current Biology*, **18**, 1–6.
- McIlroy, D., and Logan, G. A., 1999. The impact of bioturbation on infaunal ecology and evolution during the Proterozoic–Cambrian transition. *Palaaios*, **14**, 58–72.
- Meysman, F. J. R., Middelburg, J. J., and Heip, C. H. R., 2006. Bioturbation: a fresh look at Darwin’s last idea. *Trends in Ecology and Evolution*, **21**, 688–695.
- Narbonne, G. M., Myrow, P., Landing, E., and Anderson, M. A., 1987. A candidate stratotype for the Precambrian–Cambrian boundary, Fortune Head, Burin Peninsula, southeastern Newfoundland. *Canadian Journal of Earth Sciences*, **24**, 1277–1293.
- Peterson, K. J., and Butterfield, N. J., 2005. Origin of the eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 9547–9552.
- Seilacher, A., 1956. Der Beginn des Kambriums als biologische Wende. *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **103**, 155–180.
- Seilacher, A., 1999. Biomat-related lifestyle in the Precambrian. *Palaaios*, **14**, 86–93.
- Seilacher, A., 2007. *Trace Fossil Analysis*. Berlin: Springer.
- Seilacher, A., Buatois, L. A., and Mangano, M. G., 2005. Trace fossils in the Ediacaran–Cambrian transition: behavioral diversification, ecological turnover and environmental shift. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **227**, 323–356.
- Weber, B., Steiner, M., and Zhu, M. Y., 2007. Precambrian–Cambrian trace fossils from the Yangtze Platform (South China) and the early evolution of bilaterian lifestyles. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **254**, 328–349.

Cross-references

[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Microbial Mats](#)
[Origins of the Metazoa](#)

TUFA, FRESHWATER

Akihiro Kano
 Kyushu University, Fukuoka, Japan

Synonyms

Travertines (in definition of Pentecost, 2005)

Definition

Tufas are calcareous deposits formed by inorganic or microbiologically induced precipitation of calcium carbonate in a wide range of freshwater environments, including streams, rivers, and lakes. Calcium carbonate precipitates principally on the tufa surface, and the term excludes lacustrine marl in which carbonate particles are formed in water column. Tufas are commonly developed in limestone areas from tropical to temperate climatic zones (Pentecost, 1995) showing a variety of geometry from a huge riverine barrage to a small mound developed just beside water springs (Pedley, 1990). Following the definitions of Ford and Pedley (1996) that tufas are carbonate deposited in cool or near ambient temperature freshwater and in open-air conditions, the term excludes carbonate deposits in hydrothermal water (travertines) and in a limestone cave (speleothems). Some workers prefer the definition of the term carrying textural implication because the term tufa was originally derived from a Greek word *tophos* which refers porous deposits, such as carbonate tufa and volcanic tuff.

Processes

Water bearing the carbonate precipitation normally originates from an underground water system in carbonate rock where relatively high $p\text{CO}_2$ raises the concentration of dissolved calcium carbonate, but rarely from the mixture of hydrothermal water that contains a substantial amount of calcium and bicarbonate (Yoshimura et al., 2004). Tufa deposition requires supersaturation of water with respect to calcium carbonates, which are essentially achieved by CO_2 degassing from the water due to a large difference in $p\text{CO}_2$ between underground water and the atmosphere. The water actively degasses at sites where waterfalls or other obstructions along the passage cause water turbulence, and reaches high supersaturation (~ 10 times; Chen et al., 2004). Preferential deposition at the sites of high water turbulence often develops tufa mounds accompanied with water pools at the upcurrent side (Pedley, 1990). A tufa can grow at a much higher rate (typically several millimeter/year; Kano et al., 2003) than speleothems. However, continuous tufa growth has seldom lasted longer than 100 years because growth of the mounds changes watercourse in normal fluvial settings. Because of the circumstantial temperature and relatively low value of dissolved Mg/Ca, mineralogy of the most tufas is calcite.

Because tufas form outside in the light, unlike speleothems, phototrophic microbes (cyanobacteria, freshwater algae, and diatoms), and mosses normally live on the surface. The microbes form biofilms and influence carbonate precipitation rate and depositional textures of tufa. Especially, cyanobacteria are most common and have been considered to activate tufa deposition by photosynthetic uptake of CO_2 and bicarbonate that raises supersaturation of calcium carbonate (Mert, 1992), or by providing nucleation site of carbonate minerals (Pentecost and Riding, 1986). In such tufas, the calcite is deposited as fine-grained ($5\sim 10\ \mu\text{m}$ diameter) crystals encrusting a cyanobacteria sheath, which results in porous texture typically containing 50% of porosity (Kano and Fujii, 2000).

Annual lamination

Some tufas develop annual laminations consisting of both densely calcified and porous layers. Two different explanations have been proposed to account for the annual laminations. One is a seasonal change in the predominant microbial association seen in the biofilms covering the tufa surface (Freytet and Plet, 1996; Janssen et al., 1999; Arp et al., 2001). The tufa in the Franconian Alps, Germany, exhibits porous layers formed in winter–spring when diatom-dominated biofilm inhibits carbonate deposition on the tufa surface (Arp et al., 2001). However, the seasonal change in microbial association cannot always account for the lamination of some tufas, for instance, the ones from SW Japan dominated by cyanobacteria communities throughout the year (Kano et al., 2003). The annual laminations can be developed

due to seasonal changes in the inorganic calcite precipitation rate (Kano et al., 2003). Under a high rate during summer–autumn, the calcite crystals thickly encrust the cyanobacterial sheath until they become attached to neighboring encrusted sheathes and form a dense layer. In contrast, a low rate during winter–autumn results in the thinly encrusted sheathes and leaves abundant free space in a porous layer.

Climatic archive

Stable isotopes and trace elements of carbonate fraction of tufa deposits have been recently used for Holocene climatic studies (Zak et al., 2002; Makhnach et al., 2004; Garnett et al., 2004). Bulk $\delta^{18}\text{O}$ values primarily record latitude and altitude control on $\delta^{18}\text{O}$ of meteoric water (Andrews et al., 1997); although, evaporation largely affects the values in semiarid regions (Smith et al., 2004). On the other hand, $\delta^{13}\text{C}$ values are influenced by variable conditions including degassing of ^{12}C -enriched CO_2 (Pentecost, and Spiro, 1990), contribution of soil CO_2 (Hori et al., 2008), and vegetation (C3 vs. C4 plant; Kano et al., 2007), and therefore the interpretation is often problematic.

Because of their high depositional rate and development of annual lamination, tufas are potential archives of high-resolution paleoclimatic records (Andrews and Brasier, 2005; Andrews, 2006). Studies of modern specimens have reported cyclic changes in $\delta^{18}\text{O}$ and trace elements in tufa calcite that correspond to annually laminated textures, and therefore indicate seasonal change in water temperature (Matsuoka et al., 2001; Ihlenfeld et al., 2003). Tufa is also an excellent recorder of rainfall. A specimen from SW Japan exhibits a number of clay bands that were consistently correlated with heavy rainfall events of $>50\ \text{mm}$ (Kano et al., 2003). These approaches were recently applied to the Holocene tufa from Arizona (O'Brien et al., 2006).

Conclusion

Tufas are carbonate precipitates under the influence of photosynthetic microbes (cyanobacteria and algae), but the carbonate precipitation is primarily controlled by inorganic conditions, e.g., CO_2 degassing and raised supersaturation of the water. There are increasing evidences that stable isotopes and elemental geochemistry of the calcite fraction record clear environmental and climatic information.

Tufas provide high-resolution, but discontinuous paleoclimatic records from core and outcrop specimens, which can be dated by appropriate methods, such as U-Th dating of pure carbonate fraction, and radiocarbon composition of exceptionally preserved plant fragments. A promising approach in future research is combined investigation of speleothems and tufas from the same area. High-resolution temperature and rainfall records derived from tufas can add significant information to terrestrial paleoclimatology.

Bibliography

- Andrews, J. E., 2006. Palaeoclimatic records from stable isotopes in riverine tufas: synthesis and review. *Earth-Science Reviews*, **75**, 85–104.
- Andrews, J. E., and Brasier, A. T., 2005. Seasonal records of climatic change in annually laminated tufas: short review and future products. *Journal of Quaternary Science*, **20**, 411–421.
- Andrews, J. E., Riding, R., and Dennis, P. F., 1997. The stable isotope record of environmental and climatic signals in modern terrestrial microbial carbonates from Europe. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **129**, 171–189.
- Arp, G., Wedemeyer, N., and Reitner, J., 2001. Fluvial tufa formation in a hard-water creek (Deinschwanger Bach, Franconian Alb, Germany). *Facies*, **44**, 1–22.
- Chen, J., Zhang, D. D., Wang, S., Xiao, T., and Huang, R., 2004. Factor controlling tufa deposition in natural waters at waterfall sites. *Sedimentary Geology*, **166**, 353–366.
- Ford, T. D., and Pedley, H. M., 1996. A review of tufa and travertine deposits of the world. *Earth-Science Reviews*, **41**, 117–175.
- Freytet, P., and Plet, A., 1996. Modern freshwater microbial carbonates: the *Phormidium* stromatolites (tufa-travertine) of southeastern Burgundy (Paris Basin, France). *Facies*, **34**, 219–238.
- Garnett, E. R., Andrews, J. E., Preece, R. C., and Dennis, P. F., 2004. Climatic change recorded by stable isotopes and trace elements in a British Holocene tufa. *Journal of Quaternary Science*, **19**, 251–262.
- Hori, M., Hoshino, K., Okumura, K., and Kano, A., 2008. Seasonal patterns of carbon chemistry and isotopes in tufa depositing groundwaters of southwestern Japan. *Geochimica et Cosmochimica Acta*, **72**, 480–492.
- Ihlenfeld, C., Norman, M. D., Gagan, M. K., Drysdale, R. N., Maas, R., and Webb, J., 2003. Climate significance of seasonal trace element and stable isotope variations in a modern freshwater tufa. *Geochimica et Cosmochimica Acta*, **67**, 2341–2357.
- Janssen, A., Swennen, R., Podoor, N., and Keppens, E., 1999. Biological and diagenetic influence in recent and fossil tufa deposits from Belgium. *Sedimentary Geology*, **126**, 75–95.
- Kano, A., and Fujii, H., 2000. Origin of the gross morphology and internal texture of tufas of Shirokawa Town, Ehime Prefecture, southwest Japan. *Journal of Geological Society, Japan*, **106**, 397–412.
- Kano, A., Matsuoka, J., Kojo, T., and Fujii, H., 2003. Origin of annual laminations in tufa deposits, southwest Japan. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **191**, 243–262.
- Kano, A., Hagiwara, R., Kawai, T., Hori, M., and Matsuoka, J., 2007. Climatic condition and hydrological change recorded in a high-resolution stable-isotope profile of a recent laminated tufa on a subtropical island, southern Japan. *Journal of Sedimentary Research*, **77**, 59–67.
- Makhnach, N., Zernitskaja, V., Kolosov, I., and Simakova, G., 2004. Stable oxygen and carbon isotopes in Late Glacial-Holocene freshwater carbonates from Belarus and their palaeoclimatic implications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **209**, 73–101.
- Matsuoka, J., Kano, A., Oba, T., Watanabe, T., Sakai, S., and Seto, K., 2001. Seasonal variation of stable isotopic compositions recorded in a laminated tufa, SW Japan. *Earth and Planetary Science Letters*, **192**, 31–44.
- Mert, M. U. E., 1992. The biology of carbonate precipitation by cyanobacteria. *Facies*, **26**, 81–102.
- O'Brien, G. R., Kaufman, D. S., Sharp, W. D., Atudorei, V., Parnell, R. A., and Crossey, L. J., 2006. Oxygen isotope composition of modern and mid-Holocene banded travertine, Grand Canyon, Arizona, USA. *Quaternary Research*, **65**, 366–379.
- Pedley, H. M., 1990. Classification and environmental models of cool freshwater tufas. *Sedimentary Geology*, **68**, 143–154.
- Pentecost, A., 2005. *Travertine*. Heidelberg: Springer.
- Pentecost, A., and Riding, R., 1986. Calcification in cyanobacteria. In Leadbeater, B. S. C., and Riding, R. (eds.), *Biomining in Lower Plants and Animals*. The Systematics Association Special. Oxford: Oxford University Press, Vol. 30, pp. 74–90.
- Pentecost, A., and Spiro, B., 1990. Stable carbon and oxygen isotope composition of calcites associated with modern freshwater cyanobacteria and algae. *Geomicrobiology Journal*, **8**, 17–26.
- Pentecost, A., 1995. The Quaternary travertine deposits of Europe and Asia Minor. *Quaternary Science Reviews*, **14**, 1005–1028.
- Smith, J. R., Giegengack, R., and Schwarcz, H. P., 2004. Constraints on Pleistocene pluvial climates through stable-isotope analysis of fossil-spring tufas and associated gastropods, Kharga Oasis, Egypt. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **206**, 157–175.
- Yoshimura, K., Lui, Z., Cao, J., Yuan, D., Inokura, Y., and Noto, M., 2004. Deep source CO₂ in natural waters and its role in extensive tufa deposition in the Huanglong Ravines, Sichuan, China. *Chemical Geology*, **205**, 141–153.
- Zak, K., Lozek, V., Kadlec, J., Hladikova, J., and Cilek, V., 2002. Climate-induced changes in Holocene calcareous tufa formations, Bohemian Karst, Czech Republic. *Quaternary International*, **91**, 137–152.

Cross-references

[Biofilms](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbonates](#)
[Cyanobacteria](#)
[Isotopes and Geobiology](#)

- Tullborg, E.-L., Drake, H., Sandström, B., 2008. Palaeohydrogeology: A method based on fracture mineral studies. *Applied Geochemistry*, **23**, 1881–1897.
- Wallin, B., and Peterman, Z., 1999. Calcite fracture fillings as indicators of palaeohydrology at Laxemar at the Äspö Hard Rock Laboratory, southern Sweden. *Applied Chemistry*, **14**, 953–962.
- Wilson, T., 1990. *Confocal Microscopy*, London: Academic.

Cross-references

[Biosignatures in Rocks](#)
[Terrestrial Deep Biosphere](#)

REDUCTION SPHEROIDS

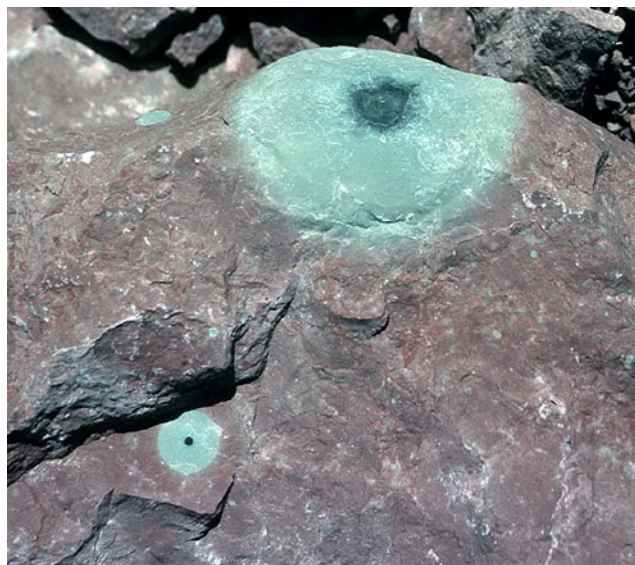
Beda A. Hofmann
 Natural History Museum Bern, Bern, Switzerland

Synonyms

Bleaching haloes; Fish eyes; Radioactive concretions; Radioactive nodules; Reduction mottling; Reduction spots; Reduktionshöfe

Definition

Reduction spheroids are small-scale (diameter: few millimeters to maximum about 20 cm) spheroidal bleached features in red-bed sediments characterized by the absence of hematite and often containing a mineralized core (center, [Figure 1](#)). Reduction spheroids result from local chemical reduction of ferric iron and other elements. Host rocks are marine and continental red beds and, more rarely, altered, hematite-stained crystalline rocks. Reduction spheroids have been described from many occurrences in red beds of widely variable age worldwide: Proterozoic of Canada (Tanton, [1948](#)); Cambro-Silurian marine red beds of the NE USA (Beutner and Charles, [1985](#)); Devonian continental red beds of Scotland (Parnell, [1985](#)); Carboniferous continental red beds of New Brunswick and Nova Scotia, Canada (Dyck and McCorkell, [1983](#)); Permian continental red beds of Germany (Eichhoff and Reineck, [1952](#); Mempel, [1960](#); Schreiter, [1930](#)), Switzerland (Hofmann, [1990](#), [1991](#)), Southwest England (Harrison, [1970](#); Perutz, [1940](#)), and Cretaceous radiolarian cherts of Oman (Hofmann, [1991](#)). Reduction spheroids characteristically consist of a spheroidal volume of rock from which hematite pigment has been dissolved. Central dark cores often display pronounced concentric zonation. The cores are strongly enriched in numerous redox-sensitive elements (dominant V, but also U, Se, As, Ni, Co, Cu, Ag, Au, PGE, and others). The dominant mineral in reduction spheroid cores is the vanadium mica roscoelite $KV_2AlSi_3O_{10}(OH)_2$. Uraninite is another very common mineral, leading to an often increased radioactivity of reduction spheroids ([Figure 2](#)). Arsenides of Ni, Co, and Cu are also quite common. Radiometric dating of roscoelite, uraninite, and

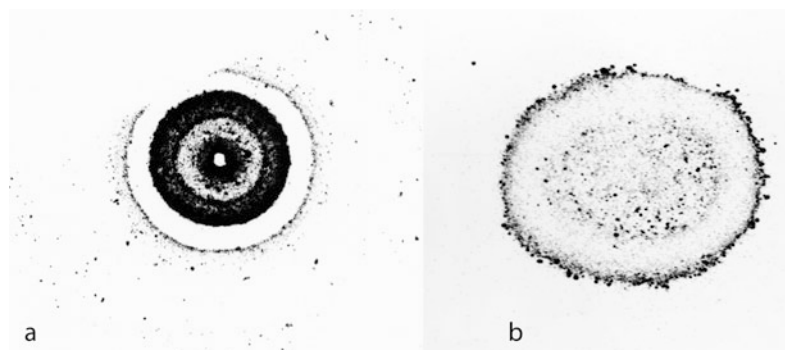


Reduction Spheroids, Figure 1 Reduction spheroids in Triassic red bed (marly dolomite), Chinle Formation, Paradox Valley, Colorado, USA. These reduction spheroids are strongly enriched in V, Cr, and Au. Diameter of larger reduction spheroid is 8 cm.

Pb-depleted haloes indicates late diagenetic ages (Hofmann, [1990](#); Hofmann and Frei, [1996](#)). Reduction spheroids often occur in large numbers in red beds and can constitute up to several vol% of the rock. Based on textural evidence, such as fracture-bound occurrences and mass-balance calculations (Hofmann, [1991](#)), reduction spheroids cannot be due to detrital organic-rich particles. Instead, the formation must be due to locally catalyzed redox reactions between a mobile reductant and oxidants. The presence of isotopically light sulfides in some cores (Hofmann, [1991](#)), indicating diagenetic sulfate reduction, as well as the need for a local catalyst, strongly favors a microbiological origin of reduction spheroids. Reductants may be mobile hydrocarbons or molecular hydrogen resulting from the radiolysis of pore water (Hofmann, [1992](#)). The formation of reduction spots is most likely due to chemolithotrophic communities. Kerogen-like organic matter is occasionally present at some localities (Curiale et al., [1983](#); Parnell, [1985](#)) but is lacking at many other occurrences (Hofmann, [1993](#)).

Conclusion

Reduction spheroids are very common in red-bed sediments, and there are strong indications for an origin due to redox reactions between a mobile reductant and oxidized elements locally catalyzed by microbial activity. Reduction spheroids are thus easily visible features resulting from redox processes catalyzed by a deep biosphere of sediments, and represent a type of biosignature that may also be present in oxidized rocks on Mars. Reduction spheroids contain small-scale



Reduction Spheroids, Figure 2 α -Autoradiographs of uranium-enriched reduction spheroid cores, Weiherfeld well, Permian of northern Switzerland. The accumulation of uranium reflects the heterogeneous distribution of reduction activity, consistent with microbial activity. Diameter of radioactive uranium-rich zone is (a) 5 mm and (b) 10 mm.

element enrichments that are in many ways analogous in element inventory, geochemistry, and mineralogy to ore deposits such as unconformity-related and roll-type uranium ores.

Bibliography

- Beutner, E. C., and Charles, E. G., 1985. Large volume loss during cleavage formation, Hamburg sequence, Pennsylvania. *Geology*, **13**, 803–805.
- Curiale, J. A., Bloch, S., Rafalska-Bloch, J., and Harrison, W. E., 1983. Petroleum-related origin for uraniferous organic-rich nodules of southwestern Oklahoma. *AAPG Bulletin*, **67**, 588–608.
- Dyck, W., and McCorkell, R. H., 1983. A study of uranium-rich reduction spheroids in sandstones from Pugwash Harbour, Nova Scotia. *Canadian Journal of Earth Sciences*, **20**, 1738–1746.
- Eichhoff, H. J., and Reineck, H. E., 1952. Uran-Vanadinkerne mit Verfärbungshöfen in Gesteinen. *Neues Jahrbuch für Mineralogie, Monatshefte*, **11/12**, 294–314.
- Harrison, R. K., 1970. Hydrocarbon-bearing nodules from Heysham, Lancashire. *Geological Journal*, **7**, 101–110.
- Hofmann, B. A., 1990. Reduction spheroids from northern Switzerland: mineralogy, geochemistry and genetic models. *Chemical Geology*, **81**, 55–81.
- Hofmann, B. A., 1991. Mineralogy and geochemistry of reduction spheroids in red beds. *Mineralogy and Petrology*, **44**, 107–124.
- Hofmann, B. A., 1992. Isolated reduction phenomena in red beds: a result of porewater radiolysis? In Maest A. S. (ed.), *7th International Symposium on Water-Rock interaction*, Balkema, Park City, Utah, USA, pp. 503–506.
- Hofmann, B. A., 1993. Organic matter associated with mineralized reduction spots in red beds. In Parnell, J., Kucha, H., and Landais, P. (eds.), *Bitumens in Ore Deposits*. Berlin: Springer, pp. 362–378.
- Hofmann, B. A., and Frei, R., 1996. Age constraints of reduction spot formation from Permian red bed sediments, northern Switzerland, inferred from U-Th-Pb systematics. *Schweizerische Mineralogische und Petrographische Mitteilungen*, **76**, 235–244.
- Mempel, G., 1960. Neue Funde von Uran-Vanadiumkernen mit Entfärbungshöfen. *Geologische Rundschau*, **49**, 263–276.
- Parnell, J., 1985. Uranium/rare earth-enriched hydrocarbons in Devonian sandstones, northern Scotland. *Neues Jahrbuch für Mineralogie—Monatshefte*, **H3**, 132–144.
- Perutz, M., 1940. Radioactive nodules from Devonshire, England. *Mineralogische und Petrographische Mitteilungen*, **51**, 141–161.
- Schreiter, R., 1930. Vanadinhaltige Kerne, Bleichungsringe und Bleichungszonen in den Schieferletten des Rotliegenden von Sachsen. *Zeitschrift der Deutschen Geologischen Gesellschaft*, **82**, 41–47.
- Tanton, T. L., 1948. Radioactive nodules in sediments of the Sibley Series, Nipigon, Ontario. *Transactions of the Royal Society of Canada*, **17**(Ser. III, Sec. 4), 69–75.

Cross-references

[Biosignatures in Rocks](#)
[Chemolithotrophy](#)
[Deep Biosphere of Sediments](#)
[Hydrogen](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Radioactivity \(Natural\)](#)

REEFS

Reefs are laterally confined submarine carbonate structures developed by the growth or activity of sessile benthic aquatic organisms, such as corals, bryozoans, sponges, algae, and microbes (Flügel and Kiessling, 2002). See entry “[Carbonate Environments](#)” for further reading and classification.

Bibliography

- Flügel, E., and Kiessling, W., 2002. A new look at ancient reefs. In Kiessling, W., Flügel, E., and Golonka, J. (eds.), *Phanerozoic Reef Patterns*. Tulsa, OK: SEPM Special Publication Series No. 72, pp. 3–20.

W

WAULSORTIAN MUD MOUNDS

Marta Rodríguez-Martínez
Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Synonyms

Waulsortian banks; Waulsortian buildups; Waulsortian mounds; Waulsortian mudbanks; Waulsortian reefs

Definition

Waulsortian mud mounds are characterized by different types of complex and highly structured fabrics of carbonate muds, texturally and genetically varied (both automicrites and allomicrites occur), with a variable content in skeletal components (heterozoan assemblages). Their origin is controversial although most authors advocate a microbial origin (calcite precipitation microbially induced). They were developed throughout the northern hemisphere in many parts of the World (Europe and North America) during the Carboniferous times (Lower Mississippian, mainly in the Tournaisian). These mud mounds grew on wider ramps and within basins in tropical “calcite seas,” immersed in a period of intense global changes (tectonic activity related with the Variscan orogeny and major sea-level changes).

Introduction

The term, from the Waulsort area, was first introduced by Dupont (1863) who studied and divided the Carboniferous Limestone of Belgium in six stratigraphic units. In the 1960s and 1970s, most of the carbonate buildups of Mississippian age were collectively named as “Waulsortian,” however, not all of them can be considered as true or classic “Waulsortian” as well as the whole Mississippian

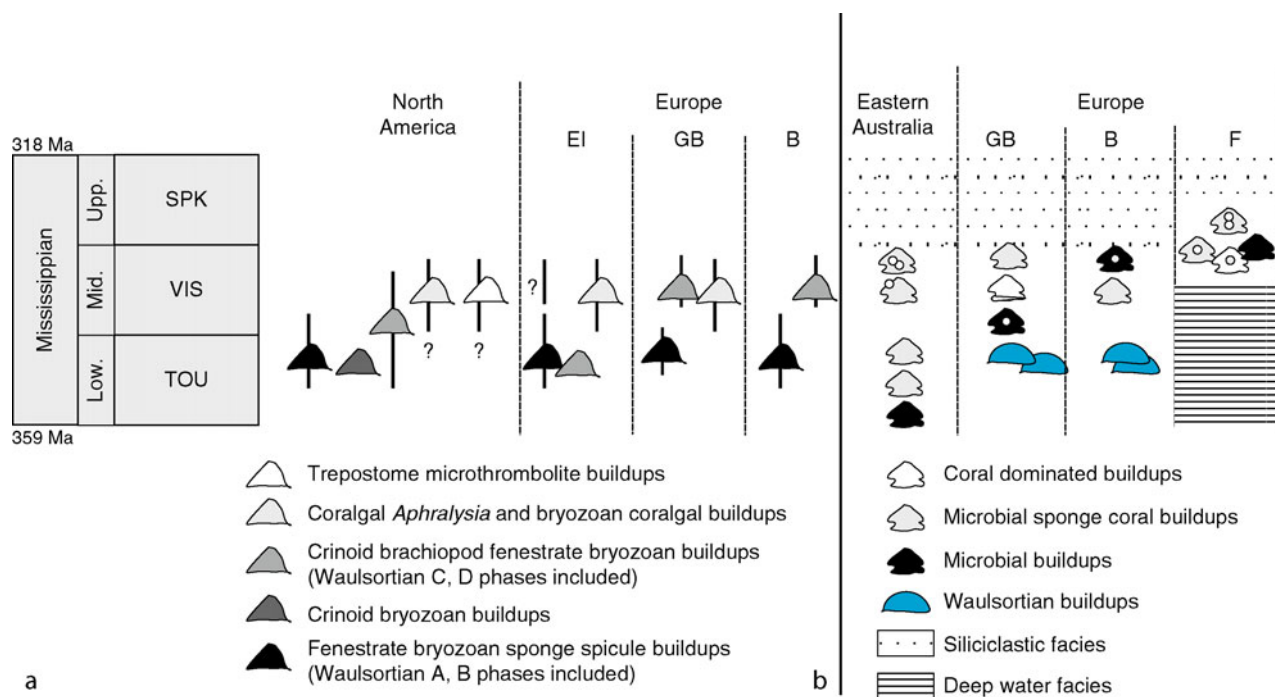
bioconstruction spectrum can not be classified or assigned to the Waulsortian mud mounds (Figure 1). Some authors believe that the application of denominations like “Waulsortian,” “Waulsortian-like,” and “non-Waulsortian” buildups is of limited value because they do not mean a fast and clear idea about the geometry, composition, and/or the possible buildup origin. In fact, the use and meanings of the term “Waulsortian” have been controversial from their origin in the 19th century and has been not always used with clarity (see historical review by Lees, 1988).

The study of the Waulsortian mud mounds have been focused in several aspects, developed not always with the same profusion: (a) facies relationships and geometries (external morphology and internal structure); (b) allochems; (c) carbonate mud types; and (d) open space structures.

Facies and geometry

Waulsortian mud mound (Mud mound is considered here as just a descriptive, nongenetic, non-morphological term, to describe a type of bioconstruction dominated by carbonate mud (>50% of the rock volume)) facies are separated normally in Waulsortian massive facies, off-mound or coeval bottom facies and flank facies, which can or not occur.

Waulsortian limestones facies are massive, although bedding intervals can occur, and characteristically pale violet-grey to dark grey in color. In the field, the major lithological variations are based on changes of the following features: (1) carbonate mud content (dominance of “muddy” fabrics); (2) presence of coarsely calcite mosaics (dominance of “sparry” fabrics); (3) in situ fenestellid bryozoan fronds; (4) skeletal debris lenses, commonly crinoids and bryozoans; and (5) other entire fossils. These characters can occur combined in different proportions,



Waulsortian Mud Mounds, Figure 1 Mississippian bioconstruction spectrum, including mud mounds: (a) Buildups type's distribution based mainly on skeletal assemblages, summarized and simplified from Bridges et al. (1995); (b) Buildups type's distribution based mainly on main type of framework, modified from Aretz and Webb (2006). *EI* Ireland; *GB* Great Britain; *B* Belgium; *F* France.

resulting in fast laterally and vertically subfacies changes in few centimeters between: (1) bryozoan cementstones (fenestellid fronds, Figure 2a); (2) bryozoan wackestones (fenestellid fronds); (3) skeletal wackestones; (4) skeletal wackestones with "sparry fabrics" (Figure 2b); and (5) crinoidal-rich packstones.

Geometrically, they were formed by the aggregation of successive, laterally extensive banks, which developed final tabular-, knoll-, or sheet-growth morphologies (Figure 3). Growth morphologies are function of many variables, some of them related with: (1) the autochthonous carbonate mud production rate; (2) the ability of the autochthonous carbonate mud to construct depositional slopes (some of them up to 50 degrees); (3) the coeval sedimentation versus carbonate mud production relationship; (4) the sea floor topography; (5) the available accommodation space, etc.

Final dimensions vary from few meters, in the simple Waulsortian mud mounds, up to hundreds of meters in thickness and thousands of square kilometers across in some Waulsortian complexes or aggregates (Figure 3). In Ireland, the biggest Waulsortian complex crops range around 1 km in thickness and spread over 30,000 km².

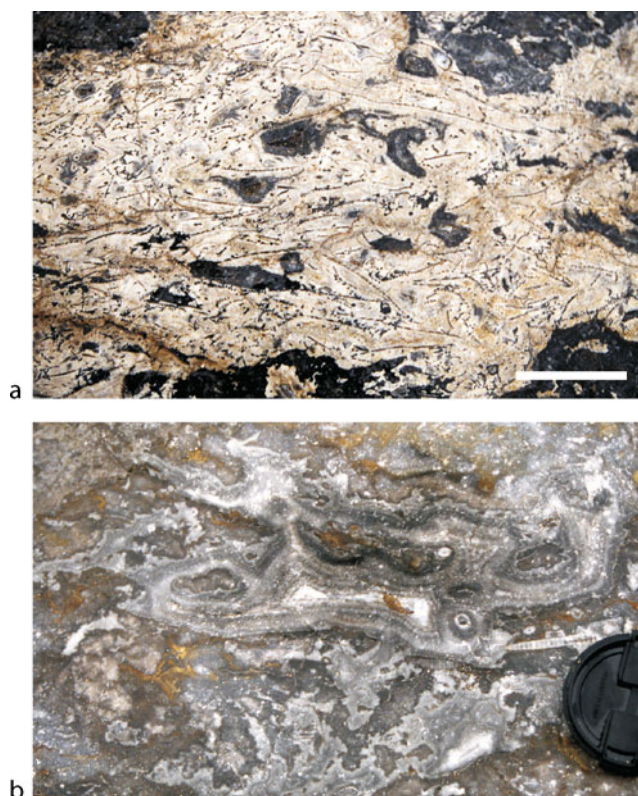
Allochems

The allochems record in the Waulsortian mud mounds, in terms of volumetric importance, is fundamentally formed

by crinoids, bryozoans (mainly fenestrate), ostracods, brachiopods, and molluscs (gastropods, goniatites, lamelli-branches, and nautiloids). Sponge spicules (hyalosteliid) are common. Minority components such as foraminifers, calcified filaments of cyanobacteria, moravamminids, aoujgaliids also occur (some of them are considered as problematica taxa by some authors). Accessory allochems are trilobites, echinoid spines, serpulids, and clorophytes. Colonial rugose corals are very rare and they occurred exceptionally in the upper part of some of these mud mounds. Others as solitary forms and heterocorals are also uncommon. Non-skeletal grains as intraclasts can appear and be locally common in some parts and small, micritized radial oolites are very unusual.

Component assemblages models

First detailed systematic petrographic analyses of Waulsortian mud mounds were carried in Belgium by Lees et al. (1985) and 25 parameters were ranked according their volumetric importance. Four depth-related phases were distinguished using correspondence analysis. Later, following the same methodology, more than 30 mud mounds from Europe and North America were analyzed and they showed also the Belgium component assemblages (results summarized in Lees and Miller, 1995). Thus, four Waulsortian Phases (A–D) were established, and they have been interpreted as the mud mound growth



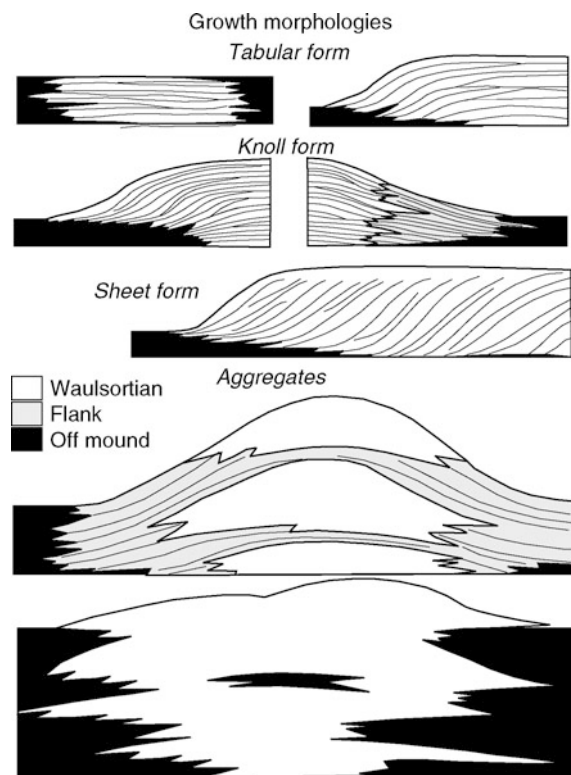
Waulsortian Mud Mounds, Figure 2 Classic Waulsortian facies: (a) bryozoan cementstones (scale bar = 5 cm) and (b) skeletal wackestone with stromatolite cavities. Ballybunnion outcrops, W Ireland.

from deep, subtidal, aphotic marine environments through to the photic zone (Figure 4):

- Phase A: fenestellids + crinoids + ostracods
- Phase B: resembles A + hyalosteliid sponge spicules
- Phase C: resembles B + plurilocular foraminifera
- Phase D: resembles C + cryptalgal coating, micritization (cement, cavity walls, grains), and calcareous algae.

In general, there is not a close correlation between the different component-assemblage phases and a determined aspect or facies at outcrop scale. Phase A represents the “classic” idea of Waulsortian facies, characterized by the fenestellid sheets with radial fibrous cements nucleated around the fronds (fenestellid cementstones) and pockets and lenses with crinoid debris. The content in fenestellid sheets tends to decrease in abundance in the rest of phases; in fact they are rare in Phase C. Sometimes they occur again in high proportions in Phase D, but bryozoans commonly were micritized before the cement nucleation.

The relationship between the mud mound biota and the off mound facies was systematically analyzed in New Mexico, where the exceptional quality of the outcrops (Figure 5a) allows this type of comparisons. Ahr and



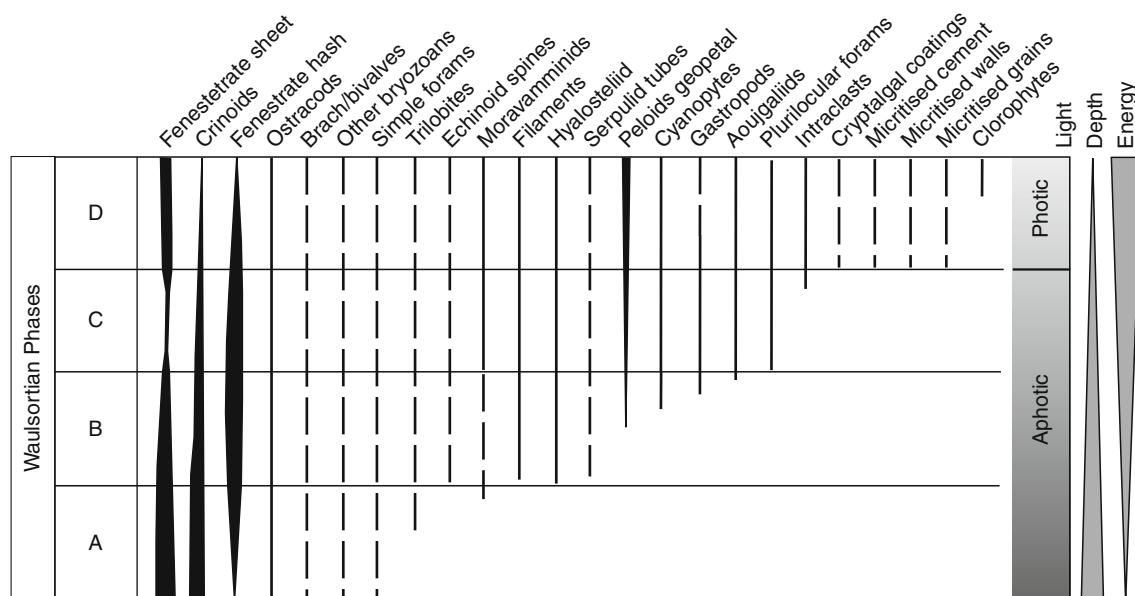
Waulsortian Mud Mounds, Figure 3 Observed growth morphologies and facies relationships in Waulsortian mud mounds, redrawn and simplified from Lees and Miller (1995). a = Waulsortian facies; b = flank facies; c = off mound facies.

Stanton (1996), analyzed the frequency occurrence of 26 parameters on thin sections from different mounds, included Muleshoe mound (Alamogordo, Nunn, and Tierra Blanca phases from the lower to the upper part) and the off mound facies (Figure 5a and b).

Crinoids, fenestellid hash, and ostracods are the most abundant and similarly distributed fossils in both facies. In fact, in the off mound facies, most frequent fossils are crinoids, ostracods, fenestellid hash, sponge spicules, and echinoid spines (>80% of the thin sections) followed by mollusc and brachiopods shells, fragments of trilobites, and hyalosteliid spicules (Figure 5b). However, the abundance of skeletal components was low in both settings, although they were volumetrically more important and diverse on the mud mounds. Most of the variations could be related with taphonomic bias and differences in available habitats (cryptic spaces, soft, firm, and hard substrates) more suitable in/on mud mounds.

The systematic analysis of the biotic content from the off mound record was developed by Jeffery and Stanton (1996), and four component assemblages were established from deep to more shallow conditions:

- Assemblage I: crinoid/echinoderm debris, fenestellid hash, and ostracods



Waulsortian Mud Mounds, Figure 4 Qualitative distribution of components through the Waulsortian Phases (A, B, C, and D). Redrawn and modified from Lees and Miller (1995).

- Assemblage II: I + stacheiins (possible algae), rare salebrids (possible bryozoan), and commonly abundant sponge spicules
- Assemblage III: II + plurilocular foraminifera
- Assemblage IV: III + green algae

Biologically controlled contribution

The dominant macrobiota in the Waulsortian mud mound was formed by heterozoan assemblages, which never constituted a skeletal framework. Photosynthetic groups such as calcareous algae were very rare and uncommon to colonize these mud mounds. Crinoids and bryozoans have played an important role when they colonized and stabilized the slopes and mound surfaces. As skeletal producers, they formed favorable substrates (encrusters, cement nucleation, etc.) and they had also relative importance as allomicrite bafflers. Thus, the endemic macrofauna was composed by crinoids, fenestrate bryozoans, and sponges (Figure 6). The metabolism and decay of siliceous sponges could also favored the bacterial activity producing organomicrites, which would be added to the main autochthonous carbonate production in these mud mounds (biologically induced mechanism are argued by most authors). Opportunistic epi- and infauna biota could colonize the locally soft mound substrates and after their death contribute to the biodegradation with the skeletal debris (Figure 6).

Carbonate muds

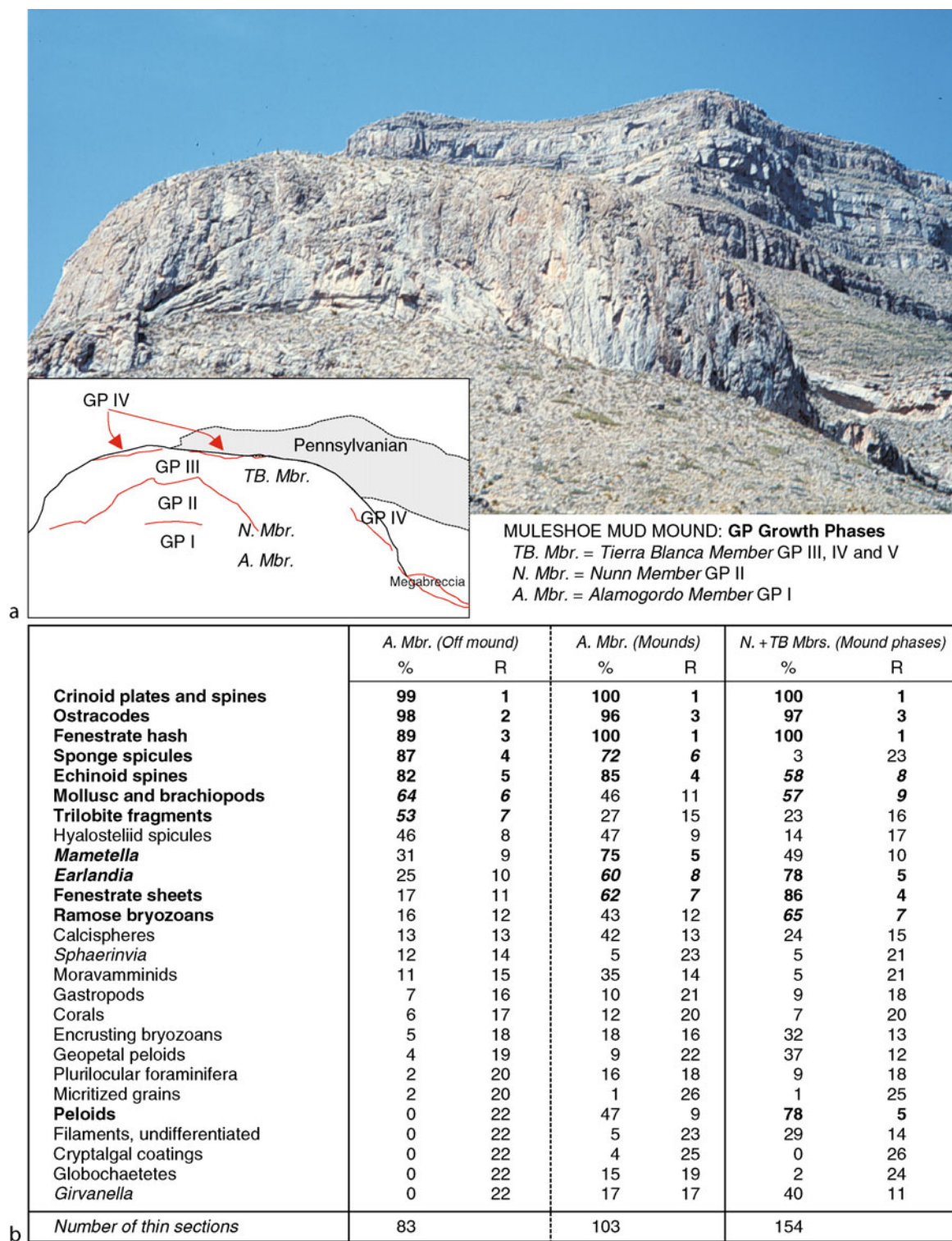
The most important component in the Waulsortian mud mounds is the carbonate mud, paradoxically during many

decades its nature has been not considered or specifically studied. In fact, the origin of the carbonate muds and its ability to maintain steep slopes has been subject of much debate during years. Some authors have advocated for an external source followed by some kind of posterior accumulation mechanisms (hydrodynamic, baffling by bryozoans and crinoids, trapping and binding by bryozoans or other organisms). From the 1980s most part of the authors suggest a probable microbial origin for the carbonate mud, and a dominant in situ carbonate mud production is assumed.

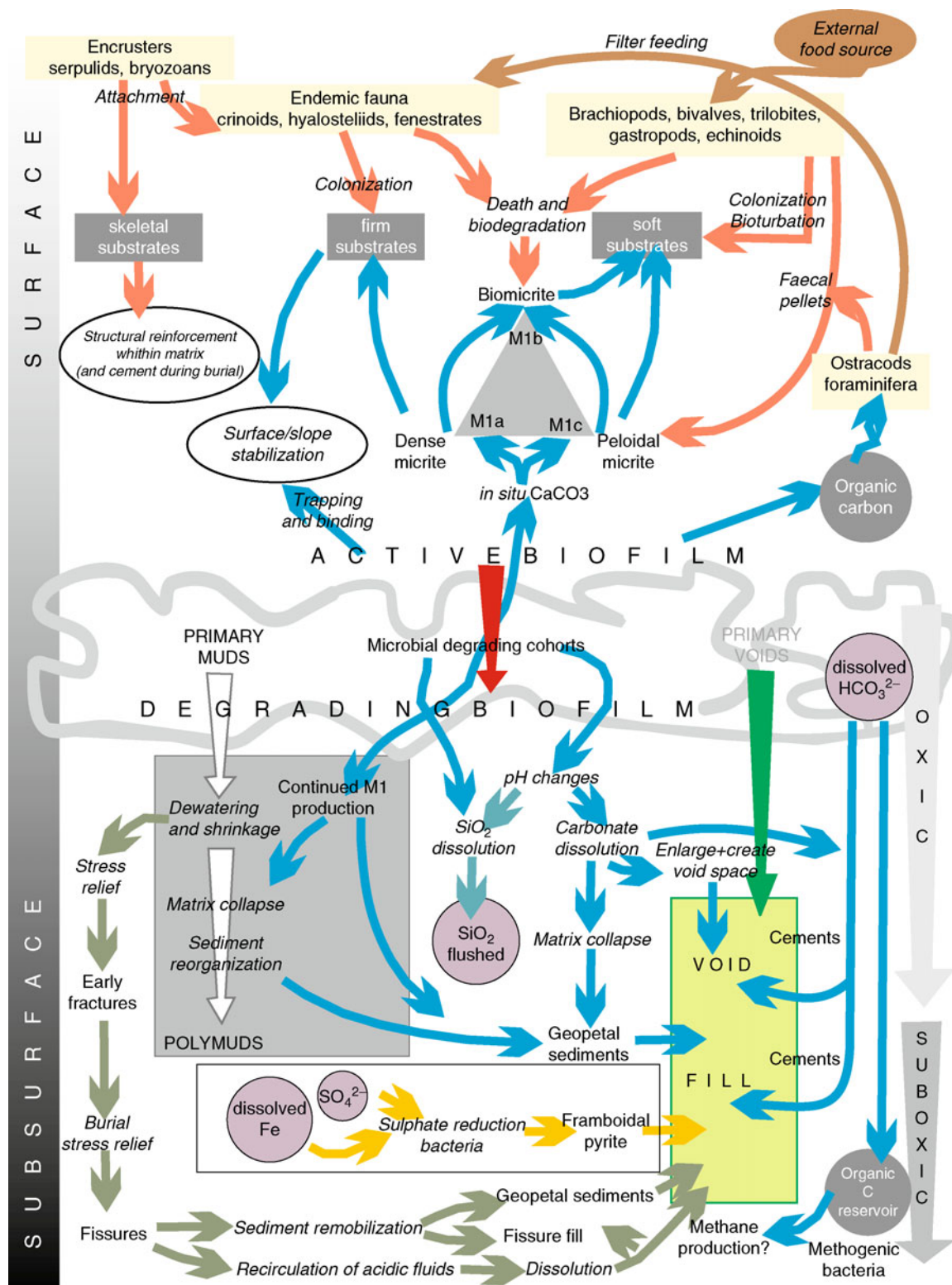
The carbonate muds appear forming mudstone to packstone, although most works coincide in wackestone as the commonest texture. They are highly organized displaying successive generations of carbonate muds in geopetal relationship. They have been separated in primary muds (M1) and later muds (M2, M3, M4, etc.), all forming together the known polymuds (name coined by Lees and Miller, 1985, after the “multicomponent mudstones” concept of Lees (1964)). The earliest mud generation (M1) can represent less than 50% of carbonate mud volume. Lees and Miller (1995) subdivided the primary muds into three different subtypes (Figure 6):

- (M1a) optically dense micrites, often with filaments
- (M1b) paler biomicrites
- (M1c) peloidal micrites

A biofilm model has been proposed for the in situ primary mud production (Figure 6). The biofilm formed by bacterial cells, cyanobacterial filaments, and bacterial extracellular polymeric substances (EPS), induced the precipitation of high-magnesium calcite micrites (dense



Waulsortian Mud Mounds, Figure 5 (a) Muleshoe mud mound, Sacramento Mountains, New Mexico. Mud mound growth phases following Kirkby and Hunt (1996). (b) Components distribution of 26 elements from both off mound levels and mud mounds (Alamogordo Member mounds and Muleshoe mound – Alamogordo, Nunn, and Tierra Blanca Members). Modified from Ahr and Stanton (1996). % Frequency of occurrence; *R* = rank position.



Waulsortian Mud Mounds, Figure 6 Biofilm model with the process-response mechanisms proposed to Waulsortian mud mounds. (Simplified and modified after Lees and Miller, 1995.)

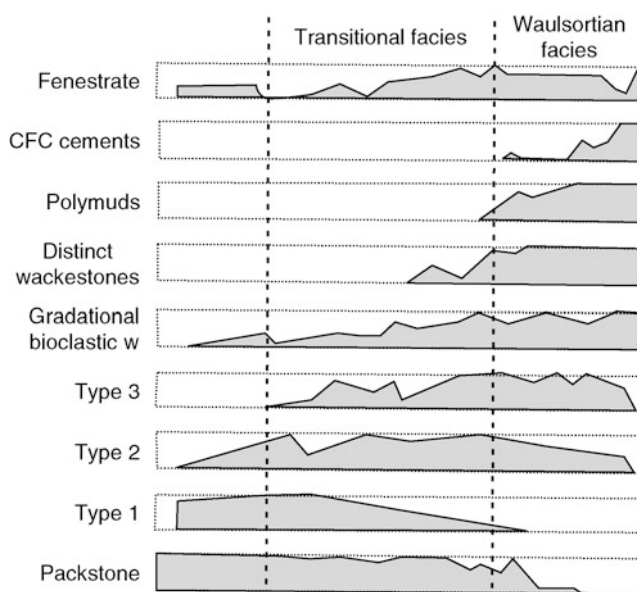
and peloidal ones). Biomicrites were formed by both reworking of dense or/and peloidal micrites and the skeletal debris input (Figure 6). Additionally, the biofilm could be used by other mud mound colonizers as a source of organic carbon. Carbonate mud production was not only reduced to the active biofilm mound surface, but also continued below the surface favored by the biofilm degradation during early burial (Figure 6). Dewatering, shrinkage, carbonate dissolution, and reworking processes produced the collapse of carbonate mud matrix. The collapse and successive reorganizations of the carbonate muds through the cavity system resulted in the final polymud fabrics. Later fractures and fissures could also have favored sediment inputs through the mound void system (Figure 6).

There are no available data on the quantitative distribution/contribution of the different primary muds through the Waulsortian phases. Filaments, characteristic in dense micrites (M1a) do not occur in phase A, whereas they are abundant in the rest of phases. As well as, cavities from phase D contain important volumes of geopetal peloids intercalated with early calcite cements. Thus an early, deep, nonfilamentous, non-photosynthetic cyanobacterial community has been suggested for the carbonate mud production in the Waulsortian phase A (Miller, 1986). In the same way, several microbial communities may have controlled the distribution/production of the different primary non-reworked mud textures (dense or peloidal micrites).

The distribution and typologies of carbonate mud, from the transitional facies to Waulsortian mud mound nucleation, were analyzed by Devusyst and Lees (2001), in western Ireland. *Precursor muds* are differentiated in function by their grumous or non-grumous character, bioclastic content, and contact relationship. They were grouped into two major types with six subtypes:

- Grumous types on basis of their apparent optical density, with three intergrading subtypes (codified as 1, 2 and 3). Types 1 and 2 are normally poor in skeletal debris; however, the dark optically dense type 3 contains abundant sponge spicules. All of them have been interpreted as automicrites. The grumous types are equivalent to the peloidal micrites (M1c) of Lees and Miller (1985, 1995).
- Non-grumous types: (1) gradational (into grumous muds) bioclastic wackestones; (2) distinct bioclastic wackestones; and (3) packstone matrix. The first subtype is also interpreted as automicrite whereas the others are seen as loose sediment.

The different precursor muds and other features were analyzed by correspondence analysis and a compositional gradient or trend was detected (Figure 7). The precursor muds display different textural and genetic types and culminate in the Waulsortian *polymuds* that contain both grumous and non-grumous muds (mainly non-grumous).



Waulsortian Mud Mounds, Figure 7 Observed relay pattern resulting from correspondence analysis of the precursor muds, polymuds, and associated features. Simplified and modified from Devusyst and Lees (2001). Vertical scales of boxes ranges from 0 up to 1, thus grey areas represent the frequency of presence. CFC = crypto-fibrous calcite; w = wackestone.

Open space structures

Waulsortian mud mounds were originally interpreted as reefs by Dupont (1881) who coined the term *Stromatactis* in 1881 to describe and interpret the “sparry masses” from Devonian mud mounds as recrystallized fossils. The stromatactis are a special type of cavities whose origin is still controversial. They have a characteristic shape, with a flat to undulose floor and irregular digitate roof and are filled by centripetal calcite cement, although internal sediment can also occur. Other terms like stromatactoid or stromatactis-like cavity are commonly found in the literature to describe similar features. They are common through the Precambrian up to the Jurassic mud mounds, (Bosence and Bridges, 1995) although can also appear in other types of limestones not just in muddy ones. A different hypothesis has been proposed to explain the stromatactis, which can be grouped in organic or inorganic models (see reviews by Bathurst, 1982; Flajs and Hüssner, 1993; Monty, 1995; Flügel, 2004, p. 194).

In Belgium, some mud mounds facies contain those described as undulating or tortuous “vein bleues” which were used, for many years, as a traditional criterion to characterize the Waulsortian facies and but was later abandoned (Lees, 1988).

Sheet-form cavities, similar as known zebra cavities, just few centimeters thick but several meters in length, are also typical in Waulsortian mud mounds. Their roofs

are not clearly associated with skeletal support and they have been interpreted as the result of dewatering and shear stress processes in the firm, gel-like consistence muds.

Stromatactis, the sheet-form and irregular shelter cavities have been considered early-formed cavity systems (Lees and Miller, 1995). Dissolution as well as physical processes like fracturing and internal erosion produce secondary cavities included fissures (Figure 6).

The size of the cavities tends to decrease through B and C phases and gets much complex in the D than in the others. Thus, "sparry" fabrics are common in phase A, whereas in B and C they tend to be muddier.

The shape and heterogeneity of the cavities seem to mainly be related with the skeletal content as well as the different carbonate mud textures and their distribution. Fillings as polymuds, cements (marine fibrous calcites are common), and their diagenetic sequences are also diverse (Meyers, 1974; Miller, 1986).

Conclusions

The understanding of the Waulsortian reefs, carbonate mudbanks, mounds, buildups, or mud mounds has evolved from the last two centuries.

The application of Waulsortian term is generally related to a group of diagnosis criteria: complex carbonate mud fabrics (polymuds), stratigraphic position (Middle-Upper Tournaisian-Lower Viséan), facies, component assemblages (Waulsortian phases), and the associated macrofaunas.

The Mississippian seas were colonized by a wide bioconstruction spectrum and not by Waulsortian mud mounds alone. Bacteria and cyanobacteria are suggested as primary producers in Waulsortian microbial mud mounds. The hypothesis about the distribution of Waulsortian mud mounds has been related with upwelling areas and methane seeps. Carbon sources and convincing models to explain such large volumes of radial fibrous calcite cements and continuous regional production of polymuds are still unrevealing.

Most part of the studies has been focussed on the allochems distribution that has been considered in detail by many authors. Few works are specifically dedicated to the analysis of the carbonated mud, main component in the Waulsortian mud mounds. However, more work would be necessary to analyze, quantify, and decipher the relationship between the allomicrite input and the automicrite production in mud mounds, particularly in Waulsortian ones, where clear microbialite frameworks are not always evident.

Summary

Waulsortian mud mounds are probably the most famous carboniferous bioconstructions. They were described first from Belgium outcrops, in the locality of Waulsort, two centuries ago. Later, they have been recognized and analyzed from several areas of Europe and North America.

The growth of these bioconstructions was controlled by the dominant autochthonous production of carbonate mud which has been explained by the activity of marine microbial benthic communities. The associated biota which colonized mud mound substrates was composed by heterozoan assemblages dominated by fenestrate bryozoans and crinoids. Detailed studies of these component assemblages have been a key to reconstruct regional gradients from deep subtidal, aphotic marine environments through the photic zone.

Today, there are no equivalent analogs to compare with such a spectacular mud mound development during the Tournaisian-lowermost Viséan period. Thus, they represent an excellent record to reconstruct and understand the paleoenvironmental and paleoecological relationships between microbial and nonmicrobial benthic communities through deep ramp to basin settings along the northern hemisphere during Lower Mississippian times.

Bibliography

- Ahr, W. M., and Stanton, R. J. Jr., 1996. Constituent composition of early Mississippian carbonate buildups and their level-bottom equivalents, Sacramento Mountains, New Mexico. In Strogon, P., Somerville, I. D., and Jones, G. L. (eds.), *Recent Advances in Lower Carboniferous Geology*. London: Geological Society Special Publication, Vol. 107, pp. 83–95.
- Aretz, M., and Webb, G. E., 2006. Western European and eastern Australian Mississippian shallow-water reefs: a comparison. In Wong, Th. E., (ed.), *Proceedings of the XVth International Congress on Carboniferous and Permian Stratigraphy*. Utrecht, the Netherlands: Royal Netherlands Academy of Arts and Sciences, pp. 433–441.
- Bathurst, R. G. C., 1982. Genesis of stromatactis cavities between submarine crusts in Paleozoic carbonate mud mounds. *Journal Geological Society of London*, **139**, 165–181.
- Bosence, D. W. J., and Bridges, P. H., 1995. A review of the origin and evolution of carbonate mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt B. R. (eds.), *Carbonate Mud-mounds: Their Origin and Evolution*. Oxford: Blackwell Science for the International Association of Sedimentologists, Special Publication, **23**, pp. 3–9.
- Bridges, P. H., Gutteridge, P., and Pickard, N. A. H., 1995. The environmental setting of early carboniferous mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds their Origin and Evolution*. Special Publication International Association of Sedimentologists, **23**, pp. 171–190.
- Devusyst, F.-X., and Lees, A., 2001. The initiation of Waulsortian buildups in Western Ireland. *Sedimentology*, **48**, 1121–1148.
- Dupont, E., 1863. Sur le Calcaire Carbonifère de la Belgique et du Hainaut français. *Bulletin de l'Académie Royale des Sciences des Lettres et des Beaux-arts de Belgique*, 2^e série, **15**, 86–137.
- Dupont, E., 1881. Sur l'origine des calcaires de la Belgique. *Académie Royale des Sciences de Belgique*, 3 serie, **5**, 264–280.
- Flajs, G., and Hüssner, H., 1993. A microbial model for the Lower Devonian stromatactis mud mounds of the Montagne Noire (France). *Facies*, **29**, 179–194.
- Flügel, E., 2004. *Microfacies of Carbonate Rocks, Analysis, Interpretation and Application*. Berlin: Springer, pp. 976.
- Jeffery, D. L., and Stanton, R. J. Jr., 1996. Biotic gradients on a homoclinal ramp: the Alamogordo member of the Lake Valley

- formation, Lower Mississippian, New Mexico, USA. In Strogon, P., Somerville, I. D., and Jones, G. L. (eds.), *Recent Advances in Lower Carboniferous Geology*. Geological Society Special Publication, 107, pp. 111–126.
- Kirkby, K. C., and Hunt, D., 1996. Episodic growth of a Waulsortian buildup: the Lower Carboniferous Muleshoe Mound, Sacramento Mountains, New Mexico, USA. In Strogon, P., Somerville, I. D., and Jones, G. L. (eds.), *Recent Advances in the Lower Carboniferous Geology*. Geological Society of London Special Publication, 107, pp. 97–110.
- Lees, A., 1964. The structure and origin of the Waulsortian (Lower Carboniferous) “reefs” of west-central Eire. *Philosophical Transactions of the Royal Society of London, Series B*, **247**, 483–531.
- Lees, A., 1988. Waulsortian reefs: the history of a concept. *Mémoires de l'Institut Géologique de l'Université de Louvain*, **34**, 43–55.
- Lees, A., Hallet, V., and Hibo, D., 1985. Facies variation in Waulsortian buildups. Part 1. A model from Belgium. *Geological Journal*, **20**, 133–158.
- Lees, A., and Miller, J., 1985. Facies variation in Waulsortian buildups. Part 2. Mid-Dinantian buildups from Europe and North America. *Geological Journal*, **20**, 159–180.
- Lees, A., and Miller, J., 1995. Waulsortian banks. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-mounds, their Origin and Evolution*. International Association of Sedimentologists Special Publication, 23, pp. 191–271.
- Meyers, W. J., 1974. Carbonate cement stratigraphy of the Lake Valley Formation (Mississippian), Sacramento Mountains, New Mexico. *Journal of Sedimentary Petrology*, **44**, 837–861.
- Miller, J., 1986. Facies relationships and diagenesis in Waulsortian mud mounds from the Lower Carboniferous of Ireland and N England. In Schroeder, J. H., and Purser, B. H. (eds.), *Reef Diagenesis*. Berlin: Springer, pp. 311–335.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, their Origin and Evolution*. International Association of Sedimentologists Special Publication, 23, pp. 11–48.

Cross-references

Bacteria
 Biofilms
 Calcite Precipitation, Microbially Induced
 Cyanobacteria
 Extracellular Polymeric Substances (EPS)
 Microbial Communities, Structure, and Function
 Microbialites
 Mud Mounds
 Reefs

WHALE AND WOOD FALLS

Steffen Kiel
 University of Göttingen, Göttingen, Germany

Synonyms

Biogenic substrates; Food falls; Nekton falls; Organic falls; Sunken wood

Definition

Whale fall: Carcass of a large marine mammal of the order Cetacea (whales) that has sunken to the seafloor.

Wood fall: A piece of wood that has sunken to the seafloor.

History

The first examples of specialized invertebrates inhabiting bones and wood in the *deep sea* (qv) were recognized during the deep-sea expeditions in the early to mid 1900s. Because these samples were recovered by dredging, the complexity of whale- and wood-fall ecosystems was not recognized before the advent of deep-sea research vessels. Pioneering experimental work on wood-falls was carried out in the early 1970s by Ruth Turner (1914–2000), using the deep-sea research vessel *Alvin*. This research came to a halt in the late 1970s when deep-sea *hydrothermal vent systems* (qv) and their fascinating fauna were discovered and *Alvin* was occupied by exploring these systems. The first deep-sea whale-fall ecosystem was accidentally discovered by Craig Smith during an *Alvin* dive in 1987 and consisted of a whale skeleton associated with many chemosymbiotic taxa that were also known from hydrothermal vents (Smith et al., 1989). Nowadays, whale-fall ecosystems are mainly investigated by sinking stranded whales which are then studied by submersibles and/or ROVs. The last major discovery in this field was *Osedax*, a “bone-eating” siboglinid worm living in symbiosis with bacteria that degrade bone lipids and cartilage (Rouse et al., 2004).

Bivalve-bored wood has been known from the fossil record for centuries, but reports on other invertebrate groups that utilized wood falls in the geologic past are rare, and entire fossil wood-fall ecosystems have only been described very recently (Kiel and Goedert, 2006b; Kiel et al., 2009). Fossil examples of deep-sea whale-fall ecosystems were discovered soon after the discovery of the modern ones and are especially common in uplifted deep-water sediments around the North Pacific margin (Squires et al., 1991; Kiel and Goedert, 2006a). Similar ecosystems were recently found on two late Cretaceous plesiosaur (a marine reptile) skeletons in Japan (Kaim et al., 2008).

Whale falls

Whale falls are the basis for the most species-rich deep-sea ecosystem, with more than 400 species known to date (Baco and Smith, 2003). Whale falls pass through three ecologic stages that vary in length and can overlap. At first the flesh of the whale carcass is removed by sleeper sharks, hagfish, and other scavenging deep-sea fishes. In the second stage small annelids and crustaceans consume the remaining particulate organic matter. The main source of nutrients during the third stage is the hydrogen sulfide (H₂S) generated by the anaerobic decay of bone lipids, which is utilized by species harboring chemotrophic endosymbionts or by species that graze on sulfur-oxidizing

Z

ZINC

Matthias Labrenz¹, Gregory K. Druschel²
¹IOW-Leibniz Institute for Baltic Sea Research, Section
Biology, Rostock-Warnemuende, Germany
²University of Vermont, Burlington, VT, USA

Synonyms

Spelter (nonscientific)

Definition

Physicochemical characteristics

Zinc (Zn) is the 23rd most abundant element in the Earth's crust, and exists as a blue-whitish metal relatively weak with a melting point of 419.5°C and a boiling point of 907°C, with a density of 7.133 g/cm³ (Henkin, 1984). Zn consists of a mixture of the five stable isotopes ⁶⁴Zn (48.6%, atomic mass 63.9), ⁶⁶Zn (27.9%, atomic mass 65.9), ⁶⁷Zn (4.1%, atomic mass 66.9), ⁶⁸Zn (18.8%, atomic mass 67.9), and ⁷⁰Zn (0.6%, atomic mass 69.9) (Coplen et al., 2002). Moreover, six synthetic radioactive isotopes are known: ⁶²Zn, ⁶³Zn, ⁶⁵Zn, ⁶⁹Zn, ⁷²Zn, and ⁷³Zn. The atomic number is 30. Zinc metal is highly reactive and produces various different salts, but it is only stable in water as the Zn²⁺ ion and associated complexes and minerals. Its sulfates and chlorides are water-soluble and its sulfides, oxides, carbonates, phosphates, silicates, as well as organic complexes are water-insoluble (Henkin, 1984). Zinc, like many other metals, also exists as dissolved metal-sulfide cluster complexes (particularly Zn₃S₃ and Zn₄S₆⁴⁻), which can exist even in oxic river waters for extended periods and account for up to 20% of the total dissolved Zn in solution (Rozan et al., 2000). Zinc as an element is not available as a metabolic component as it only exists in the Zn²⁺ state in water, but the strong bonds formed with dissolved sulfide ions result in

highly insoluble ZnS mineral formation, which is strongly linked to sulfur-based microbial activity.

Abundance and distribution in the environment

Zinc is one of the world's principle ores and stands fourth among all metals in world production (behind iron, aluminum, and copper), used in a variety of ways from metal products to rubber production to medicines (USGS, 2008). The world's total reserve of this widely distributed metal is approximately 460 million tons in the year 2006 (Cohen, 2007). More than 80 zinc minerals are known; ZnS (as cubic sphalerite and hexagonal wurtzite polymorphs) and its weathering products are the most important minerals for commercial use (Henkin, 1984), with smithsonite (ZnCO₃) and willemite (Zn₂SiO₄) being important non-sulfide forms.

Zn ore deposition

The genesis of many types of ZnS ore deposits are often attributed to purely physicochemical processes, and the distinction between abiotic and potentially biotic process is often made based on the temperature of sulfide formation and subsequent ZnS mineral formation. At low temperatures (below 150–175°C), thermochemical sulfate reduction (TSR) is very slow (Goldhaber and Orr, 1995; Ohmoto and Goldhaber, 1997; Thom and Anderson, 2008), whereas the upper temperature limit for microbial sulfate reduction overlaps with the formation of many low-temperature deposits (Ledín and Pedersen, 1996; Druschel et al., 2002). ZnS ore depositional processes without microbial influence were, for example, probably due to depositional environments outside the range of temperatures where microorganisms can survive, and TSR is sufficiently fast for significant ore formation, such as observed for the formation of high temperature hydrothermal Pb–Zn ore deposits as, e.g., described for the Rhodopian metallogenic region

(Tarkian and Breskovska, 1989; Kaiser-Rohrmeier et al., 2004). On the other hand, Mississippi Valley-Type (MVT) deposits also form from highly saline brines, between 50°C and 200°C, and span depositional environments where abiotic and biotic factors may be responsible for zinc sulfide mineralization (Bastin, 1926; Druschel et al., 2002; Thom and Anderson, 2008). Siebenthal (1915) suggested, as early as 1915, that microbial sulfate reduction contributed to the formation of low-temperature strata-bound zinc sulfide deposits. However, there is considerable controversy associated with the interpretation of the complex paragenetic sequences responsible for Zn ore deposit formation, and it is entirely possible that some deposits experience both biological and abiotic ZnS formation at different times and in different places. Trudinger et al. (1972) reviewed the feasibility of biogenic ore formation and addressed three issues: (1) the environmental limits of biogenic sulfate reduction; (2) whether the age of biological sulfate reduction was coextensive with the ages of ancient deposits; and (3) whether the rates of sulfate reduction are sufficient for ore formation. Trudinger et al. (1972) concluded that the information at the time was not adequate to address these questions. However, in the last 30 years significant progress has been made on each of these issues to better evaluate the role of microorganisms in ore deposit formation (Druschel et al., 2002; Spangenberg and Herlec, 2006). Organic geochemical and isotopic indicators of biological sulfate reduction have been recently reported from some Pb and Zn deposits (Hu et al., 1998; Bechtel et al., 1998, 1999; Spangenberg and Herlec, 2006). Moreover, modern in situ formation of pure nanocrystalline ZnS deposits at low temperature from complex groundwater solutions by SRB biofilms has been documented (Labrenz et al., 2000; Labrenz and Banfield, 2004; Moreau et al., 2004). Druschel et al. (2002) modeled the source-to-sink geochemistry of this modern ZnS-forming system and reviewed the relevance of the findings to ZnS ore deposit formation. Aggregation of those 1–5 nm sphalerite and wurtzite crystals additionally emphasizes a size-dependent stability shift in sphalerite–wurtzite stability and the role of extracellular proteins in the aggregation of ZnS nanocrystals (Zhang et al., 2003; Moreau et al., 2007). Luther et al. (1999) also showed the importance of zinc-sulfide molecular clusters (particularly Zn_3S_3 and $\text{Zn}_4\text{S}_6^{4-}$) on the speciation of dissolved zinc and their importance in ZnS nanocrystal formation.

Oxidation of ZnS minerals can occur abiotically in the presence of oxygen or other oxidants (Rimstidt et al., 1994), but is oxidized much faster via the activity of oxidizing microbes. Oxidative dissolution of ZnS and other metal sulfides is the principle cause of acid mine drainage (AMD), forming acidic, metal-rich waters (Nordstrom, 2000; Druschel et al., 2004). ZnS mineral oxidation releases dissolved Zn^{2+} ions and associated complexes into aqueous solutions. When AMD waters containing Zn^{2+} are neutralized, zinc can precipitate as a carbonate or oxide form, but is more commonly associated with iron oxide and oxyhydroxide minerals due to sorption of Zn^{2+}

to those mineral surfaces, which can remove the majority of dissolved zinc from solution at circumneutral pH (Jönsson et al., 2006).

Biological effects of Zinc: Zn contamination and bioremediation

As a micronutrient Zn(II) is an ubiquitous essential metal ion playing an important role in organisms throughout the three domains *Bacteria*, *Archaea*, and *Eukarya*. It is essential for numerous physiological processes, serves as a cofactor in members of all six major functional classes of enzymes and is especially important in the maintenance of protein structure (Blencowe and Morby, 2003). In excess it can have toxic effects, but usually Zn deficiency is more critical for most of the higher organisms (Henkin, 1984).

Usually due to anthropogenic activity zinc has been shown to exist at significantly elevated levels in groundwaters and in soil and sediments (Sani et al., 2001). It has repeatedly been demonstrated by cultivation-dependent as well as independent methods that this contamination can have ecotoxicological effects on, e.g., soil microorganisms, and can change the microbial community composition and activity (Hanbo et al., 2004; Smolders et al., 2004). Unfortunately, critical thresholds for toxic effects of Zn on microorganisms are not easy to develop because these can be influenced by the exposure time of Zn to the environment (Mertens et al., 2006) and the potential adaptation of microbial populations to changing Zn concentrations over time.

Great interest in Zn–microbe interactions has arisen in recent years as scientists and engineers try to remove, recover, or stabilize Zn in soils, contaminated water, or waste streams (Sani et al., 2001). In general, specific metabolic pathways leading to precipitation of heavy metals as metal sulfides, phosphates, or carbonates possess significance for possible biotechnology applications (Kotrba and Ruml, 2000) and, analogous to their potential role in low-temperature Zn ore deposition, SRB are already commonly used for the bioremediation of metal-contaminated soil or water. White et al. (1998), for instance, described an integrated microbial process for the bioremediation of soil contaminated with toxic metals using microbially catalyzed reactions. In this process, bioleaching of Cd, Co, Cr, Cu, Mn, Ni, and Zn via sulfuric acid produced by sulfur-oxidizing bacteria was followed by precipitation of the leached metals as insoluble sulfides by the action of SRB.

Conclusion

Zinc is a common element throughout the world, mainly concentrated in zinc sulfide deposits, and has an important biological role as an essential metal ion in organisms of all three kingdoms. Though microorganisms do not directly metabolize forms of zinc, the mobility and in part the deposition of Zn^{2+} as ZnS minerals is strongly dependent on the activity of sulfur-reducing and sulfur-oxidizing microorganisms. Indications exist for the active

involvement of ancient sulfate-reducing bacteria in the genesis of low-temperature Zinc ore deposits and a potential biosignature capacity of nanocrystalline sphalerite for this process; however, these aspects will need further investigation.

Bibliography

- Bastin, E. S., 1926. A hypothesis of bacterial influence in the genesis of certain sulphide deposits. *Journal of Geology*, **34**, 773–792.
- Bechtel, A., Pervaz, M., and Puttmann, W., 1998. Role of organic matter and sulphate-reducing bacteria for metal sulphide precipitation in the Bahloul Formation at the Bou Grine Zn/Pb deposit (Tunisia). *Chemical Geology*, **144**, 1–21.
- Bechtel, A., Savin, S. M., and Hoernes, S., 1999. Oxygen and hydrogen isotopic composition of clay minerals of the Bahloul Formation in the region of the Bou Grine zinc–lead ore deposit (Tunisia): evidence for fluid–rock interaction in the vicinity of salt dome cap rock. *Chemical Geology*, **156**, 191–207.
- Blencowe, D. K., and Morby, A. P., 2003. Zn(II) metabolism in prokaryotes. *FEMS Microbiology Reviews*, **27**, 291–311.
- Cohen, D., 2007. Earth's natural wealth: an audit. *New Scientist*, May 23, 2007, 34–41.
- Coplen, T. B., Böhlke, J. K., De Bièvre, P., Ding, T., Holden, N. E., Hopple, J. A., Krouse, H. R., Lambert, A., Peiser, H. S., Révész, K., Rieder, S. E., Rosman, K. J. R., Roth, E., Taylor, P. D. P., Vocke, R. D., and Xiao, Y. K., 2002. Isotope-abundance variations of selected elements (IUPAC Technical Report). *Pure and Applied Chemistry*, **74**, 1987–2017.
- Druschel, G. K., Labrenz, M., Thomsen-Ebert, T., Fowle, D. A., and Banfield, J. F., 2002. Biogenic precipitation of monomineralic nanocrystalline sulfides: implications of observed and modeled processes to ore deposition. *Economic Geology*, **97**, 1319–1329.
- Druschel, G. K., Baker, B. J., Gihring, T. H., and Banfield, J. F., 2004. Acid mine drainage biogeochemistry at Iron Mountain, California. *Geochemical Transactions*, **5**, 13–32.
- Goldhaber, M. B., and Orr, W. L., 1995. Kinetic controls on the thermochemical sulfate reduction as a source of sedimentary H₂S. In Vairavamurthy, M. A., and Schoonen, M. A. A. (eds.), *Geochemical Transformations of Sedimentary Sulfur*. ACS Symposium Series 612. Washington, DC: American Chemical Society, pp. 412–425.
- Hanbo, Z., Changqun, D., Qiyong, S., Weimin, R., Tao, S., Lizhong, C., Zhiwei, Z., and Bin, H., 2004. Genetic and physiological diversity of phylogenetically and geographically distinct groups of *Arthrobacter* isolated from lead–zinc mine tailings. *FEMS Microbiology Ecology*, **49**, 333–341.
- Henkin, R. J., 1984. Zinc. In Merian, E. (ed.), in *Gemeinschaft mit Geldmacher-v. Mallinckrodt, Machata, G., Nürnberg, H. W., Schlipköter, H. W., Stumm, W., Metalle in der Umwelt: Verteilung, Analytik, und biologische Relevanz*. Weinheim: Verlag Chemie, pp. 597–629.
- Hu, M. A., Disnar, J. R., Barbanson, L., and Suarez-Ruiz, I., 1998. Processus d'altération thermique, physico-chimique et biologique de constituants organiques et genèse d'une minéralisation sulfurée: le gîte Zn–Pb de La Florida (Cantabria, Espagne). *Canadian Journal of Earth Sciences*, **35**, 936–950.
- Jönsson, J., Jönsson, J., and Lövgren, L., 2006. Precipitation of secondary Fe(III) minerals from acid mine drainage. *Applied Geochemistry*, **21**, 437–445.
- Kaiser-Rohrmeier, M., Handler, R., Quadt, v. A., and Heinrich, C., 2004. Hydrothermal Pb–Zn ore formation in the Central Rhodopian Dome, south Bulgaria: review and new time constraints from Ar–Ar geochronology. *Schweizerische Mineralogische und Petrographische Mitteilungen*, **84**, 37–58.
- Kotrba, P., and Ruml, T., 2000. Bioremediation of heavy metal pollution exploiting constituents, metabolites and metabolic pathways of livings. A review. *Collection of Czechoslovak Chemical Communications*, **65**, 1205–1247.
- Labrenz, M., and Banfield, J. F., 2004. Sulfate-reducing bacteria dominated biofilms that precipitate ZnS in a subsurface circum-neutral pH mine drainage system. *Microbial Ecology*, **47**, 205–217.
- Labrenz, M., Druschel, G. K., Thomsen-Ebert, T., Gilbert, B., Welch, S. A., Kemner, K. M., Logan, G. A., Summons, R. E., De Stasio, G., Bond, P. L., Lai, B., Kelly, S. D., and Banfield, J. F., 2000. Sphalerite (ZnS) deposits forming in natural biofilms of sulfate-reducing bacteria. *Science*, **290**, 1744–1747.
- Ledin, M., and Pedersen, K., 1996. The environmental impact of mine wastes—roles of microorganisms and their significance in treatment of mine wastes. *Earth-Science Review*, **41**, 67–108.
- Luther, G. W., Theberge, S. M., and Rickard, D. T., 1999. Evidence for aqueous clusters as intermediates during zinc-sulfide formation. *Geochimica et Cosmochimica Acta*, **63**, 3159–3169.
- Mertens, J., Springael, D., De Troyer, I., Cheyns, K., Wattiau, P., and Smolders, E., 2006. Long-term exposure to elevated zinc concentrations induced structural changes and zinc tolerance of the nitrifying community in soil. *Environmental Microbiology*, **8**, 2170–2178.
- Moreau, J. W., Webb, R. I., and Banfield, J. F., 2004. Ultrastructure, aggregation-state, and crystal growth of biogenic nanocrystalline sphalerite and wurtzite. *American Mineralogist*, **89**, 950–960.
- Moreau, J. W., Weber, P. K., Martin, M. C., Gilbert, B., Hutcheon, I. D., and Banfield, J. F., 2007. Extracellular proteins limit the dispersal of biogenic nanoparticles. *Science*, **316**, 1600.
- Nordstrom, D. K., 2000. Advances in the hydrogeochemistry and microbiology of acid mine waters. *International Geology Review*, **42**, 499–515.
- Ohmoto, H., and Goldhaber, M. B., 1997. Sulfur and carbon isotopes. In Barnes, H. L. (ed.), *Geochemistry of Hydrothermal Ore Deposits*, 3rd edn. New York: Wiley, pp. 517–611.
- Rimstidt, J. D., Cermak, J. A., and Gagen, P. M., 1994. Rates of reaction of galena, sphalerite, chalcopryrite, and arsenopyrite with Fe(III) in acidic solutions. In Alpers, C. N., and Blowes, D. W. (eds.), *Environmental Geochemistry of Sulfide Oxidation*. ACS Symposium Series 550. Washington DC: American Chemical Society, pp. 2–13.
- Rozan, T. F., Lassman, M. E., Ridge, D. P., and Luther, G. W., 2000. Evidence for iron, copper, and zinc complexation as multinuclear sulphide clusters in oxic rivers. *Nature*, **406**, 879–882.
- Sani, R. K., Peyton, B. M., and Brown, L. T., 2001. Copper-induced inhibition of growth of *Desulfovibrio desulfuricans* G20: assessment of its toxicity and correlation with those of zinc and lead. *Applied and Environmental Microbiology*, **67**, 4765–4772.
- Siebenshal, C. E., 1915. Origin of the lead and zinc deposits of the Joplin region. *U.S. Geological Survey Bulletin*, **606**, 283.
- Smolders, E., Buekers, J., Oliver, I., and McLaughlin, M. J., 2004. Soil properties affecting microbial toxicity of zinc in laboratory-spiked and field-contaminated soils. *Environmental Toxicology and Chemistry*, **23**, 2633–2640.
- Spangenberg, J. E., and Herlec, U., 2006. Hydrocarbon biomarkers in the Topla-Mezica zinc–lead deposits, northern Karavanke/Drau Range, Slovenia: paleoenvironment at the site of ore formation. *Economic Geology*, **101**, 997–1021.
- Tarkian, M., and Breskovska, V., 1989. Greenockite from the Madjarovo Pb–Zn ore district, Eastern Rhodope, Bulgaria. *Mineralogy and Petrology*, **40**, 137–144.
- Thom, J., and Anderson, G. M., 2008. The role of thermochemical sulphate reduction in the origin of Mississippi Valley-type deposits. I. Experimental results. *Geofluids*, **8**, 16–26.

- Trudinger, P. A., Lambert, I. B., and Skyring, G. W., 1972. Biogenic sulfide ores: a feasibility study. *Economic Geology*, **67**, 1114–1127.
- USGS (United States Geological Survey), 2008. Zinc statistics and information. Available from: <http://minerals.usgs.gov/minerals/pubs/commodity/zinc/>.
- White, C., Sharman, A. K., and Gadd, G. M., 1998. An integrated microbial process for the bioremediation of soil contaminated with toxic metals. *Nature Biotechnology*, **16**, 572–575.
- Zhang, H., Huang, F., Gilbert, B., and Banfield, J. F., 2003. Molecular dynamics simulations, thermodynamic analysis and experimental study of phase stability of zinc sulphide nanoparticles. *Journal of Physical Chemistry B*, **107**, 13051–13060.

Cross-references

[Biosignatures in Rocks](#)
[Divalent Earth Alkaline Cations in Seawater](#)
[Isotopes](#)
[Metalloenzymes](#)
[Microbial Communities, Structure, and Function](#)
[Nanocrystals, Microbially Induced](#)
[Sulfate-Reducing Bacteria](#)
[Sulfur Cycle](#)

ICHOLOGY

Murray K. Gingras, Kurt O. Konhauser
University of Alberta, Edmonton, Alberta, Canada

Definition

Ichnology is the study of fossilized, generally macroscopic, animal–sediment interactions. This includes morphological, textural, and local compositional effects related to the animal’s activities. Similar studies in modern sediments are referred to as neoichnology.

Overview

Ichnological studies comprise a wide range of animal–sediment interactions. The scope of animal–sediment interactions was broadly established by Seilacher (1967). He showed that various animal activities, including motility, dwelling, grazing, farming, scavenging, predation, and sessile feeding, are represented by ichnofossils (also referred to as trace fossils).

Bioturbation is the displacement of sediment by animals. In marine environments, common mediators of bioturbation include worm-form animals (such as nemerteans, polychaetes, and hemichordates), marine crustaceans, bivalves, motile echinoderms, sponges, and sea anemones.

Ichnofossils can form from impressions on the sediment surface, intrusion into the sediment, or from excavation of sediment. The former can be thought of as tracks and trails, whereas intrusions and excavations are generally represented by burrows, shafts, and tunnels. Biodeformation structures may result from intrusive activities in loose or fluid-rich sediment. Intrusive, excavatory, and biodeformational structures impact the sedimentary and geochemical character of the surrounding sediment. Traces also incorporate, rework, and redistribute organics

in sediment. This influences the geochemical zonations associated with sedimentary surfaces, and in this regard, bioturbation can be viewed as a diagenetic process.

Biomechanical alteration of sediment texture

Burrowing organisms significantly alter the physical character of a substrate. The changes involved include redistribution of grain sizes (vertically and laterally), modification of grain size, sediment compaction, and sorting (summarized in Bromley, 1996). The physical manifestations of bioturbation are variable depending on the intrusive process employed by the animal (i.e., inversion, compression, excavation, or backfill).

In clastic sediments, the most important result of bioturbation is the rearrangement of coarse *versus* fine grains. This results in an alteration of permeable pathways in the sedimentary media that can influence diagenetic reactions. In carbonate sediment, grain-size reduction probably represents the most significant substrate modification (Chow and Longstaffe, 1995). Reduction of substrate grain size permits a larger surface area to interact with diagenetic fluids and provides more nucleation sites in a given volume.

Biochemical alteration of sediment texture

An important result of bioturbation is the incorporation of localized, concentrated organic material in the form of mucus or feces. Burrow-associated organics induce a geochemical microenvironment that may extend several centimeters, or even decimeters, into the substrate. In well-aerated sediments, burrow linings comprising agglutinated, fecal or primary organic material contain the most concentrated local sources of organic material. These burrows therefore provide excellent substrates for bacterial colonization. Grazing and mining of intact organic remains further lead to their disintegration and introduce larger surface areas for microbial respiration. Moreover,

the ingestion of refractory organic carbon can lead to the excretion of more labile fecal pellets (Lee, 1992).

With most intrusive bioturbation, the sediment–water interface increases in surface area as a result of infaunal animal colonization. The larger (and deepened) sediment–water interface alters chemical fluxes between the sediment and water column. Animals that pump water through their burrow (bioirrigation) increase the rate of chemical flux. Most notably, irrigation of worm burrows with oxygenated seawater results in increased downward and lateral diffusion of O₂, which then affects the oxidation of redox-sensitive elements. For the aforementioned reasons, the accepted scheme of biogeochemical zones in sediment is an oversimplification. Bioturbation influences the solid phase and pore-water properties of sediment by increasing the transport of diagenetic reactants and products across the sediment–water interface. Consequently, surficial sediments cannot be considered a homogeneous medium characterized by one-dimensional vertical diffusion. They are, instead, heterogeneous and intensively mixed with a complex spatial distribution of biogeochemical zones that are difficult to accurately resolve (Aller, 1980).

Metal ion enrichment in ichnofossils

Cation enrichment in burrow linings is the result of sorptive processes. Metals concentrate in burrow linings in three ways: (1) adsorption, as oxide or oxyhydroxide coatings on to the high-energy surfaces provided by fine-grained particles (silt and clay); (2) as sulfide or phosphate phases under reducing conditions; or (3) associated with organic material (as adsorbed coatings or forming organo-metallic complexes) (Over, 1990). The relative importance of each type of enrichment mechanism varies with the depth of burrow penetration, the grain size/composition of the sediment, and bottom-water and pore-water composition.

Ichnofossils and cementation

Trace fossils commonly influence local cementation patterns by changing the sediment texture, its redistribution, and local composition (Gingras et al., 2004). They also facilitate the accumulation of microbial communities, and through their chemoheterotrophic metabolisms, the microbes can alter pore-water chemistry and saturation states. Ichnofossils also possess higher metal concentrations which can serve as nucleation sites for authigenic mineral formation. Although ichnofossils have been strongly linked to the distribution of microorganisms, there is no existing model associating trace fossil diagenesis to microorganic process.

Summary

The presence of ichnofossils/bioturbation in a sedimentary substrate has the effect of altering sediment distribution and permeability pathways. Moreover, the redistribution and stowage of organics in and around

ichnofossils strongly influence the distribution of microorganisms associated with the sedimentary surface. This has an impact on the nature and distribution of biochemical zonations in sediment. The mechanical and (bio)chemical nature of trace fossils influences diagenetic processes.

Bibliography

- Aller, R. C., 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. *Geochimica et Cosmochimica Acta*, **44**, 1955–1965.
- Bromley, R. G., 1996. *Trace Fossils: Biology, Taphonomy and Applications*, 2nd edn. London: Chapman and Hall, p. 349.
- Chow, N., and Longstaffe, F., 1995. Dolomites of the Middle Devonian Elk Point Formation, Southern Manitoba: intrinsic controls on early dolomitization. *Bulletin of Canadian Petroleum Geology*, **43**, 214–225.
- Gingras, M. K., Pemberton, S. G., Muelenbachs, K., and Machel, H., 2004. Conceptual models for burrow-related, selective dolomitization with textural and isotopic evidence from the Tyndall Stone, Canada. *Geobiology*, **2**, 21–30.
- Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochimica et Cosmochimica Acta*, **56**, 3323–3335.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Seilacher, A., 1967. Bathymetry of trace fossils. *Marine Geology*, **5**, 413–428.

Cross-references

[Biosignatures in Rocks](#)
[Mat-Related Sedimentary Structures](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Organomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Trace Fossils: Neoproterozoic](#)

IMMUNOLocalization

Michael Hoppert, Christoph Wrede
 University of Göttingen, Göttingen, Germany

Synonyms

Immunocytochemistry; Immunodetection; Immunohistochemistry; Immunolabeling

Definition

Immunolocalization: Technique using specific antibodies to localize macromolecules (proteins, polysaccharides) within biological material (subcellular fractions, cells, tissues, biofilms).

Introduction

Immunolocalization is based on the detection of specific targets (“antigens”; mostly proteins or polysaccharides) in biological samples (cells, tissue, biofilms) by antibodies

- Fein, J. B., Scott, S., and Rivera, N., 2002. The effect of Fe and Si adsorption by *Bacillus subtilis* cell walls: insights into non-metabolic bacterial precipitation of silicate minerals. *Chemical Geology*, **182**, 265–273.
- Fournier, R. O., 1985. The behaviour of silica in hydrothermal solutions. *Reviews in Economic Geology*, **2**, 45–61.
- Ihler, R. K., 1979. *The Chemistry of Silica*. New York: Wiley-Interscience.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1997. Biogenicity of silica precipitation around geysers and hot-spring vents, North Island, New Zealand. *Journal of Sedimentary Research*, **67**, 88–104.
- Jones, B., Renaut, R. W., and Rosen, M. R., 1998. Microbial biofacies in hot-spring sinters: a model based on Ohaaki Pool, North Island, New Zealand. *Journal of Sedimentary Research*, **68**, 413–434.
- Jones, B., Renaut, R. W., and Rosen, M. R., 2001. Taphonomy of silicified filamentous microbes in modern geothermal sinters – implications for identification. *Palaios*, **16**, 580–592.
- Jones, B., Konhauser, K. O., Renaut, R., and Wheeler, R., 2004. Microbe silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society of London*, **161**, 983–993.
- Konhauser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial-silica interactions in modern hot spring sinter: possible analogues for Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–435.
- Konhauser, K. O., Jones, B., Phoenix, V. R., Ferris, F. G., and Renaut, R. W., 2004. The microbial role in hot spring silicification. *Ambio*, **33**, 552–558.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A.-L., and Ferris, F. G., 2005. Thermophilic silicification: the role of Aquificales in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Phoenix, V. R., Adams, D. G., and Konhauser, K. O., 2000. Cyanobacterial viability during hydrothermal biomineralization. *Chemical Geology*, **169**, 329–338.
- Phoenix, V. R., Martinez, R. E., Konhauser, K. O., and Ferris, F. G., 2002. Characterization and implications of the cell surface reactivity of the cyanobacteria *Calothrix* sp. *Applied and Environmental Microbiology*, **68**, 4827–4834.
- Phoenix, V. R., Konhauser, K. O., and Ferris, F. G., 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe-Si systems: implications for microbial silicification in hot-springs. *Canadian Journal of Earth Sciences*, **40**, 1669–1678.
- Walter, M. R., Bauld, J., and Brock, T. D., 1972. Siliceous algal and bacterial stromatolites in hot spring and geyser effluents of Yellowstone National Park. *Science*, **178**, 402–405.
- Yee, N., Phoenix, V. R., Konhauser, K. O., Benning, L. G., and Ferris, F. G., 2003. The effect of cyanobacteria on silica precipitation at neutral pH: implications for bacterial silicification in geothermal hot springs. *Chemical Geology*, **199**, 83–90.

Cross-references

[Cherts](#)
[Cyanobacteria](#)
[Diatoms](#)
[Hydrothermal Environments, Terrestrial](#)
[Microbial Biomineralization](#)
[Microbial Surface Reactivity](#)
[Radiolaria](#)

MICROBIAL SURFACE REACTIVITY

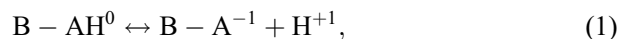
David A. Fowle, Kurt O. Konhauser
 University of Alberta, Edmonton, Alberta, Canada

Definition

The chemical charge on a cell surface caused by the deprotonation/protonation of organic functional groups.

Overview

Similar to an aqueous organic acid, bacterial cell surfaces can either bind or release protons (H^+) into solution depending upon the solution pH. In the case of the latter, this deprotonation process, which accurately mimics a number of the functional groups associated with cell surfaces, leads to the formation of an organic anion, or ligand. Unlike an aqueous organic acid, which exhibits proton buffering over a narrow pH range (± 1 –2 units), bacterial cell walls have continuous buffering capacity over a wide range of pH values which span most geologic settings (pH 2–10). Ultimately, most surface reactivity of bacteria with its external environment results from the enhanced electronegativity of the cell wall as these functional groups deprotonate. In its most simplistic form, deprotonation can be expressed by the following equilibrium reaction:



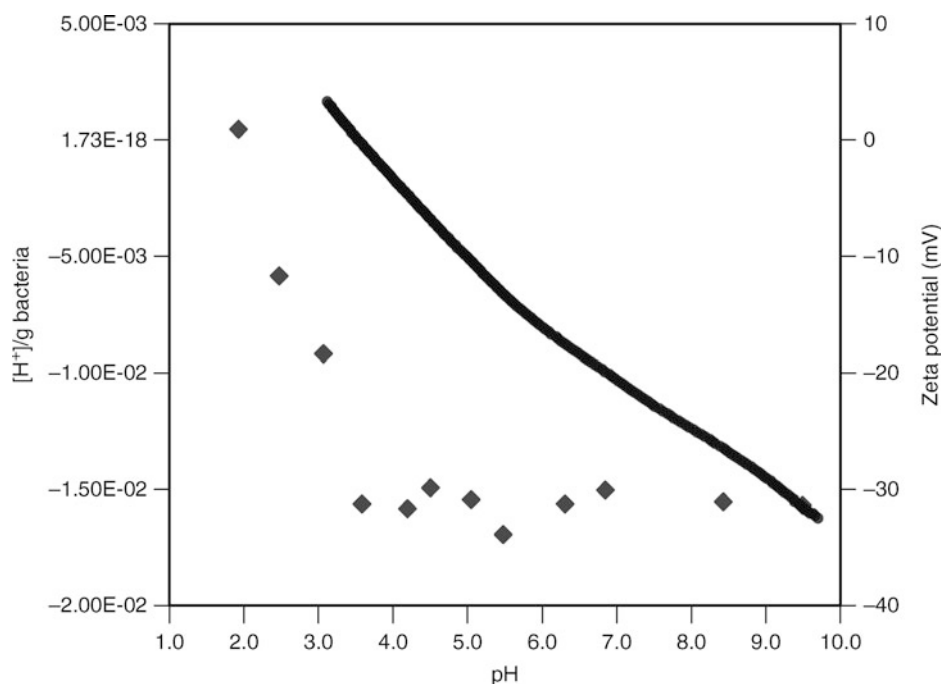
where B denotes a bacterium to which each protonated ligand type, A, is attached. The distribution of protonated and deprotonated sites can be quantified with the corresponding mass action equation:

$$K_a = \frac{[B - A^{-1}][H^+]}{[B - AH^0]}, \quad (2)$$

where K_a is the dissociation constant; $[B - A^{-1}]$ and $[B - AH^0]$ represent the concentration of exposed deprotonated and protonated ligands on the bacterium, respectively (in mol l^{-1}); and $[H^+]$ represents the activity of protons in solution. Each functional group has its own K_a , and based on equation (2), the pH at which $[B - A^{-1}]$ and $[B - AH]$ are equivalent is known as the pK_a value, where $pK_a = -\log_{10} K_a$. At $pH < pK_a$ surfaces are protonated and at $pH > pK_a$ they are deprotonated.

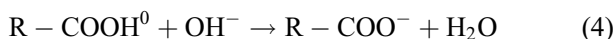
The ionization of functional groups provides an electrical charge ($[L]_T$ represented in mol mg^{-1}), which in the laboratory can be calculated as a function of pH from the difference between total base or acid added to a suspension of organic material and the equilibrium H^+ and OH^- ion activities (Reaction 3):

$$[L]_T = \frac{C_a - C_b \pm [OH^-] - [H^+]}{S} \quad (3)$$



Microbial Surface Reactivity, Figure 1 Experimental data demonstrating the buffering capacity of *Bacillus subtilis* during acid–base titration (circles; molality of protons added normalized with respect to the wet weight of bacteria present) and zeta potential (diamonds) gathered from titrations of *Bacillus subtilis* in 0.01 M NaNO₃ that were cultured in tryptocase soy broth.

The components, C_a and C_b , are the concentrations of acid and base added, respectively; n_{OH^-} and n_{H^+} are the number of moles of OH^- and H^+ in the solution at the measured pH, respectively; while “S” is the quantity of the solid (usually expressed in dry weight, i.e., mg). When such calculations are done over an entire pH range, an acid–base titration curve is created that shows the pH range over which some functional groups are chemically active and how the net surface charge of the organic surface varies with pH (Figure 1). A large difference in the total base or acid added and the free H^+ ion activity indicates significant pH buffering and a high concentration of surface functional groups. On the titration curve this is shown by a steep slope. A small difference in the total base or acid added and the free H^+ ion activity indicates weak pH buffering and a low concentration of surface functional groups. This translates into a gentle slope on a titration curve. In terms of microbial biomass, carboxyl groups account for the buffering capacity from pH 2–6 (Reaction 4), phosphate groups from pH 5–8 (Reaction 5), amino groups at pH > 8 (Reaction 6), and hydroxy groups deprotonate at pH values above 10 (Reaction 7) (see Konhauser, 2007).



As a consequence of the deprotonation reactions, the microbial surface develops an electrical charge. Therefore, when subjected to an electric field, microorganisms move in the direction, and at a rate, commensurate with the polarity and density of the overall surface charge. This process is termed electrophoresis, and the electrophoretic mobility of a microbial suspension can be quantified by measuring their velocity in an electric field. This measurement can, in turn, be used to calculate the zeta potential (ζ), using the Smoluchowski equation below (Hunter, 2001):

$$\zeta = \frac{\eta \mu}{\epsilon_0 \epsilon} \quad (8)$$

The component “ μ ” is the electrophoretic mobility of a particle; ϵ_0 and ϵ are the relative dielectric constants of the vacuum and solution, respectively; and η is the viscosity of the solution. The zeta potential reflects the electrical potential at the interfacial region (viewed as a shear plane) separating an inner layer, where cations are held tightly in place and move with the bacterium, and an outer layer where ions are mobile (Wilson et al., 2001). The greater the absolute value of the ζ potential, the greater the charge

density on the surface (Blake et al., 1994). The electrophoretic mobility (and zeta potential) is pH dependent because the activity of protons in solution controls the ionization reactions of functional groups at the microbial surface (Ahimou et al., 2001) (Figure 1). This leads us to the concept of isoelectric point, which is defined as the pH value where the net surface charge of a solid is zero. The isoelectric point can be estimated from acid–base titrations, but it can also be directly measured with electrophoretic mobility experiments because at the isoelectric point microbes do not exhibit motion in an electric field. The isoelectric point of bacterial cell walls is typically between pH 2–4 (Harden and Harris, 1953). This means that at low pH, when the surface functional groups are fully protonated, bacteria are either neutral or positively-charged, the latter being the result of a cell possessing abundant amino groups. Meanwhile, at the growth pH of most bacteria, cells inherently display a net negative charge and the magnitude of negativity increases with higher pH values. As a result, under low pH conditions, most bacterial surfaces behave hydrophobically, but with an increasingly hydrophilic nature with increasing pH.

One of the major challenges faced by the researchers today is how to translate acid–base titration data in terms of cell wall biochemistry. Ascribing pK_a values from a titration curve to specific functional groups can be problematic because there is often significant variation in pK_a values for the same functional group. This occurs because the magnitude of the dissociation constant is controlled by the structure of the molecule to which it is attached (see Martell and Smith, 1977 for details). Consequently, a single carboxyl group in two different organic acids will have different pK_a values, as will an organic acid with multiple carboxylic groups. As might then be expected, the pK_a for a carboxyl group on one microbial species versus another could yield widely different values simply because of subtle conformational variations within the wall macromolecules. Furthermore, titration experiments are only able to resolve those groups that contribute significant amounts of protons to solution. Minor groups are simply undetectable with the resolution of current techniques. It is thus important to keep in mind that the model-derived binding sites do not directly represent the functional groups of the cell surface; their identity can only be inferred by comparison of the functional group pK_a values with pK_a values of model compounds. Unequivocal identification of the types of functional groups responsible for acid–base buffering may be obtained by spectroscopic techniques, such as Fourier transform infrared spectroscopy (FTIR), calorimetry or gas/liquid chromatography of cell wall extracts. For instance, Yee et al. (2004) and Jiang et al. (2004) used infrared spectroscopy to examine the pH dependence of deprotonation of bacterial cell-wall functional groups. Although not all pH conditions were studied, it was clear that the vibrational frequencies of carboxyl type groups increased with increasing pH. Calorimetric measurements of protonation reactions on bacterial surfaces indicate

these reactions are exothermic with relatively small site-specific entropies (Gorman-Lewis et al., 2006). This provides more evidence that (1) hydrogen bonding between protonated and deprotonated sites likely stabilizes the cell wall, and (2) bacterial cell wall functionalities are similar to multifunctional organic acids rather than simple organic acids.

Summary

Microbial surface reactivity results from the protonation and deprotonation of the organic moieties of microbial cell surfaces including carboxyl, phosphate, amino, and potentially hydroxyl groups. These groups behave similarly to multifunctional organic acids – they hydrogen bond with each other which leads to negative charge on the cell wall as pH increases. At most environmentally relevant pH values, microbial cell surfaces will possess a net negative charge, and therefore, will interact with positively charged cations or mineral surfaces.

Bibliography

- Ahimou, F., Paquot, M., Jacques, P., Thonart, P., and Rouxhet, P. G., 2001. Influence of electrical properties on the evaluation of the surface hydrophobicity of *Bacillus subtilis*. *Journal of Microbiological Methods*, **45**, 119–126.
- Blake, R. C., Shute, E. A., and Howard, G. T., 1994. Solubilization of minerals by bacteria: electrophoretic mobility of *Thiobacillus ferrooxidans* in the presence of iron, pyrite, and sulfur. *Applied and Environmental Microbiology*, **60**, 3349–3357.
- Gorman-Lewis, D., Fein, J. B., and Jensen, M. P., 2006. Enthalpies and entropies of proton and cadmium adsorption onto *Bacillus subtilis* bacterial cells from calorimetric measurements. *Geochimica et Cosmochimica Acta*, **70**, 4862–4873.
- Harden, V. P., and Harris, J. O., 1953. The isoelectric point of bacterial cells. *Journal of Bacteriology*, **65**, 198–202.
- Hunter, R. J., 2001. *Foundations of Colloid Science*. New York: Oxford University Press.
- Jiang, W., Saxena, A., Song, B., Ward, B. B., Beveridge, T. J., and Myneni, S. C. B., 2004. Elucidation of functional groups on Gram-positive and Gram-negative bacterial surfaces using infrared spectroscopy. *Langmuir*, **26**, 11433–11442.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Publishing.
- Martell, A. E., and Smith, R. M., 1977. *Critical Stability Constants. III. Other Organic Ligands*. New York: Plenum Press.
- Wilson, W. W., Wade, M. M., Holman, S. C., and Champlin, F. R., 2001. Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. *Journal of Microbiological Methods*, **43**, 153–164.
- Yee, N., Benning, L. G., Phoenix, V. R., and Ferris, F. G., 2004. Characterization of metal-cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. *Environmental Science and Technology*, **38**, 775–782.

Cross-references

[Metalloenzymes](#)
[Metals, Acquisition by Marine Bacteria](#)
[Microbial Biomineralization](#)
[Microbial-Metal Binding](#)
[Organomineralization](#)
[Siderophores](#)

- formation, Lower Mississippian, New Mexico, USA. In Strogon, P., Somerville, I. D., and Jones, G. L. (eds.), *Recent Advances in Lower Carboniferous Geology*. Geological Society Special Publication, 107, pp. 111–126.
- Kirkby, K. C., and Hunt, D., 1996. Episodic growth of a Waulsortian buildup: the Lower Carboniferous Muleshoe Mound, Sacramento Mountains, New Mexico, USA. In Strogon, P., Somerville, I. D., and Jones, G. L. (eds.), *Recent Advances in the Lower Carboniferous Geology*. Geological Society of London Special Publication, 107, pp. 97–110.
- Lees, A., 1964. The structure and origin of the Waulsortian (Lower Carboniferous) “reefs” of west-central Eire. *Philosophical Transactions of the Royal Society of London, Series B*, **247**, 483–531.
- Lees, A., 1988. Waulsortian reefs: the history of a concept. *Mémoires de l’Institut Géologique de l’Université de Louvain*, **34**, 43–55.
- Lees, A., Hallet, V., and Hibo, D., 1985. Facies variation in Waulsortian buildups. Part 1. A model from Belgium. *Geological Journal*, **20**, 133–158.
- Lees, A., and Miller, J., 1985. Facies variation in Waulsortian buildups. Part 2. Mid-Dinantian buildups from Europe and North America. *Geological Journal*, **20**, 159–180.
- Lees, A., and Miller, J., 1995. Waulsortian banks. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-mounds, their Origin and Evolution*. International Association of Sedimentologists Special Publication, 23, pp. 191–271.
- Meyers, W. J., 1974. Carbonate cement stratigraphy of the Lake Valley Formation (Mississippian), Sacramento Mountains, New Mexico. *Journal of Sedimentary Petrology*, **44**, 837–861.
- Miller, J., 1986. Facies relationships and diagenesis in Waulsortian mud mounds from the Lower Carboniferous of Ireland and N England. In Schroeder, J. H., and Purser, B. H. (eds.), *Reef Diagenesis*. Berlin: Springer, pp. 311–335.
- Monty, C. L. V., 1995. The rise and nature of carbonate mud-mounds: an introductory actualistic approach. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud Mounds, their Origin and Evolution*. International Association of Sedimentologists Special Publication, 23, pp. 11–48.

Cross-references

Bacteria
 Biofilms
 Calcite Precipitation, Microbially Induced
 Cyanobacteria
 Extracellular Polymeric Substances (EPS)
 Microbial Communities, Structure, and Function
 Microbialites
 Mud Mounds
 Reefs

WHALE AND WOOD FALLS

Steffen Kiel
 University of Göttingen, Göttingen, Germany

Synonyms

Biogenic substrates; Food falls; Nekton falls; Organic falls; Sunken wood

Definition

Whale fall: Carcass of a large marine mammal of the order Cetacea (whales) that has sunken to the seafloor.

Wood fall: A piece of wood that has sunken to the seafloor.

History

The first examples of specialized invertebrates inhabiting bones and wood in the *deep sea* (qv) were recognized during the deep-sea expeditions in the early to mid 1900s. Because these samples were recovered by dredging, the complexity of whale- and wood-fall ecosystems was not recognized before the advent of deep-sea research vessels. Pioneering experimental work on wood-falls was carried out in the early 1970s by Ruth Turner (1914–2000), using the deep-sea research vessel *Alvin*. This research came to a halt in the late 1970s when deep-sea *hydrothermal vent systems* (qv) and their fascinating fauna were discovered and *Alvin* was occupied by exploring these systems. The first deep-sea whale-fall ecosystem was accidentally discovered by Craig Smith during an *Alvin* dive in 1987 and consisted of a whale skeleton associated with many chemosymbiotic taxa that were also known from hydrothermal vents (Smith et al., 1989). Nowadays, whale-fall ecosystems are mainly investigated by sinking stranded whales which are then studied by submersibles and/or ROVs. The last major discovery in this field was *Osedax*, a “bone-eating” siboglinid worm living in symbiosis with bacteria that degrade bone lipids and cartilage (Rouse et al., 2004).

Bivalve-bored wood has been known from the fossil record for centuries, but reports on other invertebrate groups that utilized wood falls in the geologic past are rare, and entire fossil wood-fall ecosystems have only been described very recently (Kiel and Goedert, 2006b; Kiel et al., 2009). Fossil examples of deep-sea whale-fall ecosystems were discovered soon after the discovery of the modern ones and are especially common in uplifted deep-water sediments around the North Pacific margin (Squires et al., 1991; Kiel and Goedert, 2006a). Similar ecosystems were recently found on two late Cretaceous plesiosaur (a marine reptile) skeletons in Japan (Kaim et al., 2008).

Whale falls

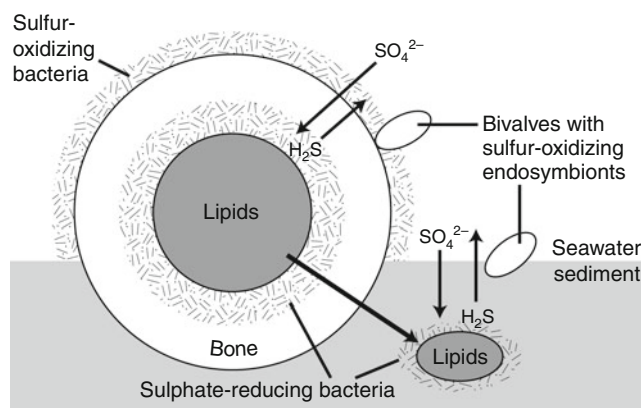
Whale falls are the basis for the most species-rich deep-sea ecosystem, with more than 400 species known to date (Baco and Smith, 2003). Whale falls pass through three ecologic stages that vary in length and can overlap. At first the flesh of the whale carcass is removed by sleeper sharks, hagfish, and other scavenging deep-sea fishes. In the second stage small annelids and crustaceans consume the remaining particulate organic matter. The main source of nutrients during the third stage is the hydrogen sulfide (H₂S) generated by the anaerobic decay of bone lipids, which is utilized by species harboring chemotrophic endosymbionts or by species that graze on sulfur-oxidizing

bacteria (Smith and Baco, 2003). While this “sulfophilic stage” can last up to 100 years in oxygen-poor ocean basins, it can be as short as a few years in areas where *Osedax* rapidly consumes and destroys the bones (Braby et al., 2007; Fujiwara et al., 2007).

Whale bones consist of up to 65 wt% of lipids. These lipids are consumed by *sulfate-reducing bacteria* (qv) at the oxic–anoxic interface in the bone, using seawater as sulfate source and emitting H_2S as metabolic byproduct. This H_2S is then consumed by *Beggiatoa* (qv) and other sulfur-oxidizing bacteria on the surface of the skeleton (Figures 1 and 2). Bone lipids and other whale remains entering the, or covered by, sediment are also consumed in this way. The resulting H_2S is either consumed by bacterial mats surrounding the whale carcass, or by invertebrates harboring sulfur oxidizing bacteria. These invertebrates include siboglinid tube worms, solemyid,



Whale and Wood Falls, Figure 1 Whale skeleton in the deep sea, covered by a large bacterial mat; photo by Craig R. Smith.



Whale and Wood Falls, Figure 2 Generalized geobiologic processes at whale falls. (Modified from Smith and Baco, 2003.)

lucinid, and vesicomyid clams, and bathymodiolin mussels. Bacteria-grazing taxa include provannid, limpet-like and various other small-sized gastropods (“skeneimorphs”), and polynoid annelids (Smith and Baco, 2003).

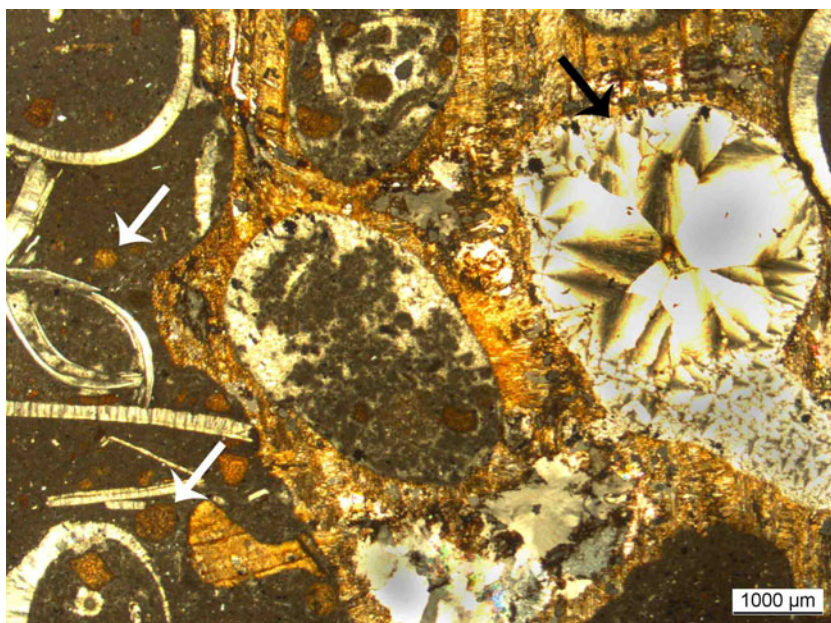
Fossil whale fall communities are known from to Pliocene sediments. They preserve mollusk associations resembling those at modern whale falls, and fossil traces of *Osedax* (Kiel et al., 2010).

Wood falls

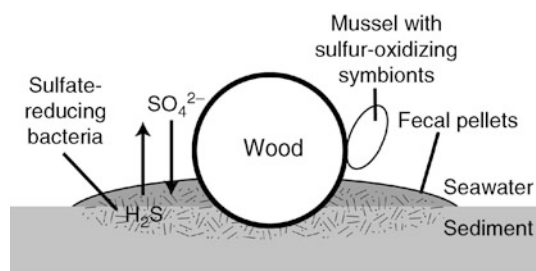
Wood falls in the deep sea support complex ecosystem of 40 or more invertebrate species, some of which are endemic to this habitat. The wood is utilized by several xylophagous (wood-eating) invertebrate groups. Most important are wood-boring bivalves of the heterodont families Teredinidae (also known as shipworms; mainly in shallow water) and Xylophaginae (mainly in deep water below 100 m) which rapidly consume and destroy the wood, presumably aided by cellulolytic symbiotic bacteria (Turner, 1973; Distel and Roberts, 1997). Shallow water wood falls are also utilized by wood-boring isopods of the family Limnoriidae. In deeper water exists a small number of xylophagous gastropod limpets and chitons (polyplacophorans), which are preyed upon by gastropods, annelids, and crustaceans. The microbial fauna on wood falls consists of a variety of fungi and bacteria (Kohlmeyer and Kohlmeyer, 1979; Palacios et al., 2006) and these microbes are grazed upon by gastropods and chitons. Chemosymbiotic species which are phylogenetically related to those at whale falls are also found on sunken wood. They probably rely on sulfide from the anaerobic decay of fecal pellets of the shipworms, or from the decaying wood itself (Figures 3 and 4; Kiel and Goedert, 2006b; Pailleret et al., 2007). The fossil record of deep-sea wood-fall communities extends back into the late Cretaceous.

Summary

Whale and wood falls in the deep sea harbor species-rich ecosystems that rely to a certain extent on H_2S from the decay of the bones and the wood. Many of the species inhabiting these habitats are endemic. Especially the chemosymbiotic and bacteria-grazing taxa are phylogenetically related to those living at *hydrothermal vents* (qv) and *cold seeps* (qv). The main H_2S source for the chemosymbiotic taxa at whale falls is the anaerobic breakdown of bone lipids by sulfate-reducing bacteria. In the case of the wood falls, it is the anaerobic decay of excrements of wood-boring bivalves (Xylophaginae). Wood-fall communities with a fauna that is taxonomically comparable to the modern wood-fall fauna are known from the late Cretaceous. Whale-fall communities are as old as ocean-going whales (late Eocene). Similar ecosystems with bacteria-grazing gastropods on plesiosaur bones are known from the late Cretaceous.



Whale and Wood Falls, Figure 3 Thin section image of a fossil wood fall. *White arrows* indicate fecal pellets of wood-boring xylophagous bivalves, *black arrow* indicates borehole filled by authigenic carbonate.



Whale and Wood Falls, Figure 4 Generalized geobiologic processes at wood falls. (Redrawn from Kiel, 2008.)

Bibliography

- Baco, A. R., and Smith, C. R., 2003. High species richness in deep-sea chemoautotrophic whale skeleton communities. *Marine Ecology Progress Series*, **260**, 109–114.
- Braby, C. E., Rouse, G. W., Johnson, S. B., Jones, W. J., and Vrijenhoek, R. C., 2007. Bathymetric and temporal variation among *Osedax* boneworms and associated megafauna on whale-falls in Monterey Bay, California. *Deep-sea Research*, **1**, 54, 1773–1791.
- Distel, D. L., and Roberts, S. J., 1997. Bacterial endosymbionts in the gills of the deep-sea wood-boring bivalves *Xylophaga atlantica* and *Xylophaga washingtona*. *Biological Bulletin*, **192**, 253–261.
- Fujiwara, Y., Kawato, M., Yamamoto, T., Yamanaka, T., Sato-Okoshi, W., Noda, C., Tsuchida, S., Komai, T., Cubelio, S. S., Sasaki, T., Jacobsen, K., Kubokawa, K., Fujikura, K., Maruyama, T., Furushima, Y., Okoshi, K., Miyake, H., Miyazaki, M., Nogi, Y., Yatabe, A., and Okutani, T., 2007. Three-year investigations into sperm whale-fall ecosystems in Japan. *Marine Ecology*, **28**, 219–232.
- Kaim, A., Kobayashi, Y., Echizenya, H., Jenkins, R. G., and Tanabe, K., 2008. Chemosynthesis-based associations on Cretaceous plesiosaurid carcasses. *Acta Palaeontologica Polonica*, **53**, 97–104.
- Kiel, S., 2008. Fossil evidence for micro- and macrofaunal utilization of large nekton-falls: examples from early Cenozoic deep-water sediments in Washington State, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **267**, 161–174.
- Kiel, S., and Goedert, J. L., 2006a. Deep-sea food bonanzas: early Cenozoic whale-fall communities resemble wood-fall rather than seep communities. *Proceedings of the Royal Society B*, **273**, 2625–2631.
- Kiel, S., and Goedert, J. L., 2006b. A wood-fall association from Late Eocene deep-water sediments of Washington State, USA. *Palaios*, **21**, 548–556.
- Kiel, S., Amano, K., Hikida, Y., and Jenkins, R. G., 2009. Wood-fall associations from Late Cretaceous deep-water sediments of Hokkaido, Japan. *Lethaia*, **42**, 74–82.
- Kiel, S., Goedert, J. L., Kahl, W.-A., and Rouse, G. W., 2010. Fossil traces of the bone-eating worm *Osedax* in early Oligocene whale bones. *Proceedings of the National Academy of Sciences of the USA*, **107**, 8656–8659.
- Kohlmeyer, J., and Kohlmeyer, E., 1979. *Marine Mycology: The Higher Fungi*. New York: Academic Press.
- Pailleret, M., Haga, T., Petit, P., Privé-Gill, C., Saedlou, N., Gaill, F., and Zbinden, M., 2007. Sunken wood from the Vanuatu Islands: identification of wood substrates and preliminary description of associated fauna. *Marine Ecology*, **28**, 233–241.
- Palacios, C., Zbinden, M., Baco, A. R., Treude, T., Smith, C. R., Gaill, F., Lebaron, P., and Boetius, A., 2006. Microbial ecology of deep-sea sunken woods: achieving quantitative measurements for bacterial biomass and cellulolytic activities. *Cahiers de Biologie Marine*, **47**, 415–420.

- Rouse, G. W., Goffredi, S. K., and Vrijenhoek, R. C., 2004. *Osedax*: Bone-eating marine worms with dwarf males. *Science*, **305**, 668–671.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology: An Annual Review*, **41**, 311–354.
- Smith, C. R., Kukert, H., Wheatcroft, R. A., Jumars, P. A., and Deming, J. W., 1989. Vent fauna on whale remains. *Nature*, **341**, 27–28.
- Squires, R. L., Goedert, J. L., and Barnes, L. G., 1991. Whale carcasses. *Nature*, **349**, 574.
- Turner, R. D., 1973. Wood-boring bivalves, opportunistic species in the deep sea. *Science*, **180**, 1377–1379.

Cross-references

[Beggiatoa](#)
[Cold Seeps](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Hydrothermal Environments, Marine](#)
[Sulfate-Reducing Bacteria](#)

deposition of this material to the sediments. Furthermore, input of nutrients to the global coastal ocean region have also significantly increased, which may stimulate increased production of organic matter and subsequent deposition of this material to the sediments within this region. As long as surface seawaters are supersaturated with respect to most carbonate phases, microbial decomposition of organic material is the main driver of carbonate dissolution. Consequently, even if the organic material deposited to the sediment–water remained constant, but the microbial activity increased, for example, owing to increasing temperature, carbonate dissolution would also increase. For the next several decades, human activities are likely to continue to lead to acidification of surface seawater and continuous increases in the amount of organic material being deposited to shallow-water sediments, and concurrently with increasing atmospheric concentrations of CO₂, surface and sea surface temperatures are likely to increase with potential increases in microbial and endolithic activity. As a result, dissolution of calcium carbonate minerals, particularly in coastal ocean environments, probably will increase due to all of these forcings. It is possible that under a business as usual scenario of fossil fuel CO₂ emissions, the production of carbonates in the global coastal ocean will be exceeded by their dissolution by year 2150 or earlier.

Summary

Diagenesis refers to those physical, chemical and biological processes that alter the original physical and chemical characteristics and properties of minerals and sediments. Calcium carbonate minerals constitute a substantial fraction of shallow-water sediments and may be subject to significant diagenetic alterations after deposition including dissolution and precipitation reactions. These reactions are largely mediated by microbial processes including oxidation of organic matter under both oxic and anoxic conditions as well as by endoliths, which actively penetrate carbonate substrates. The extent of calcium carbonate dissolution is to a large degree controlled by the amount of organic matter decomposed. Carbonate dissolution is likely to increase in the future in response to human-induced ocean acidification arising from the burning of fossil fuels, but also due to increased riverine transport of organic matter and nutrients to the coastal ocean, enhanced production in situ of organic matter in coastal waters, and subsequent deposition and microbial decomposition of the organic matter in marine sediments.

Acknowledgments

This work was supported by the US National Science Foundation grant OCE-0749401.

Bibliography

Aller, R. C., 1980. Diagenetic processes near the sediment–water interface of Long Island Sound. I. Decomposition and nutrient element geochemistry (S, N, P). *Advances in Geophysics*, **22**, 237–350.

- Andersson, A. J., Bates, N. R., and Mackenzie, F. T., 2007. Dissolution of carbonate sediments under rising pCO₂ and ocean acidification: Observations from Devil's Hole, Bermuda. *Aquatic Geochemistry*, **13**, 237–264.
- Balzer, W., and Wefer, G., 1981. Dissolution of carbonate minerals in a subtropical shallow marine environment. *Marine Chemistry*, **10**, 545–558.
- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.
- Berner, R. A., 1980. *Early Diagenesis, A Theoretical Approach*. Princeton, NJ: Princeton University Press.
- Burdige, D. J., and Zimmerman, R. C., 2002. Impact of sea grass density on carbonate dissolution in Bahamian sediments. *Limnology and Oceanography*, **47**, 1751–1763.
- Caldeira, K., and Wickett, M. E., 2003. Anthropogenic carbon and ocean pH. *Nature*, **425**, 365.
- Mackenzie, F. T., Ristvet, B. L., Thorstenson, D. C., Lerman, A., and Leeper, R. H., 1981. Reverse weathering and chemical mass balance in a coastal environment. In Martin, J. M., Burton, J. D., and Eisma, D. (eds.), *River Inputs to Ocean Systems*. Switzerland: UNEP-UNESCO, pp. 152–187.
- Morse, J. W., and Mackenzie, F. T., 1990. *Geochemistry of Sedimentary Carbonates*. Amsterdam: Elsevier.
- Moulin, E., Jordens, A., and Wollast, R., 1985. Influence of the aerobic bacterial respiration on the early dissolution of carbonates in coastal sediments. In *Proceedings Progress in Belgium Oceanographic Research*, Brussels, Belgium, pp. 196–208.
- Neumann, A. C., 1965. Processes of recent carbonate sedimentation in Harrington Sound Bermuda. *Bulletin of Marine Science*, **15**, 987–1035.

Cross-references

[Alkalinity](#)
[Bioerosion](#)
[Calcite Precipitation, Microbially Induced](#)
[Carbon \(Organic, Degradation\)](#)
[Carbonate Environments](#)
[Carbonates](#)
[Endoliths](#)
[Iron Sulfide Formation](#)
[Microbial Biomineralization](#)
[Pore Waters](#)
[Sediment Diagenesis – Biologically Controlled](#)
[Sulfate-Reducing Bacteria](#)

BIOLOGICAL VOLCANIC ROCK WEATHERING

Charles S. Cockell
 Open University, Milton Keynes, UK

Definition

The biologically induced breakdown of volcanic rocks, including crystalline rocks and glasses.

Volcanic rock weathering and global processes

The weathering of volcanic minerals is known to make a significant contribution to the global silicate weathering budget (Dessert et al., 2003), thus influencing carbon dioxide drawdown and climate control, since carbon

dioxide is consumed in mineral weathering reactions. For example, on land, the Deccan Traps in India, with an estimated area of 10^6 km², is thought to account for about 5% of the global silicate weathering flux (Dessert et al., 2003). In total, basalt rocks may account for over 30% of the global carbon dioxide drawdown in silicate weathering (Gaillardet et al., 1999). The weathering of volcanic rocks also contributes a significant flux of nutrients into the biosphere, and through the weathering of rocks and the formation of clays and secondary minerals, microorganisms accelerate the rate of production of volcanic soils (Andisols) from their parent minerals. Thus, understanding how volcanic rocks weather and what role a biota might play in these processes has importance for understanding climate control, soil formation, and the mechanisms governing the release and distribution of nutrients in the biosphere.

The biological role

The role of plants in accelerating the weathering of volcanic rocks has been established, primarily from studies in Hawaii and Iceland. In Hawaii, plants have been estimated to increase weathering rates by over an order of magnitude compared to unvegetated areas (Cochran and Berner, 1996). In geological terms, the acceleration of weathering caused by plants following their early evolution has been proposed as one mechanism for carbon dioxide drawdown in the late Silurian, resulting in a general cooling trend (Berner, 1992). Plants increase weathering rates by, *inter alia*, physically disaggregating the rock with their roots, the production of rock weathering organic acids, the removal of ions from the subsurface (which changes chemical equilibrium in favor of rock dissolution), and by indirectly producing nutrients and favorable microenvironments for prokaryotes and fungi within the rhizosphere, which themselves weather rocks (see below).

A microbiological involvement in volcanic rock weathering has been established. The primary effect of microorganisms is to accelerate the breakdown of the rock both physically and chemically, often with the formation of secondary minerals such as poorly ordered aluminosilicates and clays (Figure 1). Early studies on the biological weathering of volcanic rocks focused on the role of lichens (Jackson and Keller, 1970; Adamo and Violante, 1991, 2000; Banfield et al., 1999). The earliest study of biological volcanic rock weathering was the seminal study by Fry (1927) who showed that lichens can cause etching, fragmentation, and weakening of obsidian, a silica-rich volcanic glass. Lichens accelerate the physical disaggregation of volcanic rocks as the lichen thallus penetrates the rock substrate. The production of organic acids, organic complexing agents, and the excretion of oxalic acids are just some of the mechanisms by which lichens contribute to the chemical dissolution of the rock substrate (Adamo and Violante, 2000). Oxalic acids are produced by the fungal component in the lichen symbiosis. Calcium oxalate at the lichen–rock interface, although not



Biological Volcanic Rock Weathering, Figure 1 A 1913 basalt flow in Iceland covered in lichens, which play an important role in rock weathering. Image is 20 cm across.

ubiquitous, is one widely reported compound formed on lichen-weathered rocks, which suggests the role of oxalic acids reacting with mineral-derived calcium, although other oxalates, such as copper- and manganese-rich compounds, have been reported.

Lichens have been shown to induce the formation of considerably thicker weathering rinds on crystalline basalts in Hawaii compared to control rocks, which in addition to directly contributing to weathering, may secondarily accelerate the rate of abiotic chemical weathering (Jackson and Keller, 1970). Lichens may also accelerate the dissolution of the silicate from the rock matrix, causing it to be more susceptible to being washed away, promoting the preferential formation of oxides and other secondary minerals from iron. Iron oxide formation is reported at the interface between lichens and basaltic rocks in Hawaii and near Vesuvius, Italy.

Early studies on lichen weathering of volcanic rocks were the first to elucidate two important factors in microbial rock weathering in general: (1) the role of changes in the microenvironment induced by microorganisms at the rock surface, which include changes in pH, water retention, and redox state that alter the equilibrium chemistry of rock dissolution or secondary mineral formation, accelerating rock weathering; and (2) the differential susceptibility of minerals to microbial rock weathering. Calcium-containing plagioclase tends to weather faster than minerals, such as K-feldspars, and these differential effects are determined by the susceptibility of minerals to weathering agents produced by microorganisms or the changes in chemical equilibria.

Since early studies on lichens, many other microorganisms have been examined and found to be capable of accelerating rock weathering. Mechanisms of microbial weathering include: the production of organic acids; the secretion of protons, which changes the local pH at the

rock surface and accelerates weathering; and the production of complexing ligands, such as siderophores, which chelate oxidized iron. It is also becoming clear that these are not merely serendipitous processes resulting from microbial metabolic activity, but that microorganisms actively excrete substances to gain access to nutrients within rocks that might otherwise be limiting, such as major nutrients, including Fe, P, Na, and Ca and trace elements such as K, Ni, or Mn. In addition to the secretion of weathering-promoting substances, by oxidizing or reducing metals during energy acquisition microorganisms can accelerate chemical dissolution or alteration of the rock surface, particularly important examples being iron-oxidizing or reducing bacteria, which use iron as an electron donor or acceptor respectively, and may therefore change the oxidation state of the iron in iron-containing minerals, including olivines and pyroxenes, which form an important component of basaltic mineralogies.

The considerations above apply to both fungi and prokaryotes. In the case of fungi, the penetration of the rock by the hyphae is one primary means by which fungi can invade the interior of a rock and accelerate weathering. Fungi, in particular, are well-known to produce organic acids by which they contribute to rock weathering (Mehta et al., 1978; Gadd, 2006).

Microorganisms do not merely accelerate the weathering of all elements from volcanic rocks. A number of studies have shown that biotic weathering can cause preferential leaching or enrichment of elements at the weathering front or within the sediments produced by volcanic rock weathering. Staudigel et al. (1998), in a study of basaltic glass weathering using a microbial enrichment culture from Loihi seamount, Hawaii, showed that the population of microorganisms, which included heterotrophic bacteria, cyanobacteria, and diatoms, induced an enrichment of calcium in the sediments produced by weathering, but a loss of magnesium, in contrast to the controls which showed the opposite trend. These results, and many others, show that microorganisms can cause a differential rate of leaching of different elements from rocks. Bacteria on their own have been shown to induce the release of iron and manganese. Investigations using a single bacterial species (*Burkholderia fungorum*) (Wu et al., 2007) showed that iron release is induced by changes in the pH at the rock surface. Phosphorus released from the apatite within the basalt rock apparently also helped sustain bacterial growth. All of these experiments show that microorganisms potentially have an important role in modulating the release of different elements from the crust. The oxidation of iron by bacteria in the deep-sea probably gives them an important role in iron cycling and thus weathering of the basalt crust (Edwards et al., 2003).

These data also highlight the general point that although the role of microorganisms in enhancing mineral dissolution has been a major focus of research, it is also important to understand that the weathering of elements from rocks might be influenced by the retardation of elemental release caused by microbial action. For example,

the production of oxidized iron from reduced iron in basaltic minerals such as olivine has been suggested to retard leaching of elements from the rocks (Santelli et al., 2001). Thus, through changes in oxidation states of elements and the production of secondary minerals at rock surfaces, the microbial involvement in volcanic rock weathering may be much more complex than merely enhancing weathering rates, instead changing the leaching rates of specific elements in ways that are either retarded or accelerated compared to abiotic conditions.

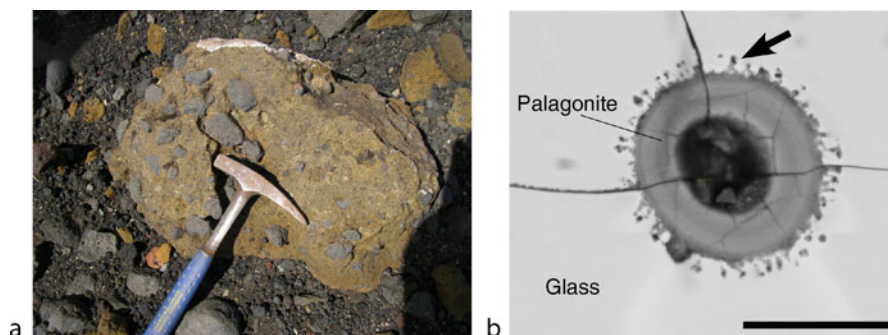
The formation of clays and poorly ordered aluminosilicates, such as smectites and allophane-like materials, has been reported around bacterial aggregates in volcanic ash near Sakurajima volcano, Japan (Kawano and Tomita, 2001). The authors, by carrying out batch culture experiments in the laboratory using bacteria isolated from the ash, suggest that the natural populations are involved in biomineralization and therefore play an important part in bacterial alteration of the weathered volcanic ash. In this case mineral formation is likely to have been facilitated by the bacterial cell surface. Although this work did not show active weathering of the primary rock minerals, it illustrates that the formation of clays and other minerals from weathered volcanic ash by microorganisms can play an important role in defining the chemical characteristics of the weathering environment, which itself will alter the environment for other members of the microbial community which may be directly involved in weathering. The work underlines the important link between rock weathering (dissolution and alteration) and mineral formation (biomineralization) during the transition of primary volcanic minerals to soils such that both processes must be considered to truly understand biological volcanic rock weathering and soil neogenesis.

As a logical development of the work of Fry, Herrera et al. (2008) examined bacterial communities within obsidian in Iceland and showed a diverse population of bacteria, which by the use of hybridization probes were shown to be associated with weathering alteration fronts in the rock. Basaltic rocks in terrestrial environments, as with the oceanic environments described above, have been shown to harbor a high prokaryotic diversity (Gomez-Alvarez et al., 2007).

The mechanisms by which microorganisms weather rocks are still based primarily on chemical measurements such as organic acid production or changes in solution pH. New molecular methods are likely to elucidate pathways by which microorganisms can weather rocks and strengthen our understanding of the link between the organisms and the weathering process.

Microbial borings in volcanic glass

Other evidence for a direct role of microorganisms in volcanic rock weathering has been presented in the form of channels or pitted and corroded granular textures, in reactive volcanic glasses. The most commonly studied of these are endolithic borings into volcanic glasses, including



Biological Volcanic Rock Weathering, Figure 2 (a) An outcrop of subglacial basalt glass at Valafell, Iceland. The glass turns conspicuous brown as it transforms into palagonite, a weathering product, (b) “elongate” (tunnellike) weathering features in a vesicle within the outcrop, features (arrow) are at the interface between the palagonite weathering rind (center) and the glass (scale bar 40 μm). The biological origin of such features in different environments is an ongoing controversy.

deep ocean pillow lavas (Fisk et al., 1998; Torsvik et al., 1998; Thorseth et al., 2003; Storrie-Lombardi and Fisk, 2004). Furnes and Staudigel (1999) suggest a dominant role for bioalteration of volcanic glasses in the upper 300 m of oceanic crust. Glass recovered by drilling from more than 250 m below the sea floor shows channels into altered zones that are 1–10 μm wide and penetrate to a depth of 150 μm . In other settings, larger tubes up to 20 μm in diameter and with a depth of 0.5 mm have been observed. The provenance of some of these borings has been controversial since the organisms responsible for the borings have not been cultured. Abiotic ambient inclusion trails, generated by the pressure forcing of mineral grains, such as sulfides, through glass have been suggested as a mechanism for the production of channel features (Figure 2). To address this possibility a number of approaches have been used to show an involvement of biological activity. These include isotopic and elemental analysis. Binding of the nucleic acid stain DAPI (4',6-diamidino-2-phenylindole) to DNA and fluorescence signals obtained using fluorescent in situ hybridization (FISH) probes against both archaea and bacteria in the channels suggest that organisms were responsible for the weathering. Microprobe analysis shows the presence of carbon not associated with carbonates. Low carbon isotope values were also suggested as evidence supporting a biological role in glass alteration.

Early Earth studies and astrobiology

Volcanic terrains would have dominated the landscape of the Archean Earth when life emerged about 3.5 billion years ago. One reason for the keen interest in weathering textures in volcanic glass is the discovery of similar features in ancient Archean volcanic rocks (Furnes et al., 2004). Insofar as channels are morphologically more distinctive than, for example, irregular pitted features, then they might offer evidence for life in early Earth environments. The age dating of materials with biological isotopic signatures has been used to argue that fossils of putative

ancient microbial volcanic glass borers are the same as the age of the rocks (Banerjee et al., 2006). The study of microbial volcanic rock weathering and the textures and minerals left by such interactions may also offer a means to assay ancient Martian terrains for evidence of life (McLoughlin et al., 2007), particularly since Mars, in the absence of plate tectonics, is a planet dominated by volcanic terrains. On a more general level, understanding the interaction of microorganisms with volcanic rocks may provide a means to understand whether the formation of clays and other secondary minerals from basalts on Mars is a purely abiotic process or whether it could ever have been biologically mediated.

Conclusion

Both field and laboratory observations show that plants and microorganisms play an important role in volcanic weathering. The mechanisms by which they achieve this are diverse, but reduce to some common principles which include physical disruption, production of compounds that attack minerals, alteration of chemical equilibria in favor of dissolution, and changes in redox states of metals which favors dissolution. Through their actions, the biota generally accelerates rock weathering, contributing to the release of rock nutrients into the environment and silicate weathering reactions which themselves contribute to long-term carbon dioxide drawdown.

Bibliography

- Adamo, P., and Violante, P., 1991. Weathering of volcanic rocks from Mt. Vesuvius associated with the lichen *Stereocaulon vesuvianum*. *Pedobiologia*, **35**, 209–217.
- Adamo, P., and Violante, P., 2000. Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science*, **16**, 229–256.
- Banerjee, N. R., Furnes, H., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2006. Preservation of ~3.4–3.5 Ga microbial biomarkers in pillow lavas and hyaloclastites from the Barberton Greenstone Belt, South Africa. *Earth and Planetary Science Letters*, **241**, 707–722.

- Banfield, J. F., Barker, W. W., Welch, S. A., and Taunton, A., 1999. Biological impact on mineral dissolution: application of the lichen model to understanding mineral weathering in the rhizosphere. *Proceedings of the National Academy of Sciences*, **96**, 3404–3411.
- Berner, R. A., 1992. Weathering, plants and the long-term carbon cycle. *Geochimica et Cosmochimica Acta*, **56**, 3225–3231.
- Cochran, M. F., and Berner, R. A., 1996. Promotion of chemical weathering by higher plants: field observations on Hawaiian basalts. *Chemical Geology*, **132**, 71–77.
- Dessert, C., Dupré, B., Gaillardet, J., François, L. M., and Allègre, C. J., 2003. Basalt weathering laws and the impact of basalt weathering on the global carbon cycle. *Chemical Geology*, **202**, 257–273.
- Edwards, K. J., Bach, W., and Rogers, D. R., 2003. Geomicrobiology of the ocean crust: a role for chemoautotrophic Fe-bacteria. *Biological Bulletin*, **204**, 180–185.
- Fisk, M. R., Giovannoni, S. J., and Thorseth, I. H., 1998. Alteration of oceanic volcanic glass: textural evidence of microbial activity. *Science*, **281**, 978–980.
- Fry, E. J., 1927. The mechanical action of crustaceous lichens on substrata of shale, schist, gneiss, limestone, and obsidian. *Annals of Botany*, **151**, 437–460.
- Furnes, H., and Staudigel, H., 1999. Biological mediation in ocean crust alteration: how deep is the deep biosphere? *Earth and Planetary Science Letters*, **166**, 97–103.
- Furnes, H., Banerjee, N. R., Muehlenbachs, K., Staudigel, H., and de Wit, M., 2004. Early life recorded in Archean pillow lavas. *Science*, **304**, 578–581.
- Gadd, G. M., 2006. *Fungi in Biogeochemical Cycles*. Cambridge: Cambridge University Press.
- Gaillardet, J., Dupre, P., Louvat, C. J., and Allègre, C. J., 1999. Global silicate weathering and CO₂ consumption rates deduced from the chemistry of the large rivers. *Chemical Geology*, **159**, 3–30.
- Gomez-Alvarez, V., King, G. M., and Nüsslein, K., 2007. Comparative bacterial diversity in recent Hawaiian volcanic deposits of different ages. *FEMS Microbiology Ecology*, **60**, 60–73.
- Herrera, A., Cockell, C. S., Self, S., Blaxter, M., Reitner, J., Arp, G., Dröse, W., Thorsteinsson, T., and Tindle, A. G., 2008. Bacterial colonization and weathering of terrestrial obsidian in Iceland. *Geomicrobiology Journal*, **25**, 25–37.
- Jackson, T. A., and Keller, W. D., 1970. A comparative study of the role of lichens and “inorganic” processes in the chemical weathering of recent Hawaiian lava flow. *American Journal of Science*, **269**, 446–466.
- Kawano, M., and Tomita, K., 2001. Microbial biomineralization in weathered volcanic ash deposit and formation of biogenic minerals by experimental incubation. *American Mineralogist*, **86**, 400–410.
- McLoughlin, N., Brasier, M. D., Wacey, D., Green, O. R., and Perry, R. S., 2007. Formulating biogenicity criteria for endolithic microborings on early Earth and beyond. *Astrobiology*, **7**, 10–26.
- Mehta, A., Torma, A. E., Murr, L. E., and Berry, V. K., 1978. An SEM characterization of biodegradation of an aluminium-bearing (basalt) rock by fungi. *Scanning Electron Microscopy*, **1**, 171–176.
- Santelli, C. M., Welch, S. A., Westrich, H. R., and Banfield, J. F., 2001. The effect of Fe-oxidizing bacteria on Fe-silicate mineral dissolution. *Chemical Geology*, **180**, 99–115.
- Staudigel, H., Yayanos, A., Chastain, R., Davies, G., Verdurmen, E. A. T., Schiffman, P., Boucier, R., and de Baar, H., 1998. Biologically mediated dissolution of volcanic glass in seawater. *Earth and Planetary Science Letters*, **164**, 233–244.
- Storrie-Lombardi, M. C., and Fisk, M. R., 2004. Elemental abundance distributions in suboceanic basalt glass: evidence of biogenic alteration. *Geochemistry, Geophysics and Geosystems*, **5**, 1–15.
- Thorseth, I. H., Pedersen, R. B., and Christie, D. M., 2003. Microbial alteration of 0–30-Ma seafloor and sub-seafloor basaltic glasses from the Australian Antarctic discordance. *Earth and Planetary Science Letters*, **215**, 237–247.
- Torsvik, T., Furnes, H., Muehlenbachs, K., Thorseth, I. H., and Tumyr, O., 1998. Evidence for microbial activity at the glass-alteration interface in oceanic basalts. *Earth and Planetary Science Letters*, **162**, 165–176.
- Wu, L., Jacobson, A. D., Chen, H., and Hausner, M., 2007. Characterisation of elemental release during microbe-basalt interactions at $T = 28^{\circ}\text{C}$. *Geochimica et Cosmochimica Acta*, **71**, 2224–2239.

Cross-references

[Astrobiology](#)
[Basalt \(Glass, Endoliths\)](#)
[Biodeterioration \(of Stone\)](#)
[Bioerosion](#)
[Biosignatures in Rocks](#)
[Fluorescence In Situ Hybridisation \(FISH\)](#)
[Glass](#)

BIOMARKERS (MOLECULAR FOSSILS)

Jochen J. Brocks¹, Kliti Grice²

¹The Australian National University, Canberra, ACT, Australia

²Curtin University of Technology, Perth, WA, Australia

Synonyms

Biological marker molecules; Geochemical fossils; Molecular fossils

Definition

Biomarkers are the molecular fossils of lipids and other natural products. In sedimentary environments, lipids that escape the remineralization process are commonly chemically reduced to hydrocarbon skeletons. Encased in sedimentary rocks, these skeletons can remain intact over hundreds of millions of years. The structure of biomarkers is often directly related to their precursor lipids and may be diagnostic for a specific group or groups of organisms. Diagnostic biomarkers are used to obtain information about the composition of past (microbial) ecosystems or to determine the first occurrence of organisms in the geological record. As many organisms prefer specific habitats, and as lipid compositions of individual organisms are frequently adjusted to changing physical and chemical conditions, biomarkers may also serve as paleoenvironmental proxies, for example for salinity, temperature, and oxygen availability. In the petroleum industry, biomarkers are utilized to elucidate the extent of biodegradation of crude oil and to obtain information about the thermal history, lithology, depositional environment, and geological age of hydrocarbon source rocks.

- Bertini, I., Ciurli, S., and Luchinat, C., 1995. The electronic structure of FeS centers in proteins and models. A contribution to the understanding of their electron transfer properties. *Structure and Bonding*, **83**, 1–53.
- Carrell, T. G., Tyryshkin, A. M., and Dismukes, G. C., 2002. An evaluation of structural models for the photosynthetic water-oxidizing complex derived from spectroscopic and X-ray diffraction structures. *Journal of Biological Inorganic Chemistry*, **7**, 2–22.
- Chaudhuri, P., Wieghardt, K., Weyhermüller, T., Paine, T. K., Mukherjee, S., and Mukherjee, C., 2005. Biomimetic metal-radical reactivity: aerial oxidation of alcohols, amines, aminophenols and catechols catalyzed by transition metal complexes. *Biological Chemistry*, **386**, 1023–1033.
- Cowan, J. A., 2002. Structural and catalytic chemistry of magnesium-dependent enzymes. *BioMetals*, **15**, 225–235.
- Crans, D. C., Smee, J. J., Galdamauskas, E., and Yang, L., 2004. Chemistry and biology of vanadium and the biological activities exerted by vanadium compounds. *Chemistry Reviews*, **104**, 849–902.
- Degtyarenko, K., 2000. Bioorganic motifs: towards functional classification of metalloproteins. *Bioinformatics Review*, **16**, 851–864.
- Degtyarenko, K., and Contrino, S., 2004. COMe: the ontology of bioinorganic proteins. *BMC Structural Biology*, **4**, 3.
- Ermler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer R. K., 1998. Active sites of transition-metal enzymes with a focus on nickel. *Current Opinon Structural Biology*, **8**, 749–758.
- Hille, R., 2002. Molybdenum and tungsten in biology. *Trends in Biochemical Sciences*, **27**, 360–367.
- Holm, R., Kennepohl, P., and Solomon, E. I., 1996. Structural and functional aspects of metal sites in biology. *Chemistry Reviews*, **96**, 2239–2314.
- Kobayashi, M., and Shimizu, S., 1999. Cobalt proteins. *European Journal of Biochemistry*, **261**, 1–9.
- Lipscomb, W. N., and Sträter, N., 1996. Recent advances in zinc enzymology. *Chemistry Reviews*, **96**, 2375–2433.
- Mauzerall, D. C., 1998. Evolution of porphyrins. *Clinics Dermatology*, **16**, 195–201.
- McCall, K. A., Huang, C.-C., and Fierke, C. A., 2000. Function and mechanism of zinc metalloenzymes. *Journal of Nutrition*, **130**, 1437S–1446S.
- Michibata, H., Yamaguchi, N., Uyama, T., and Ueki, T., 2003. Molecular biological approaches to the accumulation and reduction of vanadium by ascidians. *Coordination Chemistry Reviews*, **237**, 41–51.
- Nordlund, P., and Eklund, H., 1995. Diiron-carboxylate proteins. *Current Opinion in Structural Biology*, **5**, 758–766.
- Ogo, S., Kabe, R., Uehara, K., Kure, B., Nishimura, T., Menon, S. C., Harada, R., Fukuzumi, S., Higuchi, Y., Ohhara, T., Tamada, T., and Kuroki, R., 2007. A dinuclear Ni(μ -H)Ru complex derived from H₂. *Science*, **316**, 585–587.
- Page, M. J., and Di Cera, E., 2006. Role of Na⁺ and K⁺ in enzyme function. *Physiological Reviews*, **86**, 1049–1092.
- Que, L. Jr., and Ho, R. Y. N., 1996. Dioxygen activation by enzymes with mononuclear nonheme iron active sites. *Chemistry Reviews*, **96**, 2607–2624.
- Raymond, J. R., Siefert, J. L., Staples, C. R., and Blankenship, R. E., 2004. The natural history of nitrogen fixation. *Molecular Biology and Evolution*, **21**, 541–554.
- Russell, M. J., and Martin, W., 2004. The rocky roots of the acetyl-CoA pathway. *Trends in Biochemical Sciences*, **29**, 358–363.
- Sigel, R. K. O., and Pyle, A. M., 2007. Alternative roles for metal ions in enzyme catalysis and the implications for ribozyme chemistry. *Chemistry Reviews*, **107**, 97–113.
- Song, L.-C., Yang, Z.-Y., Bian, H.-Z., Liu, Y., Wang, H.-T., Liu, X.-F., and Hu, Q.-M., 2005. Diiron oxadithiolate type models for the active site of iron-only hydrogenases and biomimetic hydrogen evolution catalyzed by Fe₂(μ -SCH₂OCH₂S- μ)(CO)₂. *Organometallics*, **24**, 6126–6135.
- Szpunar, J., 2005. Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics. *Analyst*, **130**, 442–465.
- Wächtershäuser, G., 1990. Evolution of the first metabolic cycles. *Proceedings of the National Academy of Sciences of the United States of America*, **87**, 200–204.
- Wieghardt, K., 1994. A structural model for the water-oxidizing manganese cluster in photosystem II. *Angewandte Chemie International Edition in English*, **33**, 725–728.
- Williams, R. J. P., and Frausto da Silva, J. J. P., 2002. The involvement of molybdenum in life. *Biochemical and Biophysical Research Communications*, **292**, 293–299.
- Wilson, C. J., Apiyo, D., and Wittung-Stafshede, P., 2004. Role of cofactors in metalloprotein folding. *Quarterly Review of Biophysics*, **37**, 285–314.
- Yang, W., Hsiao-Wei, L., Hellings, H., and Yang, J. D., 2002. Structural analysis, identification, and design of calcium-binding sites in proteins. *Proteins Structure, Function, and Genetics*, **47**, 344–356.
- Yocum, C. F., and Pecoraro, V. L., 1999. Recent advances in the understanding of the biological chemistry of manganese. *Current Opinon Structural Biology*, **3**, 182–187.
- Zbaida S., and Kariv, R., 1989. Biomimetic models for monooxygenases. *Biopharmaceutics and Drug Disposition*, **10**, 431–442.
- Zerkle, A. L., House, C. H., Cox, R. P., and Canfield, D. E., 2006. Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle. *Geobiology*, **4**, 285–297.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Methane, Organ](#)
[Methane Oxidation \(Aerobic\)](#)
[Nickel, Biology](#)
[Siderophores](#)
[Zinc](#)

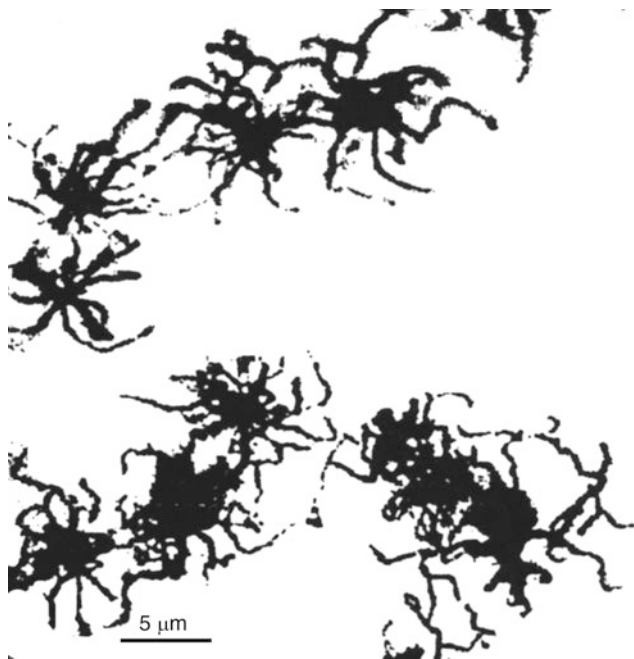
METALLOGENIUM

Joachim Reitner
 University of Göttingen, Göttingen, Germany

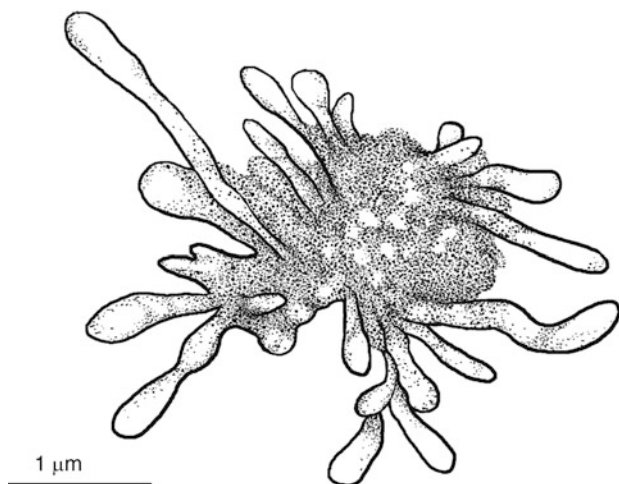
Definition

Metallogenium s.str. is a star-shaped enigmatic bacterium oxidizing Mn and iron. It preferentially lives in limnic environments.

Metallogenium is a relatively large (2–10 μ m) microorganism with bacterial affinities that concentrates manganese and iron oxides in significant amounts. The genus *Metallogenium* has not yet been assigned to any taxonomic group. The cells lack rigid walls and develop flexible, tapering threads from which new cells probably arise. The organism is therefore, star-shaped and is found in various facies, predominantly in limnic environments (Figures 1 and 2). The threads are often heavily encrusted with oxides of iron and/or manganese. The oldest



Metallogenium, Figure 1 *Metallogenium personatum*. (Redrawn from Perfil'ev and Gabe, 1961.)



Metallogenium, Figure 2 *Metallogenium personatum*. (Redrawn from Klaveness, 1999.)

Metallogenium-like fabrics are known from the 1.88 Gy old Paleoproterozoic Gunflint Formation (Barghorn and Tyler, 1965).

The first detailed description of *Metallogenium* was carried out by Perfil'ev and Gabe (1961) who called the type species *Metallogenium personatum*. There is an enormous record of publications about this microorganism (Zavarin, 1964a, b; Dubinia, 1970; Schmidt

and Overbeck, 1984), and many speculations have been made about its systematic position, for example, within the Mycoplasmatales (Zavarin and Hirsch, 1974), Chlamydobacteria (Klaveness, 1977), and Hyphomicrobiales (Perfil'ev and Gabe, 1961). Clasen (1969), Schmidt and Overbeck (1984), and Zavarin (1989), suggested a relationship with the iron bacterium *Leptothrix echinata* (Beger, 1935) which shows a considerable morphological similarity to *Metallogenium*. Unfortunately, valid molecular biological examinations presently do not exist to clarify the phylogenetic position of this organism, although it is common especially in many freshwater lakes (e.g., Klaveness, 1999; Schulte, 1994). Besides *Metallogenium personatum*, which is undoubtedly a prokaryotic organism, "*Metallogenium symbioticum*" was described (Zavarin, 1964b), which is also an Mn-oxidizing prokaryote preferentially growing on fungal microorganisms (Schweisfurth, 1971).

Bibliography

- Barghorn, E. S., and Tyler, S. A., 1965. Microorganisms from the Gunflint Chert. *Science*, **147**, 563–577.
- Beger, H., 1935. *Leptothrix echinata*, ein neues, vorwiegend Mangan fällendes Eisenbakterium. *Zentralbl. Bakteriol. Abt.*, **II** 92, 401–406.
- Clasen, J., 1969. *Leptothrix echinata* und die Mangankonzentration in der Wahnachtalsperre. *Städtehygiene*, **20**, 171–174.
- Dubinia, G. A., 1970. Untersuchungen über die Morphologie von *Metallogenium* und die Beziehungen zu Mycoplasma. *Zeitschrift für Allgemeine Mikrobiologie*, **10**, 309–320.
- Klaveness, D., 1977. Morphology, distribution and significance of the manganese-accumulating microorganism *metallogenium* in lakes. *Hydrobiologica*, **56**, 25–33.
- Klaveness, D., 1999. *Metallogenium* – a microbial enigma. In Seckbach, J. (ed.), *Enigmatic Microorganisms and Life in Extreme Environments*. Dordrecht: Kluwer, pp. 539–548.
- Perfil'ev, B. V., and Gabe, D. R., 1961. Studies of microlandscape. In *Capillary Methods of Investigating Micro-organisms* (Engl transl. Shewan, J. M., 1969). Toronto: University of Toronto Press, pp. 55–58.
- Schmidt, W. D., and Overbeck, J., 1984. Studies of "iron-bacteria" from Lake Pluss.I. Morphology, finestructure and distribution of *Metallogenium* sp. and *Siderocapsa geminata*. *Zeitschrift für Allgemeine Mikrobiologie*, **24**, 329–339.
- Schulte, N., 1994. Untersuchungen zur Ökologie, Morphologie und Entwicklung von *Metallogenium personatum* in einer Talsperre, Diss., Techn. Univ. Carolo-Wilhelmina Braunschweig.
- Schweisfurth, R., 1971. Manganoxidierende Pilze. *Zeitschrift für Allgemeine Mikrobiologie*, **11**, 415–430.
- Zavarin, G. A., 1964a. Structure of *Metallogenium*, *Mikrobiologiya* (Engl. Transl.), **32**, 864–867.
- Zavarin, G. A., 1964b. *Metallogenium symbioticum*. *Zeitschrift für Allgemeine Mikrobiologie*, **4**, 390–395.
- Zavarin, G. A., 1989. Genus "*Metallogenium*" Perfil'ev and Gabe, 1961. In Staley, J. T., Bryant, M. P., Pfennig, N., and Holt, J. G. (eds.), *Bergey's Manual of Systematic Bacteriology*. Baltimore, MD: Williams & Wilkins, Vol. 3.
- Zavarin, G. A., and Hirsch, P., 1974. Genus *Metallogenium* Perfil'ev and Gabe 1961. In *Bergey's Manual of Determinative Bacteriology*, 8th edn. Baltimore, MD: Williams & Wilkins, pp. 163–165.

Cross-references

Bacteria
 Biofilms
 Leptothrix
 Microbial Biomineralization
 Microbial-Metal Binding
 Ores, Microbial Precipitation and Oxidation
 Subsurface Filamentous Fabrics
 Terrestrial Deep Biosphere

METALS, ACQUISITION BY MARINE BACTERIA

Alison Butler, Vanessa V. Homann
 University of California, Santa Barbara, CA, USA

Definition

Oceanic bacteria produce siderophores to acquire iron. Given that the iron concentration in surface ocean water is very low, siderophores produced by marine bacteria are tailored to the physical and chemical constraints of the marine environment.

Introduction

Microbial iron uptake

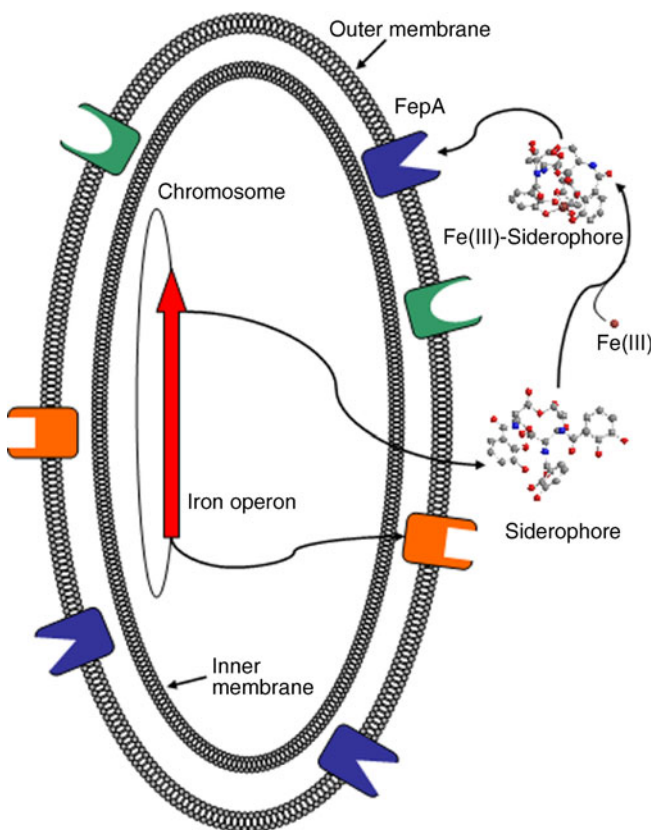
Virtually, all bacteria require iron for growth. Bacteria growing under aerobic conditions utilize a variety of pathways to acquire iron, but under conditions of iron starvation, bacteria often produce siderophores (Crosa et al., 2004). Siderophores are low molecular weight, chelating compounds produced by bacteria to facilitate iron(III) uptake (Crosa et al., 2004; Kraemer et al., 2005; Butler, 2007). The Fe(III)-siderophore complex is recognized by a specific outer membrane protein receptor, which catalyzes the transport of the Fe(III)-siderophore complex across the outer membrane (Figure 1).

The study of marine microbial siderophores is relatively new (Butler and Martin, 2005), yet certain characteristic structural features have already begun to emerge: suites of amphiphilic siderophores and photoreactive Fe(III)-siderophores.

Marine Siderophores

Amphiphilic marine siderophores

One characteristic of marine siderophores is the predominance of suites of amphiphilic siderophores, comprised of a hydrophilic head group, which coordinates with Fe(III), and one of a series of hydrophobic fatty acid appendages, which confers amphiphilicity (Figure 2). Two types of suites of amphiphilic siderophores are produced by marine bacteria. The aquachelins, marinobactins, and amphibactins are amphiphilic peptidic siderophores produced by distinct genera of marine bacteria (Figure 2) (Martinez et al., 2000, 2003). The relative amphiphilicity of each of these siderophores is



Metals, Acquisition by Marine Bacteria, Figure 1 Siderophore-mediated iron uptake by a bacterium.

defined by both the number of amino acids in the peptide fraction, compared to the fatty acid chain length (e.g., C12–C18). The amphibactins with only four amino acids and relatively long C18 fatty acids are the most hydrophobic of the amphiphilic peptide siderophores. As a result, they remain cell associated and are extracted from the bacterial pellet during the isolation process (Martinez et al., 2003). In contrast, the marinobactins and aquachelins, with five and six amino acids in the head group and a predominance of the shorter fatty acids, are more hydrophilic and are thus isolated from the supernatant following centrifugation of the bacterial culture to pellet the cells (Martinez et al., 2000).

The synechobactins and ochrobactins (Figure 2) are different suites of amphiphilic siderophores, in which the head group is based on citric acid, as opposed to a peptide. The head group is thus smaller than in the peptidic amphiphilic siderophores. The fatty acids are also shorter than observed for the peptidic amphiphilic siderophores. Nevertheless, the ochrobactins are quite hydrophobic as a result of two fatty acid appendages; these siderophores remain associated with the bacterial pellet (Martin et al., 2006). The synechobactins, with

- Wood, R. A., Grotzinger, J. P., and Dickson, J. A. D., 2002. Proterozoic modular biomineralized metazoan from the Nama Group, Namibia. *Science*, **296**, 2383–2386.
- Wooldbridge, S. A., 2008. Mass extinctions past and present: a unifying hypothesis. *Biogeosciences Discussion*, **5**, 2401–2423.
- Xian-guang, H., Aldridge, R. J., Bergström, J., Sivetter, D. J., Siveter, D. J., and Xiang-hong, F., 2004. *The Cambrian Fossils Of Chengjiang, China. The Flowering Of Early Animal Life*. Oxford: Blackwell.
- Xiao, S., and Kaufman, A. J. (eds.), 2007. Neoproterozoic geobiology and paleobiology. *Topics in Geobiology*, **27**, 300.
- Xiao, S., Yuan, X., and Knoll, A. H., 2000. Eumetazoan fossils in terminal Proterozoic phosphorites? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 13684–13689.
- Young, S. A., Saltzman, M. R., Foland, K. A., Linder, J. J., and Kump, L. R., 2009. A major drop in seawater $^{87}\text{Sr}/^{86}\text{Sr}$ during the Middle Ordovician (Darrivilian): links to volcanism and climate? *Geology*, **37**, 951–954.
- Zhuravlev, A. Y., and Riding, R. (eds.), 2001. *The Ecology of the Cambrian Radiation*. New York: Columbia University Press.
- Zhuravlev, A. Y., and Wood, R. A., 2008. Eve of biomineralization: controls on skeletal mineralogy. *Geology*, **36**, 923–926.

Cross-references

Algae (Eukaryotic)
 Anaerobic Oxidation of Methane with Sulfate
 Animal Biocalcification, Evolution
 Animal Skeletons, Advent
 Archaea
 Asteroids and Comet Impacts
 Bacteria
 Banded Iron Formations
 Early Precambrian Eukaryotes
 Ediacaran Biota
 Isotope Fractionation (Metal)
 Mass Extinctions, Phanerozoic
 Mat-Related Sedimentary Structures
 Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Nickel, Biology
 Origin of Life
 Photosynthesis
 Salinity History of the Earth's Ocean
 Snowball Earth
 Soda Ocean Hypothesis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle
 Trace Fossils: Neoproterozoic

CRYOBIOSPHERE

The term “cryobiosphere” refers to organisms living in extremely cold environments. For detailed reading, see entries “*Permafrost Microbiology*” and “*Extreme Environments*.”

CYANOBACTERIA

Kathrin I. Mohr^{1,2}, Nicole Brinkmann¹, Thomas Friedl¹
¹University of Göttingen, Göttingen, Germany
²Helmholtz Centre for Infection Research, Braunschweig, Germany

Synonyms

Blue-green algae; Chloroxybacteria; Cyanophyta; Cyanoprokaryota

Definition

Cyanobacteria are a large and morphologically rather diverse group of photoautotrophic prokaryotes and are classified as a monophyletic phylum within Domain Bacteria. Cyanobacteria share oxygenic photosynthesis with the eukaryotic algae. Cyanobacteria belong to the oldest organisms on earth. Their broad environmental tolerance range makes cyanobacteria well suited to live in habitats of extreme and fluctuating conditions where they can outcompete eukaryotic algae.

Many cyanobacteria have large cells like the eukaryotic algae with which they share the same life-style: Consequently, they are often treated as prokaryotic algae or, formerly, blue-green algae. Some cyanobacteria even form macroscopic colonies from the size of a few millimeters to up to 30 cm (Dodds and Castenholz, 1988). Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony-forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. Photosynthetic cyanobacteria produced earth's first oxygen atmosphere, which fostered the rise of eukaryotes. In addition, ancient cyanobacteria produced massive undersea carbonate deposits, thereby reducing atmospheric carbon dioxide levels and influencing global climate. The first plastids arose from cyanobacteria through a process called primary endosymbiosis (McFadden, 2001; Keeling, 2004; Palmer, 2003; Reyes-Prieto et al., 2007). Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Nitrogen-fixing cyanobacteria increase soil and water fertility and foster the growth of certain plants and fungi in symbiotic associations. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years (Rasmussen and Willemotte, 2008) and with an evolutionary history stretching over at least 1.5 and possibly 2.7 billion years (Blank, 2004). Cyanobacteria are able to live in some of the most extreme habitats on earth (e.g., Seckbach, 2007 for an overview). The broad environmental tolerance range of cyanobacteria in extreme habitats is in contrast to most other prokaryotic and eukaryotic organisms from extreme environments that occur only within a small window of almost constant conditions. From an evolutionary point of view, it appears that cyanobacteria which are

among the oldest organisms on earth may have been forced by the “modern” eukaryotes to withdraw themselves into habitats of extreme and fluctuating conditions (Whitton, 1992; Bhattacharya et al., 1999).

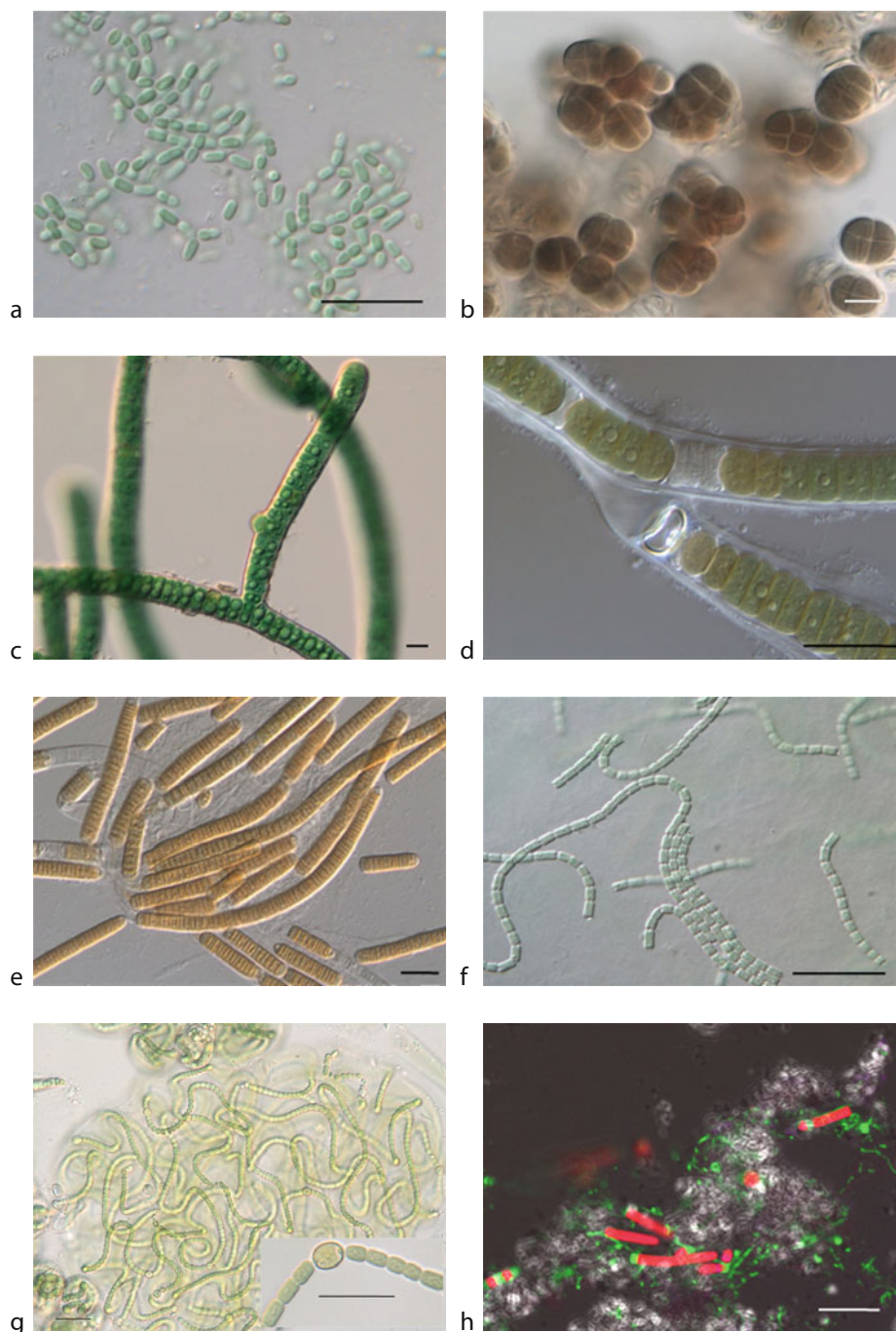
Cell structure

Unlike most groups of prokaryotes, there is substantial morphological and life history variation within the cyanobacteria (Hayes et al., 2007). Although some cyanobacteria occur as solitary unicells, in most species the cells adhere at each other to form at least pairs of cells (representing the products of a recent cell division, *Synechococcus* Figure 1a) or group of cells are held together to form colonies (e.g., in *Myxosarcina*, Figure 1b). Very common growth forms are filaments (trichomes) where the cells are joined end to end through regular divisions into one plane (Figure 1c–g). Such filaments can be even joined into bundles or can be branched (true-branched, Figure 1b or false-branched, Figure 1d). Many filamentous cyanobacteria produce specialized cells known as heterocytes (formerly heterocysts; Figure 1d and g) that function in nitrogen fixation. Such cyanobacteria are usually capable to produce specialized cells for dormancy, known as akinetes, under adverse environmental conditions. Some filamentous cyanobacteria are capable of gliding, a slow uniform motion on a solid substrate. The gliding occurs by an interaction of slime secretion and certain proteinaceous fibers (Hoiczky, 2000). Cyanobacteria lack sexual reproduction, but gene exchange may be realized through viral transduction by specialized cyanophages (Coleman et al., 2006). The only mode of reproduction is by cell division (binary fission, resulting in two equal-sized cells) or simply by fragmentation of a colony. In unicellular cyanobacteria, baeocytes (endospores) are formed by the division of the cytoplasm several times and in different planes whereby the mother does not increase in size. Filamentous cyanobacteria form hormogonia (Figure 1e), which are short filaments resulting from a breakup of a longer filament, promoted by the controlled death or collapse of certain cells. Hormogonia exhibit gliding motility for several hours, are positively phototactic and lack heterocytes. Light is absorbed by thylakoids, photosynthetic membranes, which are arranged concentrically at the cell periphery, not stacked and not connected to the plasma membrane. They contain the chlorophylls *a* and *d*, in some cyanobacteria also chlorophyll *b*, as well as carotenoids and phycobilins as accessory pigments to capture light energy and convey it to the photosynthetic reaction centers located inside the thylakoid membranes. Phycobilins are blue or red water-soluble pigments and are present in large amounts. They are bound to proteins that form up hemispherical phycobilisomes which occur on the outer thylakoid surfaces. Several particles in the cytoplasm of cyanobacteria may represent storage products, that is, cyanophycin (a polymer of the nitrogen-rich amino acids asparagine and arginine), cyanophycan starch

(a polyglucan), and polyphosphate. In addition, lipid droplets, carboxysomes (polygonal aggregations of *Rubisco*, the key enzyme in CO₂ fixation), and gas vesicles may be present in the cytoplasm. Gas vesicles (assemblies of hollow, pointed cylinders, but not delimited by membranes) occur frequently in aquatic cyanobacteria and enable them for buoyancy and to confer vertical mobility in the water column. The cell wall of cyanobacteria is basically the same as for Gram-negative bacteria, that is, a thin peptidoglycan layer (a polymer composed of sugar derivatives and amino acids) is outside of the cell membrane (cytoplasm membrane). Outside of the peptidoglycan layer is a space surrounded by another membrane, termed outer membrane. A very characteristic feature of cyanobacteria is that they usually are surrounded by a mucilaginous sheath (extracellular polymeric substances [EPS], see entry “*Extracellular Polymeric Substances (EPS)*”), mostly composed of polysaccharides, which protects the cells from drying and enables them to attach to substrates.

Ecology

Cyanobacteria are widely distributed in aquatic and terrestrial environments, including such extreme habitats as hot springs, deserts, and polar regions (Whitton and Potts, 2000). Many cyanobacteria are diazotrophic, that is, they can fix atmospheric molecular nitrogen gas (N₂) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen fixation is an inducible process and is exceptionally energy demanding. The environmental levels of ammonium or nitrate regulate it and it can only be performed if sufficient ATP (produced by photosynthesis) is available. The key enzyme of nitrogen fixation is nitrogenase, which is irreversibly inhibited by oxygen and, therefore, nitrogen can only be fixed under anaerobic conditions. Cyanobacteria that produce heterocytes are capable to fix nitrogen even in aerobic habitats, because several processes within the heterocytes (e.g., intracellular respiration) consume oxygen and the heterocytes’ thick cell walls reduce diffusion of oxygen into the cells. Cyanobacteria without heterocytes may fix nitrogen in the dark. Bloom-forming filamentous cyanobacteria devoid of heterocytes are responsible for fixing one-quarter of the total nitrogen in the oceans (Bergman and Carpenter, 1991). These cyanobacteria fix nitrogen in randomly distributed vegetative cells adjacent to each other within the filaments. Here and inside the colonies, the filaments may be depleted from oxygen. In addition, these species occur in tropical seas that are relatively low in dissolved oxygen. The ability to fix molecular nitrogen makes cyanobacteria attractive as symbionts for many eukaryotes because eukaryotes are not able to fix nitrogen. Very common occurrences of nitrogen-fixing cyanobacteria as extracellular symbionts are lichens, that is, symbioses with certain fungi (Friedl and Büdel, 2008, see entries “*Symbiosis*” and “*Fungi and Lichens*”). In the marine environment, cyanobacterial symbioses are known



Cyanobacteria, Figure 1 Different cyanobacterial morphotypes from pure culture. Figure a, d–g show strains recovered from tufa stromatolite biofilms of hardwater creeks: (a) coccoid unicellular *Synechococcus* sp. The cells adhere at each other to form at pairs representing the products of a recent cell division (Photograph taken by A. Hodačova); (b) *Myxosarcina* sp. (strain SAG 30.84) with adhering groups of cells forming irregular colonies (Photograph taken by A. Hodačova); (c) filamentous, true-branched *Stigonema* sp. (strain SAG 49.90) (Photograph taken by A. Hodačova); (d) filamentous, false-branched *Tolypothrix* sp.; (e) thick unbranched filaments of *Lyngbya* sp. forming hormogonia; (f) thin unbranched filaments of *Pseudanabaena* sp. which are fragmenting (Photograph taken by A. Hodačova); (g) filamentous *Nostoc* sp. forming gelatinous colonies. Insert, filament with a heterocyte located within the filament (intercalary) (Photographs taken by A. Hodačova); (h) Fluorescence in situ hybridization using an oligonucleotide probe specific for bacteria at a cryosection of the top of a tufa-forming biofilm. The red filaments represent cyanobacteria (*Phormidium* sp.). Cyanobacterial autofluorescence is shown in red and calcite in white (Photograph taken by G. Arp, printed with permission of the publisher). Scale bar, 20 μm .

with sponges, ascidians (sea squirts), and echiuroid worms in the benthos and diatoms, as well as dinoflagellates in the plankton. These symbioses can be significant in terms of the biogeochemistry of coastal and open-ocean areas (Carpenter, 2002). Planktonic diatom–cyanobacteria symbioses play a significant role in the ecology and biogeochemistry of the surface ocean (see entry *Algae, Eukaryotic*). Symbiotic associations with cyanobacteria are also known from plants. In the water fern *Azolla* and cycads where filamentous cyanobacteria with heterocytes occur within certain cavities inside the leaf and intercellular spaces inside the root, respectively (Lee, 2008; Graham et al., 2009). N_2 -fixing cyanobacteria are common in waterlogged rice fields and the importance of these naturally occurring prokaryotes in the nitrogen economy of rice cultivation has long been known (Whitton, 2000). To support the demand for nitrogen in rice cultivation, input of chemical N fertilizers is a prerequisite, but the inoculation with free-living N_2 -fixing cyanobacteria as biofertilizers has been shown to increase growth and crop yield of rice. An example for intracellular associations involving cyanobacteria can be found in certain diatoms (see entry *Diatoms*).

The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles (Hayes et al., 2007). There is an enormous variety of habitats where cyanobacteria play important roles, but to discuss this comprehensively is beyond the scope of this article. Therefore, we confine the discussion of cyanobacteria ecology just to a few selected examples. Other examples may be equally intriguing and important. In the littoral zone of marine environments, cyanobacteria form thin adhering films on porous or soft granular rocks within the spray zone. Cyanobacteria of the littoral zone make a significant contribution to the productivity of rocky shores (Lee, 2008). In the open-ocean minute unicellular cyanobacteria (less than 2 μm in diameter) may occur in high concentrations due to their adaptation to low light and effective nutrient uptake kinetics. Here, cyanobacteria represent the major organisms of the picophytoplankton (Ferris and Palenik, 1998) that dominates biomass and production of these oligotrophic habitats. Massive development of nitrogen-fixing filamentous cyanobacteria occur in certain tropical waters and the large surface area produced by the cyanobacterial filaments forms miniature ecosystems (Carpenter et al., 1992; Lee, 2008). Also in freshwaters blooms of cyanobacteria are common. Bloom-forming cyanobacteria often release significant toxic substances, termed cyanotoxins, with physiologically two types, neurotoxins and hepatotoxins (e.g., Codd et al., 1999). Cyanotoxins may function as anti-herbivore chemicals and can inhibit the growth of other algae. Cyanobacteria are also well known from hot acidic springs throughout the world (e.g., Ward and Castenholz, 2000). Many physiological properties that make the cyanobacteria capable to adapt to a large variety of extreme and environmental conditions may also indicate

their long evolutionary history. Many cyanobacteria are able to tolerate low oxygen conditions and even free sulfide at levels much higher than those tolerated by most eukaryotic algae. Many terrestrial cyanobacteria can tolerate high UVB and -C radiations, which may also be reminiscent of an adaption to early earth's conditions. Some cyanobacteria can even confer anoxygenic photosynthesis where H_2S is utilized as an electron donor. Cyanobacteria are also very successful primary colonizers in terrestrial habitats due to their tolerance of desiccation and to water stress. In biological soil crusts of semideserts, for example, cyanobacteria play a key role in maintaining the stability of the surface (e.g., Büdel et al., 2009). As they bind sand and soil particles, cyanobacteria prevent erosion. They help to maintain moisture in the soil and contribute nitrogen to it through nitrogen fixation. That may even assist higher plant growth by supplying growth substances (Lee, 2008). The cyanobacterial genus *Arthrospira* (formerly *Spirulina*) is used as human food supplement; the *Spirulina* industry is of increasing economic importance. Large filamentous colonies of *Nostoc* are used in China as “hair vegetable” (Facai).

Many species of cyanobacteria have calcium carbonate in the enveloping mucilage of the cells; crystals of calcite may also be trapped in the mucilage excreted by the cells (see entry *Calcite Precipitation, Microbially Induced*). In the marine environment, trapping and binding of the sediments as well as carbonate precipitation by cyanobacteria led to the formation of stromatolites (see entries *Microbialites, Modern*, and *Microbialites, Stromatolites, and Thrombolites*). The production of laminae in the stromatolites depends on fluctuations ultimately derived from movements of the earth, sun, and moon, and requires rhythmicity such as daily photosynthetic cycle of the organisms in the stromatolites. Up to 2,000 million years ago, there were no grazers and boring organisms and, therefore, stromatolites grew uncontested. The occurrence and size of stromatolites declined dramatically after the evolution of grazing and boring organisms. Today stromatolites grow only in warm hypersaline waters inhospitable to grazers and borers (e.g., Shark Bay, Australia). Extant cyanobacteria do not calcify in open-ocean sea water because this water is normally not supersaturated sufficiently with calcium carbonate to allow calcification (Riding, 2000; see entry *Calcified Cyanobacteria*). However, even in extant freshwaters, calcification processes somehow similar to the stromatolite formation in marine environments occur, for example, in tufa-forming biofilms of karstwater streams (Arp et al., 2010). Epilithic cyanobacterial species may enhance calcium carbonate deposition, either by trapping particles or by contributing to communities where calcite and aragonite (in freshwaters) or other carbonates are precipitated. Endolithic species mainly occur in soft and porous limestone (Figure 1h) where they grow actively into the limestone at sites where removal of surface material occurs (Pentecost and Whitton,

2000; see entry *Endoliths*). Whether the cell surface polymers or the degradation of cyanobacterial sheath by bacterial metabolism promote the calcification is still unclear. The calcification seems to be a side effect of the cyanobacteria metabolism related to the alkalization of the surroundings during photosynthesis (Verrecchia et al., 1995). The living cyanobacteria try to limit the mineralization of the surface of their cells by some means. Nevertheless, when the calcification in cyanobacteria occurs, it is connected with their mucilaginous sheath. However, significant influence of cyanobacterial photosynthesis in the regulation of tufa-forming processes has been found recently (Bisset, 2008a, b; Shiraishi et al., 2008).

Fossil history and classification

Fossil evidence indicates that major lineages of cyanobacteria had originated 2.3–2.45 billion years ago (Eigenbrode and Freeman, 2006; Tomitani et al., 2006; Graham et al., 2009), well before the establishment of eukaryotes 1.5 billion years ago (Javaux et al., 2001). Even older fossils resembling cyanobacteria have been found in ancient stromatolites (e.g., Schopf, 2006) and geochemical evidence may also indicate an even earlier origin of cyanobacteria, but this is not left undisputed. Collectively, the available data suggest that oxygenic photosynthesis arose sometime between 3.4 and 2.3 billion years ago (Allan and Martin, 2007). For classification of the cyanobacteria, more recently a combination of phenotypic and genotypic characters has been used (Castenholz, 2001). The fact that cyanobacteria have an algal way of life – cyanobacteria “usually behave like algae” (Wilmotte, 1994) – led to treat them as plants under the botanical code of nomenclature until the late 1970s (e.g., Anagnostidis and Komárek, 1985). However, as cyanobacteria are essentially prokaryotes, their treatment under the bacteriological code of nomenclature seems more appropriate (e.g., Castenholz, 2001). For a review of the difficulties of cyanobacteria classification, see Hayes et al. (2007). Based on the most recent broad-based description of cyanobacterial taxonomy (Castenholz, 2001), cyanobacteria are classified into five subsections (Subsections I–V) which correspond to the five orders Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales which have been erected under the botanical code of nomenclature. Some of the groups described by this current classification scheme are known to be polyphyletic in molecular phylogenies (for reviews see, e.g., Hayes et al., 2007). This shows that taxonomy of cyanobacteria is still in a state of flux. Still more work using multiple gene analyses as well as correction of misidentified strains in DNA sequence databases and culture collections is required to achieve progress toward a more robust taxonomy of the cyanobacteria.

Summary

Cyanobacteria form a large and morphological diverse group of photoautotrophic Gram-negative bacteria. Many cyanobacteria have large cells like the eukaryotic algae with which they share oxygenic photosynthesis. Cyanobacteria occur in a broad variety of forms, from solitary unicells over colony forming and undifferentiated filamentous types to more complex forms that even display the hallmarks of multicellularity. There is strong evidence that cyanobacteria are the oldest microorganisms performing oxygenic photosynthesis, dating back to about 2.45 billion years. Cyanobacteria exhibit a broad environmental tolerance range, which makes them well adapted to live under extreme and fluctuating conditions. From an evolutionary point of view, it appears that cyanobacteria may have been forced by the “modern” eukaryotes to withdraw themselves into such habitats. Modern cyanobacteria are recognized for their ability to occupy diverse aquatic and terrestrial habitats, cyanobacteria produce organic compounds used by other organisms, and they stabilize sediments and soils. Many cyanobacteria can fix atmospheric molecular nitrogen gas (N₂) into ammonia which is then further assimilated into amino acids and proteins. Nitrogen-fixing cyanobacteria increase soil and water fertility and are particularly attractive as symbionts, for example, to form lichens with fungi or being endosymbionts in plants, some metazoa, dinoflagellates and diatoms. The cyanobacteria metabolic activities make quantitatively important contributions to the carbon, nitrogen, sulfur, and other biogeochemical cycles.

Bibliography

- Allan, J. G., and Martin, W., 2007. Out of thin air. *Nature*, **445**, 610–612.
- Anagnostidis, K., and Komárek, J., 1985. Modern approach to the classification system of the Cyanophytes. I. Introduction. *Algalological Studies*, **38/39**, 291–302.
- Arp, G., Bissett, A., Brinkmann, N., Cousin, S., De Beer, D., Friedl, T., Mohr, K. I., Neu, T. R., Reimer, A., Shiraishi, F., Stackebrandt, E., and Zippel, B., 2010. *Tufa-Forming Biofilms of German Karstwater Streams: Microorganisms, Exopolymers, Hydrochemistry and Calcification*. London: Geological Society. Special Publications, Vol. 336, pp. 83–118.
- Bhattacharya, D., Friedl, T., and Schmidt, H., 1999. The phylogeny of thermophiles and hyperthermophiles and the three domains of life. In Seckbach, J. (ed.), *Enigmatic Microorganisms and Life in Extreme Environments*. Dordrecht, The Netherlands: Kluwer, pp. 291–304.
- Blank, C. E., 2004. Evolutionary timing of the origin of mesophilic sulphate reduction and oxygenic photosynthesis: a phylogenetic dating approach. *Geobiology*, **2**, 1–20.
- Bergman, B., and Carpenter, E. J., 1991. Nitrogenase confined to randomly distributed trichomes in the marine cyanobacterium *Trichodesmium thiebautii*. *Journal of Phycology*, **27**, 158–165.
- Bissett, A., de Beer, D., Schoon, R., Shiraishi, F., Reimer, A., and Arp, G., 2008a. Microbial mediation of stromatolite formation in karst-water creeks. *Limnology and Oceanography*, **53**, 1159–1168.

- Bissett, A., Reimer, A., de Beer, D., Shiraishi, F., and Arp, G., 2008b. Metabolic micro-environmental control by photosynthetic biofilms under changing macroenvironmental temperature and pH conditions. *Applied and Environmental Microbiology*, **74**, 6306–6312.
- Büdel, B., Darienko, T., Deutschewitz, K., Dojani, S., Friedl, T., Mohr, K., Salisch, M., Reisser, W., and Weber, B., 2009. Southern African biological soil crusts are ubiquitous and highly diverse in drylands, being restricted by rainfall frequency. *Microbial Ecology*, **57**, 229–247.
- Carpenter, E. J., 2002. Marine cyanobacterial symbioses. Biology and environment. *Proceedings of the Royal Irish Academy*, **102B1**, 15–18.
- Carpenter, E. J., Capone, D. C., and Reuter, J. (eds.), 1992. *Marine Pelagic Cyanobacteria: Trichodesmium and Other Diazotrophs*, Dordrecht: Kluwer.
- Castenholz, R. W., 2001. Cyanobacteria. In Boone, D. R., and Castenholz, R. W. (eds.), *Bergey's Manual of Systematic Bacteriology*, 2nd edn, Vol. 1. New York: Springer, pp. 474–487.
- Coleman, M. L., Sullivan, M. B., Martiny, C. A., Steglich, C., Barry, K., DeLong, E., and Chisholm, S. W., 2006. Genomic islands and the ecology and evolution of *Prochlorococcus*. *Science*, **311**, 1768–1770.
- Codd, G. A., Bell, S. G., Kaya, K., Ward, C. J., Beattie, K. A., and Metcalf, J. S., 1999. Cyanobacterial toxins, exposure routes and human health. *European Journal of Phycology*, **34**, 405–415.
- Dodds, W. K., and Castenholz, R. W., 1988. The biological effects of nitrate fertilization and water replacement in an oligotrophic cold water pond. *Hydrobiologia*, **162**, 141–146.
- Eigenbrode, J. L., and Freeman, K. H., 2006. Late Archaean rise of aerobic microbial ecosystems. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15759–15764.
- Ferris, M. J., and Palenik, B., 1998. Niche adaptation in ocean cyanobacteria. *Nature*, **396**, 226–228.
- Friedl, T., and Büdel, B., 2008. Photobionts. In Nash, T. III (ed.), *Lichen Biology*. Cambridge: Cambridge University Press, pp. 9–26.
- Graham, L. E., Graham, J. M., and Wilcox, L. W., 2009. *Algae*, 2nd edn. San Francisco: Person Benjamin Cummings.
- Hayes, P. K., El Semary, N. A., and Sánchez-Baracaldo, P., 2007. The taxonomy of cyanobacteria: molecular insights into a difficult problem. In Brodie, J., and Lewis, J. (eds.), *Unravelling the Algae: The Past, Present, and Future of Algal Systematics*. CRC Press/Taylor & Francis, pp. 93–101, ISBN-13: 978-0-8493-7989-5.
- Hoiczky, E., 2000. Gliding motility in cyanobacteria: observation and possible explanations. *Archives of Microbiology*, **174**, 11–17.
- Javaux, E. J., Knoll, A. H., and Walter, M. R., 2001. Morphological and ecological complexity in eukaryotic ecosystems. *Nature*, **412**, 66–69.
- Keeling, P. J., 2004. Diversity and evolutionary history of plastids and their hosts. *American Journal of Botany*, **91**, 1481–1493.
- Lee, R. E., 2008. *Phycology*, 4th edn. New York: Cambridge University Press, ISBN 978-0521-68277-0.
- McFadden, G. I., 2001. Primary and secondary endosymbiosis and the origin of plastids. *Journal of Phycology*, **37**, 951–959.
- Palmer, J., 2003. The symbiotic birth and spread of plastids: how many times and whodunit? *Journal of Phycology*, **39**, 4–12.
- Pentecost, A., and Whitton, B. A., 2000. Limestones. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 233–255.
- Rasmussen, U., and Wilmotte, A., 2008. Diversity and host specificity of *Azolla* cyanobionts. *Journal of Phycology*, **44**, 60–70.
- Reyes-Prieto, A., Weber, A. P. M., and Bhattacharya, D., 2007. The origin and establishment of the plastid in algae and plants. *Annual Review of Genetics*, **41**, 147–168.
- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Seckbach, J., 2007. *Algae and Cyanobacteria in Extreme Environments*. Berlin: Springer.
- Schopf, J. W., 2006. Fossil evidence of Archaean life. *Philosophical Transactions of the Royal Society B*, **361**, 869–885.
- Shiraishi, F., Bissett, A., de Beer, D., Reimer, A. and Arp, G., 2008. Photosynthesis, respiration and expolymer calcium-binding in biofilm calcification. *Geomicrobiology Journal*, **25**, 83–94. (Westerhöfer and Deinschwanger Creek, Germany) London.
- Tomitani, A., Knoll, A. H., Cavanaugh, C. M., and Ohno, T., 2006. The evolutionary diversification of cyanobacteria: Molecular-phylogenetic and paleontological perspectives. *PNAS*, **103**, 5442–5447.
- Verrecchia, E. P., Freytet, P., Verrecchia, K. E., and Dumont, J. L., 1995. Spherulites in calcrete laminar crusts: biogenic CaCO₃ precipitation as a major contributor to crust formation. *Journal of Sedimental Research*, **65**, 690–700.
- Ward, D. M., and Castenholz, R. W., 2000. Cyanobacteria in geothermal habitats. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 37–59.
- Whitton, B. A., 1992. Diversity, ecology and taxonomy of the cyanobacteria. In Mann, N. H., and Carr, N. G. (eds.), *Photosynthetic Prokaryotes*. New York: Plenum, pp. 1–51.
- Whitton, B. A., 2000. Soils and rice-fields. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 233–255.
- Whitton, B. A., and Potts, M., 2000. Introduction to the Cyanobacteria. In Whitton, B. A., and Potts, M. (eds.), *The Ecology of Cyanobacteria*. Dordrecht, The Netherlands: Kluwer, pp. 1–11.
- Wilmotte, A., 1994. Molecular evolution and taxonomy of the cyanobacteria. In Bryant, D. A. (ed.), *The Molecular Biology of the Cyanobacteria*. Dordrecht: Kluwer, pp. 1–25.

Cross-references

Algae (Eukaryotic)
 Biomarkers (Molecular Fossils)
 Calcified Cyanobacteria
 Calcite Precipitation, Microbially Induced
 Chroococciopsis
 Critical Intervals in Earth History
 Diatoms
 Endoliths
 Extracellular Polymeric Substances (EPS)
 Fungi and Lichens
 Microbial Biomineralization
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites
 Photosynthesis
 Stromatolites
 Symbiosis

Cross-references

Beggiatoa
Chemolithotrophy
Cold Seeps
Hydrothermal Environments, Marine
Pyrite Oxidation
Sulfate-Reducing Bacteria
Sulfur Cycle
Thiomargarita

THROMBOLITES

Thrombolites (Greek: *thrombos*, clot; *lithos*, stone) are “cryptalgal structures related to stromatolites, but lacking lamination and characterized by a macroscopic clotted fabric” (Aitken, 1967, p. 1164).

Bibliography

Aitken, J. D., 1967. Classification and environmental significance of cryptalgal limestones and dolomites, with illustrations from the Cambrian and Ordovician of southwestern Alberta. *Journal of Sedimentary Petrology*, **37**, 1163–1178.

Cross-references

Microbialites, Stromatolites, and Thrombolites

TIDAL FLATS

Meinhard Simon
University of Oldenburg, Oldenburg, Germany

Synonyms

Wadden Sea: name of the world’s largest tidal flat system in Central Europe

Definition

Tidal Flat. A wide and almost flat coastal area between the mean upper and lower tidal seawater levels (eulitoral) exposed to the rhythmic tidal water movements. It consists of unconsolidated sediments and is bordered toward the land by permanently emerged areas and toward the sea by the permanently submerged sublittoral zone.

Introduction

Tidal flats occur worldwide in shallow coastal regions: in the tropics, subtropics, temperate zones, and subpolar, and polar regions. In the tropics and subtropics, such as at various regions of the east coast of South America, in Africa, Bangladesh, Indonesia, and Australia, tidal flats are closely associated with mangrove ecosystems. In other places, such as Mauretania, the Persian Gulf, and the coast of Texas, USA, tidal flats are either wide and open coastal stretches or protected by barrier islands. Large tidal flat

areas adjacent to marshlands exist on the Atlantic coast of the USA (North/South Carolina, Georgia) and Canada (Nova Scotia, Bay of Fundy), Korea, and in Europe along the Atlantic coast (Portugal, Spain, France, and Great Britain), and the North Sea. At the southern coast of the North Sea, the worldwide largest tidal flat system, the Wadden Sea ranges from The Netherlands (den Helder) through Germany to Denmark (Esbjerg). Subpolar and polar tidal flat systems are found in southern Chile, Alaska, and Siberia. Tidal flats may also develop in shallow estuaries, and thus their sediments may partially exhibit riverine properties.

A special type of tidal flats is wind-induced; these wind flats form along shallow coastlines with very little tidal water movements but occasionally persistently strong winds. The winds lead to the emergence or submergence of shallow areas for irregular periods, and the flats are characterized by microbial mats and sediments similar to those of regular tidal flats.

Formation and shaping forces

Along shallow coastlines with little wave action, the rhythmic incoming and outgoing tidal currents are the main driving forces for establishing and structuring tidal flats. A critical tidal range of at least 1 m is a prerequisite for the formation of a tidal flat. The incoming tide imports dissolved nutrients and particulate inorganic and organic matter, which settle out during reduced water movement at slack tide. This hydrographic energy gradient leads to a fractionation of the suspended material according to its settling properties. The finest material, the mud fraction (>50% of the material with a particle size <63 µm and the highest content of organic carbon up to 10% of dry weight) settles at the lowest current velocity and thus in the shallowest and least energetic zones. The other fractions consist of sandy (<5% mud) and mixed sediments (5–50% mud) and form the bulk of the sediment in highly and intermediate energetic areas (Chang et al., 2006). As a consequence, material and nutrients accumulate in tidal flats and provide the basis for highly productive ecosystems (Postma, 1981). Additional input of particulate organic and inorganic matter and nutrients may come from the adjacent coastal areas permanently emerged and/or by pore water (Kasten, this volume) and groundwater via tidal pumping (only inorganic nutrients and dissolved organic matter). Export of matter occurs mainly in the dissolved form and net export of particulates occurs only when re-suspension exceeds sedimentation for more than a tidal cycle, during storm events. The interplay of coastal and tidal currents, settling, re-suspension, and fractionation of suspended matter and sediment, results in the formation of typical depositional features, such as banks, islands, barrier island systems with distinct sediment structures, and drainage channels of different sizes and depths (Figure 1). Because of the ample nutrient supply, tidal flats are hot spots of marine productivity and biodiversity.

- Flajs, G., and Hüssner, H., 1993. A microbial model for the Lower Devonian stromatactis mud mounds of the Montagne Noire (France). *Facies*, **29**, 179–194.
- Flajs, G., Hüssner, H., and Vigener, M., 1996. Stromatactis mud mounds in the Upper Emsian of the Montagne Noire (France): formation and diagenesis of stromatactis structures. In Reitner, J., Neuweiler, F., and Gunkel, F. (eds.), *Global and Regional Controls on Biogenic Sedimentation. I. Reef Evolution*. Research reports. *Gött. Arb. Geol. Paläont.*, Sb2, 345–348.
- Heckel, P. H., 1972. Possible inorganic origin for stromatactis in calcilitite mounds in the Tully Limestone, Devonian of New York. *Journal of Sedimentary Petrology*, **42**, 7–18.
- Hladil, J., 2005. The formation of stromatactis-type fenestral structures during the sedimentation of experimental slurries – a possible clue to a 120-year-old puzzle about stromatactis. *Bulletin of Geosciences*, **80**, 193–211.
- Hladil, J., Růžicka, M. C., and Koptíková, L., 2006. Stromatactis cavities in sediments and the role of coarse-grained accessories. *Bulletin of Geosciences*, **81**, 123–146.
- Hladil, J., Koptíková, L., Růžicka, M., and Kulaviak, L., 2007. Experimental effects of surfactants on the production of stromatactis-shaped cavities in artificial carbonate sediments. *Bulletin of Geosciences*, **82**, 37–50.
- James, N. P., and Gravestock, D., 1990. Lower Cambrian shelf and shelf margin buildups, Flinders Ranges, South Australia. *Sedimentology*, **37**, 455–480.
- Jansa, L. F., Pratt, B. R., and Dromart, G., 1989. Deep water thrombolite mounds from the Upper Jurassic of offshore Nova Scotia. In Geldsetzer, H. H., James, N. P., and Tebutt, G. E. (eds.), *Reefs, Canada and Adjacent Areas*. *Canadian Society of Petroleum Geologists*, **13**, 725–735.
- Kauffman, E. G., Arthur, M. A., Howe, B., and Scholle, P. A., 1996. Widespread venting of methane-rich fluids in Late Cretaceous (Campanian) submarine springs (Tepee Buttes), Western Interior seaway, USA. *Geology*, **24**(9), 799–802.
- Krause, F. F., 2001. Genesis and geometry of the Meiklejohn Peak lime mud-mound, Bare Mountain Quadrangle, Nevada, USA. *Sedimentary Geology*, **145**, 189–213.
- Kukal, Z., 1971. Open-space structures in the Devonian limestones of the Barrandian (Central Bohemia). *Časopis pro mineralogii a geologii*, **16**, 345–362.
- Logan, B. W., and Semeniuk, V., 1976. Dynamic metamorphism; processes and products in Devonian carbonate rocks, Canning Basin, Western Australia. *Geological Society of Australia Special Publication*, **6**, 1–138.
- Lowenstam, H. A., 1950. Niagaran reefs of the Great Lakes area. *Journal of Geology*, **58**, 430–487.
- Matyszkiewicz, J., 1993. Genesis of stromatactis in an Upper Jurassic carbonate buildup (Mlynka, Cracow region, Southern Poland): internal reworking and erosion of organic growth cavities. *Facies*, **28**, 87–96.
- Matyszkiewicz, J., 1997. Stromatactis cavities and stromatactis-like cavities in the Upper Jurassic carbonate buildups at Mlynka and Zabierzów (Oxfordian, southern Poland). *Annales, Société Géologique de Pologne*, **67**, 45–55.
- Neumann, A. C., Kofoed, J. W., and Keller, G. H., 1977. Lithohermes in the straits of Florida. *Geology*, **5**, 4–10.
- Neuweiler, F., Bourque, P.-A., and Boulvain, F., 2001. Why is stromatactis so rare in Mesozoic carbonate mud mounds? *Terra Nova*, **13**, 338–346.
- Neuweiler, F., Gautret, P., Thiel, V., Langes, R., Michaelis, W., and Reitner, J., 1999. Petrology of Lower Cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Orme, G. R., and Brown, W. W. M., 1963. Diagenetic fabrics in the Avonian Limestones of Derbyshire and North Wales. *Proceedings of the Yorkshire Geological Society*, **34**, 51–66.
- Pascal, A., and Przybyla, A., 1989. Processus biosédimentaires et diagénétiques précoces dans les mud-mounds (Thrombolite-mounds) urgoniens d'Espagne du Nord (Aptien-Albien) et leur signification. *Géologie Méditerranée*, **16**(2–3), 171–183.
- Philcox, M. E., 1963. Banded calcite mudstone in the Lower Carboniferous “reef” knolls of the Dublin Basin, Ireland. *Journal of Sedimentary Petrology*, **33**, 904–913.
- Pratt, B. R., 1982. Stromatolitic framework of carbonate mud-mounds. *Journal of Sedimentary Petrology*, **52**, 1203–1227.
- Pratt, B. R., 1995. The origin, biota and evolution of deep-water mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-Mounds: Their Origin and Evolution*. IAS special publication 23, pp. 49–123.
- Ross, R. J. Jr., Jaanusson, V., and Friedman, I., 1975. Lithology and origin of Middle Ordovician calcareous mudmounds at Meiklejohn Peak, southern Nevada. *U.S.G.S. Professional Paper*, **871**, 1–48.
- Schwarzacher, W., 1961. Petrology and structure of some Lower Carboniferous reefs in northwestern Ireland. *AAPG Bulletin*, **46**, 1481–1503.
- Shinn, E. A., 1968. Burrowing in Recent lime sediments of Florida and the Bahamas. *Journal of Paleontology*, **42**, 879–894.
- Textoris, D. A., 1966. Algal cap for a Niagaran (Silurian) carbonate mud mound of Indiana. *Journal of Sedimentary Petrology*, **36**, 455–461.
- Textoris, D. A., and Carozzi, A. V., 1964. Petrography and evolution of Niagaran (Silurian) reefs, Indiana. *AAPG Bulletin*, **48**, 397–426.
- Tsien, H. H., 1985. Origin of Stromatactis - a replacement of colonial microbial accretion. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 274–289.
- Wallace, M. W., 1987. The role of internal erosion and sedimentation in the formation of stromatactis mudstones and associated lithologies. *Journal of Sedimentary Petrology*, **57**, 695–700.

Cross-references

[Calcite Precipitation, Microbially Induced Carbonates Mud Mounds](#)
[Origins of the Metazoa Reefs](#)
[Sediment Diagenesis – Biologically Controlled Sponges \(Porifera\) and Sponge Microbes](#)
[Waulsortian Mud Mounds](#)

STROMATOLITES

Stromatolites are laminated benthic microbial deposits (Riding, 1991).

Bibliography

Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous algae and stromatolites*. Berlin: Springer-Verlag, pp. 21–51.

Cross-references

[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thombolites](#)

and humics. Furthermore, *G. metallireducens*, *G. psychrophilus*, and *G. sulfurreducens* were shown to transfer electrons from the oxidation of organic compounds onto the surface of electrodes. All *Geobacter* species utilize acetate as electron donor and carbon source; other electron donors that are utilized by the majority of *Geobacter* species include ethanol, pyruvate, lactate, hydrogen, and formate. *Geobacter* species are unable to grow by fermentation. Ecological studies indicate that *Geobacter* species predominate under iron-reducing conditions. Furthermore, the combination of ecological and physiological studies implies that *Geobacter* species might play important roles in bioremediation processes such as the oxidation of aromatic compounds, the reduction of tetrachloroethene, or the immobilization of U(VI).

Species: *G. argillaceus*, *G. bemidjensis*, *G. bremensis*, *G. chapellei*, *G. grbiciae*, *G. humireducens*, *G. hydrogenophilus*, *G. lovleyi*, *G. metallireducens*, *G. pelophilus*, *G. pickeringii*, *G. psychrophilus*, *G. sulfurreducens*, *G. thiogenes*, *G. uraniireducens*

Among the validly described *Geobacter* species, *G. metallireducens* strain GS-15 and *G. sulfurreducens* strain PCA are the most intensively studied in terms of physiology, biochemistry, and genetics. The genomes of both species were sequenced and a genetic system was established for *G. sulfurreducens* (Méthé et al., 2003).

Bibliography

- Lovley, D. R., Giovannoni, S. J., White, D. C., Champine, J. E., Phillips, E. J., Gorby, Y. A., and Goodwin, S., 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. *Archives of Microbiology*, **159**, 336–344.
- Méthé, B. A., Nelson, K. E., Eisen, J. A., Paulsen, I. T., Nelson, W., Heidelberg, J. F., Wu, D., Wu, M., Ward, N., Beanan, M. J., Dodson, R. J., Madupu, R., Brinkac, L. M., Daugherty, S. C., DeBoy, R. T., Durkin, A. S., Gwinn, M., Kolonay, J. F., Sullivan, S. A., Haft, D. H., Selengut, J., Davidsen, T. M., Zafar, N., White, O., Tran, B., Romero, C., Forberger, H. A., Weidman, J., Khouri, H., Feldblyum, T. V., Utterback, T. R., Van Aken, S. E., Lovley, D. R., and Fraser, C. M., 2003. Genome of *Geobacter sulfurreducens*: metal reduction in subsurface environments. *Science*, **302**, 1967–1969.

Cross-references

Fe(II)-Oxidizing Prokaryotes
Fe(III)-Reducing Prokaryotes
Gallionella

GEOCHRONOLOGY

Volker Liebetrau
Leibniz Institute of Marine Sciences, IFM-GEOMAR,
Kiel, Germany

Synonyms

Age determination; Dating

Definition

Geochronology. Determination of ages and time intervals for geologic materials and processes on geologic, archeologic, and historic time scales. The science of investigating and reflecting the chronology of the earth constituents as induced from geologic data, based on absolute and relative dating methods.

Age, absolute. Age determination based on radioactive elements, their rates of decay and physical measurements, resulting in an actual age given in years for the analyzed geologic material (e.g., mineral, rock, fossil, fluid).

Age, relative. Age information based on stratigraphic anomalies (e.g., lithostratigraphic and biostratigraphic principles, field observations) or utilizing chemical reaction rates (e.g., diffusion, exchange, oxidation, hydration) as a measure of time.

Units. Geochronologic data are presented as yr BP (years before present), as ka (age date in thousands of years), as kyr (time interval in thousands of years, e.g., between distinct age data), following the same principle as Ma and Myr (millions of years), respectively. Alternatively, the mathematical expression in years, times order of magnitude is used and favored for age data and time intervals in the range of billions of years and the description of half-lives (e.g., 4.47×10^9 years, half-life of the ^{238}U isotope).

Introduction

The term “geochronology” may reasonably be used in reference to all principles and methods employed in geology to determine the relative and numerical ages of all kinds of rocks (Walsh, 2001). The immense age of the Earth and the long-term duration of processes changing the morphology and composition of the Earth’s crust and interior were progressively recognized throughout the last centuries. Geoscientists developed and established a relative chronological systematic based on lithostratigraphic and biostratigraphic principles, including global correlations and ongoing refinement. The actual state of the art is presented by the International Commission on Stratigraphy (ICS, 2009; <http://www.stratigraphy.org/upload/ISChart2009.pdf>).

In contrast, the direct determination of absolute ages required fundamental new physical approaches and analytical methods in the field of geochemistry. The basis for “geochronology” as a new geoscientific discipline was provided by the early description of radioactive transformations more than 100 years ago (Rutherford, 1906). An actual status in combining detailed knowledge of relative age successions with precise absolute ages and the impact of resulting chronostratigraphy on the understanding of the Earth’s geologic evolution is presented in concise manner by Ogg et al. (2008). A still valid and systematic overview about the broad variety, principles, scopes, potentials, and limitations of physical and chemical dating methods is given in a comprehensive way by Geyh and Schleicher (1990). This work is especially

useful for non-geochronologists on search for analytical methods most suitable for their specific demand in geologic time series data, providing fundamental knowledge for collaborative approaches.

Geochronology in geobiology

The science of geochronology provides the fourth dimension of geological and geobiological processes as a crucial requirement for their reconstruction in space and time, e.g., the determination of changes in biochemical fluxes, habitat conditions, and their comparison through Earth's geological and biological evolution.

Due to the fast developing field of geobiology, the increase in recognition and understanding of biological mediated formation and destruction of minerals, stones, and rock sequences the demand for high resolution, and absolute age data is continuously evolving.

In the following some essential, very different, and often in geobiologic approaches preferred concepts of age determination are explained.

Some essential concepts

Counting of structural and chemical anomalies

(High resolution, high precision, robust, low analytical effort, short time scales, strict prerequisites on growth systematic for high reliability):

Well known is the counting of growth, sedimentation, and precipitation cycles in correlation to short time intervals of orbital cycles in a broad variety of archives. Typical examples are changes of tree-ring structures (season-depending density, dendrochronology), layering of varves in sediments (seasonal change in detrital and organic input), sclerochronology of corals (annual growth bands), chemical lamination within sclerosponges, and fast growing Fe-Mn precipitates (reflecting seasonal changes in temperature and oxygenation, respectively).

Assuming the youngest part of a succession as recent reference point, this concept allows very precise and reliable age data, as long as continuous growth is ensured and no hiatus is observed. The latter would interrupt and extend the age record to unknown length, until independent radiometric age data provide new reference points. Alternatively, the identification of characteristic markers for over-regional to global events allows the combination of such archives from different locations and their sequential connection to longer time series (e.g., regionally resolved reconstruction of the Little Ice Age; Mann, 2001).

Radiometric age determination/radiochemistry

(Precise absolute ages, independent from growth structure and stratigraphy, decadal to billions of years time scales, robustness, and uncertainties depending on method and age, high analytical effort, on highest level of precision often depending on physico-chemical models, and correction assumptions):

Radiochemistry and related methods for radiometric age determination are the most powerful tools in geochronology, providing independent absolute ages for samples throughout the Earth's evolution. Their principles are based on changes of the isotopic composition of an element in the sample, due to the decrease of radioactive parent isotopes by decay and/or the increase of radiogenic isotopes as product of decay. Both processes are depending on isotope-specific half-lives, therefore, providing directly chronologic information. Radiometric methods belong to the broad field of Isotope Geochemistry. Detailed information about isotope systematic, analytical procedures, data interpretation, and geologic and geochemical background is presented by Dickin (2005), Faure and Mensing (2005), and Allègre (2008).

Two major geochronological principles, **deficiency** and **in-growth**, are dominating these methods and are explained in examples given below. In general, the deficiency principle can be described as determining the age by calculating the time required for the decay of a radioactive isotope from an assumed starting concentration or ratio down to the actually measured value. The starting value is equivalent to an excess concentration of a radioactive isotope above its equilibrium activity within its decay chain. The initial degree of excess depends on the investigated environment and the isotope system of interest. Required assumptions on the initial values are generally based on the measurements of recent analogues. Deduced ages are often presented by adding "excess" or the abbreviation (exc.) to the related isotope for method identification, especially if this isotope or isotope ratio is relevant for different geochronological methods.

In contrast, if a sample is strongly depleted in a decay product down to negligible or well-known initial concentration, e.g., by element fractionation during sample growth or crystallization, the in-growth principle can be used for direct age determination.

The activity ratio between decay product and parent isotope is increasing with time until equilibrium is reached. The time span required to reach the actually measured concentration or ratio is directly related to the half-life.

The maximum limit of age determination is reached for both principles in samples reflecting equilibrium activity within the analytical uncertainty, just reached from different directions (decay/in-growth). As a rule of thumb, for the robust estimation of the dynamic range of a geochronological approach, a factor of 4–8 (depending on the analytical technique and its precision) could be applied on the half-life of the relevant isotope. Best control on age reliability and accuracy is given where both principles could be applied on the absolute determination of a sample age or duration of a process.

It should be considered that radiometric methods can be easily overestimated and misinterpreted due to manifold requirements in sample quality, preparation, and analytical

effort. As more precise modern analytical techniques allow the determination of isotope ratios and concentrations, more attention should be given to the clear distinction between precision of a measurement and the accuracy of the deduced age. Especially in terms of realistic age uncertainties, the range of whole procedure blanks, reproducibility, and applied correction factors must be well known.

A general requirement of radiometric dating methods is a closed-system behavior of the sample material after formation until preparation for measurement. Differential analyses combining **relative age** successions with subsequent absolute age determinations are fundamental to achieve the highest level of accuracy, as, e.g., required for international chronostratigraphic calibration approaches. Relative age determination is generally based on the field and the microscopic observation and biostratigraphic classification. Absolute age determination is most reliable if independent measurements could be applied. In any case, the expensive isotope analyses should be combined with mineralogical and/or chemical pre-investigations for verification or estimation of potential secondary perturbations. Main issues are mineral composition and purity, the detection of secondary recrystallization processes, and chemical exchange reactions. For robust results of isotope approaches, a useful guiding principle includes to (1) look carefully, then to (2) select well-documented samples, before (3) crushing and homogenizing these materials for lab procedures.

Radiometric example A: cosmogenic ^{14}C /radiocarbon method/deficiency principle

Substrates for geochronological approaches in geobiology often consist of carbonic components, therefore the radiocarbon method, based on the cosmogenic nuclide ^{14}C , is especially advantageous and often suitable. ^{14}C is the least abundant of the three natural carbon isotopes (^{12}C , 98.98%; ^{13}C , 1.11%; ^{14}C , 1.176×10^{-12} atoms per ^{12}C atom) and decays with a half-life of 5,730 years. Due to its general dependence on cosmic activity and processes, it belongs to a group of cosmogenic and geochronologic relevant nuclides like ^{10}Be (Beryllium), ^{36}Cl (Chlorine), and ^{129}I (Iodine) of very different chemical affinity and dating potential.

^{14}C is mainly produced by the collision of low-energy cosmic ray neutrons with nitrogen atoms (^{14}N) in the upper atmosphere, rapidly oxidized to CO_2 and available for metabolic processes and mineral precipitation. A complex system of various exchange processes between the atmosphere, the biosphere, and the hydrosphere results in a kind of dynamic equilibrium resulting in rather constant $^{14}\text{C}/^{12}\text{C}$ ratios.

As soon as organic matter (e.g., by death of an organism) or a mineral (e.g., by biomineralization) is excluded from equilibration processes with the ambient carbon, the carbon isotopic composition changes systematically

by decay of ^{14}C . The decreasing ^{14}C concentration is ideally transferable to direct age information, i.e., about the date since the system was closed.

The rather short half-life of ^{14}C limits this method in general to samples younger than 50 ka. Extremely challenging is the application on samples of heterogeneous carbon supply, like marine cold seep and AOM (anaerobic oxidation of methane)-related carbonates, which tend to be influenced by varying proportions of buried methane-driven ^{14}C from older sources. Due to the low abundance, precise, and high resolution, ^{14}C measurements on small samples or growth increments (e.g., 0.002 g of a coral fragment) are restricted on very expensive and rare accelerator mass spectrometry (AMS) facilities. High-resolution analyses and chronological profiles are restricted to mechanical preparation methods using handheld mini- and computer-controlled micro-drilling devices.

Radiometric example B: Uranium decay series/ $^{230}\text{Th}/^{234}\text{U}$ /in-growth principle

The U decay series offers a wide range of established geochronological methods, suitable from marine mineral precipitation processes of the last decade ($^{230}\text{Th}/^{234}\text{U}$) back to the oldest rocks and minerals ($^{238}\text{U}/^{206}\text{Pb}$) on Earth, billions of years in age. Bourdon et al. (2003) presented an outstanding selection of papers covering the broad scope of Uranium series geochemistry.

Considering geobiological approaches, the affinity of many organisms to build directly calcium carbonate skeletons and indirectly remnants of biologically mediated precipitation processes (chemohierms) favors the formation of high-resolution archives, enriched in U and poor in Thorium.

An important and often applied approach for carbonate samples of the last 350 kyr is the in-growth of ^{230}Th (half-life of 7.5×10^4 years) by the decay of primary incorporated ^{234}U (half-life of 2.45×10^5 years). The change of their isotopic ratio with time during closed-system conditions provides direct geochronologic information. In regard to precision, reliability, and robustness it is crucial to select and prepare pristine sample parts without secondary overprint and with lowest ^{232}Th -content. The latter reflects the degree of initial Th incorporation which controls the influence of correction factors for initial element and isotope ratios on the final age calculation.

The state-of-the-art analytical procedure is based on multicollector – inductively coupled plasma – mass spectrometry (MC-ICP-MS) combined with low-blank clean-lab preparation procedures. A direct comparison of sclerochronology and U-series analyses on smallest sample amounts of a continuous drill core from a tropical reef demonstrated the analytical potential of extending this technique to multi-static multiple ion counting (MIC)-ICP-MS (Fietzke et al., 2005). Another promising step toward high-resolution U-Th age data was presented by Potter et al. (2005) who used laser-ablation-MC-

ICP-MS. Unfortunately, this in situ approach is still hampered by the high uncertainties of low-counting statistics on samples younger than 10 ka. Meanwhile, recent efforts in conventional data precision and sample selection pushed the maximum limit of age determination with the U-Th method to the successful dating of extraordinarily well preserved 600-ka-old corals (Andersen et al., 2008).

Summary

Geochronology in geobiologic approaches offers detailed insights into the time scales of biological processes, changes in biochemical fluxes, and the identification of driving mechanisms. Still open challenges are the rather low sample throughput of conventional clean-lab preparation procedures, the necessary achievements in precision for in situ approaches, and the methodical extension toward reliable dating tools covering the last millions of years.

Bibliography

- Allègre, C. J., 2008. *Isotope Geology*. New York: Cambridge University Press.
- Andersen, M. B., Stirling, C. H., Potter, E.-K., Halliday, A. N., Blake, S. G., McCulloch, M. T., Ayling, B. F., and O'Leary, M., 2008. High precision U-series measurements of more than 500,000 year old fossil corals. *Earth and Planetary Science Letters*, **265**, 229–245.
- Bourdon, B., Turner, S., Henderson, G. M., and Lundstrom, C. C., 2003. *Reviews in Mineralogy and Geochemistry 52: Uranium Series Geochemistry*. Washington: The Mineralogical Society of America.
- Dickin, A. P., 2005. *Radiogenic Isotope Geology*, 2nd edn. New York: Cambridge University Press.
- Faure, G., and Mensing, T. M., (2005). *Isotopes: Principles and Applications*, 3rd edn. New Jersey: Wiley.
- Fietzke, J., Liebetrau, V., Eisenhauer, A., and Dullo, Chr., (2005). Determination of uranium isotope ratios by multi-static MIC-ICP-MS: Method and implementation for precise U- and Th-series isotope measurements. *Journal of Analytical Atomic Spectrometry*, **20**, 395–401.
- Geyh, M. A., and Schleicher H., 1990. *Absolute Age Determination: Physical and Chemical Dating Methods and Their Application*. Berlin, Heidelberg, New York: Springer.
- International Commission on Stratigraphy, 2009. International Stratigraphic Chart <http://www.stratigraphy.org/upload/ISChart2009.pdf>
- Mann, M. E., 2001. Little ice age. In MacCracken, M. C., and Perry, J. S. (eds.), *Encyclopedia of Global Environmental Change*. Chichester: Wiley.
- Ogg, J. G., Ogg, G., and Gradstein, F. M., 2008. *The Concise Geologic Time Scale*. Cambridge, New York, Melbourne: Cambridge University Press. doi:10.1017/S0016756809006207.
- Potter, E.-K., Stirling, C. H., Wichert, U. H., Halliday, A. N., and Spötl, C., 2005. Uranium-series dating of corals in situ using laser-ablation MC-ICPMS. *International Journal of Mass Spectrometry*, **240**, 27–35.
- Rutherford, E., 1906. *Radioactive Transformations*. New York: Scribner.
- Walsh, S. L., 2001. Notes on geochronologic and chronostratigraphic units. *Geological Society of America*, **113**, 704–713. doi: 10.1130/0016–7606.

Cross-references

Anaerobic Oxidation of Methane with Sulfate
Chemolithotrophy
Cold Seep
Reefs

GEOMYCOLOGY

Geoffrey M. Gadd
University of Dundee, Dundee, Scotland, UK

Definition

Geomycology. The scientific study of the roles of fungi in processes of fundamental importance to geology

Introduction

Fungi are chemoorganotrophic (heterotrophic) organisms relying on organic carbon sources for energy and metabolism, and ubiquitous in aquatic and terrestrial environments. They are important as decomposers, animal and plant mutualistic symbionts and pathogens, and spoilage organisms of natural and manufactured materials (Gadd, 1993a, 1999, 2006; Burford et al., 2003a). They also have a role in the maintenance of soil structure due to their predominantly filamentous branching growth habit (each filament is called a hypha; plural = hyphae; all the hyphae constitute the mycelium) and frequent exopolymer production. Fungi have important roles in the biogeochemical cycling of the elements (e.g., C, N, P, S, metals), and this is linked with their ability to adopt a variety of growth, metabolic, and morphological strategies, their adaptive capabilities to environmental extremes and, their mutualistic associations with animals, plants, algae, and cyanobacteria (Gadd and Griffiths, 1980; Burford et al., 2003a; Gadd, 2004a, 2007a, b; Braissant et al., 2004; Gadd et al., 1984, 2005; Fomina et al., 2005a, 2006a, b) (Table 1). Fungal polymorphism and reproduction by huge numbers of spores underpins successful colonization of many different environments. Most fungi exhibit a filamentous growth habit which enables explorative or exploitative growth strategies, and many form linear organs of aggregated hyphae for protected translocation of nutrients (Jacobs et al., 2002, 2004; Boswell et al., 2002, 2003, 2006; Fomina et al., 2005a, b). Some fungi are polymorphic, occurring as both filamentous mycelium and unicellular yeasts or yeast-like cells, as in the black microcolonial fungi colonizing rocks (Gadd and Griffiths, 1980; Sterflinger, 2000; Gorbushina et al., 2002a, b, 2003). Some fungi are predominantly unicellular such as the yeasts, exemplified by *Saccharomyces cerevisiae*.

A broad appreciation of fungi as agents of biogeochemical change is lacking within geobiology, and apart from obvious connections with the carbon cycle, they are frequently neglected within broader geomicrobiological and geochemical contexts. While the geochemical activities

Bibliography

- Crawford, J. W., Harris, J. A., Ritz, K., and Young, J. M., 2005. Towards an evolutionary ecology of life in soil. *Trends in Ecology and Evolution*, **20**, 81–87.
- Driessen, P., Deckers, J., Spaargaren, O., and Nachtergaele, F., 2001. *Lecture Notes on the Major Soils of the World*. World Soil Resources Reports 94, Rome: FAO.
- FAO-UNESCO, 2002. *Digital Soil Map of the World and Derived Soil Properties*. Land and Water Digital Media Series No. 1 Rev. 1. Rome: FAO.
- Fiedler, S. H., Jungkunst, P. F., Kleber, M., Sommer, M., Jahn, R., and Stahr, K., 2002. Linking soil classification and soil dynamics – pedological and ecological perspectives. *Journal of Plant Nutrition and Soil Science*, **165**, 517–529.
- IUSS Working Group WRB, 2006. *World Reference Base for Soil Resources: Atlas*. Leuven, The Netherlands: ISRIC-FAO-ISSS-Acco, p. 63.
- Jones, D. L., and Bassington, D. S., 1998. Sorption of organic acids in acid soils and its implication in the rhizosphere. *European Journal of Soil Science*, **49**, 447–455.
- Kögel-Knabner, I., Hatcher, P. G., and Zech, W., 1990. *Decomposition and Humification Processes in Forest Soils: Implications from Structural Characterization of Forest Soil Organic Matter*. Kyoto, Japan: Trans. 14th Int. Congr. Soil Sci., pp. 218–223.
- Meir, P., Cox, P., and Grace, J., 2006. The influence of terrestrial ecosystems on climate. *Trends in Ecology and Evolution*, **21**, 254–260.
- O'Donnell, A. G., Young, J. M., Rushton, S. P., Shirley, M. D., and Crawford, J. W., 2007. Visualization, modeling and prediction in soil microbiology. *Nature Reviews Microbiology*, **5**, 689–699.
- Rillig, M. C., and Mummey, D. L., 2006. Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.
- Schaumann, G. E., 2006. Soil organic matter beyond molecular structure, part I: macromolecular and supramolecular characteristics. *Journal of Plant Nutrition and Soil Science*, **169**, 145–156.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: Wiley.
- van der Heijden, M. G., Bardgett, R. D., and van Straalen, N. M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.
- Wardle, D. A., 2006. The influence of biotic interactions on soil biodiversity. *Ecology Letters*, **9**, 870–886.
- Watt, M., Silk, W. K., and Passioura, J. B., 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of Botany*, **97**, 839–855.
- Watteau, F., and Berthelin, J., 1994. Microbial dissolution of iron and aluminum from soil minerals: efficiency and specificity of hydroxamate siderophores compared to aliphatic acids. *European Journal of Soil Biology*, **30**, 1–9.
- Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., and Pütz, T., 2007. In situ soil water extraction: a review. *Journal Of Environmental Quality*, **36**, 1735–1748.

Cross-references

Bacterial Clay Autigenesis
 Biogeochemical Cycles
 Carbon Cycle
 Denitrification
 Microbial Degradation
 Microbial-Metal Binding
 Mycorrhizae
 Nitrogen Fixation
 Siderophores

SPECIES (MICROBIAL)

Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “*Microbial Communities, Structure, and Function*” for further reading.

SPELEOTHEMS

Roman Aubrecht
 Comenius University, Bratislava, Slovakia

Synonyms

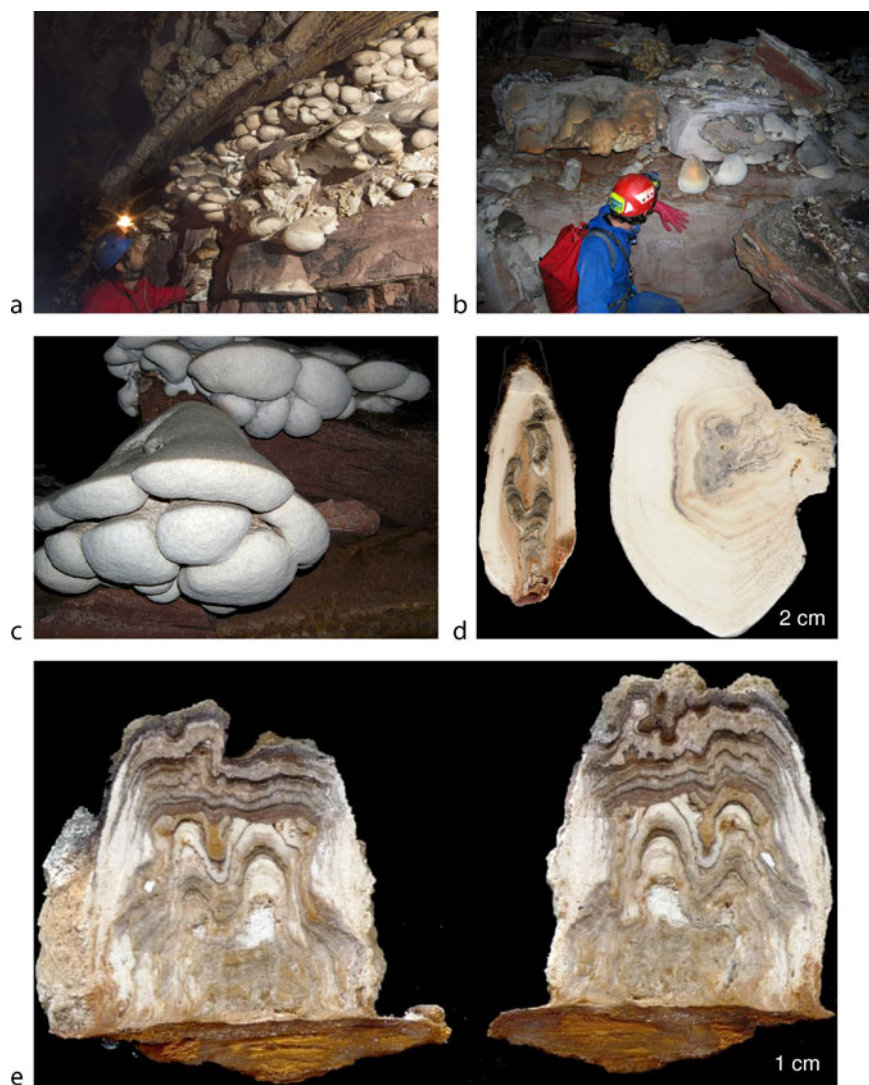
Cave deposits; Cave sinters

Definition

The word “speleothem” is from Greek and means “cave deposit.” According to its origin, this term can be used for any cave deposits, but it is used mostly for secondary mineral deposits formed (precipitated) in caves. In this entry, speleothem is understood as constructively formed, new cave material. Materials originated by destructive phenomena, such as erosion, weathering, etching, biocorrosion, or bioerosion are not subjects of this entry.

Biogenic and abiogenic speleothems

Most of the speleothems originate inorganically by precipitation of various minerals from solutions. The minerals are dissolved by undersaturated fluids and precipitate when the solutions become supersaturated, e.g., by evaporation, outgassing, or pH changes. Mineralogical compositions of speleothems vary depending on the environment in which the caves are formed. The most common speleothem-forming mineral is calcite (as most of the caves are formed of limestones), forming well-known sinter forms, such as dripstones (stalactites, stalagmites) and flowstones. Locally, mostly in hydrothermally influenced cavern systems, speleothems may be formed by its rhombic modification – aragonite. Gypsum and other sulfate minerals commonly form speleothems in gypsum karst but are widespread also in other types of caves. Speleothems formed in silicate rocks (e.g., sandstones, quartzites, granites, etc., [Figure 1](#)) are relatively rare, composed mostly of various forms of silica (from opal-A to microquartz depending on its recrystallization). Other speleothems can be represented by ferroan and manganese oxides and hydroxides forming cave flowstones.



Speleothems, Figure 1 Examples of microbial speleothems – cave stromatolites encrusted by silica in the sandstone cave Cueva Charles Brewer, Chimantá Plateau, Venezuela. (a–c) Mushroom-like stromatolites growing on the cave walls. (d) Slabs of stalactitic and mushroom-like stromatolites. Columnal stromatolites (*dark*) are formed by filamentous microbes; *white*, chalk-like parts represent stromatolites formed by tiny spherical microbial colonies. (e) Other shape of stromatolites with prevailing laminae formed by filamentous microbes.

Overall inventory of cave minerals is very large (for a summary see Hill and Forti, 1986).

The formation of some speleothems is influenced by organisms. The organisms can be passively covered (encrusted) by speleothems or they can directly biologically mediate their formation. In some cases it is not easy to distinguish between the active and passive role of the organisms. Biological mediation of mineral precipitation usually involves biomineralization (biomineralization (general aspects); microbial biomineralization; silica biomineralization (sponges)) and/or organomineralization. Biomineralization means direct precipitation of mineral skeleton (either internal or external) or encrustation by the organism itself (*syn vivo*). Organomineralization in

which the precipitation of minerals is mediated by organic matters produced by the organisms and by changing the ambient conditions (e.g., pH, enrichment or depletion of the solution by metabolism, etc.). They can occur either *syn vivo* (when the organisms are alive – they influence the ambient environment actively) or *postmortem* (after the organisms are dead – the ambient environment is influenced by the decay products of the organisms).

Organisms which mediate speleothem formation

The speleothem-forming organisms are mostly microorganisms (Archaea, Bacteria including Cyanobacteria, and Algae); more evolved multicellular organisms are

mostly represented by Fungi, mosses and lichens. Also roots of higher plants, penetrating to caves can form speleothems. All the mentioned organisms form speleothems from the autochthonous cave material (mostly taken from solutions). Excrements of animals (e.g., bats) can turn to well-known cave deposit – guano. Unlike the previously mentioned speleothems, guano and its minerals (rich group of phosphates, nitrates, and organic minerals) are completely allochthonous in caves, introduced originally from outside.

Microbes

Microbes inhabiting caves are mostly chemolithoautotrophic or chemoheterotrophic, i.e., their nutrition is not dependent on sunlight (for review see Konhauser, 2007). The exception is the so-called lampenflora, represented by phototrophic cyanobacteria and other algae which live on the lightened places in show caves. Phototrophic microorganisms normally inhabit the entrance areas and the number of microorganisms declines rapidly toward the twilight areas. Unicellular algae – diatoms – were found to contribute to the formation of speleothems, which occurred close to the entrance (Kashima et al., 1987; Kashima and Ogawa, 1995). Some cyanobacteria, although originally phototrophic, can adapt themselves to darkness and change their life mode to heterotrophic. For instance, the genera *Fisherella* and *Calothrix* were found to be able to change their mode of life to slow heterotrophic in complete darkness (Whitton, 1987). Some cyanobacteria were found to inhabit dark cave environment e.g., *Geitleria calcarea* and *Scytonema julianum* (Friedman, 1955; Bourrelly and Depuy, 1973) and contribute to speleothem formation. *Nostoc* is also easily adaptable to a heterotrophic mode of life, and is a common symbiont (endobiont) in lichens and higher plants. In addition to photosynthesis, *Nostoc* is able to perform nitrogen fixation (symbiosis of higher plants with *Nostoc* provides them with nitrate). *Nostoc* has also been reported from caves in Israel, growing in various zones, from well illuminated to complete darkness (Vinogradova et al., 1998). Moreover, *Nostoc* is also supposed to form large opal stromatolites in the Venezuelan sandstone caves (Figure 1; Aubrecht et al., 2008).

The most common in cave environments are chemolithoautotrophic and chemoheterotrophic microorganisms. Similar to other microorganisms, they use CO₂ as their carbon source but their source of energy comes from different chemical reactions. Chemoheterotrophs utilize preformed organic compounds, whereas chemolithoautotrophs utilize mostly inorganic compounds (Konhauser, 2007). These organisms and processes they utilize are numerous but only some of them are important speleothem-forming factors. Sulfur-oxidizing bacteria, such as *Beggiatoa* or *Thiothrix*, are responsible for deposition of elemental sulfur and sulfates in caves. They are particularly important in the caves with sulfur-rich waters, e.g., Parker Cave, Kentucky (Barton

et al., 2001). Bacteria also mediate formation of iron oxide and manganese oxide flowstones (Gradziński et al., 1995). Finally, they may also mediate formation of the carbonate speleothems, which are the most widespread speleothems in the world (Taboroši, 2006).

Fungi

Fungi are ubiquitous in all caves, being mostly chemoheterotrophs. They not only inhabit the caves but they actively contribute to the formation of some carbonate speleothems, such as a particular, soft form of sinter – moonmilk (Gradziński and Szulc, 1997; Gradziński et al., 1997).

Mosses

Mosses (often together with lichens) occur mostly in the near-entrance areas and are mostly passively encrusted by minerals (mostly calcite), thus forming moss tufas (Taboroši, 2006).

Plant roots

In shallow-placed galleries, plant roots sometimes penetrate from the surface. They are often mineralized, forming the so called rootsicles (Taboroši, 2006). It is not yet known whether they represent passive encrustations, or the roots actively mediate the precipitation. This is also true for other plant (and animal) remnants which are locally transported to the caves from the surface.

Marine organisms forming speleothems

Marine organisms represent a special chapter in the genesis of organic-mediated speleothems. Marine water, bringing nutrition to submarine caves, enables many organisms to live in this cryptic environment. Sessile organisms overgrowing the cave walls easily turn into speleothems. Very common are cryptic stromatolites (Reitner, 1993) formed by nonphotosynthetic organisms or serpulid buildups (Taboroši, 2006; Schlögl et al., 2008) formed by these filter feeders in submarine caves.

Methods of recognition of the speleothem-forming microorganisms

Cave-dwelling microorganisms, including those which contribute to speleothem formation, are considered to be extremophiles. That means they are organisms living in and adapted to environments which are not prolific for many organisms and which lack many aspects necessary for life; in caves, it is mainly the lack of sunlight and generally oligotrophic character of the environment. Adaptation of these organisms to the cave environment makes them uneasy to be determined by classical microbiological methods (Barton, 2006). For instance, for the complete identification of cyanobacteria, about 37 characteristics are needed, e.g., cell morphology, ultrastructure, morphology of colonies or filaments, genetic characteristics (DNA), cultivation conditions, and life conditions (Castenholz, 2001). Cultivation was so far the most

important tool in microbiology. This tool was developed mostly for medical purposes and was optimized for the research of microbes inhabiting animal bodies. However, only a small portion of free-living microbes is cultivable from environmental samples, including caves. Therefore, new culture-independent *in situ* methods have been developed for the detection of microbial groups in natural microbial assemblages, based on the molecular analysis of their DNA (Barton, 2006). Among these methods, one of the most important is *in situ* hybridization (FISH). Biomarker analysis may also be a suitable tool for the analysis of speleothems, in which only organic matter remained after dead, decayed organisms.

Conclusion

Speleothems are secondary mineral deposits formed in caves. They can form either inorganically or originate biologically. The inorganic formation pathway is based on physical precipitation from solutions after they become supersaturated. Biologically mediated precipitation is influenced by organic matter produced either by living or dead organisms and does not necessarily involve supersaturated solutions. This kind of precipitation acts via biomineralization or organomineralization, and may result in various sorts of minerals, such as calcite, elementary sulfur, sulfates, iron and manganese oxides, etc. The main biological protagonists forming speleothems are microbes. They are mostly chemolithoautotrophic and chemoheterotrophic microorganisms, or phototrophic organisms that changed their life modes and adapted themselves to the cave environment. Normally, the phototrophs are active only in near-entrance parts of caves. Other organisms forming speleothems are mosses, lichens, higher plants, or animals. These are however, mostly passively encrusted by minerals; their potential for active biological mediation to form speleothems is still the matter of research.

Bibliography

- Aubrecht, R., Brewer-Carías, Ch., Šmída, B., Audy, M., and Kováčik, L., 2008. Anatomy of biologically mediated opal speleothems in the world's largest sandstone cave Cueva Charles Brewer, Chimantá Plateau, Venezuela. *Sedimentary Geology*, **203**(3–4), 181–195.
- Barton, H. A., 2006. Introduction to cave microbiology: a review for the non-specialists. *Journal of Cave and Karst Studies*, **68**(2), 43–64.
- Barton, H. A., Spear, J. R., and Pace, N. R., 2001. Microbial life in the underworld: biogenicity in secondary mineral formations. *Geomicrobiology Journal*, **18**, 359–368.
- Bourrelly, P., and Depuy, P., 1973. Quelques stations françaises de *Geitleria calcarea*, Cyanophycée cavernicole. *Schweizerische Zeitschrift für Hydrologie*, **35**, 136–140.
- Castenholz, R. W., 2001. Phylum BX. Cyanobacteria. Oxygenic photosynthetic bacteria. In Boone, D. R., and Castenholz, R. W. (eds.), *Bergey's Manual of Systematic Bacteriology*, The Archaea and the deeply branching and phototrophic Bacteria, 2nd edn. New York: Springer, Vol. 1, pp. 473–599.
- Friedman, I., 1955. *Geitleria calcarea* n.gen. et n.sp., a new atmophytic lime-encrusting blue-green alga. *Botaniska Notiser*, **108**, 439–445.
- Gradziński, M., and Szulc, J., 1997. Recent microbial speleothems ("moonmilk" deposits). 3rd IFAA Regional Symposium & IGCP 380 International Meeting Guidebook, Cracow, Sept. 14–20, 1997, pp. 38–39.
- Gradziński, M., Banaś, M., and Uchman, A., 1995. Biogenic origin of manganese flowstones from Jaskinia Czarna Cave, Tatra Mts., Western Carpathians. *Annales Societatis Geologorum Poloniae*, **65**, 19–27.
- Gradziński, M., Szulc, J., and Smyk, B., 1997. Microbial agents of moonmilk calcification. In Jeannin, P.-Y. (ed.), *Proceedings of the 12th International Congress of Speleology*. Basel, Switzerland: IUS, Vol. 1, pp. 275–278.
- Hill, C. A., and Forti, P., 1986. *Cave Minerals of the World*. Huntsville: National Speleological Society, 238 pp.
- Kashima, N., and Ogawa, T., 1995. A note on biogenic effect of coralloid speleothems in Round Mountain Lava Cave, Oregon, U.S. *A. Journal of Speleological Society of Japan*, **19**, 8–12.
- Kashima, N., Teruo, I., and Kinoshita, N., 1987. Diatom, contributors of coralloid speleothems, from Togawa-Sakaidani-do Cave in Miyasaka prefecture, Central Kyushu, Japan. *International Journal of Speleology*, **16**, 95–100.
- Konhauser, K., 2007. *Introduction to Geomicrobiology*. Malden: Blackwell Publishing, 425 pp.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40.
- Schlögl, J., Michalik, J., Zágoršek, K., and Atrops, F., 2008. Early Tithonian serpulid-dominated cavity-dwelling fauna, and the recruitment pattern of the serpulid larvae. *Journal of Paleontology*, **82**(2), 382–392.
- Taboroši, D., 2006. Biologically influenced carbonate speleothems. In Harmon, R. S., and Wicks, C. M. (eds.), *Perspectives on Karst Geomorphology, Hydrology and Geochemistry*. GSA Special Paper 404, pp. 307–317.
- Vinogradova, O. N., Kovalenko, O. V., Wasser, S. P., Nevo, E., and Weinstein-Evron, M., 1998. Species diversity gradient to darkness stress in blue-green algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel. *Israel Journal of Plant Sciences*, **48**, 229–238.
- Whitton, B. A., 1987. The biology of *Rivulariaceae*. In Fay, P., and van Baalen, C. (eds.), *The Cyanobacteria – A Comparative Review*. Amsterdam: Elsevier, pp. 513–534.

Cross-references

[Algae \(Eukaryotic\)](#)
[Archaea](#)
[Bacteria](#)
[Beggiatoa](#)
[Biomarkers \(Organic, Compound-Specific Isotopes\)](#)
[Calcite Precipitation, Microbially Induced](#)
[Chemolithotrophy](#)
[Cyanobacteria](#)
[Diatoms](#)
[Extreme Environments](#)
[Fungi and Lichens](#)
[Karst Ecosystems](#)
[Microbial Biomineralization](#)
[Microbial Ecology of Submarine Caves](#)
[Microbialites, Modern](#)
[Moonmilk](#)
[Nitrogen Fixation](#)
[Organomineralization](#)
[Sediment Diagenesis – Biologically Controlled](#)

Silica Biomineralization, Sponges
Sinter
Stromatolites
Sulfate-Reducing Bacteria

SPONGES (PORIFERA) AND SPONGE MICROBES

Friederike Hoffmann¹, Marie-Lise Schläppy²

¹Sars International Centre for Marine Molecular Biology, Bergen, Norway

²Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

Sponge-associated bacteria; Sponge-associated microbes; Sponge-associated microbiota; Sponge bacteria; Sponge-microbe systems; Sponge symbionts

Definition

Sponges are sessile multicellular aquatic animals (Lat. Porifera = bearing pores) whose bodies have a typical body plan which allows water to pass through the animals. Sponges are characterized by a skeleton made of calcareous, siliceous spicules or horny fibers.

Sponge microbes: Bacteria and archaea that live in close and permanent association with sponges, usually in the mesohyl between sponge cells.

Introduction

Sponges are sedentary benthic organisms found in shallow waters on tropical coral reefs to the Arctic and the deep sea. Some species, however, are restricted to freshwater ecosystems. Sponges are characterized by a typical body plan built around a system of water canals and chambers (Figure 1a). Within the chambers, flagellated cells called choanocytes produce a water current that enters the sponge body through surface pores (ostia) and leaves through larger openings called oscula. Hence, sponges are filter feeders and incorporate particles from the water as energy and nutrient source. An external cell layer (pinacoderm) encloses the sponge mesohyl, a glycosidic matrix containing several cell types which perform a variety of functions. Sponge cells show a low degree of specialization and a high degree of independence so that the sponge body in some respect resembles a protozoan colony. However, sponges are, without any doubt, placed as true members of the Metazoan (Ax, 1995; Müller, 1998a, b).

Sponges have been grouped into three classes: Calcarea, Hexactinellida, and Demospongiae. Calcarea are characterized by the presence of calcareous spicules. The upgrade of this class to the phylum level has recently been proposed by Borchiellini et al. (2001). Hexactinellida are characterized by siliceous spicules of hexactine structure and syncytial tissue organization.

Demospongiae, the most numerous and diverse class, is a nonmonophyletic group (Boury-Esnault, 2006). They generally have a mineral skeleton made of siliceous spicules, but several lineages, like the common bath sponge, have no mineral skeleton but a network of fibers instead. Modern sponge phylogeny is now based on a combination of molecular methods and sponge morphological features, but sponge spicules are still important features for species determination and identification of sponges in the fossil record.

Sponges form one of the deepest radiations of the Metazoa and can be regarded as one of the oldest animal phylum still alive. The spicule record for sponges starts in the late Proterozoic (Reitner and Wörheide, 2002), while chemofossil records even indicate the presence of sponges – or their direct ancestors – already in the early Proterozoic: specific C₃₀ steranes (24-isopropylcholestanes), which are unambiguous biomarkers for demosponges, were found in 1,800 Ma old stromatolites (McCaffrey et al., 1994; Moldowan et al., 1994).

Choanoflagellates, a monophyletic group of protists which can show a colonial lifestyle, are the closest sister group of the Metazoa (Medina et al., 2001) and show a morphological resemblance to the flagellated choanocytes of sponges. It is, therefore, widely agreed that the “urmetazoa,” the last common ancestors of all Metazoa, would have many traits found in sponges (see review in Gaidos et al., 2007).

Numerous sponge species host vast amounts of microbes in their mesohyl matrix (Figure 1b and c) or sometimes in specialized cells, while others contain only few or no microorganisms at all. The former have been termed bacteriosponges (Reiswig, 1981) or high-microbial-abundance sponges (Hentschel et al., 2003) while the latter are referred to as “low-microbial-abundance sponges.” Bacterial population densities in bacteriosponges may reach 10⁸–10¹⁰ microbes per gram of sponge wet weight, while those in low-microbial-abundance sponges are within the range of natural seawater.

The first studies exploring the microbial community of sponges were based on electron microscopy and isolation of microbes and yielded invaluable information about the microorganism morphotypes and their specific location within the sponge (e.g., Wilkinson, 1978). Microbes were found to exist extra- and intra-cellularly (e.g., Vacelet and Donadey, 1977) and even inside the nucleus of sponge cells (Vacelet, 1970). More recently, the advent of molecular techniques have enabled a detailed phylogenetic description of the microorganisms associated with sponges (Hentschel et al., 2006), whereas environmental genomics have given insights into their metabolic and physiological properties (Grozdanov and Hentschel, 2007).

Phylogenetically complex, yet highly sponge-specific microbial communities were identified in numerous species over large geographical ranges (Hentschel et al., 2002). Sequences representing 16 bacterial phyla and both major archaeal lineages have been recovered to date (see

S

SALINE LAKES

Carol D. Litchfield
George Mason University, Manassas, VA, USA

Synonyms

Salt Lakes

Definition

Salt: chloride, sulfate, phosphate, carbonate, bicarbonate salts of primarily sodium, magnesium, calcium, and potassium.

Lake: an enclosed body with or without water entering it from a stream, rain, or snow melt.

Ephemeral lake: a lake formed only following rain or snow melt, which dries up during the rest of the year.

Thalassohaline: salts in a lake derived from the evaporation of seawater with the dominant ions of sodium and chloride as found in the ocean.

Athalassohaline: salts in a lake derived from rocks and geological weathering and dominated by magnesium, calcium, and sulfate.

Alkaliphilic lake: also called soda lake. A lake in which the dominant ions are sodium and bicarbonate and carbonate. This causes the lake to have an alkaline pH.

General description of saline lakes

Saline lakes are a natural worldwide phenomenon. They can be found on all continents and in most countries, and they are frequently recognizable by their name – for example, Salt Lick, Salt Lake, Salt Pond, and Salt Marsh. They range in salinity from just above the salt content of sea water (>3.2% salts) to hypersaline lakes (>20% and up to saturation with respect to salts). Examples of saline lakes include, at the lower end, salt lakes in Antarctica, the Caspian Sea, which borders five countries and has

a salinity slightly less than that of seawater, and the Salton Sea in California. At the upper end in salt concentrations are the Dead Sea bordering Israel and Jordan, the soda lakes of North Africa, and Quinghai Lake in China. A general characteristic of saline lakes is that they are terminal lakes, which means that although rivers may flow into them, they have no significant outflow. Therefore, as the water evaporates, the lakes become more salty and in some cases may dry up completely forming salt basins or playas especially in extremely arid environments. In Australia, for example, occasional rains may cause the dry salt basins to become lakes, and these then are called ephemeral salt lakes.

Saline lakes are typically divided into two main types: athalassohaline and thalassohaline. The former refers to lakes which have a different chemical ratio of the main cations from what is found in seawater, while the latter refers to lakes with the cation ratio similar to seawater. [Table 1](#) lists the sodium + potassium ion to calcium + magnesium ion ratios and summarizes the chemical composition of a variety of saline lakes thus showing the wide range of lake types possible.

The data in [Table 1](#) indicate that a subset of the athalassohaline lakes exists. These lakes have a high concentration of carbonate and therefore a high pH. These are the soda lakes or alkaline lakes. They, too, are found worldwide and are described below. No Antarctic lakes are listed in [Table 1](#) because the surface waters are generally only very slightly saline (less than seawater) and it is only with depth, often greater than 10 m, that true saline waters are found. In general, it is the bottom waters that are hypersaline with little turnover to bring the saline waters to the surface. The chemical compositions of Antarctic lakes have been reviewed by Matsubaya et al. (1979).

The reason for the differences in the chemical composition lies in the origins of the various types of lakes. The thalassohaline lakes were generally formed during the

Saline Lakes, Table 1 Examples of the chemical compositions of saline lakes. Concentrations in g/L

Lake name	Location	Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ⁻²	HCO ₃ ⁺	pH	Ratio ^a	References
Small lake near Koombekine	Australia	9.36	0.065	0.077	0.38	15.7	0.67	0	3.0	20.6	Geddes et al. (1981)
Mar Chiquita Lake	Argentina	12.5	0.14	0.35	0.25	16.7	5.1	0.21	8.53	21.1	Martinez (1995)
Laguna Amarga	Chile	24	0.33	0.002	1.7	8.2	38	4.4	9.4	14.3	Saijo et al. (1995)
Great Salt Lake – North Arm	Utah, USA	101	6.9	0.28	8.5	175	22	<0.05	7.9 ^b	12.3	Utah Geol. Survey (unpublished)
Great Salt Lake – South Arm	Utah, USA	59	3.4	0.26	4.6	95	13	<0.05	7.3–8.1 ^b	12.8	Utah Geol. Survey (unpublished)
Dead Sea	Israel	34.3	8.0	18.3	47.1	228.6	0.4	0.3	6.3	0.65	Gavrieli et al. (2005)
Urmia Lake	Iran	88	1.1	1.21	6.6	153	14.2	284	6.75	11.4	Alipour (2006), Ghaheiri et al. (1999)
Mono Lake	California, USA	29.5	1.5	0.004	0.033	17.6	10.3–12.5	30.1	9.8	838	Oren (2002)
Wadi El Natrun	Egypt	279.7	NL ^c	ND ^d	ND	86.4	43.4	5.3	8.9	ND	Taher (1999)
Laguna Colorado	Bolivia	18.1	1.85	NL	NL	33.3	3.84	NL	8.4	ND	Mancinelli (personal communication)
Muskiki	Saskatoon, Canada	62.2	NL	NL	29	11.4	237	1.47	7.9	ND	Javor (1989)
Oygon	Gobi Desert	18.0 ^e		0.02	0.41	16.9	6.25	8.91	NL	41.9	Egorov (1993)
Seawater	Mongolia	11	0.39	0.42	1.32	19.7	2.76	0.145	8.0	6.5	ZoBell (1963)

^aCalculation for this paper: (Na⁺ + K⁺)/(Ca⁺⁺ + Mg⁺⁺)^bTaylor et al. (1980)^cNot listed^dNot determined^eCombined Na⁺ + K⁺

Pleistocene period (Hammer, 1986) and are the result of seawater intrusion before tectonic activity created the mountains which cut the bodies of seawater off from the ocean. Brine springs are formed from the dissolution of salts deposited during evaporation of the seawater. As surface water seeps into the ground, the water percolates downward and eventually encounters the salt, dissolves it, and forms underground saline lakes. These form the basis of often small salty ponds, springs, licks, and rivers.

Athalassohaline lakes are most generally formed by the weathering of rocks by wind, rain, and freshwater and were formed during the Pleistocene period and later by volcanic and faulting activity (Hammer, 1986). These are also terminal lakes, and they are typically close to saturation with respect to salt. They may also be ephemeral – found only occasionally after the rainy season – or permanent containing water year round.

The soda lakes are considered athalassohaline because they were formed from high carbonate rocks due to an absence of rocks of sedimentary origins. Consequently, they have low concentrations of calcium and magnesium ions and high concentrations of carbonate or carbonate complexes (Jones et al., 1998).

Biology of saline lakes

Despite the differences in chemical composition, all saline lakes have certain features in common. In general, fish are not found in lakes with salinities greater than 4‰ (40‰). Two major reasons for this are egg survival (Gerking and Lee, 1980) and loss of osmoregulation (Morris, 1960). One exception is the European sea bass, *Dicentrarchus labrax*, which tolerates salinities up to twice that of seawater (Jensen et al., 1998).

Other eukaryotic organisms are found in the higher salinity lakes, specifically algae, brine flies, and brine shrimp. The diversity of the algae depends on the total salt concentration. At salinities lower than around 10–12‰ salt, a wide variety of diatoms and green algae is found. At salinities greater than 12–15‰ and up to saturation, the main algae are from the genus *Dunaliella*. It is also at these higher salinities that the brine fly and brine shrimp are major biomass contributors. These small eukaryotes provide the food sources for the extensive bird populations that are frequently associated with saline lakes. However, other crustaceans such as the mussel shrimp cannot survive in lakes with salinities greater than 18‰ (Deckker, 1981).

Another group of eukaryotic organisms found throughout the range of saline lakes is the fungi. It is only recently that fungi have been isolated from the higher salinity lakes. The state of our understanding of halotolerant and halophilic fungi was summarized by Frisvad (2005). The presence of protozoans and flagellates in saline lakes has also been reviewed and documented by Hauer and Rogerson (2005) and Cho (2005), respectively.

Just as lower salinities allow for a greater diversity of algae, the same is true for the bacteria. Members of the

Domain *Bacteria* predominate in the salinity range of 3–15‰ salt (Ventosa et al., 1998). Above this, the members of the archaeal kingdom domain, the salt-loving *Halobacteriaceae* become dominant though several members of the domain *Bacteria* grow at these elevated salinities. The predominant *Bacteria* genera in elevated salinities are *Halomonas* and *Salinibacter*. As of August 2007, there were 26 described genera of the haloarchaea and 85 species (Oren et al., 2007), and most, but not all, grow over salinity ranges from approximately 12‰ to close to saturation. Microbes found in most salt lakes are therefore, salt-loving and prefer a circumneutral pH.

Among those haloarchaea genera, the “Natrono-” genera are primarily found in soda lakes. As mentioned above, soda lakes have an alkaline pH; therefore, bacteria which grow under those conditions are considered alkaliphiles (alkaline pH-loving). There are many genera of the kingdom *Bacteria* which also contain genera and species that are alkaliphiles. Thus, all of these bacteria are both salt- and high pH-loving organisms. This distinguishes them from the bacteria described in the previous paragraph.

Halophages are viruses that attack bacteria living in saline lakes. The viruses may incorporate into the bacterial cells or they may cause the lysis of the cells. In either case, halophages, like other bacteriophages, tend to be host-specific and can be found wherever their host organism lives. Dyall-Smith et al. (2005) have recently summarized the state of knowledge about the halophages which are specific for the haloarchaea.

Saline lake sediments

Sediments of saline lakes have been the subject of numerous studies because of the potential for determining stable isotope ratios hence sediment age (Piovano et al., 2004) and using lipid biomarkers (Rontani and Volkman, 2005; Pearson et al., 2007) to indicate the types of biota in the sediments. The exposure history of Dead Sea sediments was determined by examining the various minerals formed within the interstitial waters again improving the understanding of the age and depositional history of saline lake sediments (Yechieli and Ronen, 1997). As the salinity of the overlying water increases, this results in similar increases in the salinity of the interstitial waters within the shallow sediments, and the reverse is also true that decreases in the salinity of the lake due to rainfall or snow melt will decrease the salinity of the pore waters.

These postdepositional changes have also been noted for the alkaline Soda Lake in Washington State in the USA (Mantuani, 1973) and in playa Lake Salines in southeastern Spain (Queralt et al., 1997). Such changes can also affect the biology of the sediments which are anaerobic (low to no oxygen) and are dominated by bacterial mats which can produce sulfides and methane (Miller et al., 1993). These mats are composed of layers of bacteria with the cyanobacteria on the surface and various green and purple sulfur bacteria followed by the sulfate-reducing bacteria that produce the sulfides, and the obligately

anaerobic methane-producing bacteria. More recently, Jioang et al. (2007) have noted, in a core from Lake Chaka on the Tibetan Plateau that the distribution of bacteria within the core is determined by the salinity in the pore waters rather than by the mineralogy, and that the biomass decreased most around 150–200 cm depth and increased slightly at 600 cm with a maximum at 900 cm as determined by phospholipid fatty acid analyses. However, the direct counts remained steady with a slight increase at 900 cm. Most of the organisms identified at these lower depths were anaerobic bacteria, again confirming the importance of oxygen to the types of microorganisms found in any sediment.

Value of saline lakes

Saline lakes are nature preserves and serve as home to extensive permanent and migratory bird populations. Great Salt Lake, Utah, USA, hosts over five million birds annually (and this is true in Africa, Australia, the Caribbean, etc.). They may also act as hatching grounds for flamingoes and other birds at the lower salinities. Thus, saline lakes are important in maintaining the biodiversity of our planet.

A second major value, which is related to the first, is that these environments with their large numbers of microorganisms, brine flies, and brine shrimp provide an important gene pool. Although there are numerous studies on the brine shrimp, very little is known about the brine flies, the algae, or the bacteria found in these lakes. Unknown enzymes, potential antibiotics, drug delivery systems, or mechanisms for adaptation to stress could be present but unknown for lack of research into these areas.

Another value of the lower salinity lakes is their potential for aquaculture. Gamito (1997) successfully showed that the gilthead, *Sparus aurata*, could be grown in the Ria Formosa because of the high benthic productivity (1997). Undoubtedly, there are other fish species that could be successfully grown in the lower salinity saline lakes.

A major economic value of saline lakes is their salt. The ephemeral or dried salt beds are a source of salt to many groups of people. This is especially true in Africa with the Taureg people, and in Tibet which also has salt caravans, in the Atacama region of Chile, in Australia, and in Bolivia to name just a few of the places where salt is harvested from dry saline lake beds.

Bibliography

- Alipour, S., 2006. Hydrogeochemistry of seasonal variation of Urmia Salt Lake, Iran. *Saline Systems*, **2**, 9–28.
- Cho, B. C., 2005. Heterotrophic flagellates in hypersaline waters. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 541–549.
- Decker, P. de., 1981. Ostracods of athalassic saline lakes: a review. *Hydrobiologia*, **81**, 131–144.
- Dyall-Smith, M., Burns, D. G., Camakaris, H. M., Janssen, P. H., Russ, B. E., and Porter, K., 2005. Haloviruses and their hosts. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 553–563.
- Egorov, A. N., 1993. Mongolian salt lakes: some features of their geography, thermal patterns, chemistry and biology. *Hydrobiologia*, **267**, 13–21.
- Frisvad, J. C., 2005. Halotolerant and halophilic fungi and their extrolite production. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 425–439.
- Gamito, S., 1997. Sustainable management of a coastal lagoonal system (Ria Formosa, Portugal): an ecological model for extensive aquaculture. *International Journal of Salt Lake Research*, **6**, 145–173.
- Gavrieli, I., Bein, A., and Oren, A., 2005. The expected impact of the “Peace Conduit” project (The Red Sea-Dead Sea pipeline) on the Dead Sea. *Mitigation and Adaptation Strategies for Global Change*, **10**, 3–21.
- Geddes, M. C., De Decker, P., Williams, W. D., Morton, D. W., and Topping, M., 1981. On the chemistry and biota of some saline lakes in Western Australia. *Hydrobiologia*, **82**, 201–222.
- Gerking, S. D., and Lee, R. M., 1980. Reproductive performance of the desert pupfish (*Cyprinodon n. nevadensis*) in relation to salinity. *Environmental Biology of Fishes*, **5**, 375–378.
- Ghaheri, M., Baghal-Vayjooee, M. H., and Naziri, J., 1999. Lake Urmia, Iran: a summary review. *International Journal of Salt Lake Research*, **8**, 19–22.
- Hammer, U. T., 1986. *Saline Lake Systems of the World*. Boston: Dr. W. Junk, p. 46.
- Hauer, G., and Rogerson, A., 2005. Heterotrophic protozoa from hypersaline environments. In Gunde-Cimerman, N., Oren, A., and Plemenitaš, A. (eds.), *Adaptations to Life at High Salt Concentrations in Archaea, Bacteria, and Eukarya*. New York: Springer, pp. 519–539.
- Javor, B., 1989. *Hypersaline Environments*. New York: Springer.
- Jensen, M. K., Madsen, S. S., and Kristiansen, K., 1998. Osmoregulation and salinity effects on the expression and activity of Na⁺, K⁺-ATPase in the gills of European sea bass, *Dicentrarchus labrax* (L.). *Journal of Experimental Zoology*, **282**, 290–300.
- Jioang, H., Dong, H., Yu, B., Liu, X., Li, Y., Ji, S., and Zhang, C., 2007. Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau. *Environmental Microbiology*, **9**, 2603–2621.
- Jones, B., Grant, W. D., Duckworth, A. W., and Owenson, G. G., 1998. Microbial diversity of soda lakes. *Extremophiles*, **2**, 191–200.
- Mantuani, M. A., 1973. Postdepositional osmotic adjustments in sediments from Soap Lake, Washington. *Limnology and Oceanography*, **18**, 403–413.
- Martinez, D. E., 1995. Changes in the ionic composition of a saline lake, Mar Chiquita, Province of Córdoba, Argentina. *International Journal of Salt Lake Research*, **4**, 25–44.
- Matsubaya, O., Sakal, H., Toril, T., Burton, H., and Kerry, K., 1979. Antarctic saline lakes – stable isotopic ratios, chemical compositions and evolution. *Geochimica et Cosmochimica Acta*, **43**, 7–25.
- Miller, L. G., Jellison, R., Oremland, R. S., and Culbertson, C. W., 1993. Meromixis in hypersaline Mono Lake, California. 3. Biogeochemical response to stratification and overturn. *Limnology and Oceanography*, **38**, 1040–1051.
- Morris, R. W., 1960. Temperature, salinity, and southern limits of three species of Pacific cottid fishes. *Limnology and Oceanography*, **5**, 175–179.
- Oren, A., 2002. *Halophilic Microorganisms and their Environments*. Dordrecht: Kluwer Academic Publishers.
- Oren, A., Vreeland, H., and Ventosa, A., 2007. International Committee on Systematics of prokaryotes; subcommittee on the

- taxonomy of halobacteriaceae and subcommittee on the taxonomy of Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology*, **57**, 2975.
- Pearson, E. J., Farrimond, P., and Juggins, S., 2007. Lipid geochemistry of lake sediments from semi-arid Spain: relationships with source inputs and environmental factors. *Organic Geochemistry*, **38**, 1169–1195.
- Piovano, E. L., Ariztegui, D., Bernasconi, S. M., and McKenzie, J. A., 2004. Stable isotopic record of hydrological changes in subtropical Laguna Mar Chiquita (Argentina) over the last 230 years. *The Holocene*, **14**, 525–535.
- Queral, I., Juliá, R., Plana, F., and Bischoff, J. L., 1997. A hydrous Ca-bearing magnesium carbonate from playa lake sediments, Salines Lake, Spain. *American Mineralogist*, **82**, 812–819.
- Rontani, J. -F., and Volkman, J. K., 2005. Lipid characterization of coastal hypersaline cyanobacterial mats from the Camargue (France). *Organic Geochemistry*, **36**, 251–272.
- Saijo, Y., Mitamura, O., and Tanaka, M., 1995. A note on the chemical composition of lake water in the Laguna Amarga, a saline lake in Patagonia, Chile. *International Journal of Salt Lake Research*, **4**, 165–167.
- Taher, A. G., 1999. Inland saline lakes of Wadi El Natrun depression. *International Journal of Salt Lake Research*, **8**, 149–169.
- Taylor, P. L., Hutchinson, L. A., and Muir, M. K., 1980. Heavy metals in the Great Salt Lake, Utah. In Gwynn, J. W. (ed.), *Great Salt Lake A Scientific, Historical and Economic Overview*. Salt Lake City, Utah: Utah Geological and Mineral Survey. Bulletin 116, pp. 195–200.
- Ventosa, A., Nieto, J. J., and Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. *Microbiology and Molecular Biology Reviews*, **62**, 504–544.
- Yechieli, Y., and Ronen, D., 1997. Early diagenesis of highly saline lake sediments after exposure. *Chemical Geology*, **138**, 93–106.
- ZoBell, C. E., 1963. Domain of the marine microbiologist. In Oppenheimer, C. H. (ed.), *Symposium on Marine Microbiology*. Springfield, IL: Charles C. Thomas, pp. 3–24.

Cross-references

Alkalinity
 Archaea
 Bacteria
 Biomarkers (Organic Compound-Specific Isotopes)
 Extreme Environments
 Halobacteria – Halophiles
 Hypersaline Environments
 Microbial Ecology of Submarine Caves
 Microbial Mats
 Pore Waters
 Sediment Diagenesis – Biologically Controlled
 Soda Lakes

SALINITY HISTORY OF THE EARTH'S OCEAN

L. Paul Knauth
 Arizona State University, Tempe, AZ, USA

Definition

Salinity refers to the total quantity of dissolved salts in water and is normally about 35 parts per thousand (‰) in today's ocean. Due to problems with making accurate measurements of total salts, salinity is now normally

expressed as a ratio of the electrical conductivity of the seawater to that of a standard concentration of KCl solution. The concentration of the standard solution is set such that the ratio of the approximate average salinity of the ocean today is equal to 35. The previous concentration value (‰) is thus unnecessary, but it is still widely used to highlight the basic meaning of the term. The relative proportions of the dissolved constituents are approximately constant, so the amount of Cl^- , the major component, is a proxy for salinity. Salinity history refers to past changes in the amount of dissolved salts in sea water over geologic time.

Significance

Salinity is an important environmental variable affecting chemical balances and osmotic pressures in all living cells. It particularly affects metazoans, because the amount of dissolved oxygen available for respiration depends on oxygen solubility that decreases as salinity increases. Besides its direct effects on cellular biochemistry, salinity governs the temperature of maximum density of sea water and thus the global thermohaline circulation that influences ecosystems. Models of geochemical cycles yield no constraints on past salinity levels, and reliable geochemical proxies, isotopic records, or other possible indicators of ancient oceanic salinity are currently unknown. However, Na^+ and Cl^- constitute about 85% of the salt in the sea, so the geologic record of halite deposits allows insights into how ocean salinity may have varied with time.

Causes of salinity changes with time

Early ideas that steady riverine input of salt to the oceans causes salinity to increase with time failed to recognize the geologic processes that intermittently cause salt to be removed from the sea and then later released following tectonic uplift. Nor did they correctly assess the probable initial origin of salt in the sea or consider possible small changes in ocean volume over time. Salt in rivers is derived largely from groundwater dissolution of ancient marine salt beds deposited when sea water evaporated under arid conditions in low latitude, geographically restricted arms of the sea. Deposition of these “saline giants” depletes the ocean in salt and thus lowers salinity. Subsequently, these enormous deposits may be tectonically lifted above sea level. Depending upon elevation, descending groundwaters can eventually dissolve the deposit and debouch into streams or lodge as dense, saline brines deeper in the continental subsurface. In addition to the saline giants, carbonate depositional environments in shallow seas that flooded the continents in past times typically contained vast tidal flats and supratidal areas in which sea water intermittently evaporated. Partially evaporated sea water from these environments as well as their ephemeral salts dissolved by falling rains descended into the subsurface and lodged in sedimentary basins even where large saline giants were absent. Most large

sedimentary accumulations on continents thus have highly saline waters in the deep subsurface. The salts in these brines were also in sea water once and their sequestration on continents thus also served to lower salinity of sea water. The presence of salt beds and brines hundreds of millions of years old illustrates the enormous lengths of time salt extracted from the sea can remain isolated.

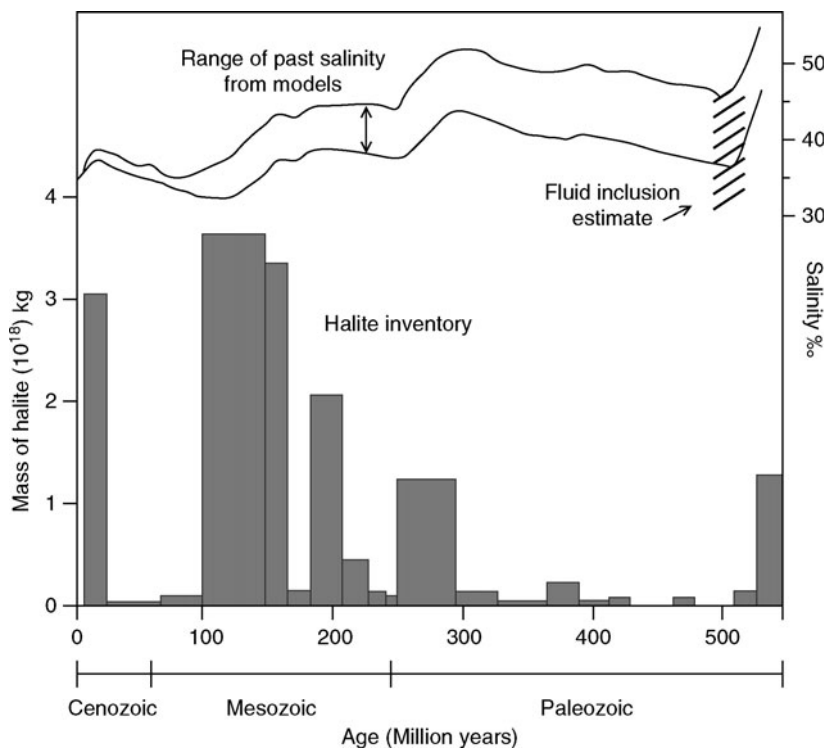
Salinity history for the past 540 Ma

Estimates of the salt inventory in sedimentary rocks have steadily grown as more and more subsurface deposits are discovered. The latest estimate by Hay et al. (2006) confirms that deposition of the saline giants is strikingly episodic (Figure 1). Over half of the salt currently identified was deposited between 100 and 300 million years ago following breakup of the supercontinent Pangea. Presumably, this breakup and subsequent drift of the continental fragments produced the unusual array of geographic, geologic, climatic, and ocean circulation conditions that Sonnenfeld (1984) argued were necessary to produce saline giants. It follows that ocean salinity should have declined during these times of exceptional withdrawal of salts from the sea. The amounts shown in Figure 1 are surely minimal estimates of the amount of salt withdrawn because some significant percentage has been dissolved by groundwaters to become brines in the strata beneath

the salt. Additionally, salt depositional basins are rimmed with clastic deposits adjacent to the surrounding highlands and these are conduits to the subsurface for the prodigious amounts of evaporite brine present when the salt was being deposited. Combined geochemical and isotopic studies have shown that deep basin brines are probably mixtures of partially evaporated sea water and brine created by fresh water dissolution of salt (Knauth, 1988).

Salinity should increase during periods when there are no giant salt extractions underway and when older, uplifted deposits are eroding into the sea. This is the current situation, so the old arguments that the oceans are increasing in salinity via riverine input of salt are understandable. There is no direct evidence yielding the amount of salt returned to the sea at any given past time. Instead, it is necessary to use models for continental sediment erosion that are based on an array of assumptions. Hay et al. (2006) estimate that the return of salt to the sea has varied by a factor of three over the past 540 Ma, reaching maxima in the Upper Cambrian (515 Ma), at the Permian/Triassic boundary (245 Ma), and during the Lower Cretaceous (135 Ma).

Using the inventory of currently sequestered salt and the calculated estimate of salt return, a possible salinity history of sea water for the past 540 Ma can be inferred if the volume history of sea water can be estimated. Fresh basalt on the ocean floors becomes hydrated during subsea



Salinity History of the Earth's Ocean, Figure 1 Salt deposition in the Phanerozoic and estimates of past salinity variations. Ocean salinity declines during deposition of large salt masses. Model estimates by Hay et al. (2006) yield surprisingly high salinity values for the Paleozoic which are not incompatible with values inferred from fluid inclusion measurements by Johnson and Goldstein (1993). Salt masses from estimates by Hay et al. (2006).

weathering and hydrous sedimentary minerals are deposited as the basalt rides tectonic plates to subduction zones. At issue is how much of the hydrated water returns to the surface as it gets progressively heated during subduction. Some models predict a net loss to the mantle over geologic time, so it is not unreasonable to suggest that ocean volumes may have decreased somewhat with time. Combining all these uncertain estimates and models, Hay et al. (2006) have suggested the possible salinity history for the oceans for the past 540 Ma shown in Figure 1. The inferred salinity levels prior to 200 Ma are 50% higher than modern values. Inasmuch as life in the sea is stressed by current salinity values, the idea that Paleozoic life could thrive at such high inferred salinity is likely to be resisted by marine biologists and paleontologists. The amount of brine sequestered during initial deposition and later dissolution remain uncertain and could severely affect such calculated salinity histories. Nevertheless, the provocative Hay et al. (2006) estimates indicate that ocean salinities in the past were likely higher.

Direct salinity measurements in fossil sea water

Tiny fluid inclusions in carbonates or other minerals precipitated directly from ancient seawater may be fossil sea water that could be analyzed for salinity. The difficulty is that the initial precipitates typically undergo dissolution/reprecipitation episodes during subsequent burial and metamorphism. Actual attempts at making this difficult analysis on inferred primary precipitates yield salinities of 31–47‰ at about 500 Ma (Johnson and Goldstein, 1993). These measurements suggest past salinities not too dissimilar from the modern value, but the large error bars do overlap the Hay et al. (2006) estimates (Figure 1). While reliable ancient salinity values remain elusive, it seems demonstrated that significant variations should have occurred in the past 540 Ma and that salinity was likely higher rather than lower in the past.

Salinity history of the early ocean

With regard to deeper time, past salinity can be estimated if it is assumed that the oceans outgassed early in Earth history (Holland, 1978) and that the plate tectonic cycle long ago reached an approximate steady state with regard to hydrosphere cycling through the crust and mantle. Cl^- does not fit readily into any common silicate mineral and thus should have outgassed as HCl along with water following accretion of the Earth. The earliest hydrosphere would thus contain the entire Cl inventory and been at maximum salinity. The only way this high initial salinity can be reduced is by sequestration of salts and brines in the saline giants deposited on long-lived continental cratons. Large continents may have formed shortly after the Earth accreted 4.5 billion years ago (Ga), but recent studies confirm the likelihood that objects in the inner solar system were largely resurfaced by the Late Heavy Bombardment (LHB) at about 3.8–4.0 Ga (Gomes et al., 2005). Continents may have started to reaggregate

following the LHB in the interval between 3.8–2.5 Ga, but the kind of long-lived, continental cratons upon which evaporatively deposited salt and brine could be sequestered following its removal from sea water do not appear until about 2.5 Ga (Rogers, 1996). It follows that all the salt and brine currently lodged on the continents was in the Archean sea following the LHB and prior to the development of these stable cratons.

In the absence of a paleosalinity indicator in the rock record, a reasonable assessment of the earliest salinity could be made if the total amount of salt and brine currently lodged on the continents were known. While the inventory of salt in strata younger than 540 Ma is becoming increasingly better quantified (Figure 1), the amount of salt in older strata is yet to be inventoried. Enormous deposits are known in Neoproterozoic strata (540–800 Ma) across the Middle East, through Pakistan, and into Australia. These apparently accumulated in restricted basins that developed following the breakup of supercontinent Rodinia, analogous to those developed following the breakup of Pangea. The geographic extent of these enormous salt deposits exceeds that of the Cenozoic deposits in the Gulf of Mexico, so it might even be the largest accumulation in Earth history. The amount of subsurface brine associated with the saline giants and with innumerable ancient platform carbonate deposits is also unknown. Land (1995) used estimates of the amount and average salinity of pore fluids to suggest that sequestered brines could hold an amount of salt equal to or greater than that of the rock salt. Adding these amounts of possible total salt and brine currently on the continents back into modern ocean water raises salinity by over a factor of 2. Assuming that the Cl inventory and the oceans were outgassed early in Earth history and have not changed their amounts significantly with time, Archean salinities were therefore much higher and could have exceeded 70‰ (Knauth, 2005).

Implications

The geologic history of salt deposition clearly seems to suggest significant past salinity variations with values possibly much higher than current levels. If so, the impact on biology and biologic evolution must have been significant. An assessment of the paleontologic record in relation to the emerging possible salinity history for the past 540 Ma (Figure 1) remains to be done. The inferred very high salinity of the earliest ocean combined with the probable hot climatic conditions of the early Earth may have limited early marine biologic diversity to the appropriate extremophiles. If the great Neoproterozoic salt deposits were really the first saline giants, the prospect arises that high salinity was prevalent throughout the Precambrian. The great salinity decline likely accompanying deposition of the Neoproterozoic salt combined with declining temperatures associated with probable glaciations may have allowed oxygenation of the ocean for the first time. The Cambrian explosion of life in the sea at 540 Ma may have been, in part, a response of respiring organisms evolving

rapidly in this vast new array of oxygenated marine habitats (Knauth, 2005).

Summary

Salinity is not fixed by any equilibria, feedbacks, or geochemical cycles and appears to have been generally higher in past times. It is apparently a free variable dependent upon the vagaries of intermittent salt (and brine) sequestration on long-lived continental cratons. Ocean salinity declines when the salt and brine accumulate and rises when they are eroded back into the sea. Sequestration seems to have been enhanced following breakup of the two supercontinents in Earth history. These breakups are, in turn, driven by the vagaries of mantle convection. The combination of processes therefore necessary to lower the initial, pre-continent high salinity and to affect the rise and fall of ocean salinity involve a long chain of independent variables. The role of chance and contingency in biologic evolution has long been recognized. The low odds that a rerun of the tape of evolution would yield the same results have been duly noted (Gould, 1990). The same might be said about the evolution of oceanic salinity. Salinity history should therefore probably be added to all those chance occurrences that have likely affected the course of biologic evolution.

Bibliography

- Gomes, R., Levison, H. F., Tsiganis, K., and Morbidelli, A., 2005. Origin of the cataclysmic Late Heavy Bombardment period of the terrestrial planets. *Nature*, **435**, 466–469.
- Gould, S. J., 1990. *Wonderful Life: The Burgess Shale and the Nature of History*. New York: W.W. Norton.
- Hay, W. W., Migdisov, A., Balukhovskiy, A. N., Wold, C. N., Fogel, S., and Soding, E., 2006. Evaporites and the salinity of the ocean during the Phanerozoic: implications for climate, ocean circulation and life. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **240**, 3–46.
- Holland, H. D., 1978. *The Chemistry of the Atmosphere and Oceans*. New York: Wiley.
- Johnson, W. J., and Goldstein, R. H., 1993. Cambrian sea water preserved as inclusions in marine low-magnesium calcite cement. *Nature*, **362**, 335–337.
- Knauth, L. P., 1988. Origin and mixing history of brines, Palo Duro Basin, Texas, USA. *Applied Geochemistry*, **3**, 455–474.
- Knauth, L. P., 2005. Temperature and salinity history of the Precambrian ocean: implications for the course of microbial evolution. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **219**, 53–69.
- Land, L. S., 1995. The role of saline formation water in crustal cycling. *Aquatic Chemistry*, **1**, 137–145.
- Rogers, J. J. W., 1996. A history of continents in the past three billion years. *The Journal of Geology*, **104**, 91–107.
- Sonnenfeld, P., 1984. *Brines and Evaporites*. Orlando, FL: Academic Press.

Cross-references

[Critical Intervals in Earth History](#)
[Evaporites](#)
[Hypersaline Environments](#)
[Pore Waters](#)
[Soda Ocean, Hypothesis](#)

SCANNING PROBE MICROSCOPY (INCLUDES ATOMIC FORCE MICROSCOPY)

Michael Hoppert

University of Göttingen, Göttingen, Germany

Synonyms

Atomic force microscopy; Atomic probe microscopy; Force microscopy; Scanning force microscopy

Definition

Scanning probe microscopy uses a nanoscopic probe that is scanned over a solid surface. The interaction between the probe and the surface may be a mechanical or electromagnetic force. The force signal is enhanced and then composed to a force diagram of the surface. Under ideal conditions, an atomic-scale resolution may be achieved.

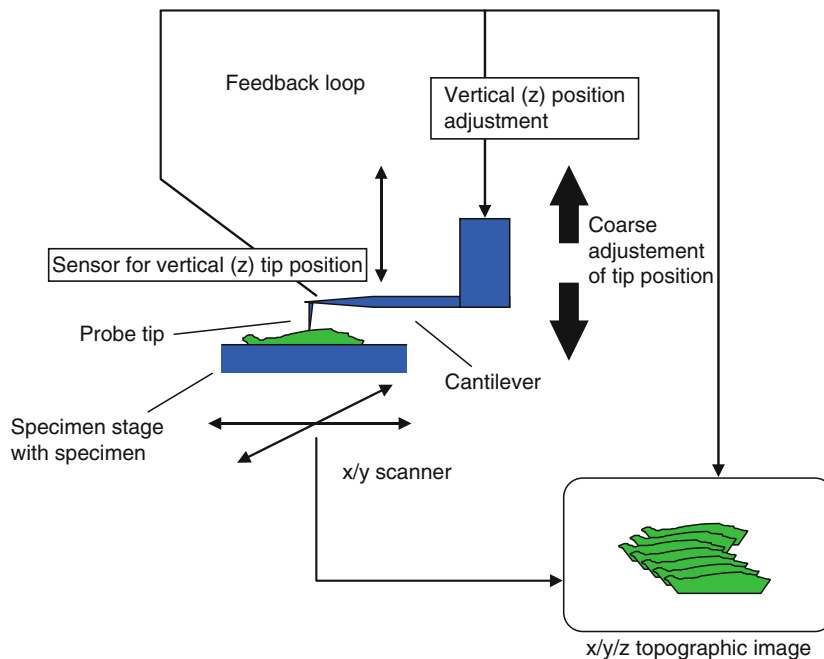
Introduction

Since their development during the 1970s and early 1980s, scanning probe microscopes (SPMs) have developed into instruments suitable for the analysis of surface topography (and other surface properties) down to near-atomic resolutions. All instruments belonging to this family contain essential components as depicted in [Figure 1](#). In the scanning process, surface material properties are mapped in all three directions. The essential parts of the system are the sharp scanning probe tip, which moves in the *z* direction, a piezoelectric scanner that moves the specimen, or, alternatively, the tip, in the *x/y* directions, and a sensor for tip positioning. Sensor and tip are connected in a feedback loop for the correction of *z* positioning. The *x/y/z* positions are used to generate a topographic image of the specimen. The resolution of SPMs is only limited by the size of the probe tip (or the probe-sample interaction volume). Since the tip apex ends up in one single atom and the piezoelectric actuators execute motions down the range of angstroms, resolutions down to atomic scale are possible. In contrast to electron microscopes, the sample can be observed under ambient conditions, which makes the technique potentially attractive for life science, including geobiological research.

The scanning tunneling microscope (STM)

The scanning tunneling microscope (STM) is the ancestor of all SPMs (Binnig et al., 1982). The specimen needs to be conductive or semiconductive. STM is predominantly used in materials sciences and chemistry. Though not ideally suited for biological specimens, the technique has also been used in life sciences (see, e.g., Guckenberger et al., 1988). The instrument provides a surface topography down to atomic resolution.

STMs use a sharpened conducting tip with a bias voltage (in mV to V-range) applied between the tip and the sample. If the distance between the tip and the sample is large, no current flows. When, however, the tip is brought



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 1 Basic setup of a scanning force microscope.

very close (1 nm), but without physical contact, a small current (pico- to nano-amperes) flows across the gap between the tip and the sample. This current is the result of the overlapping of wave functions between the tip atoms and the surface atoms. Electrons can “tunnel” across the vacuum barrier separating the tip and sample in the presence of the bias voltage. The magnitude of the tunneling current increases exponentially when the tip approaches the specimen surface. This results in high system sensitivity: when the separation between the tip and the specimen changes by 10%, the tunneling current changes by an order of magnitude. This provides, in the end, surface images with lateral atomic resolution. The image of the tunneling current depicts approximately the topography of the sample. Actually, rather than physical topography, the STM measures a surface of constant electron tunneling probability.

Atomic force microscopy and related techniques

Atomic force microscopy (AFM) comprises several derivatives and is applicable for measurement of numerous physical and chemical parameters of sample surfaces. AFM in the strict sense (without a modified probe tip) is used to study the material properties of specimens (stiffness, plasticity, and viscosity; see, e.g., Rotsch et al., 1997). For biological specimens, on the cellular level, the properties of the cytoskeleton and (induced) cytoskeletal changes become visible (see Fung, 1993; Goldmann et al., 1998 for some applications). With the aid of chemically modified tips, AFM is useful for quantification of binding forces between receptors and ligands, protein

complexes and cells (Lehenkari and Horton, 1999; Yip, 2001; Allison et al., 2002; Dufrene, 2008). Since AFM is generally suited for solid surfaces, it is especially a versatile tool for the observation of biomineral formation down to nanoscale in situ (see, e.g., Hildebrand et al., 2008).

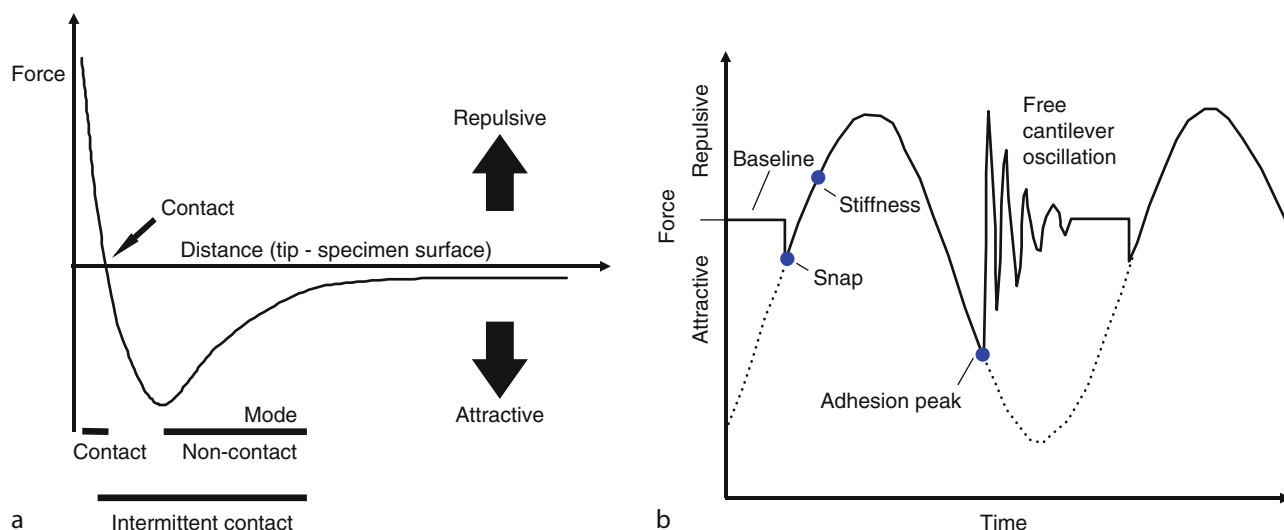
Image formation in AFM is generally determined by sensing of interatomic forces, making the technique applicable to all solid specimens. Similar to STM, the AFM probes the surface of a sample with a sharp tip, less than 10 nm in diameter. The tip is located at the free end of a 100–500 μm long cantilever. The cantilever is deflected by forces between the tip and the sample surface, and a detector measures the cantilever deflection as the tip is scanned over the sample (or the sample is scanned under the tip). The measured cantilever deflections are used to generate a map of surface topography (Figure 1). Conductors, semiconductors, and insulators may all be scanned. Resolution in the atomic range requires rigid specimens; hence, for most biological samples (except for biominerals), maximum resolution is not achievable.

The topographic data set necessary for image formation can be directly obtained from the measurement of cantilever deflection. When a laser beam is projected onto the cantilever, the position of the reflected beam is shifted, which results in detection of even sub-nm movement at the tip surface. Other methods of signal enhancement are based upon optical interference (e.g., by a Michelson interferometer). A STM tip may also be used to read the cantilever deflection. If the cantilever is made from a piezoresistive material, its deflection can also be detected electrically.

When the cantilever deflection is detected, this can be used to generate the topographic data set directly. This mode (constant height) is often used for producing atomic-scale images of atomically flat surfaces, for which the cantilever deflections and variations in applied force are small. The constant height-mode is also essential for recording real-time images of changing surfaces, which requires high scan speeds. Alternatively, the deflection of the cantilever can be used in a feedback loop that moves the scanner up and down in the z direction, responding to the topography, by keeping the cantilever deflection constant. In this case, the image is generated from the scanner's motion. With the cantilever deflection held constant, the total force applied to the sample is constant. This mode (constant force) is generally preferred for most applications.

Figure 2a depicts the typical force–distance curve for AFM. As the atoms of the tip and the specimen surface are gradually brought together, they first weakly attract each other by van der Waals forces. This attraction increases until the electron clouds of the atoms are so close together that their electrostatic repulsion progressively weakens the attractive force. The force becomes zero when the distance between the atoms reaches a couple of ångströms, about the length of a chemical bond. In the *contact mode*, the cantilever is held less than a few ångströms from the sample surface, and the interatomic force between the cantilever and the sample is repulsive. For biological (i.e., soft and wet) objects, the contact mode may induce several artifacts. This is avoided by application of modes in which less force is applied to the specimen. However, smaller forces also have to be detected, which in practice reduce the final resolution. In the *noncontact mode*, the cantilever is held of the order of tens to hundreds of ångströms from the sample surface, and the

interatomic force between the cantilever and sample is attractive (largely as a result of long-range van der Waals interactions). In this mode, the cantilever is oscillated with a frequency of 100–400 kHz (the resonance frequency of the cantilever) with an amplitude of a few tens of nanometers. The resonance frequency of the cantilever varies with the force gradient acting on the cantilever. These variations can be used as a measure of changes in the force gradient, which in turn reflects the changes in the tip-to-sample distance, or sample topography. The resonant frequency or vibrational amplitude of the cantilever is monitored and kept constant with the aid of the feedback loop that moves the scanner up and down in the z direction. The system also keeps the average tip-to-sample distance constant. As in contact AFM (in constant-force mode), the motion of the scanner is used to generate the topographic data set. It thus detects changes in the resonant frequency or vibration amplitude as the tip comes near the sample surface. *Intermittent-contact* (or tapping mode) AFM is similar to noncontact AFM, but the vibrating tip is brought closer to the sample in such a way that the sample is intermittently contacted or “tapped.” As in noncontact AFM, the oscillation amplitude of the tip also changes in response to the distance between tip and sample. Intermittent-contact AFM is also less likely to damage the sample than contact AFM. Though it touches the specimen, lateral forces (friction or drag) between the tip and the sample are minimal. The *pulsed force mode* (PFM) is used to monitor, besides sample topography, stiffness and adhesion simultaneously (Krotil et al., 1999). In PFM, the probe scans the surface in contact mode. When the cantilever is oscillated with a frequency of about 1 kHz, it is possible to analyze the force output as a function of the distance between the tip and the surface. The force output is depicted in Figure 2b. During an oscillation



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 2 (a) Force–distance curve in atomic force microscopes. (b) Force curve in the pulsed force mode (PFM). Important features of the curve are marked with blue dots.

cycle the probe tip is attracted by the surface and “snaps” into contact at a certain distance. The tip is then pushed further to the sample, the force signal increases, whereby the slope of the curve depends on the specimen stiffness. When the distance increases, force signal decreases, the tip loses contact (adhesion peak) and oscillates freely until the signal is damped to the baseline. Characteristic points of these curves are taken for processing to generate simultaneous topographic, stiffness (deformation of the specimen under an applied force), and adhesion maps. In the lateral force mode (LFM), lateral deflections (twisting) of the cantilever are measured. These are caused by forces on the cantilever parallel to the plane of the sample surface. LFM studies are used to detect surface friction arising from inhomogeneity in surface material; an artificial enhancement of edges in the image may also be achieved (Overney et al., 1992). In the force modulation mode (FMM), the elastic properties of a specimen are mapped. The cantilever tip is scanned in contact with the sample surface. As in constant-force contact AFM, the feedback loop uses the cantilever deflection signal to maintain a constant force between the tip and the sample and to generate a topography image. When a periodic oscillation is applied to the tip or the sample, the sample surface resists and the cantilever bends according to the elastic properties of the sample surface. The system generates an image, a map of sample stiffness, from the changes in the amplitude of cantilever modulation (Troyon et al., 1997).

Scanning force microscopy is a highly variable technique, because forces between a specimen and an AFM tip may be caused by a multitude of underlying factors. A modified scanning tip may therefore be used to measure a variety of parameters other than the described surface forces. In life sciences, the detection of chemical properties on biological surfaces is of outstanding interest. Some derivatives of AFM, mainly based on a modified scanning tip, are listed below.

Magnetic force microscopy (MFM) is used for measuring the magnetic force between the tip and the sample. For MFM, the tip is coated with a thin ferromagnetic film. The system mostly operates in noncontact mode and detects magnetic domains down to a resolution of approximately 20 nm (de Lozanne, 2006).

Electrostatic force microscopy (EFM) measures the forces originating from an electrical field between the AFM tip and the sample. When a voltage is applied between the tip and the sample (the tip is not in contact with the surface), the cantilever deflects when it scans over charged particles on the sample surface. In this way, EFM maps locally charged domains on the sample surface (Girard, 2001).

Scanning thermal microscopy (SThM) measures the specimen temperature and thermal conductivity of the sample surface. The SThM tip may be made from a thermocouple temperature sensor: a pair of dissimilar metals joined at the tip (platinum/silver Wollaston wires). The temperature difference between the tip and the open end generates a proportional net voltage. In this way, thermal

data and surface topography (with the conventional AFM force feedback mechanism) can be measured at the same time. Probes made of Wollaston wires also operate as active heat sources. The probe resistance is proportional to the probe temperature. Changes in the current required to keep the probe at constant temperature produce a thermal conductivity map. When the tip is held at a constant current, the specimen temperature is mapped by changes in the probe resistance (Price et al., 1999).

Photothermal microspectroscopy (PTMS) combines scanning probe microscopy at a submicron spatial resolution with an IR interferometer. IR radiation from a Fourier-transform spectrometer is focused on a specimen. IR absorption causes stretching and bending of molecular bonds. Upon relaxation of these molecular vibrations, evanescent thermal waves are emitted. This signal is detected with a Wollaston wire (SThM) probe. The resulting temperature fluctuations are converted to electrical signals, recorded as a function of time and amplified to produce an “interferogram” which can be transformed back to give a conventional spectrum as in other forms of FTIR spectroscopy. In PTMS, resolutions down to 10 μm are achievable, whereas in conventional FTIR microspectrometric devices, only 100 μm are possible (Grude et al., 2007).

Phase detection microscopy (PDM) is another variation for mapping of surface properties such as elasticity, adhesion, and friction. Unlike FMM (see above), the technique operates in the noncontact mode, which is more suitable for less rigid (biological) samples. The technique monitors the phase lag between the signal causing the cantilever to oscillate and the cantilever oscillation output signal. Changes in the phase lag reflect changes in the mechanical properties of the sample surface. The feedback loop in PDM operates in the usual manner, using changes in the cantilever’s deflection or vibration amplitude to measure sample topography. The phase lag is monitored while the topographic image is being taken, so that images of the topography and material properties can be collected simultaneously.

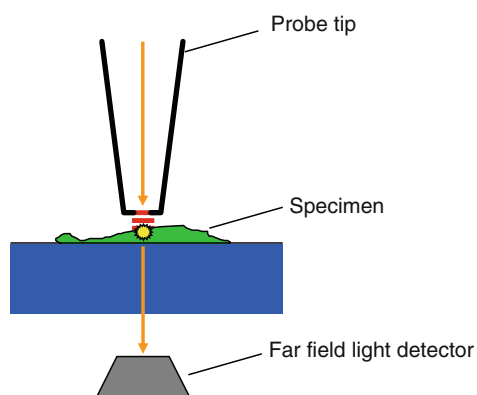
Scanning near-field acoustic microscopy (SNAM) utilizes an ultrasonic quartz oscillator as resonator and at the same time as a sensor of the damping force exerted by the specimen. Damping increases when the tip approaches the surface. The instruments have been especially developed for scanning of larger areas, with a surface relief in the millimeter range. For low resolution, wide probing tips are used. At resolutions of 100 nm, both hard and soft specimens are well suited for mapping. Higher resolution is obtained with sharp AFM tips (Murdfield et al., 1996).

With chemical force microscopy (CFM) it is possible to obtain modified force curves based on specific interactions between molecules on the specimen surface and a sensor molecule on the probe tip. The tip (normally made from silicon or silicon nitride) is modified by coating a very thin metallic film (normally a 5 nm thick chromium layer followed by a 50 nm thick gold layer) onto the probe, followed by immersion of the probe in a solution of an

organic thiol. The molecules form a monolayer, with the thiol group bound to the gold surface. The distal end of thiol molecule contains an appropriate functional group for interaction with the sample surface. Besides small methyl, carboxyl or amino groups, macromolecules may also be used as specific probes. Coupled enzyme proteins may be used to measure enzyme–substrate interactions. In a similar experimental setup, even whole bacterial cells, coupled to the cantilever, were used to measure distance–force curves between the cell surface and biominerals (Lower et al., 2001; Dufrene, 2008 and references therein).

Near-field scanning optical microscopes (NSOMs) are based upon the AFM operating in the noncontact mode. At the same time, the probe tip generates nonpropagating (evanescent) electromagnetic wave fields to excite a specimen at a small (down to tens of nm) surface spot. Most NSOM setups use an aluminum-coated glass fiber with a sub-light wavelength aperture (25–100 nm in diameter) at the tip of an AFM-like scanning probe (Figure 3). Light, guided through the fiber optics, causes an evanescent wave field that decays exponentially with distance from its origin. Evanescent waves interact with the specimen surface, e.g., by excitation of fluorophores. Fluorescence light emitted from the specimen is scattered from the sample, but only from the evanescent wave field region (at distances from the probe tip of approximately one fifth of the light wavelength). The fluorescence signal is conventionally propagating far-field light. This signal may be collected by a conventional optics below the specimen (e.g., an inverted microscope). Because of the extremely small excitation volume determined by the evanescent wave, together with the high-resolution topographical map generated by the AFM tip, a high-resolution map of the fluorescent targets in the specimen is achieved.

With NSOM, the AFM topographic information is combined with optical properties of a specimen, such as



Scanning Probe Microscopy (Includes Atomic Force Microscopy), Figure 3 Hollow fiber tip in a near-field scanning optical microscope (NSOM). At the tip, the evanescent wave field (red) excites a fluorophore marker (yellow). The resulting (far-field) light signal is detected by an external detector. Propagating far-field light (orange arrows).

refraction index, reflectivity, transparency, polarization Raman shift, and fluorescence (Gheber et al., 1998; de Lange et al., 2001; Edidin, 2001).

Conclusion

Scanning probe microscopy introduced a purely surface-related technique with atomic resolution into the field of material sciences and also life science. For the first time it was possible to visualize macromolecules directly, under physiological conditions. Even more important is the application potential for mapping all kinds of intermolecular interactions. The fact that just signals from a surface can be collected may be a drawback for the observation and documentation of most biological processes because they are inside cells and other compartments. Most mechanisms in material science, chemistry and biology are, however, surface-related. It is just the question how a surface, which may also be a membrane or the interface of another compartment, can be prepared in a way that it is reachable by an SPM.

Other limitations are the low scanning speed of most modes and the small obtainable image area. Hence, it is straightforward to combine scanning probe microscopy with other imaging techniques. Correlation of low-resolution techniques including fast image acquisition (fluorescence light microscopy or confocal microscopy) with the high-resolution AFM image may be versatile for a multitude of applications, especially concerning the interaction of organisms with solid surfaces (e.g., Arnold et al., 2004; overview in Haupt et al., 2006).

Bibliography

- Allison, D. P., Hinterdorfer, P., and Han, W., 2002. Biomolecular force measurements and the atomic force microscope. *Current Opinion in Biotechnology*, **13**, 47–51.
- Arnold, J. W., Boothe, D. H., Suzuki, O., and Bailey, G. W., 2004. Multiple imaging techniques demonstrate the manipulation of surfaces to reduce bacterial contamination and corrosion. *Journal of Microscopy*, **216**, 215–221.
- Binnig, G., Rohrer, H., Gerber, C., and Weibel, E., 1982. Surface studies by scanning tunneling microscopy. *Physical Review Letters*, **49**, 57–61.
- de Lange, F., Cambi, A., Huijbens, R., de Bakker, B., Rensen, W., Garcia-Parajo, M., van Hulst, N., and Figdor, C. G., 2001. Cell biology beyond the diffraction limit: near-field scanning optical microscopy. *Journal of Cell Science*, **114**, 4153–4160.
- de Lozanne, A., 2006. Application of magnetic force microscopy in nanomaterials characterization. *Microscopy Research and Technique*, **69**, 550–562.
- Dufrene, Y. F., 2008. Towards nanomicrobiology using atomic force microscopy. *Nature Reviews Microbiology*, **6**, 674–680.
- Edidin, M., 2001. Near-field scanning optical microscopy, a siren call to biology. *Traffic*, **2**, 797–803.
- Fung, Y. C., 1993. *Biomechanics: Mechanical Properties of Living Tissues*. New York: Springer.
- Gheber, L., Hwang, J., and Edidin, M., 1998. Design and optimization of a near-field scanning optical microscope for imaging biological samples in liquid. *Applied Optics*, **37**, 3574–3581.
- Girard, P., 2001. Electrostatic force microscopy: principles and some applications to semiconductors. *Nanotechnology*, **12**, 485–490.

- Goldmann, W. H., Galneder, R., Ludwig, M., Xu, W., Adamson, E. D., Wang, N., and Ezzell, R. M., 1998. Differences in elasticity of vinculin-deficient F9 cells measured by magnetometry and atomic force microscopy. *Experimental Cell Research*, **239**, 235–242.
- Grude, O., Hammiche, A., Pollock, H., Bentley, A. J., Walsh, M., Martin, F. L., and Fullwood, N. J., 2007. Near-field photothermal microspectrometry for adult stem-cell identification and characterization. *Journal of Microscopy*, **228**, 366–372.
- Guckenberger, R., Kösslinger, C., Gatz, R., Breu, H., Levai, W., and Baumeister, W., 1988. A scanning tunneling microscope (STM) for biological applications. Design and performance. *Ultramicroscopy*, **25**, 795–802.
- Haupt, B. J., Pelling, A. E., and Horton, M. A., 2006. Integrated confocal and scanning probe microscopy for biomedical research. *The Scientific World Journal*, **6**, 1609–1618.
- Hildebrand, M., Doktycz, M. J., and Allison, D. P., 2008. Application of AFM in understanding biomineral formation in diatoms. *Pflügers Archiv-European Journal of Physiology*, **456**, 127–137.
- Krottil, H.-U., Stifter, T., Waschipky, H., Weishaupt, K., Hild, S., and Marti, O., 1999. Pulsed force mode: a new method for the investigation of surface properties. *Surface and Interface Analysis*, **27**, 336–340.
- Lehenkari, P. P., and Horton, M. A., 1999. Single integrin molecule adhesion forces in intact cells measured by atomic force microscopy. *Biochemical and Biophysical Research Communications*, **259**, 645–650.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Schewanella* and α -FeOOH. *Science*, **292**, 1360–1363.
- Murdfeld, T., Fischer, U. C., and Fuchs, H., 1996. Acoustic and dynamic force microscopy with ultrasonic probes. *Journal of Vacuum Science and Technology B*, **14**, 877–881.
- Overney, R. M., Meyer, E., Frommer, J., Brodbeck, D., Lüthi, R., Howald, L., Güntherodt, H.-J., Fujihira, M., Takano, H., and Gotoh, Y., 1992. Friction measurements on phase-separated thin films with a modified atomic force microscope. *Nature*, **359**, 133–134.
- Price, D. M., Reading, M., Hammiche, A., and Pollock, H. M., 1999. Micro-thermal analysis: scanning thermal microscopy and localised thermal analysis. *International Journal of Pharmaceutics*, **192**, 85–96.
- Rotsch, C., Braet, F., Wisse, E., and Radmacher, M., 1997. AFM imaging and elasticity measurements on living rat liver macrophages. *Cell Biology International*, **21**, 685–696.
- Troyon, M., Wang, Z., Pastre, D., Lei, H. N., and Hazotte, A., 1997. Force modulation microscopy for the study of stiff materials. *Nanotechnology*, **8**, 163–171.
- Yip, C. M., 2001. Atomic force microscopy of macromolecular interactions. *Current Opinion in Structural Biology*, **11**, 567–572.

Cross-references

[Raman Microscopy \(Confocal\)](#)

SEDIMENT DIAGENESIS – BIOLOGICALLY CONTROLLED

Kurt O. Konhauser¹, Murray K. Gingras¹, Andreas Kappler²

¹University of Alberta, Edmonton, Alberta, Canada

²University of Tübingen, Tübingen, Germany

Definition

The biological processes that modify unconsolidated sediment after initial deposition and burial.

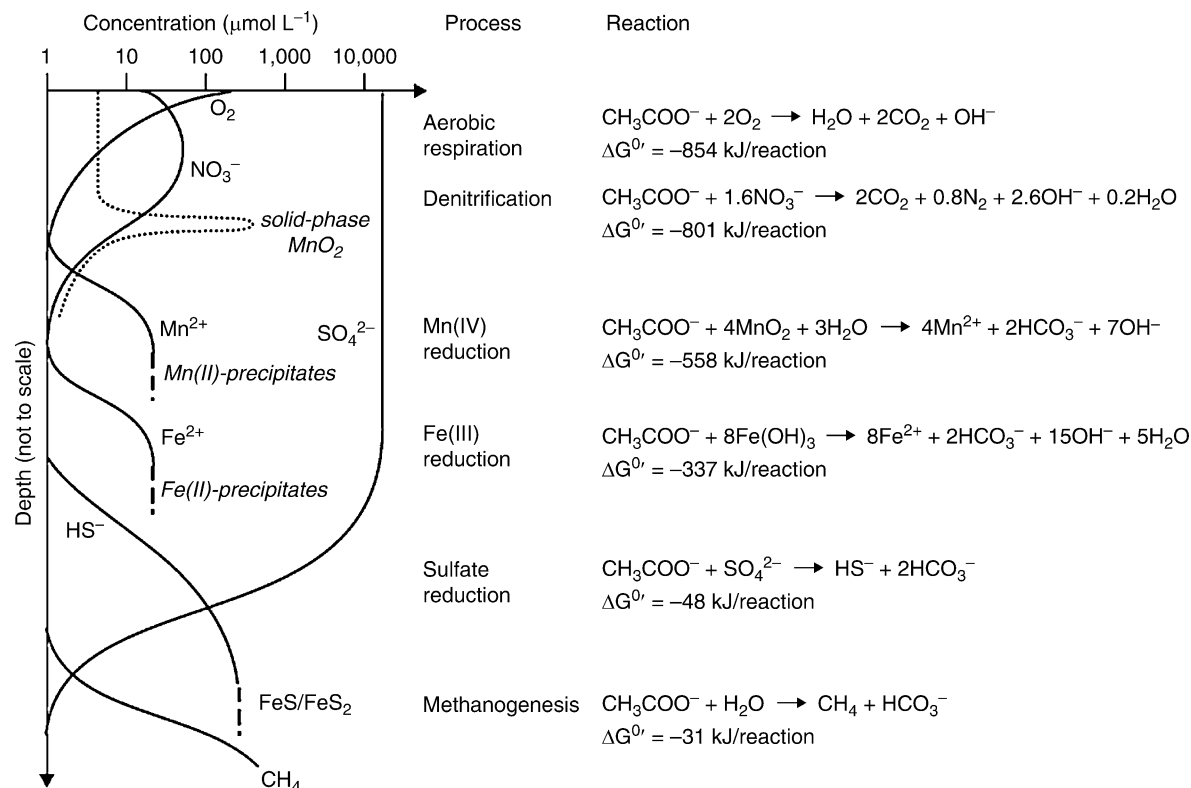
Overview

Throughout the sediment column, up to the temperature threshold of life, microbial activity plays an integral role in diagenesis. It is widely recognized that through their various chemoheterotrophic pathways, microorganisms are ultimately responsible for the conversion of organic carbon to CO₂ and CH₄ at temperatures <100°C. Some aerobic bacteria use hydrolytic enzymes to break down complex molecules into simple monomers such as sugars, amino acids, and fatty acids that they can then utilize, while most anaerobes are restricted to simple organic compounds (e.g., acetate, lactate, H₂) that are the by-products of fermentation (Lovley and Chapelle, 1995). Typically, the more labile materials (e.g., proteins, carbohydrates) are degraded in near-surface sediments on time scales of days to years, and more refractory materials (e.g., lipids) are broken down deeper in the sediment on time scales of decades, while the most resistant materials (lignins) are transformed only on timescales of thousands to millions of years. The amount of refractory material left after microbial attack will depend on the nature of the primary producers and the degree of processing that has taken place in the river catchment area or oceanic water column. In the end, for example, <1% of the original material buried into marine sediment may ultimately contribute to the sedimentary organic geochemical record (Emerson and Hedges, 1988).

Pore water and mineralogical changes during diagenesis are directly related to bacterial reduction of soluble (O₂, NO₃[−], SO₄^{2−}, CO₂) or solid-phase (MnO₂ and ferric oxyhydroxides) components in the sediment. The terminal electron accepting processes (TEAPs) that occur at any given layer depends on oxidants that are available and, in the situation when multiple electron acceptors are present (as in the uppermost sediment layers), on the free energy yield of the specific reaction. Thus, the decomposition of freshly deposited organic material in sediments proceeds in a continuous sequence of redox reactions, with the most electropositive oxidants being consumed at, or near the surface, and progressively energetically poorer oxidants being consumed at depth until the labile organic fraction is exhausted and the deeper sediments are left with a composition very different from that of the sediments originally deposited (Figure 1). Inorganic by-products of chemoheterotrophy (e.g., HCO₃[−], Mn²⁺, Fe²⁺, NH₄⁺, NO₂[−], HS[−], HPO₃^{2−} and CH₄) seldom accumulate. Physical and macrofaunal processes will cause the net transport of these reduced species from the deeper layers toward the sediment surface, where residing anaerobic and aerobic chemolithoautotrophic bacteria use them as metabolic reactants. Alternatively, their presence in pore waters may trigger important abiotic reactions between the solid and dissolved phases leading to secondary mineral formation, i.e., cementation.

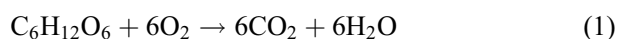
Oxic zone reactions

The sediment–water interface is the site of most intense heterotrophic activity. Aerobic respiration (Reaction 1) is



Sediment Diagenesis – Biologically Controlled, Figure 1 Idealized pore water and solid-phase profiles based on the successive utilization of terminal electron acceptors during the decomposition of marine sedimentary organic matter. (From Konhauser 2007.)

the first used metabolic pathway for the degradation of organic matter, not only because it has the greatest energy yield per amount of organic carbon oxidized, but the aerobes are equipped with a full suite of extracellular enzymes capable of degrading complex organic polymers into simpler substrates (e.g., cellulose into glucose).



$$\Delta G^0 = -2872 \text{ kJ/mol glucose or } -240 \text{ kJ/2e}^-$$

It is estimated that on a global scale, the majority of all particulate organic carbon (POC) deposited is aerobically respired; the percentage varies among different marine environments, with aerobic respiration accounting for >90% of the organic carbon oxidized in deep sea sediments (e.g., Bender and Heggie, 1984), while O_2 is the TEA in only 45% of coastal marine settings and freshwater lakes (e.g., Jones, 1985).

In marine sediment, one of the primary factors controlling the patterns above is the composition of the organic material and the rate at which it is deposited. Plankton-derived organic matter, rich in lipids and cellulose, exhibit significantly higher reactivities than land-derived organic material consisting of vascular plants rich in lignins (Hedges et al., 1988). Thus, in coastal settings, a significant fraction of POC may be relatively difficult

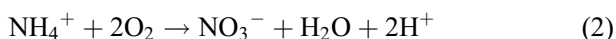
to decompose. Furthermore, under the high deposition rates characteristic of coastal settings (1 mm year^{-1}), O_2 diffusion is very shallow and the oxic zone may only extend down a few millimeters in depth, although physical stirring of surface sediments by waves and currents, circulation of overlying water through animal burrows that are connected to the sediment–water interface (known as irrigation), and bioturbation of surface sediments may locally increase the depth to several centimeters (e.g., Hammond et al., 1985). Therefore, with high accumulation rates, freshly deposited organic matter passes through the oxic zone on the orders of 1–10s of years, leaving a significant amount of carbon intact to support fermentative and anaerobic heterotrophic populations (Canfield, 1993). By contrast, the influx rate of POC and nutrients decreases with distance from land and water depth. This results in a progressive diminishment of macrofaunal and planktonic species, as well as the benthic bacterial populations, leading to low organic matter accumulation rates in the deep sea. Coupled with the low sedimentation rates associated with the open ocean ($<0.01 \text{ mm year}^{-1}$), the organic fraction will remain exposed to oxygenated waters for thousands of years, leading to almost complete oxidation by O_2 – only the most refractory fractions will remain available for other TEAs in the deeper sediments (Murray and Grundmanis, 1980).

O₂ is also consumed by a vast number of chemolithoautotrophic bacteria that metabolize upwardly diffusing reductants (such as HS⁻, NH₄⁺, Fe(II)). Therefore, as reduced metabolites from anaerobic respiratory pathways diffuse upward, a fraction of the oxygen consumed in the surface layers is diverted away from aerobic respiration, toward reoxidation of the reduced species. As a consequence, the depth of O₂ penetration correspondingly decreases. In fact, some diagenesis models show that the amount of O₂ partitioned to organic carbon oxidation in coastal sediments can be <1%, with nitrification or hydrogen sulfide oxidation comprising the major O₂ sinks (e.g., Blackburn and Blackburn, 1993).

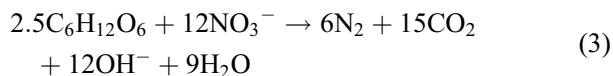
Suboxic zone

Nitrification–denitrification

Nitrogen cycling in aquatic sediments is relatively complex, with nitrate pore water concentrations governed by the balance between nitrification in the oxic zone and denitrification in the suboxic zone. The amount of nitrification is regulated by the concentration of pore water ammonium (NH₄⁺), which largely depends on the C:N ratio of the sedimentary organic carbon being oxidized, and the amount of O₂ penetration (Blackburn and Blackburn, 1993). In the presence of O₂, ammonium is first oxidized to nitrite by ammonium-oxidizing bacteria (e.g., *Nitrosomonas* sp.), and the nitrite is then further oxidized to nitrate by nitrite oxidizers (e.g., *Nitrobacter* sp.). Collectively, these processes (Reaction 2) cause a subsurface peak of nitrate in the pore water (Mortimer et al., 1999).



The opposing processes in marine N-cycling is denitrification (loss of NO₃⁻ to N₂) and nitrate ammonification (loss of NO₃⁻ to NH₄⁺). Denitrifiers most closely resemble aerobic respirers in that they are capable of completely degrading complex organic matter to carbon dioxide (Reaction 3).

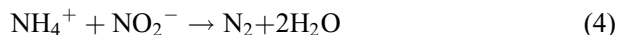


$$\Delta G^0 = -2715 \text{ kJ/mol glucose or } -226 \text{ kJ/2e}^-$$

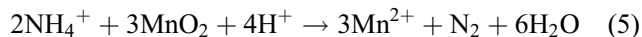
In coastal sediments (and lakes), these bacteria reduce nitrate to negligible levels within 1–10 cm of the sediment–water interface. No nitrate occurs below this depth except where burrows, lined with nitrifying bacteria, irrigate oxygenated water to depths well below the oxic–suboxic boundary (Hansen et al., 1981). In deep sea sediment, the vertical zone of all terminal electron acceptors (TEAs) are expanded, and for nitrate, concentrations may not become negligible until depths of >1 m. Denitrification fluxes range over several orders of magnitude in marine sediments, between values as low as 1 μmol m⁻² day⁻¹ in deep sea sediments (Bender and Heggie, 1984)

and over 1 mmol m⁻² day⁻¹ in some estuarine and coastal waters (e.g., Devol, 1991).

The flux of N₂ escaping from sediments is often higher than predicted biological or inorganic denitrification rates based solely on pore water nitrate concentrations. This means that in the sediments, excess N₂ is being formed independent of a nitrate intermediate. One way this occurs is through the anammox process, where shunting nitrogen directly from ammonium to N₂ (Reaction 4) can promote ammonium deficiencies in sediments where this process plays a key role in nitrogen cycling (Thamdrup and Dalsgaard, 2002).

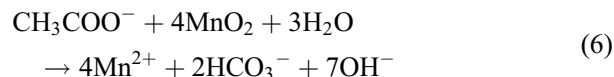


Ammonium can also react inorganically with MnO₂ to form N₂ instead of nitrate (Reaction 5). The Mn(II) formed in this reaction is biologically oxidized by Mn(II)-oxidizing bacteria (see below), with O₂ as the TEA, generating more reactive MnO₂ to continue the oxidation of the soluble ammonium (Luther et al., 1997). Field evidence suggests that this reaction can outcompete the direct oxidation of NH₄⁺ by O₂ to NO₃⁻ in Mn-rich coastal sediments, and in doing so, potentially account for 90% of N₂ formation. In essence, this process short-circuits the traditionally considered nitrification–denitrification process.



Manganese cycling

Detrital mineral phases that survive transport and deposition are for the most part an unreactive component of the bottom sediment. The oxides and hydroxides of manganese and iron are the exceptions. These phases are stable under the oxygenated conditions encountered in the water column, but they quickly become unstable after burial below the oxic layers of sediment. Following denitrification, reduction of Mn(IV) oxides (typically written as MnO₂) to dissolved Mn²⁺ becomes the most energy-efficient bacterial respiratory process (Reaction 6).



$$\Delta G^0 = -558 \text{ kJ/mol acetate or } -139 \text{ kJ/2e}^-$$

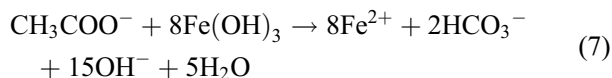
In most sediments, the limited amount of manganese (and iron) oxides deposited in sediment, as well as the slower rates of burial of these oxides compared to the downward diffusion of O₂, NO₃⁻ and SO₄²⁻, generally makes their reduction of minor importance (<10%) in terms of the amount of total organic carbon oxidized (Burdige, 1993). There are, however, exceptions to this generalization, i.e., some lakes and marine hydrothermal settings where high rates of metal oxide reduction are driven by a combination of relatively high metal inputs and active macrofaunal activity that mix suboxic sediment with

oxygenated bottom waters, thereby reoxidizing dissolved Mn^{2+} (and Fe^{2+}) to MnO_2 or $\text{Fe}(\text{OH})_3$ (Aller, 1990).

In typical marine sediments, the concentration of Mn^{2+} is negligible at the sediment surface, but at some depth between the NO_3^- maximum and zero NO_3^- , it begins to increase toward its maximum values. Despite forming near the base of the nitrate reduction zone, its concentration is never highest at that depth because the $\text{Mn}(\text{II})$ that diffuses upward is biologically reoxidized and precipitated as fresh MnO_2 at the sediment surface, suggesting that the TEA can be either nitrate or oxygen (Froelich et al., 1979). A characteristic feature of $\text{Mn}(\text{II})$ oxidation is the upward convexity of the pore water Mn^{2+} profile and the discrete layer of MnO_2 forming at the base of the oxic zone (recall Figure 1). By contrast, downward diffusion of $\text{Mn}(\text{II})$, and its reaction with HCO_3^- , most commonly results in the formation of manganese carbonates, such as rhodochrosite (MnCO_3).

Iron cycling

Below the zone of dissimilatory manganese reduction, and at the depth of complete nitrate removal from pore waters, is where $\text{Fe}(\text{III})$ reduction takes place (Reaction 7). Ferrihydrite [$\text{Fe}(\text{OH})_3$] is the least crystalline, most reactive, and easily reducible iron oxide phase, with rates of $\text{Fe}(\text{III})$ reduction in sediment declining rapidly with depth as these poorly crystalline phases become depleted (Lovley and Phillips, 1986).



$$\Delta G^0 = -337 \text{ kJ/mol acetate or } -84 \text{ kJ}/2\text{e}^-$$

Despite the lower reactivity of crystalline phases, in sediment where ferrihydrite has either been depleted or where crystalline iron oxides are inherently more abundant, the latter can serve as significant source of reducible iron that couples the oxidation of buried organic material. According to experimental observations, $\text{Fe}(\text{III})$ -reducing microorganisms developed three different strategies to cope with the difficulty of transferring electrons from the cell to the surface of a barely soluble electron acceptor (see Lovley et al., 2004 for review). First, physical contact between the cell surface and ferric iron allows direct delivery of electrons. Second, iron chelators increase the solubility of $\text{Fe}(\text{III})$ and hence, alleviate the need for $\text{Fe}(\text{III})$ reduction. Third, electron-shuttling compounds transfer electrons from the cell to $\text{Fe}(\text{III})$ oxide surface without the necessity of physical contact between cells and mineral. Considering the complexity of natural environments and the wealth of microbial capabilities, it is not surprising that different organisms, as well as single organisms, developed different strategies in order to reduce diverse $\text{Fe}(\text{III})$ compounds under varying conditions. For example, some evidence indicates that *Geothrix fermentans* produce and release both $\text{Fe}(\text{III})$ chelators and electron

shuttles (Nevin and Lovely, 2002). Furthermore, studies on *Geobacter* sp. indicate that different cellular compounds are involved in reduction of dissolved $\text{Fe}(\text{III})$ -citrate and barely soluble ferrihydrite (e.g., Leang et al., 2005).

The reduction of ferric iron minerals increases the concentration of $\text{Fe}(\text{II})$ in suboxic sediment pore waters, with a peak in concentration at the boundary between the $\text{Fe}(\text{III})$ and sulfate reduction zones. Some of this ferrous iron may diffuse upward to be reoxidized to ferric hydroxide inorganically by either NO_2^- , MnO_2 , or O_2 (e.g., Myers and Nealson, 1988), and it is likely that in some lakes with near-surface sediment, phototrophic $\text{Fe}(\text{II})$ oxidation should take place. Some $\text{Fe}(\text{II})$ also precipitates as mineral phases. In iron-rich sediments, the high reactivity of Fe^{2+} and the general availability of HCO_3^- in suboxic and anoxic pore waters tend to cause ferrous iron to precipitate quickly as micritic cement in the form of siderite (FeCO_3). By contrast, when sulfate reduction rates exceed $\text{Fe}(\text{III})$ reduction rates (see below), enough HS^- is produced to react preferentially with any ferrous iron to precipitate iron monosulfide minerals instead (FeS has lower solubility than FeCO_3). Therefore, the precise juxtaposition of iron reduction and sulfate reduction in marine sediments controls the formation of siderite and/or pyrite (e.g., Coleman, 1985). The formation of vivianite [$\text{Fe}_3(\text{PO}_4)_3$] in many ways resembles that of siderite, namely $\text{Fe}(\text{II})$ concentrations must exceed those of HS^- . It also requires that soluble phosphate is made available through oxidation of organic matter, the dissolution of phosphorous-bearing solid phases, or through reduction of phosphorous-adsorbing $\text{Fe}(\text{III})$ oxides (Krom and Berner, 1980).

Anoxic zone

Sulfate reduction

Below the suboxic layers is a highly reducing, oxygen-free zone under which sulfate reduction predominates. In marine sediments, this is the principal process by which simple fermentation products are oxidized (Reaction 8), accounting for approximately 50% of the carbon oxidation in coastal marine sediments (Jørgensen, 1982), but <10% in deep sea and freshwater environments (Bender and Heggie, 1984; Jones, 1985).



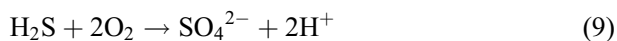
$$\Delta G^0 = -49 \text{ kJ/mol acetate or } -12 \text{ kJ}/2\text{e}^-$$

Its relative importance in marine environments compared to freshwater environments is based simply on the higher concentrations of dissolved SO_4^{2-} (~28 mM) at the sediment–water interface, some 50 times greater than the combined sum of all other electron acceptors with higher electrode potentials. Moreover, external electron acceptors that yield more energy than SO_4^{2-} typically disappear within the first few centimeters of sediment depth, leaving sulfate as the dominant TEA for most of the sediment column (D'Hondt et al., 2002).

The rates of dissimilatory sulfate reduction are proportional to the quantity and reactivity of organic matter entering the anoxic sediments – the greater the amount of aerobic decomposition, the less labile organic matter remains available for sulfate reduction, and the more refractory the residual material becomes (Westrich and Berner, 1984). As a result of natural variations in the above, sulfate reduction rates in marine sediments can vary by six orders of magnitude, with the highest in rapidly deposited coastal and lagoonal sediments, where it occurs just below the sediment–water interface ($>1 \text{ mM cm}^{-2} \text{ year}^{-1}$) and at the lowest levels in deep sea sediments, where rates may be $<10^{-5} \text{ mM cm}^{-2} \text{ year}^{-1}$ (Canfield, 1993).

The product of sulfate reduction is hydrogen sulfide, and as mentioned above, can lead to the formation of iron sulfide minerals. The amount and reactivity of the detrital iron phases dictate the amount of pyrite that will form, commonly referred to as the “degree of pyritization.” In slowly accumulating sediments (i.e., the deep sea), little labile organic matter is buried, sulfate reduction rates are slow, less HS^- is available for reaction with detrital iron, so less ferric iron is eventually converted to pyrite. Since the saturation state of the pore waters with respect to iron monosulfides controls the pore water concentrations of dissolved iron and sulfide, limited HS^- means that pore water Fe(II) will increase in concentration. By contrast, in rapidly accumulating sediments, more labile organics are buried, near-surface sulfate reduction rates are high, HS^- is abundant, and more of it is available for reaction with dissolved Fe(II) and solid-phase ferric iron (Berner and Raiswell, 1983). Nonetheless, not all ferric iron phases are pyritized because some ferric iron minerals react only very slowly with dissolved sulfide, and complete pyritization becomes impossible in the time span over which sulfate reduction occurs in the sediment (Canfield et al., 1992).

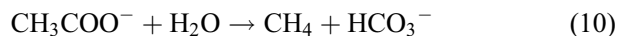
Only a fraction of the HS^- that forms via bacterial sulfate reduction is actually precipitated as pyrite sulfur (Berner, 1982). Instead, some 90% of the HS^- is reoxidized to sulfate. Although O_2 , NO_3^- , and Mn(IV)- or Fe(III)-oxides are the ultimate oxidants in sedimentary systems, there exists a dynamic S-subcycle that involves a number of sulfur intermediate products, with thiosulfate appearing to be the most significant (Jørgensen, 1990). In addition, some of the HS^- that ultimately escapes the thio-sulfate subcycle provides a useable source of reducing power for various chemolithoautotrophs, most of which gain energy from oxidizing sulfide, with O_2 as the TEAP (Reaction 9).



Methanogenesis

The terminal step in the anaerobic degradation of organic material is methanogenesis (Reactions 10 and 11). As the least energetically favorable process of organic

degradation, the reaction product, methane (CH_4), actually stores a major part of the energy available for aerobically respiring species, i.e., methanotrophs.



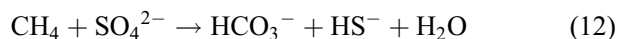
$$\Delta G^0 = -31 \text{ kJ/reaction}$$



$$\Delta G^0 = -34 \text{ kJ/reaction}$$

On average, methanogenesis is responsible for 5–10 times less organic carbon degradation than sulfate reduction, and it is only important in sediments where a significant amount of relatively fresh organic material is delivered rapidly enough to pass through the sulfate reduction zone that it takes on any prominence (Canfield, 1993). Indeed, methane does not accumulate in sediments until more than 90% of the dissolved sulfate has been reduced (Martens and Berner, 1974). This is unsurprising given that the growth of methanogens is inhibited by SRBs, because the latter have a higher affinity for the oxidizable substrates than do the former (Lovley et al., 1982). In other words, methanogens prosper when subsurface activity of SRBs is high and pore water SO_4^{2-} becomes diminished.

The CH_4 generated by methanogens in the underlying sediment subsequently diffuses upward toward the sulfate-rich sediments, where it serves as an electron donor in the sulfate–methane transition zone (SMTZ) (Reaction 12).



In some marginal marine sites, it has been estimated that nearly 100% of the downward SO_4^{2-} flux and the upward CH_4 flux can go toward anaerobic CH_4 oxidation. Comparison of these fluxes to open ocean sites even suggests that anaerobic methane oxidation might be the dominant sink for sulfate in marine sediments relative to organic carbon oxidation (D'Hondt et al., 2002). The diffusive flux of CH_4 upward is usually inadequate to completely reduce pore water sulfate, and hence, accumulate above the SMTZ. Yet, it has been observed in some rapidly depositing shallow water sediments that methane saturates pore waters and ultimately may escape the sediment by bubble ebullition, without complete oxidation by sulfate (Martens and Klump, 1984).

Sediment hydrogen concentrations

As discussed above, the dominant terminal electron accepting pathways in sediments are generally segregated into distinct zones based on the potential thermodynamic yield of the various metabolic processes. Yet, on a purely thermodynamic basis, reactions yielding less energy should also take place as long as they are energetically favorable. Instead, the segregation can be more accurately explained by competition between different types of

microorganisms for electron donors, such as the fermentation products H_2 and acetate (Lovley and Klug, 1986). Accordingly, microorganisms utilizing electropositive TEAs effectively maintain H_2 concentrations below the capabilities of microorganisms using TEAs that yield less energy, and it is only when the electropositive TEA becomes depleted that the next most electropositive TEA controls the H_2 concentrations in the pore water system (Lovley and Goodwin, 1988). In pore waters, this translates into distinct H_2 concentration gradients for each particular metabolism and a threshold level below which H_2 cannot be further metabolized. When nitrate and manganese reductions are the dominant terminal electron accepting pathways, pore water hydrogen concentrations are extremely low (<0.05 nM), with increasing levels through iron reduction (0.2 nM), sulfate reduction (1–1.5 nM), and methanogenesis (7–10 nM). Acetate concentrations follow a similar pattern (Chapelle and Lovley, 1992).

This pattern of H_2 and acetate competition can be ascribed to the physiological capabilities of the microorganisms growing in the sediments. Microorganisms that use amorphous ferric hydroxide as their TEA can metabolize H_2 or acetate to concentrations lower than those that can be utilized by sulfate reducers, and the sulfate reducers can metabolize the same substrates to concentrations below those usable by methanogens (Lovley and Phillips, 1987). These findings are consistent with observations of sediments where sulfate is not reduced, and methane is not produced until the reducible ferric iron minerals have been converted to Fe(II). Given the pattern above, it is not surprising then that the availability of NO_3^- or Mn(IV) oxides diminishes the magnitude of Fe(III) reduction in suboxic sediment, and under such conditions, some of the Fe(III) reducers may even switch over to nitrate or manganese reduction when H_2 becomes limiting for them under their normal mode of growth (Lovley and Phillips, 1988; diChristina, 1992).

Macroscopic biological influence on diagenesis

On the seafloor, recently deposited sediment is subject to grazing, burrowing, and particle reworking by relatively large animals moving over the surface of the sediment and the more numerous small organisms and microorganisms living within the upper layers of sediment. This influence may extend several decimeters (and even meters) below the sediment–water interface, but their effect is largely concentrated in the uppermost 20 cm. The benthos are typically inhibited when (1) the particulate organic supply is too low; (2) when the sediment is too fine-grained and compacted to provide sufficient space for them to live, (3) when O_2 levels reach values <0.2 mg l^{-1} , and (4) when strong ebb and current flows quickly redistribute sediment for burrows to become established (e.g., Van Cappellen and Gaillard, 1996).

Burrowing organisms biomechanically and geochemically alter sediments – a process generally referred to as

bioturbation – in spatially intricate ways. Biomechanical modification includes the redistribution of grain sizes and the modification of grain size and sediment cohesiveness. It also impacts on the organic properties of the sediment because grazing causes greater disintegration of intact organic remains, leading to higher surface areas for microbial oxidation, while burrowing mixes fresh organic matter with older, more decomposed materials, causing enhanced degradation of the latter. Ingestion of refractory organic carbon may lead to excretion of more labile fecal pellets (Lee, 1992). The style of biomechanical deformation can be variable depending on the intrusive process employed by the burrowing animal. In clastic sediments, the redistribution of sediment grain sizes strongly influences the arrangement of permeability pathways, thereby exerting a control on the flux of pore water near, and within, preserved burrows. Through abrasion and ingestion, carbonate sediments are prone to (biogenic) grain-size reduction. This provides a more reactive medium for diagenetic fluids to react with.

The biochemical modification of sediment is the result of local compositional heterogeneity imposed by biogenic structures. This includes an increase in the surface area at the sediment–water interface, which in turn, increases the rate and amount of chemical exchange between the sediment, the pore water, and the water column. For instance, irrigation of worm burrow tubes with oxygenated seawater results in increased downward and lateral diffusion of O_2 into surrounding sediment and concomitantly the loss of sulfur from the sediment by oxidation of pyrite and enhanced diffusive loss of HS^- (Bernier and Westrich, 1985). Subsurface flushing with oxygenated waters also influences nitrification, Mn(II)- and Fe(II)-oxidation patterns. In the case of iron cycling, as long as ferric iron persists, phosphate remains adsorbed to the oxide surface and is thus unavailable to the pore waters. Meanwhile, the incorporation of concentrated (and typically labile) organic material in the form of mucus or feces not only provides an oxidizable substrate for the microbial communities, but also leads to concentration of metals in, and adjacent to, burrow linings as sorbed cations or as metal oxyhydroxide, sulfide, or phosphate coatings (Over, 1990). These metals may then serve as nucleation sites for authigenic mineral formation, which provides a variable chemical stock for diagenetic fluids to interact with (i.e., dolomitization, cementation).

Collectively, the imposition of burrows at the sediment–water interface perturbs the generally accepted geochemical zonations within (subaqueous) sediments, proffering a complex distribution of geochemical zones (Aller, 1980).

Summary

Microorganisms are integral to the chemical and physical changes sediment undergoes during the process of diagenesis. Through various chemoheterotrophic pathways, microorganisms are ultimately responsible for the

conversion of organic carbon to CO_2 (or CH_4) through the coupled reduction of dissolved (O_2 , NO_3^- , SO_4^{2-} , CO_2) or Fe(III)/Mn(IV) oxyhydroxides in the sediment. The inorganic by-products of chemoheterotrophy (HCO_3^- , Mn^{2+} , Fe^{2+} , NH_4^+ , NO_2^- , HS^- , HPO_4^{2-} , CH_4) are either transported from deeper sediment toward the seafloor, where residing chemolithoautotrophic bacteria use them as reactants in their metabolism, or their presence in pore waters may trigger secondary mineral precipitation and cement formation. Macrofauna also contribute to diagenetic reactions through their bioturbating activity. This includes the direct effects of burrowing and mixing of fresh sediment with older sediment, grazing and disintegration of intact organic remains, and the excretion of labile fecal pellets. Indirectly, they influence the solid-phase and pore water properties of sediment by increasing the transport of redox-active elements across the sediment–water interface.

Bibliography

- Aller, R. C., 1980. Quantifying solute distributions in the bioturbated zone of marine sediments by defining an average microenvironment. *Geochimica et Cosmochimica Acta*, **44**, 1955–1965.
- Aller, R. C., 1990. Bioturbation and manganese cycling in hemipelagic sediments. *Philosophical Transactions of the Royal Society of London*, **331**, 51–68.
- Bender, B., and Heggge, D. T., 1984. Fate of organic carbon reaching the deep sea: a status report. *Geochimica et Cosmochimica Acta*, **48**, 977–986.
- Berner, R. A., 1982. Burial of organic carbon and pyrite sulphur in the modern ocean: its geochemical and environmental significance. *American Journal of Science*, **282**, 451–473.
- Berner, R. A., and Raiswell, R., 1983. Burial of organic carbon and pyrite sulfur in sediments over Phanerozoic time: a new theory. *Geochimica et Cosmochimica Acta*, **47**, 855–862.
- Berner, R. A., and Westrich, J. T., 1985. Bioturbation and the early diagenesis of carbon and sulphur. *American Journal of Science*, **285**, 193–206.
- Blackburn, T. H., and Blackburn, N. D., 1993. Coupling of cycles and global significance of sediment diagenesis. *Marine Geology*, **113**, 101–110.
- Burdige, D. J., 1993. The biogeochemistry of manganese and iron reduction in marine sediments. *Earth-Science Reviews*, **35**, 249–284.
- Canfield, D. E., 1993. Organic matter oxidation in marine sediments. In Wollast, R., Chou, L., and Mackenzie, F. (eds.), *Interactions of C, N, P and S Biogeochemical Cycles and Global Change*. Berlin: Springer, pp. 333–363.
- Canfield, D. E., Raiswell, R., and Bottrell, S., 1992. The reactivity of sedimentary iron minerals toward sulfide. *American Journal of Science*, **292**, 659–683.
- Chapelle, F. H., and Lovley, D. R., 1992. Competitive exclusion of sulfate reduction by Fe(III)-reducing bacteria: a mechanism for producing discrete zones of high-iron ground water. *Ground Water*, **30**, 29–36.
- Coleman, M. L., 1985. Geochemistry of diagenetic non-silicate minerals. Kinetic considerations. *Philosophical Transactions of the Royal Society of London*, **315**, 39–56.
- Devol, A. H., 1991. Direct measurement of nitrogen gas fluxes from continental shelf sediments. *Nature*, **349**, 319–321.
- D'Hondt, S., Rutherford, S., and Spivack, A. J., 2002. Metabolic activity of subsurface life in deep-sea sediments. *Science*, **295**, 2067–2070.
- diChristina, T. J., 1992. Effects of nitrate and nitrite on dissimilatory iron reduction by *Shewanella putrefaciens* 200. *Journal of Bacteriology*, **174**, 1891–1896.
- Emerson, S., and Hedges, J. I., 1988. Processes controlling the organic carbon content of open ocean sediments. *Paleoceanography*, **3**, 621–634.
- Froelich, P. N. et al., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic. Suboxic diagenesis. *Geochimica et Cosmochimica Acta*, **43**, 1075–1090.
- Hammond, D. E., Fuller, C., Harmon, D., Hartman, B., Korosec, M., Miller, L. G., Rea, R., Warren, S., Berelson, W., and Hager, S. W., 1985. Benthic fluxes in San Francisco Bay. *Hydrobiologia*, **129**, 69–90.
- Hansen, J. I., Henriksen, K., and Blackburn, T. H., 1981. Seasonal distribution of nitrifying bacteria and rates of nitrification in coastal marine sediments. *Microbial Ecology*, **7**, 297–304.
- Hedges, J. I., Clark, W. A., and Cowie, G. L., 1988. Fluxes and reactivities of organic matter in a coastal marine bay. *Limnology and Oceanography*, **33**, 1137–1152.
- Jones, J. G., 1985. Microbes and microbial processes in sediments. *Philosophical Transactions of the Royal Society of London*, **315**, 3–17.
- Jørgensen, B. B., 1982. Mineralization of organic matter in the seabed – the role of sulfate reduction. *Nature*, **296**, 643–645.
- Jørgensen, B. B., 1990. A thiosulphate shunt in the sulfur cycle of marine sediments. *Science*, **249**, 152–154.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell Publishing.
- Krom, M. D., and Berner, R. A., 1980. Adsorption of phosphate in anoxic marine sediments. *Limnology and Oceanography*, **25**, 797–806.
- Leang, C., Adams, L. A., Chin, K. J., Nevin, K. P., Methé, B. A., Webster, J., Sharma, M. L., and Lovley, D. R., 2005. Adaptation to disruption of the electron transfer pathway for Fe(III) reduction in *Geobacter sulfurreducens*. *Journal of Bacteriology*, **187**, 5918–5926.
- Lee, C., 1992. Controls on organic carbon preservation: the use of stratified water bodies to compare intrinsic rates of decomposition in oxic and anoxic systems. *Geochimica et Cosmochimica Acta*, **56**, 3323–3335.
- Lovley, D. R., and Chapelle, F. H., 1995. Deep subsurface microbial processes. *Reviews of Geophysics*, **33**, 365–381.
- Lovley, D. R., and Goodwin, S., 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochimica et Cosmochimica Acta*, **52**, 2993–3003.
- Lovley, D. R., and Klug, M. J., 1986. Model for the distribution of sulfate reduction and methanogenesis in freshwater sediments. *Geochimica et Cosmochimica Acta*, **50**, 11–18.
- Lovley, D. R., and Phillips, E. J. P., 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Applied and Environmental Microbiology*, **51**, 683–689.
- Lovley, D. R., and Phillips, E. J. P., 1987. Competitive mechanisms for inhibition of sulphate reduction and methane production in the zone of ferric iron reduction in sediments. *Applied and Environmental Microbiology*, **53**, 2636–2641.
- Lovley, D. R., and Phillips, E. J. P., 1988. Manganese inhibition of microbial iron reduction in anaerobic sediments. *Geomicrobiology Journal*, **6**, 145–155.
- Lovley, D. R., Dwyer, D. F., and Klug, M. J., 1982. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Applied and Environmental Microbiology*, **43**, 1373–1379.

- Lovley, D. R., Holmes, D. E., and Nevin, K. P., 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology*, **49**, 219–286.
- Luther, G. W., III, Sundby, B., Lewis, B. L., Brendel, P. J., and Silverberg, N., 1997. Interactions of manganese with the nitrogen cycle. Alternative pathways to dinitrogen. *Geochimica et Cosmochimica Acta*, **61**, 4043–4052.
- Martens, C. S., and Berner, R. A., 1974. Methane production in the interstitial waters of sulfate-depleted sediments. *Science*, **185**, 1167–1169.
- Martens, C. S., and Klump, J. V., 1984. Biogeochemical cycling in an organic-rich coastal marine basin. 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. *Geochimica et Cosmochimica Acta*, **48**, 1987–2004.
- Mortimer, R. J. G., Davey, J. T., Krom, M. D., Watson, P. G., Frickers, P. E., and Clifton, R. J., 1999. The effect of macrofauna on pore-water profiles and nutrient fluxes in the intertidal zone of the Humber Estuary. *Estuarine, Coastal and Shelf Science*, **48**, 683–699.
- Murray, J. W., and Grundmanis, V., 1980. Oxygen consumption in pelagic marine sediments. *Science*, **209**, 1527–1530.
- Myers, K. H., and Neelson, K. H., 1988. Microbial reduction of manganese oxides: interactions with iron and sulfur. *Geochimica et Cosmochimica Acta*, **52**, 2727–2732.
- Nevin, K. P., and Lovley, D. R., 2002. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Applied and Environmental Microbiology*, **68**, 2294–2299.
- Over, D. J., 1990. Trace metals in burrow walls and sediments, Georgia Bight, USA. *Ichnos*, **1**, 31–41.
- Thamdrup, B., and Dalsgaard, T., 2002. Production of N₂ through anaerobic ammonium oxidation coupled to nitrate reduction in marine sediments. *Applied and Environmental Microbiology*, **68**, 1312–1318.
- Van Cappellen, P., and Gaillard, J. F., 1996. Biogeochemical dynamics in aquatic sediments. In Lichtner, P. C., Steefel, C. I., and Oelkers, E. H. (eds.), *Reactive Transport in Porous Media*. Reviews in Mineralogy. Washington: Mineralogical Society of America, Vol. 34, pp. 335–376.
- Westrich, J. T., and Berner, R. A., 1984. The role of sedimentary organic matter in bacterial sulphate reduction: the G model tested. *Limnology and Oceanography*, **29**, 236–249.

Cross-references

Aerobic Metabolism
 Anaerobic Oxidation of Methane with Sulfate
 Biogeochemical Cycles
 Carbon (Organic, Cycling)
 Carbon (Organic, Degradation)
 Cold Seeps
 Deep Biosphere of Sediments
 Deep Biosphere of the Oceanic Deep Sea
 Fe(III)-Reducing Prokaryotes
 Hydrogen
 Iron Sulfide Formation
 Methane Oxidation (Aerobic)
 Methanogens
 Microbial Degradation
 Pore Waters
 Siderite
 Sulfate-Reducing Bacteria
 Sulfur Cycle

SELENIUM

John F. Stolz¹, Ronald S. Oremland²

¹Duquesne University, Pittsburgh, PA, USA

²United States Geological Survey, Menlo Park, CA, USA

Definition

Selenium, element 34, is a metalloid in row VIB of the periodic table. The main oxidation states are selenide (−2), elemental selenium (0), selenite (+4), and selenate (+6). Selenate and selenite are found in the oxic zone, while Se(0) is more abundant in the anoxic zone. Although hydrogen selenide may be produced by microorganisms, it is highly toxic. Se(−II) is more often found as organoselenium in the form of selenoproteins. The common methylated species (e.g., dimethylselenide, dimethyldiselenide) are volatile. Selenium can be a substitute for sulfur and is often found as metal selenide (e.g., Clausthalite, Crookesite). Selenium occurs in eight stable isotopes, the most common of which are Se80 (~50%) and Se78 (~24%), and nine radioactive isotopes.

The microbial oxidation of selenium is a slow process, whereas the dissimilatory reduction of selenate via selenite to Se(0) is rapid (Dowdle and Oremland, 1998). Selenate and selenite may also be methylated, and Se(0) can be further reduced to selenide (HSe^{−1}). Se(0) is insoluble, and bacteria form “nanospheres” of roughly uniform diameter (0.2–0.3 μm) (Oremland et al., 2004). These nanospheres can occur inside the cytoplasm or on the outside of the cell wall and may exhibit measurable isotope fractionation (⁸⁰Se/⁷⁶Se of −8.0‰ to −12.4‰) (Herbel et al., 2000). Selenium may be assimilated via the sulfur assimilation pathway.

Selenium is an essential element primarily needed for the synthesis of selenocysteine and selenomethionine (as reviewed in Stolz et al., 2006). Selenocysteine (Sec, U) is the 21st amino acid (encoded by UGA) and is found in a number of enzymes (e.g., glycine reductase, formate dehydrogenase). Sec requires four specific gene products: SelA, selenocysteine synthase; SelB, a special translation factor; SelC, a Sec-specific tRNA (tRNA^{Sec}); and SelD, the selenophosphate synthase. SelB recognizes the alternative coding of UGA for selenocysteine aided by a stem loop structure in the mRNA. The selenocysteine insertion sequence, or SECIS, is found within the 3′ untranslated region of the mRNA in archaea and eukaryotes and immediately downstream of the UGA codon in bacteria.

Over 20 species of selenate respiring prokaryotes are known and include members of the Crenarchaeota, low and high G + C Gram-positive bacteria, Halanaerobacter, and beta-, gamma-, and epsilon-proteobacteria; however, only a few species respire selenite. The respiratory selenate reductase (Ser) from *Thauera selenatis* is a heterotrimer (SerA, SerB, SerC) and belongs to the

DMSO reductase family of molybdenum enzymes. Two different methyltransferases have been identified. Bacterial thiopurine methyltransferase converts selenite and selenocysteine to dimethylselenide and dimethyldiselenide. MmtA (the calicheamicin-like methyltransferase) produces dimethylselenide and dimethyldiselenide from selenite and selenocysteine (as reviewed in Stolz et al., 2006).

Bibliography

- Dowdle, P. R., and Oremland, R. S., 1998. Microbial oxidation of elemental selenium in soil slurries and bacterial cultures. *Environmental Science & Technology*, **32**, 3749–3755.
- Herbel, M. J., Johnson, T. M., Oremland, R. S., and Bullen, T. D., 2000. Fractionation of selenium isotopes during bacterial respiratory reduction of selenium oxyanions. *Geochimica et Cosmochimica Acta*, **64**, 3701–3709.
- Oremland, R. S., Herbel, M. J., Switzer Blum, J., Langley, S., Beveridge, T. J., Sutto, T., Ajayan, P. M., Ellis, A., and Curran, S., 2004. Structural and spectral features of selenium nanospheres formed by Se-respiring bacteria. *Applied and Environmental Microbiology*, **70**, 52–60.
- Stolz, J. F., Basu, P., Santini, J. M., and Oremland, R. S., 2006. Selenium and arsenic in microbial metabolism. *Annual Review of Microbiology*, **60**, 107–130.

SHALES

Jürgen Schieber
Indiana University, Bloomington, IN, USA

Definition

Historically, the term shale has been used to describe fine-grained sedimentary rocks of largely terrigenous derivation, dominated by mud-size particles (0.06 mm and smaller) and containing appreciable quantities (several 10%) of clay minerals. The definition of the term has been a subject of debate (Potter et al., 2005) because of the closely allied terms mud and clay for which competing definitions may emphasize grain size, mineralogy (clay dominated), or physical properties (plasticity). In place of shale, the term mudstone is also used widely and describes the same compositional spectrum of sedimentary rocks. Because shales span the clay–silt boundary, a good many rocks that are identified as siltstones in the literature also qualify as shales (and vice versa). Although the term shale traditionally had a “terrigenous” connotation, shales that accumulated in sediment starved settings may to more than 50% consist of microbially induced early diagenetic cements (calcite, dolomite, ferroan carbonates, silica, authigenic clays, and iron sulfides), organic matter, and biogenic debris (fish bones, microfossils) upon close inspection. One could argue that the term “biogenic” would be more appropriate for these rocks.

Thus, categorizing shales is by no means a trivial exercise and the various classification schemes in existence (e.g., Ingram, 1953; Dunbar and Rodgers, 1957; Folk,

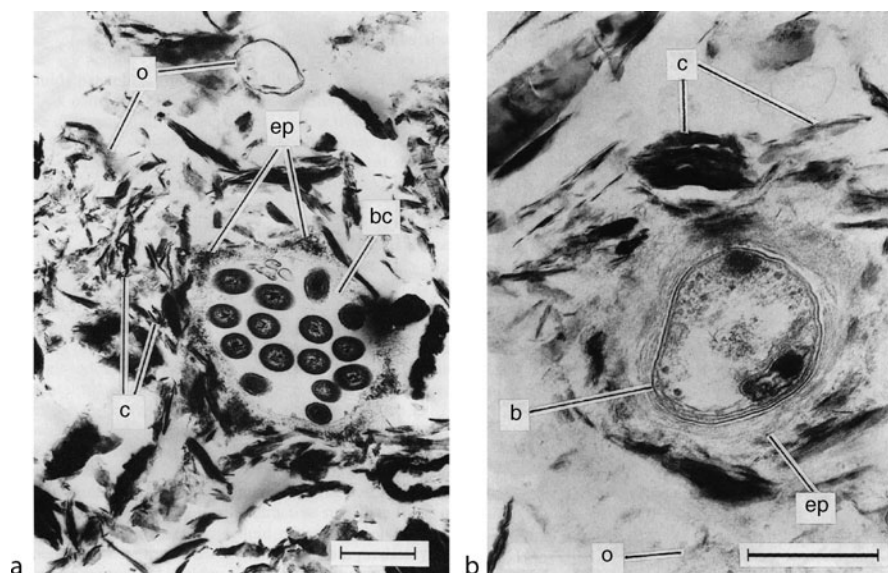
1965; Picard, 1971; Lewan, 1979; Blatt et al., 1980; Lundegard and Samuels, 1980; Potter et al., 1980; Stow and Piper, 1984; Macquaker and Adams, 2003) leave much to be desired. Many fine-grained sedimentary rocks contain variable amounts of a fine-grained carbonate component and it is common practice to use the terms shale and mudstone for rocks with less than 50% carbonate (Potter et al., 2005). Beyond that arbitrary cut-off the terms marl, micrite, limestone, and dolostone are most commonly used. The classification scheme proposed by Macquaker and Adams (2003), however, also includes fine-grained materials of non-terrigenous origin. The books by Potter et al. (1980, 2005) and Schieber et al. (1998) provide further information on this, as well as other topics concerning shales.

Fundamentally, shales span such a large compositional and textural spectrum that a comprehensive and meaningful classification will only be possible when we can build on a much larger body of case studies than currently available. At present it is probably pointless to agonize over the actual names given to shale types encountered in a given study. What matters primarily is that the rocks be dominantly fine grained, and that we report as accurately as possible their composition, texture, and fabric. The latter two are indispensable for meaningful descriptions of shales because they convey key insights into their transport and deposition (Schieber, 1999a; Schieber et al., 2007).

Significance of shales for geobiology

Although still understudied when compared to sandstones and carbonates (Schieber and Zimmerle, 1998), shales nonetheless are geologically important for multiple reasons. They constitute by volume two thirds of the sedimentary rock record and once examined systematically will contribute much to a better understanding of Earth history and the evolution of life. Because overall they accumulate slower than sandstones or carbonates, as much as 90% of geologic time recorded in sedimentary rocks may be preserved in shales.

Shales are an excellent matrix for fossil preservation, and contained fossils are comparatively easy to extract for study. Because their low permeability limits chemical exchange and access by oxygen, metastable skeletal minerals (e.g., aragonite) and even soft tissues may be preserved. It is not by accident that many of the world's most scientifically significant sites of fossil preservation (Fossil Lagerstätten; Seilacher et al., 1985) are found in shale successions. Notable examples are the Cambrian Burgess Shale (Morris, 1998), the Devonian Hunsrück Slate (Bartels et al., 1998), the Jurassic Posidonia Shale (Kauffman, 1978), and the Messel oil shale deposit (Wuttke, 1983). Preservation of fossils carries much valuable information, particularly in the case of marine shales. Fossils are useful because taphonomy (the study of organismal decay over time), spatial distribution of fossils, fossil diagenesis, morphological attributes of fossils



Shales, Figure 1 (a) TEM micrograph of modern mud from the continental slope off northern California at a burial depth of 5 cm. Illustrates the spatial relationships of a colony of bacteria (bc), its extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c) and organic matter (o). Scale bar represents 1 μm . (b) TEM micrograph of a modern mud from Eckernförde Bay, Germany. Shows the relationships between bacteria (b), their extracellular polymeric substances (ep), and the surrounding sediment matrix of clay (c), and organic matter (o). Scale bar represents 0.5 μm . (From Ransom et al., 1999.)

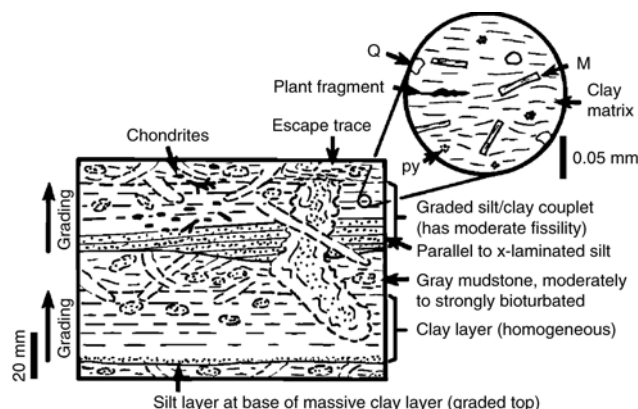
(e.g., reflecting adaptation to substrate conditions), fossil assemblages and comparison to modern counterparts, and paleocommunities (paleoecology) all can provide a wealth of information about the deposition of a shale succession (e.g., Allison, 1988; Brett and Allison, 1998).

Freshly deposited muds, the sediments destined to become shales after compaction, have an enormous internal surface area. Depending on the assumptions about particle geometry and water content, one can readily estimate that a liter of watery mud should have an internal surface area on the order of several hundred square meters (e.g., Fenchel, 1970). That is enough space to provide habitat for trillions (10^{12}) of microbial cells (Figure 1).

Deposition of shales

The sedimentology of shales has made progress over the past decades (Potter et al., 2005), and sedimentologists are gradually learning to appreciate the utility of the many small scale sedimentary structures observable in shales (e.g., Schieber, 1989, 1990a,b, 1999a; O'Brien and Slatt, 1990). When studied in polished slabs and petrographic thin sections, a wealth of information about depositional conditions and history can be gleaned from most shales (Figure 2).

Laminae are the most typically observed sedimentary feature in shales. They show a large range in thickness and lamination styles (even, discontinuous, lenticular, wrinkled etc.), and these may represent conditions that include quiet settling, sculpting of the sediment surface by bottom currents, and growth of microbial mats



Shales, Figure 2 Line drawing that summarizes features observed in a mudstone from the late Devonian Sonyea Group in New York. (From Schieber 1999a.) M = mica; Q = quartz; py = pyrite.

respectively (Schieber, 1986, 1999b). Internal lamina features, e.g., grading (a), random clay orientation (b), preferred clay orientation (c), sharp basal contacts (d), and sharp top contacts (e) may be interpreted as indicative of (a) event-sedimentation (e.g., floods, storms, turbidites), (b) flocculation or sediment trapping by microbial mats, (c) settling from dilute suspension, (d) current flow and erosion prior to deposition, and (e) current flow and erosion/reworking after deposition (Schieber, 1990a,b). Due to somewhat larger grain size, silt laminae are the most readily observed lamina type in shales and may imply

somewhat more energetic conditions. Examples are density currents (grading, fading ripples), storm reworking and transport (graded rhythmites), wave winnowing (fine even laminae with scoured bases), and bottom currents (silt layers with sharp bottom and top). Gradual compositional changes between, e.g., clay and silt dominated laminae are another commonly observed feature, and are suggestive of continuous (although slow) deposition, possibly from deltaic sediment plumes and shifting nepheloid flows.

For many years conventional wisdom has held that parallel laminae in shales are the result of settling from slow moving or still suspensions, and the common assumption was that this reflects distal deposition in comparatively deep water with only minor current activity. Recent flume experiments, however, have demonstrated conclusively that muds can be deposited from swift moving currents that are competent enough to move sand in bedload (Schieber et al., 2007). This occurs because muds have a strong tendency to flocculate, regardless of exact particle mineralogy and water composition. The floccules travel in bedload and form migrating ripples (Figure 3) that build up a contiguous mud bed if sediment supply is sufficient. In spite of accumulation via lateral accretion, after compaction these muds have a parallel laminated appearance, just like many shales in the rock record (Figure 4; Schieber et al., 2007). Obviously, it will be difficult from now on to ascribe a quiet, deep water setting to a laminated shale without corroborating evidence (e.g., paleoecology, trace fossils).

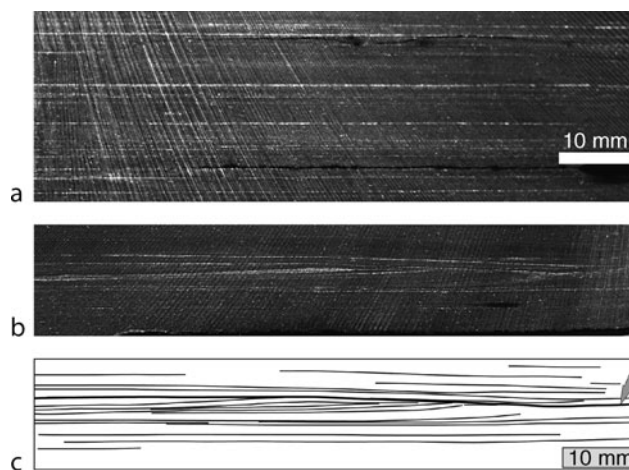
A variety of other small scale sedimentary features also occurs in shales, including mudcracks, load casts, flame structures, dewatering structures, graded rhythmites (Reineck and Singh, 1980), cross-lamination, loop structures (Cole and Picard, 1975), bioturbation (Wetzel and Uchmann, 1998), fossil concentrations and lags, all of



Shales, Figure 3 Migrating ripples of flocculated kaolinite in seawater, photographed through the bottom of a flume. Width of flume channel is 25 cm, flow direction indicated by red arrow. (From Schieber et al., 2007.) The ribbed “tails” behind the ripples are the eroded remains of foreset laminae.

which carry information about conditions of sedimentation. Among the more subtle sedimentary features are clay-filled mud cracks, brecciation due to desiccation, and sands or conglomerates that consist entirely of shale particles (Schieber, 1985). The latter can, for example, form as a result of soil erosion (pedogenic particles; Nanson et al., 1986; Rust and Nanson, 1989), erosion of cracked mud crusts, and submarine scouring of mud substrates by strong currents.

Biologic agents may produce microbial laminae and protection of mud surfaces from erosion (e.g., Schieber, 1986; O’Brien, 1990), or may manifest themselves as bioturbation and via destruction of primary fabrics (Wetzel and Uchmann, 1998). Infaunal activity that took place early in depositional history, when the muds had a high water content, may be exceedingly subtle and hard to detect after compaction (Schieber, 2003). In many instances of bioturbation sufficient proportions of primary features survive and can still be interpreted in terms of depositional processes. Careful examination of bioturbation features can provide additional information about substrate firmness, event deposits (escape traces), and rates of deposition. Fecal pellets and pelletal fabrics are another by-product of organic activity, and they are probably more abundant in mudstones and shales than commonly recognized (Pryor, 1975; Potter et al., 1980; Cuomo and Rhoads, 1987). Best seen in thin section, pellets typically differ from the shale matrix with respect to texture, color, and organic content. They range in size from 0.2–2 mm, are generally of elliptical outline, and



Shales, Figure 4 (a) Parallel-laminated black shale, New Albany Shale, Devonian, Indiana. Lighter laminae are silt enriched (the steeply inclined and slightly curved lines are saw marks). (b) A sample from the same core interval as seen in (a). In the center, we see inclined (to the left) truncated laminae, forming the outline of a compacted mud-dominated ripple. (c) Tracing of silt laminae visible in (b). Arrow marks an internal erosion surface. This is a fossil equivalent of migrating mud ripples observed in flume experiments. (From Schieber et al., 2007.)

flattened by compaction. If matrix and pellets are similar in composition and composed of particles of similar grain size, pellet identification can be challenging. Pellets of benthic versus planktonic organisms may be differentiated on the basis of composition (Cuomo and Bartholomew, 1991; Schieber, 1994).

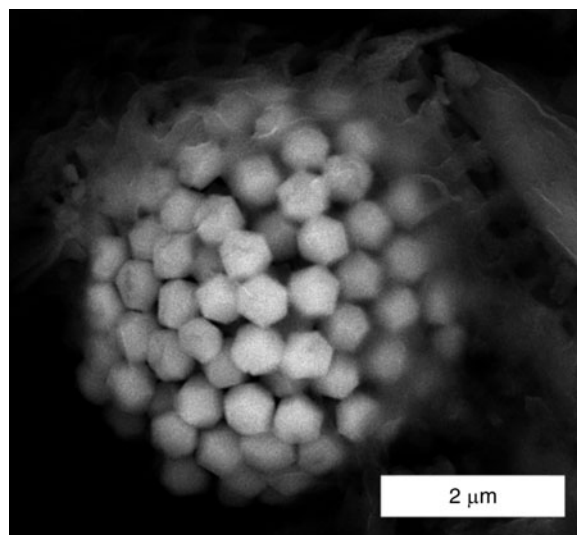
Early diagenesis of shales

It is due to the abundance of endosedimentary microbes (Figure 1) that surficial muds are very active sediments from a geochemical perspective. Much of the initially buried organic material is remineralized in these water-rich sediments (80–90% initial water content), prompting the description of surficial muds as “fluidized bed reactors” (Aller, 1998). The microbial breakdown of buried organic matter requires terminal electron acceptors (oxidizing agents) and due to limitations by diffusion, overall abundance, and differences in energy yield for the various oxidation reactions, we observe a systematic exhaustion of available electron acceptors (e.g., oxygen, nitrate, iron (III), manganese (IV), sulfate, carbon dioxide) as we move downwards from the sediment-water interface. For these reasons closely comparable microbial decay profiles (Brett and Allison, 1998; Curtis et al., 2000) are observed in many marine sediments. For a detailed description of these microbial decay profiles, see Chapters *Sediment Diagenesis – Biologically Controlled* and *Pore Waters*.

A good number of diagenetic minerals, such as pyrite (Figure 5), are readily explained by these decay-reaction schemes (Brett and Allison, 1998; Curtis et al., 2000; Allison, 1988). For others that are not, associated pH shifts can nonetheless be instrumental for their precipitation. For example, early diagenetic silica (quartz) precipitation is not uncommon in shales (Figure 6). In Phanerozoic shales it is derived from the dissolution of biogenic opal (radiolarian, diatoms, sponge spicules) and fills pore spaces that range from tens of microns to as much as a millimeter in size (Figure 7). Quite frequently these pore fills (in situ quartz) are mistaken for detrital quartz (Schieber, 1996; Schieber et al., 2000).

If phosphorous is abundant in the initial mud, such as due to accumulation of phosphatic skeletal material, phosphatic concretions, ranging in size from less than a mm to more than 100 mm's, may form (Figure 8). An early diagenetic phosphate matrix seems to be an excellent medium for preserving minute details of soft tissues, such as in the Neoproterozoic Doushantuo Formation of China (Shen et al., 2000).

Due to the rapid and systematic expulsion of pore waters and decrease of porosity during compaction, early diagenetic minerals in shales are preferentially preserved. For this reason, shales contain a rich record of geochemical processes that relate closely to the chemistry of the overlying ocean waters and paleoceanographic conditions. When studied systematically, the authigenic component of shales holds the promise to capture a snapshot of seawater and/or porewater chemistry. With the necessary

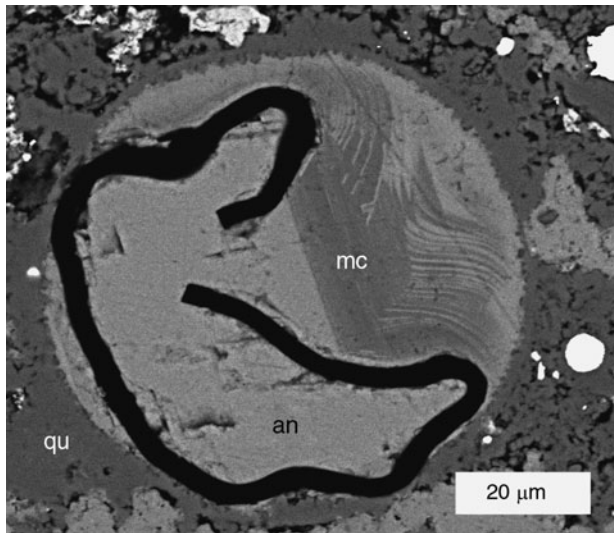


Shales, Figure 5 Pyrite framboid from bottom muds of the Santa Barbara Basin. Note regular arrangement of euhedral subcrystals. The sediment layer in which this framboid occurs is only a few years old. Pyrite formation is associated with the activity of anaerobic bacteria that utilize seawater sulfate (SO_4) as an oxygen source and generate hydrogen sulfide as a byproduct. They are among the most common and familiar of early diagenetic mineral formations in shales.

age constraints, this may enable us to develop a much more detailed record of ocean geochemical changes than currently available.

Conclusion

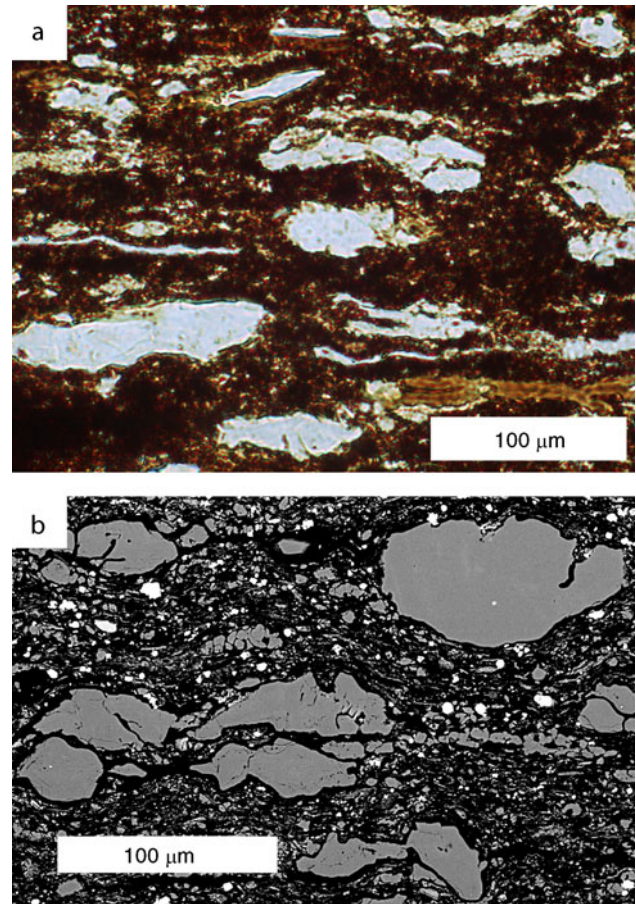
Shales result from the complex interplay of a range of geologic variables and processes, and there are no easy answers and no “one size fits all” models. Although these rocks have an (undeserved) reputation for being drab, uniform, and uninteresting, the repay the investigative challenges they pose by being a rich storehouse of information about the geologic past. Sedimentological study includes recognition and tracing of facies changes, sedimentary features, shale fabrics, erosion surfaces and internal stratigraphy, as well as information that can be derived from interbedded non-shale lithologies. Paleontological studies may provide data on paleo-oxygenation, primary production, substrate conditions, paleocurrents, bathymetry, and paleosalinity (Schieber et al., 1998). Petrographic investigations provide the basic inventory of shale constituents, and may yield clues about provenance, compaction history, chemical conditions, sedimentary processes (via small scale sedimentary structures), and the origin and maturation of organic matter (Taylor et al., 1998). Chemical analysis of major and trace elements can provide information on provenance (Roser and Korsch, 1986), and a range of proxies for paleo-oxygenation (Jones and Manning, 1994). Organic geochemistry can furnish information on the source of organic matter, its dispersal,



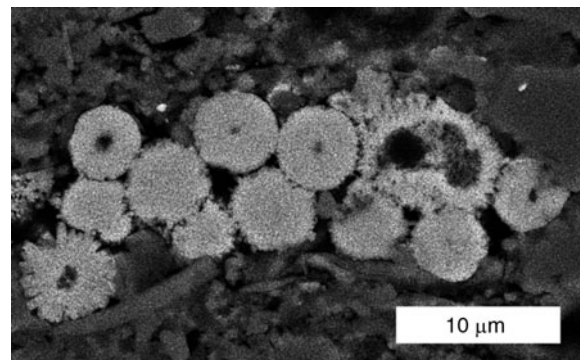
Shales, Figure 6 SEM photomicrograph (backscatter image) of a pore space in the Devonian New Albany Shale (Indiana, USA), illustrating suboxic to anoxic diagenesis. Light gray mineral **an** is ankerite (Fe-Mg-Ca-carbonate) that initially grew in the interior of a *Tasmanites* cyst (dark black line), a locally reducing and anoxic microenvironment. Very early growth is indicated by the uncompacted *Tasmanites* wall and the overall circular growth of carbonate (**an** + **mc**) cement. Outside of the protected microenvironment of the cyst we see growth of manganoan calcite (**mc**, darker gray), indicating suboxic conditions in the sediment and oxygenated bottom waters. (From Calvert et al., 1996.) The carbonate cement is surrounded by fine crystalline quartz (**qu**), derived from dissolution of radiolarian opal. (From Schieber et al., 2000). The image suggests that pore waters were initially alkaline, allowing for precipitation of carbonates and dissolution of biogenic opal, and that this was followed by a lowering of pH that led to precipitation of quartz. This image illustrates the level of insight one can derive from careful petrographic study of early diagenetic cements.

and maturation (Engel and Macko, 1993). Certain organic molecules, such as photosynthetic pigments, may survive as “carbon skeletons” (biomarkers), and may identify the original source of diagenetically altered organic matter and potential indicators of water column anoxia (Brassell, 1992).

Fundamentally, shales do not give up their secrets easily. The tools are at hand, however, to extract a wide range of data from them and allow for multiple avenues of inquiry (Schieber et al., 1998). Interpretations that rely on just one perspective (e.g., petrography, fabric study, trace element geochemistry, or organic geochemistry) typically do not match conclusions derived from a different line of inquiry. Shales need to be investigated in a multidisciplinary way and at multiple scales because of the complex interplay of variables that produces them. Conclusions derived from microscopic features must be in agreement with insights coming out of basin scale studies, as well as with findings from all scales in between.



Shales, Figure 7 Authigenic quartz (chalcedony) in the late Devonian Chattanooga Shale (Kentucky, USA). (a) Photomicrograph in transmitted light. The clear/bright grains are chalcedonic infills of algal cysts (*Tasmanites*). (b) SEM image (backscatter) from the same specimen, showing more clearly the rounded-lobate outlines of the quartz grains and the enclosing cyst walls (black rim). For a detailed discussion of these features see Schieber (1996) and Schieber et al. (2000).



Shales, Figure 8 SEM photomicrograph (backscatter image) of an example of early diagenetic phosphate precipitation. The image shows what appears to be a colony of phosphate encrusted bacteria (Mid-Proterozoic Bijaigarh Shale, India).

Bibliography

- Aller, R. C., 1998. Mobile deltaic and continental shelf muds as suboxic, fluidized bed reactors. *Marine Chemistry*, **61**, 143–155.
- Allison, P. A., 1988. The role of anoxia in the decay and mineralization of proteinaceous macrofossils. *Paleobiology*, **14**, 139–154.
- Bartels, C., Briggs, D. E. G., and Brassell, G., 1998. *The Fossils of the Hunsrück Slate - Marine Life in the Devonian*. Cambridge: Cambridge University Press, p. 309.
- Blatt, H., Middleton, G., and Murray, R., 1980. *Origin of Sedimentary Rocks*. Englewood Cliffs: Prentice Hall, 782 p.
- Brassell, S. C., 1992. Biomarkers in sediments, sedimentary rocks and petroleum. In Pratt, L. M., Brassell, S. C., and Comer, J. B. (eds.), *Geochemistry of Organic Matter in Sediments and Sedimentary Rocks*. SEPM Short Course Notes 27. Tulsa: Society for Sedimentary Geology, pp. 29–72.
- Brett, C., and Allison, P. A., 1998. Paleontological approaches to the environmental interpretation of marine mudrocks. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology, Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, pp. 301–349.
- Calvert, S. E., Bustin, R. M., and Ingall, E. D., 1996. Influence of water column anoxia and sediment supply on the burial and preservation of organic carbon in marine shales. *Geochimica et Cosmochimica Acta*, **60**, 1577–1593.
- Cole, R. D., and Picard, M. D., 1975. Primary and secondary sedimentary structures in oil shale and other fine-grained rocks, Green River Formation (Eocene), Utah and Colorado. *Utah Geology*, **2**, 49–67.
- Cuomo, M. C., and Bartholomew, P. R., 1991. Pelletal black shale fabrics: their origin and significance. In Tyson, R. V., and Pearson, T. H. (eds.), *Modern and Ancient Continental Shelf Anoxia*. London: Geological Society, Special Publication No. 58, pp. 221–232.
- Cuomo, M. C., and Rhoads, D. C., 1987. Biogenic sedimentary fabrics associated with pioneering polychaete assemblages: modern and ancient. *Journal of Sedimentary Petrology*, **57**, 537–543.
- Curtis, C. D., Cope, J. C. W., Plant, D., and Macquaker, J. H. S., 2000. "Instantaneous" sedimentation, early microbial sediment strengthening and a lengthy record of chemical diagenesis preserved in Lower Jurassic ammonitiferous concretions from Dorset. *Journal of the Geological Society, London*, **157**, 165–172.
- Dunbar, C. O., and Rodgers, J., 1957. *Principles of Stratigraphy*. New York: Wiley, 356 p.
- Engel, M. H., and Macko, S. A., 1993. *Organic Geochemistry: Principles and Applications*. New York: Plenum Press, 861 p.
- Fenchel, T., 1970. Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. *Limnology and Oceanography*, **15**, 14–20.
- Folk, R. L., 1965. *Petrology of Sedimentary Rocks*. Austin: Hemphill's Bookstore, 170 p.
- Ingram, R. L., 1953. Fissility in mudrocks. *Geological Society of America Bulletin*, **65**, 869–878.
- Jones, B., and Manning, D. A. C., 1994. Comparisons of geochemical indices used for the interpretation of paleoredox conditions in ancient mudstones. *Chemical Geology*, **111**, 111–129.
- Kauffman, E. G., 1978. Benthic environments and paleoecology of the Posidonienschiefer (Toarcian). *Neues Jahrbuch für Geologie und Paläontologie, Abhandlungen*, **157**, 18–36.
- Lewan, M. D., 1979. Laboratory classification of very fine-grained sedimentary rocks. *Geology*, **6**, 745–748.
- Lundegard, P. D., and Samuels, N. D., 1980. Field classification of fine-grained sedimentary rocks. *Journal of Sedimentary Petrology*, **50**, 781–786.
- Macquaker, J. H. S., and Adams, A. E., 2003. Maximizing information from fine-grained sedimentary rocks: an inclusive nomenclature for mudstones. *Journal of Sedimentary Research*, **73**, 735–744.
- Morris, S. C., 1998. *The Crucible of Creation: The Burgess Shale and the Rise of Animals*. Oxford: Oxford University Press, 276 p.
- Nanson, C. G., Rust, B. R., and Taylor, G., 1986. Coexistent mud braids and anastomosing channels in an arid-zone river: Cooper Creek, central Australia. *Geology*, **14**, 175–178.
- O'Brien, N. R., 1990. Significance of lamination in Toarcian (Lower Jurassic) shales from Yorkshire, Great Britain. *Sedimentary Geology*, **67**, 25–34.
- O'Brien, N. R., and Slatt, R. M., 1990. *Argillaceous Rock Atlas*. New York: Springer, 141 p.
- Picard, M. D., 1971. Classification of fine grained sedimentary rocks. *Journal of Sedimentary Petrology*, **41**, 179–195.
- Potter, P. E., Maynard, J. B., and Pryor, W. A., 1980. *Sedimentology of Shale*. New York: Springer, 303 p.
- Potter, P. E., Maynard, J. B., and Depetris, P. J., 2005. *Mud and Mudstones*. New York: Springer, 297 p.
- Pryor, W. A., 1975. Biogenic sedimentation and alteration of argillaceous sediments in shallow marine environments. *Geological Society of America Bulletin*, **86**, 1244–1254.
- Ransom, B., Bennett, R. H., Baerwald, R., Hulbert, M. H., and Burkett, P. J., 1999. In situ conditions and interactions between microbes and minerals in fine-grained marine sediments; a TEM microfabric perspective. *American Mineralogist*, **84**, 183–192.
- Reineck, H. E., and Singh, I. B., 1980. *Depositional Sedimentary Environments*. Berlin: Springer, 549 p.
- Roser, B. P., and Korsch, R. J., 1986. Determination of tectonic setting of sandstone-mudstone suites using SiO₂ content and K₂O/Na₂O ratio. *Journal of Geology*, **94**, 635–650.
- Rust, B. R., and Nanson, G. C., 1989. Bedload transport of mud as pedogenic aggregates in modern and ancient rivers. *Sedimentology*, **36**, 291–306.
- Schieber, 1985. *The Relationship between Basin Evolution and Genesis of stratiform Sulfide Horizons in Mid-Proterozoic Sediments of Central Montana (Belt Supergroup)*. PhD dissertation, University of Oregon, Eugene, 811 p.
- Schieber, J., 1986. The possible role of benthic microbial mats during the formation of carbonaceous shales in shallow Proterozoic basins. *Sedimentology*, **33**, 521–536.
- Schieber, J., 1989. Facies and origin of shales from the Mid-Proterozoic Newland Formation, Belt basin, Montana, U.S.A. *Sedimentology*, **36**, 203–219.
- Schieber, J., 1990a. Sedimentary structures, textures, and depositional settings of shales from the Lower Belt Supergroup, Mid-Proterozoic, Montana, U.S.A. In Bennett, R. H., Bryant, W. R., and M. H. Hulbert (eds.), *Microstructure of Fine-Grained Sediments*, New York, Springer, pp. 101–108.
- Schieber, J., 1990b. Significance of styles of epicontinental shale sedimentation in the Belt basin, Mid-Proterozoic of Montana, U.S.A. *Sedimentary Geology*, **69**, 297–312.
- Schieber, J., 1994. Reflection of deep vs shallow water deposition by small scale sedimentary features and microfabrics of the Chattanooga Shale in Tennessee. *Canadian Society of Petroleum Geologists, Memoir*, **17**, 773–784.
- Schieber, J., 1996. Early diagenetic silica deposition in algal cysts and spores: a source of sand in black shales? *Journal of Sedimentary Research*, **66**, 175–183.
- Schieber, J., 1999a. Distribution and deposition of mudstone facies in the Upper Devonian Sonyea Group of New York. *Journal of Sedimentary Research*, **69**, 909–925.
- Schieber, J., 1999b. Microbial mats in terrigenous clastics: the challenge of identification in the rock record. *Palaios*, **14**, 3–12.
- Schieber, J., 2003. Simple gifts and hidden treasures – implications of finding bioturbation and erosion surfaces in black shales. *The Sedimentary Record*, **1**, 4–8.
- Schieber, J., Krinsley, D., and Riciputi, L., 2000. Diagenetic origin of quartz silt in mudstones and implications for silica cycling. *Nature*, **406**, 981–985.

- Seilacher, A., Reif, W. E., Westphal, F., Riding, R., Clarkson, E. N. K., and Whittington, H. B., 1985. Sedimentological, Ecological and Temporal Patterns of Fossil Lagerstätten [and Discussion]. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, Vol. **311**, No. 1148, Extraordinary Fossil Biotas: Their Ecological and Evolutionary Significance (Oct. 17, 1985), 5–24.
- Schieber, J., and Zimmerle, W., 1998. The history and promise of shale research. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology, Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, pp. 1–10.
- Schieber, J., Zimmerle, W., and Sethi, P. (eds.), 1998. *Shales and Mudstones*. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vols. 1 and 2.
- Schieber, J., Southard, J. B., and Thaisen, K. G., 2007. Accretion of mudstone beds from migrating floccule ripples. *Science*, **318**, 1760–1763.
- Shen, Y., Schidlowski, M., and Chu, X. L., 2000. Biogeochemical approach to understanding phosphogenic events of the terminal Proterozoic to Cambrian. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **158**, 99–108.
- Stow, D. A. V., and Piper, D. J. W., 1984. Deep-water fine-grained sediments: facies models. In Stow, D. A. V., and Piper, D. J. W. (eds.), *Fine-Grained Sediments: Deep-Water Processes and Facies*. London: Geological Society, Special Publication No. 15, 611–645.
- Taylor, G. H., Teichmüller, M., Davis, A., Diessel, C. F. K., Littke, R., and Robert, P., 1998. *Organic Petrology*. Stuttgart: Borntraeger, 704 p.
- Wetzel, A., and Uchmann, A., 1998. Biogenic sedimentary structures in mudstones – an overview. In Schieber, J., Zimmerle, W., and Sethi, P. (eds.), *Shales and Mudstones*. Basin Studies, Sedimentology and Paleontology. Stuttgart: Schweizerbart'sche Verlagsbuchhandlung, Vol. 1, p. 351–369.
- Wuttke, M., 1983. 'Weichteil-Erhaltung' durch lithifizierte Mikroorganismen bei mittel-eozänen Vertebraten aus en Ölschiefern der 'Grube Messel' bei Darmstadt. *Senckenbergiana Lethaea*, **64**, 509–527.

Cross-references

Biofilms and Fossilization
 Black Shales
 Iron Sulfide Formation
 Mat-Related Sedimentary Structures

SHEWANELLA

Nadia-Valérie Quéric
 University of Göttingen, Göttingen, Germany

Classification

Bacteria – Proteobacteria – Gammaproteobacteria – Alteromonadales – Shewanellaceae (Ivanova et al., 2004), fam. nov. – *Shewanella* (MacDonell and Colwell, 1985), gen. nov.

Definition

Assumed as one of the first metabolic processes of ancient microbial life (prior to oxygen, nitrate, and sulfate respiration), microbial iron reduction is still of particular interest

in recent geobiological systems. The efficiency of iron reducing microorganisms in utilizing structural or crystalline solid-phase Fe^{3+} oxide minerals as electron acceptors with respect to nutrient availability and pH is still matter of discussion; this type of metabolism, however, is an ubiquitous phenomenon, applied by a wide range of species throughout the Archaea and Bacteria (Weber et al., 2006 and references therein). Members of the family *Geobacteraceae* and the genus *Shewanella* are among the most significant and common studied organisms with regard to dissimilatory iron reduction and oxidation of organic matter in soils, sediments, and groundwater.

According to the surface complexation model (Dzombak and Morel, 1990), Warren and Ferris (1998) report on an equilibrium between ferric iron sorption and precipitation reactions on bacterial surfaces, emphasizing iron-reducing bacteria like *Shewanella* as highly reactive key players in pristine and contaminated waters (Pedersen and Albinsson, 1991).

Actually including 50 recognized species (List of prokaryotic names with standing in nomenclature: <http://www.bacterio.cict.fr/>), the genus *Shewanella* features Gram-negative, facultatively anaerobic, dissimilatory metal-reducing, rod-shaped organisms. Due to their highly diverse physiology, they inhabit a wide range of redox-stratified environments varying in nutrient composition, salinity, temperature, redox potential, and barometric pressure (marine and freshwater, surface, ground and deep waters, sediment, mud and soils), but also comprise symbiotic, epibiotic, and pathogenic species (Venkateswaran et al., 1998; Hau and Gralnick, 2007 and references therein). Under anaerobic conditions, most *Shewanella* species can produce hydrogen sulfide when grown on thiosulfate or polysulfide, and also can grow on lactate, pyruvate, formate, and on amino acids (Nealson and Scott, 2006 and references therein). For respiratory growth, they can use oxygen as well as a variety of different terminal electron acceptors, including nitrate, elemental sulfur, fumarate, dimethyl sulfoxide, and metals like Mn^{4+} , Fe^{3+} , As^{5+} , Cr^{6+} , Co^{3+} , Tc^{7+} , and U^{6+} (Nealson and Myers, 1992; Haas et al., 2001; Guha et al., 2003; Saltikov et al., 2003). As diffusion across the cell wall is not possible for metals in their solid form, metal-reducing *Shewanella* species specifically interact with the mineral surface to facilitate the electron transfer process by active cell adhesion in combination with a unique shuttle-system of proteins reaching from the cytoplasm to the outer membrane (Lower, 2001; Korenevsky and Beveridge, 2007; Fredrickson et al., 2008 and references therein).

Near-surface habitats may get limited in oxygen but not in minerals like iron oxyhydroxides (ferrihydrite, goethite, and hematite), in large part consisting of Fe^{3+} (Urrutia et al., 1998). In case of dissimilatory metal-reducing bacteria like *Shewanella*, Fe^{3+} is reductively dissolved during oxidation of carbon substrates, affecting processes like the biogeochemical cycle of iron and phosphorus, the oxidation of natural and anthropogenic carbon sources, and

biocorrosion. The respiratory reduction of metals turns these organisms to key players in groundwater ecology, as they contribute to the mobilization of occasionally harmful metals (e.g., arsenic) from the solid to the soluble phase (Malasarn et al., 2008).

Bibliography

- Dzombak, D. A., and Morel, F. M. M., 1990. *Surface Complex Modelling: Hydrous Ferric Oxide*. New York: Wiley Interscience.
- Fredrickson, J. K., Romine, M. F., Beliaev, A. S., Auchtung, J. M., Driscoll, M. E., Gardner, T. S., Nealson, K. H., Osterman, A. L., Pinchuk, G., Reed, J. L., Rodionov, D. A., Rodrigues, J. L. M., Saffarini, D. A., Serres, M. H., Spormann, A. M., Zhulin, I. B., and Tiedje, J. M., 2008. Towards environmental systems biology of *Shewanella*. *Nature Reviews Microbiology*, **6**(8), 592–603.
- Guha, H., Jayachandran, K., and Maurrasse, F., 2003. Microbiological reduction of chromium(VI) in presence of pyrolusite-coated sand by *Shewanella alga* Simidu ATCC 55627 in laboratory column experiments. *Chemosphere*, **52**, 175–183.
- Haas, J. R., Dichristina, T. J., and Wade, R., 2001. Thermodynamics of U(VI) sorption onto *Shewanella putrefaciens*. *Chemical Geology*, **180**(1–4), 33–54.
- Hau, H. H., and Gralnick, J. A., 2007. Ecology and biotechnology of the genus *Shewanella*. *Annual Review of Microbiology*, **61**(1), 237–258.
- Ivanova, E. P., Flavier, S., and Christen, R., 2004. Phylogenetic relationships among marine *Alteromonas*-like proteobacteria: emended description of the family *Alteromonadaceae* and proposal of *Pseudoalteromonadaceae* fam. nov., *Colwelliaceae* fam. nov., *Shewanellaceae* fam. nov., *Moritellaceae* fam. nov., *Ferrimonadaceae* fam. nov., *Idiomarinaceae* fam. nov. and *Psychromonadaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, **54**(5), 1773–1788.
- Korenevsky, A., and Beveridge, T. J., 2007. The surface physicochemistry and adhesiveness of *Shewanella* are affected by their surface polysaccharides. *Microbiology*, **153**(6), 1872–1883.
- Lower, S. K., Hochella, M. F., and Beveridge, T. J., 2001. Bacterial recognition of mineral surfaces: nanoscale interactions between *Shewanella* and alpha-FeOOH. *Science*, **292**(5520), 1360–1363.
- MacDonell, M. T., and Colwell, R. R., 1985. Phylogeny of the *Vibrionaceae* and recommendation for two new genera, *Listonella* and *Shewanella*. *Systematics and Applied Microbiology*, **6**, 171–182.
- Malasarn, D., Keeffe, J. R., and Newman, D. K., 2008. Characterization of the arsenate respiratory reductase from *Shewanella* sp. Strain ANA-3. *Journal of Bacteriology*, **190**(1), 135–142.
- Nealson, K. H., and Myers, C. R., 1992. Microbial reduction of manganese and iron: new approaches to carbon cycling. *Applied and Environmental Microbiology*, **58**, 439–443.
- Nealson, K. H., and Scott, J. H., 2006. Ecophysiology of the genus *Shewanella*. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.). *The Prokaryotes: A Handbook on the Biology of Bacteria*, Proteobacteria: Gamma Subclass. New York: Springer-Verlag, Vol. 6, pp. 1133–1151.
- Pedersen, K., and Albinsson, Y., 1991. Effect of cell number, pH and lanthanide concentration on the sorption of promethium by *Shewanella putrefaciens*. *Radiochimica Acta*, **54**, 91–95.
- Saltikov, C. W., Cifuentes, A., Venkateswaran, K., and Newman, D. K., 2003. The ars detoxification system is advantageous but not required for As(V) respiration by the genetically tractable *Shewanella* species strain ANA-3. *Applied and Environmental Microbiology*, **69**, 2800–2809.
- Urrutia, M. M., Roden, E. E., Fredrickson, J. K., and Zachara, J. M., 1998. Microbial and surface chemistry controls on reduction of

synthetic Fe(III) oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga*. *Geomicrobiology Journal*, **15**(4), 269–291.

Venkateswaran, K., Dollhopf, M. E., Aller, R., Stackebrandt, E., and Nealson, K. H., 1998. *Shewanella amazonensis* sp. nov., a novel metal-reducing facultative anaerobe from Amazonian shelf muds. *International Journal of Systematic Bacteriology*, **48**(3), 965–972.

Warren, L. A., and Ferris, F. G., 1998. Continuum between sorption and precipitation of Fe(III) on microbial surfaces. *Environmental Science and Technology*, **32**, 2331–2337.

Weber, K. A., Achenbach, L. A., and Coates, J. D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. *Nature Reviews Microbiology*, **4**(10), 752–764.

Cross-references

[Banded Iron Formations](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Microbial Biomineralization](#)
[Microbial-Metal Binding](#)

SIDERITE

Volker Thiel
 University of Göttingen, Göttingen, Germany

Siderite is an iron carbonate mineral (FeCO_3 , 48% Fe) belonging to the calcite group (hexagonal crystal system). Magnesium and manganese may substitute for the iron. Siderite crystals are yellow to dark brown in color, rhombohedral in shape, and with curved and striated faces, and also occur in masses. Siderite is commonly found in bedded sedimentary iron ores, including banded iron formations (BIFs, see entry [Banded Iron Formations](#)), and in hydrothermal veins. It is also a common diagenetic mineral, forming at shallow burial depths in shales and sandstones where it tends to form concretions. The diagenetic formation of siderite strongly depends on the availability of Fe^{2+} ions in pore waters (see entry [Pore Waters](#)) and is thus a phenomenon of suboxic to anoxic environments (Berner, 1971). Diagenetic siderite precipitation has been linked to microbially mediated reactions, specifically to the release of ferrous (Fe^{2+}) iron due to dissimilatory iron reduction performed by Fe^{3+} -reducing prokaryotes (see entry [Fe\(III\)-Reducing Prokaryotes](#)) such as *Shewanella* (Roh et al., 2003; Hansel et al., 2003; see entry [Shewanella](#)). Microbially assisted siderite precipitation appears to preferentially occur in environments where the rate of bacterial iron reduction exceeds the rate of sulfate reduction performed by sulfate-reducing bacteria (Pye et al., 2006; see entry [Sulfate-Reducing Bacteria](#)). In sulfidogenic sediments, iron is largely precipitated as iron sulfide rather than as siderite (see entry [Iron Sulfide Formation](#)).

Bibliography

- Berner, R. A., 1971. *Principles of Chemical Sedimentology*. New York: McGraw-Hill.

- Hansel, C. M., Benner, S. G., Neiss, J., Dohnalkova, A., Kukkadapu, R. K., and Fendorf, S., 2003. Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. *Geochimica et Cosmochimica Acta*, **67**, 2977–2992.
- Pye, K., Dicksin, J. A. D., Schiavon, N., Coleman, M. L., and Cox, M., 2006. Formation of siderite–Mg–calcite–iron sulphide concretions in intertidal marsh and sandflat sediments, north Norfolk, England. *Sedimentology*, **37**, 325–343.
- Roh, Y., Zhang, C. L., Vali, H., Lauf, R. J., Zhou, J., and Phelps, T. J., 2003. Biogeochemical and environmental factors in Fe biomineralization: magnetite and siderite formation. *Clays and Clay Minerals*, **51**, 83–95.

Cross-references

Banded Iron Formations
Carbonates
Fe(III)-Reducing Prokaryotes
Iron Sulfide Formation
Pore Waters
Shewanella
Sulfate-Reducing Bacteria

SIDEROPHORES

Stephan M. Kraemer
University of Vienna, Vienna, Austria

Definition

Siderophores are low molecular weight iron-specific organic ligands that are exuded by iron-limited organisms as part of a high-affinity iron acquisition strategy.

Introduction

Iron is a nutrient to almost all known organisms. Even though iron is the fourth most abundant element on earth, the acquisition of this nutrient poses a serious challenge to organisms in many natural environments. A particularly iron-depleted system is the photic zone of the marine water column (Kraemer et al., 2005). Here, soluble iron is removed by biological uptake and subsequent sinking of the biomass below the mixed zone. In ocean areas with particularly low iron concentrations relative to other nutrients in the photic zone, the primary productivity is limited by the low bioavailability of iron. This marine iron limitation may affect the global climate by limiting the efficiency of the marine carbon pump. However, even in soils that contain abundant iron-bearing mineral phases, iron acquisition poses a challenge to microbial and plant life. Indeed, the low solubility and dissolution rates of iron-bearing minerals in calcareous soils are responsible for large annual agricultural losses by plant iron limitation (iron chlorosis) (Kraemer et al., 2006).

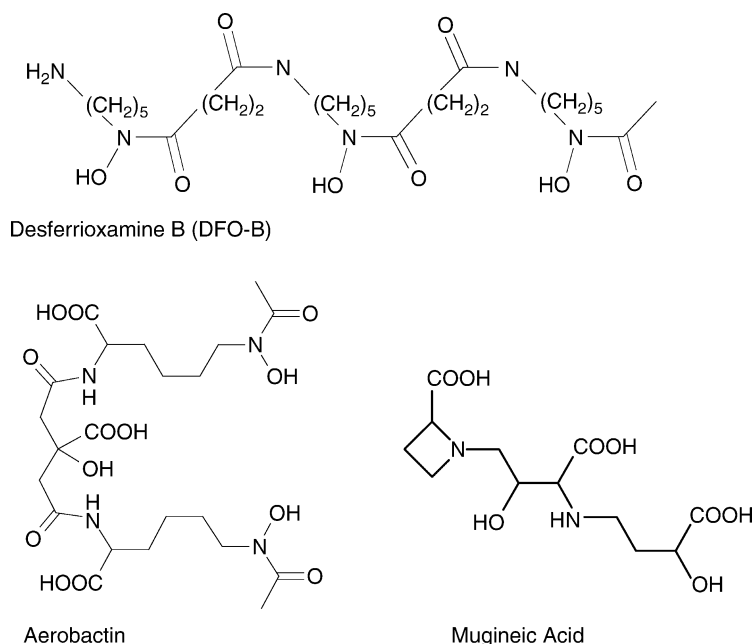
The ability of organisms to scavenge iron from their surroundings is prerequisite to controlling intracellular iron concentrations within the physiologically required range (homeostasis). An efficient mechanism of iron

uptake involves the exudation of siderophores that are designed to bind iron(III) in the extracellular space with a sufficient affinity and specificity to ensure the formation of Fe(III)-siderophore complexes even in the presence of high concentrations of other competing cations and in the appropriate pH range. This high-affinity iron uptake strategy is used by bacteria (including cyanobacteria) in marine, freshwater, and terrestrial environments, by fungi and some plant species. Other iron acquisition mechanisms include the acidification of the environment, the reduction of Fe(III) coupled to Fe(II) uptake, or the secretion of other ligands (e.g., citrate) with lower affinity for iron. High-affinity iron uptake involving siderophores is generally the most efficient system in environments with extremely low iron availability.

Siderophore structure and properties

Siderophores are not defined by their chemical structure, but by their biological function. Several hundreds of distinct and structurally diverse siderophores are known. Siderophores are low molecular mass (<1,500 Dalton) organic ligands, typically chelating iron in hexadentate octahedral coordination, although siderophores with lower denticity are known. Iron is coordinated by hard ligating groups including hydroxamate, catecholate, carboxylate, and hydroxycarboxylate groups (Figure 1). The nature of the ligating groups and the optimization of the overall siderophore structure are responsible for their outstanding affinity for iron. For example, desferrioxamine B (DFO-B), a siderophore produced by the soil bacterium *Streptomyces pilosus* forms an iron complex with a 1:1 stability constant logK of 32.02 (Martell et al., 2001). The affinity of microbial siderophores for iron binding is much higher than for competing ions that are often found in high concentrations in the extracellular environment. For example, DFO-B does not, to measurable extent, coordinate with calcium in seawater or in pore water of calcareous soils (Kraemer et al., 2005, 2006). Most siderophores excreted by terrestrial organisms are highly water soluble, allowing the diffusion of siderophores toward insoluble iron sources. This is prerequisite to their function as an iron shuttle overcoming the necessity for the organisms to directly contact the iron source (Kraemer et al., 2006). However, amphiphilic siderophores possessing fatty acid tails have been isolated from cultures of distinct genera of marine bacteria (Martinez and Butler, 2007) and pathogenic bacteria. The inhibition of ligand diffusion away from individual bacteria by anchoring of amphiphilic siderophores in the plasma membrane seems to be advantageous in marine systems where bacterial abundances are low.

Graminaceous plant species, including agriculturally important crops, such as barley, wheat, and corn, are the only plant species known to possess a high-affinity iron uptake system. They exude a distinct class of iron-specific ligands with aminocarboxylate and hydroxycarboxylate functional groups, the so-called phytosiderophores or



Siderophores, Figure 1 Chemical structures of the phytosiderophore mugineic acid and of microbial siderophores desferrioxamine B (DFO-B) and aerobactin.

mugineic acids (Takagi, 1976; Takagi et al., 1984) (Figure 1).

Uptake of Fe(III)-siderophore complexes

Gram-negative bacteria bind siderophore complexes by a receptor in the outer membrane followed by shuttling to the cytoplasmic membrane. Gram-positive bacteria have a receptor anchored to the cytoplasmic membrane. The complexes are then delivered to the cytoplasm by a transmembrane protein (ABC-transporter) (Andrews et al., 2003). Similarly, graminaceous plants possess a high-affinity uptake system specific for iron(III)-phytosiderophore complexes (Curie et al., 2001). Some microorganisms are known to be endowed with uptake systems for iron complexes of siderophores that are not produced by the same organism.

The intracellular release of iron from internalized siderophores is promoted by either hydrolysis of the siderophore or reduction of ferric iron to ferrous iron followed by competitive sequestration of the iron (Miethke and Marahiel, 2007). The biosynthesis and exudation of siderophores as well as the expression of their uptake systems are highly regulated as a response to iron limitation (Hantke, 2001).

Siderophore concentrations in natural environments

The exudation of siderophores is a costly strategy in terms of cellular nutrient and energy budgets, particularly in systems with low bacterial densities such as oligotrophic marine systems (Völker and Wolf-Gladrow, 1999).

Therefore, it is not surprising that observed siderophore concentration in the environment do not exceed iron concentrations required for efficient uptake. Powell et al. (1980) have estimated hydroxamate siderophore concentrations in soil solutions to be between 10 and 100 nM. Essen et al. (2006) found up to 2 and 12 nM of the hydroxamate siderophores ferrichrome and ferricrocin, respectively, in soil solutions of podzolic forest soils. However, the quantitative analysis of siderophores in natural waters remains challenging due to their structural diversity and low concentrations. For example, siderophores have not been detected unambiguously in ocean water even though iron complexes with conditional stability constants comparable to siderophore complexes in the nanomolar and subnanomolar concentration range have been observed by voltammetric techniques (Rue and Bruland, 1995). Römheld (1991) has estimated that phytosiderophore concentrations in the rhizosphere can reach local concentrations of up to 1 mM in the soil solution.

Sources of iron

The soluble iron concentrations in oxic soils or surface waters are limited by the solubility of iron oxides, iron hydroxides, or hydrous ferric oxide. In the neutral to slightly alkaline pH range corresponding to the pH of calcareous soil solutions and ocean water, their solubility reaches a minimum below 1 nM (Liu and Millero, 2002). The presence of iron complexing organic ligands can increase the iron solubility. Thus, iron complexes with low molecular weight organic ligands, fulvic and humic acids, as well as iron-bearing mineral phases (including

primary silicates and pedogenic iron oxides) are the most important iron sources for high-affinity uptake systems (Kraemer, 2004). Prerequisite for iron sequestration from these sources are the superior affinity of siderophores for iron compared to the stability of the iron complexes or minerals that serve as iron source and reasonably fast rates of the associated ligand exchange or dissolution reactions.

Siderophore promoted dissolution of iron oxides and iron-bearing silicates

Siderophores promote iron sequestration from iron oxides (Kraemer et al., 1999) and iron-bearing primary (e.g., hornblende [Liermann et al., 2000]) and secondary silicates [e.g., kaolinite (Rosenberg and Maurice, 2003)]. The effect of siderophores on iron dissolution rests on their ability to increase the solubility of iron and to accelerate dissolution rates. It has been shown that bacterial and plant siderophores promote iron oxide dissolution in the dark by a ligand-controlled dissolution mechanism, where the rate of dissolution is a linear function of the adsorbed siderophore concentration (Kraemer et al., 1999; Reichard et al., 2005). This mechanism is important in high-affinity iron acquisition by graminaceous plants. Considering the low concentrations of microbial siderophores observed in natural systems, their main function seems to be to increase solubility of iron oxides, generating thermodynamic driving force for other dissolution mechanisms (Cheah et al., 2003; Reichard et al., 2007).

Photochemistry of siderophore complexes

Soluble iron complexes of siderophores possessing α -hydroxycarboxylate ligating groups photolyze in seawater under irradiation by sunlight, resulting in oxidation of the ligand and formation of transient Fe(II) that is rapidly reoxidized (Barbeau, 2006). Both the Fe(II) and iron bound to the oxidized ligand are bioavailable to phototrophic phytoplankton that cannot take up Fe(III)-siderophore complexes. Moreover, siderophores promote photoreductive dissolution processes that tend to be faster than ligand-promoted dissolution reactions (Borer et al., 2005). These photochemical processes may be of key importance for algal and bacterial iron acquisition in marine systems.

Formation of siderophore complexes with other metals and their uptake

A prerequisite for the efficiency of the high-affinity iron uptake system is the ability of organisms to discriminate against uptake of other (potentially deleterious) metal ions. The selectivity of the uptake system is ensured by the high specificity of siderophores for iron binding relative to most cations in solution and by the specific recognition of iron(III)-siderophore complexes by receptors at the cell surfaces. However, some exceptions to the selectivity of iron binding and uptake are known. Siderophores have a high affinity for metal ions with an equal or higher charge to radius ratio than Fe(III). For example, the

siderophore DFO-B forms a Pu(IV) complex with a 1:1 stability constant that is orders of magnitude higher than the corresponding iron complex (Boukhalfa et al., 2007). Moreover, slow uptake of Pu(IV)-siderophore complexes by the high-affinity iron uptake system of *Microbacterium flavescens* has been observed (John et al., 2001). The formation of plutonium-siderophore complexes and their bacterial uptake illustrate that the selectivity for a metal ion other than iron does not necessarily imply that the metal ion is actively targeted by the high-affinity uptake system for the purpose of nutrition. Also, the siderophore DFO-B has a high selectivity for the trivalent manganese ion (Duckworth and Sposito, 2005). Manganese in this redox state is not commonly observed in the absence of the siderophores, but the shifted redox potential of the complex stabilizes Mn(III) under conditions that are found in natural systems. In order to appreciate the effect that siderophores may have on the geochemistry of metal ions other than iron, it is important to keep in mind the extremely low siderophore concentrations measured in natural systems (vide supra).

Uptake of phytosiderophore complexes of Fe(II), Ni(II), Zn(II), Cu(II), Mn(II), and Cd(II) by maize (*Zea mays*) has been observed (Schaaf et al., 2004), and it has been controversially discussed if their uptake by the high-affinity iron uptake system is regulated or fortuitous. A high-affinity uptake system also seems to promote the uptake of molybdenum and vanadium by the nitrogen fixing soil bacterium *Azotobacter vinelandii* (Bellenger et al., 2008). Methanotrophic bacteria use a high-affinity uptake system for copper acquisition involving the exudation of Cu(I) specific organic ligands, the so-called chalcophores. Chalcophores have a distinctly different architecture compared to siderophores involving nitrogen and sulfur as ligands (Kim et al., 2004).

Conclusions

The key role of siderophores in marine and terrestrial biological iron acquisition is well established. However, a thorough and quantitative understanding of their effect on processes involved in, e.g., global carbon cycling or crop production requires a better understanding of how siderophores influence the availability and distribution of iron among prokaryotic and eukaryotic organisms in complex ecosystems. New observations of siderophore photochemistry, their amphiphilic properties and their mineral surface chemistry as well as observations of iron speciation in natural systems are important steps in this direction. Furthermore, recent work has shown that nutrient acquisition involving the exudation of ion-specific ligands is not unique to high-affinity iron acquisition. Discoveries of other similar nutrient acquisition systems may reshape our thinking about the effect of trace metal biogeochemistry on the microbial ecology of oligotrophic natural systems and vice versa. Finally, the unique properties of siderophores and other ion-specific biogenic ligands continue to inspire technical applications in fields such as pollutant remediation or biomedicine.

Bibliography

- Andrews, S. C., Robinson, A. K., and Rodriguez-Quinones, F., 2003. Bacterial iron homeostasis. *FEMS Microbiology Ecology*, **27**, 215–237.
- Barbeau, K., 2006. Photochemistry of organic iron(III) complexing ligands in oceanic systems. *Photochemistry and Photobiology*, **82**, 1505–1516.
- Bellenger, J. P., Wichard, T., Kustka, A. B., and Kraepiel, A. M. L., 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience*, **1**, 243–246.
- Borer, P., Sulzberger, B., Reichard, P. U., and Kraemer, S. M., 2005. Effect of siderophores on the light-induced dissolution of colloidal iron(III)(hydr)oxides. *Marine Chemistry*, **93**, 179–193.
- Boukhalfa, H., Reilly, S. D., and Neu, M. P., 2007. Complexation of Pu(IV) with the natural siderophore Desferrioxamine B and the redox properties of Pu(IV)(siderophore) complexes. *Inorganic Chemistry*, **46**, 1018–1026.
- Cheah, S. F., Kraemer, S. M., Cervini-Silva, J., and Sposito, G., 2003. Steady-state dissolution kinetics of goethite in the presence of desferrioxamine B and oxalate ligands: implications for the microbial acquisition of iron. *Chemical Geology*, **198**, 63–75.
- Curie, C., Panaviene, Z., Loulergue, C., Dellaporta, S. L., Briat, J. -F., and Walker, E. L., 2001. Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. *Nature*, **409**, 346–349.
- Duckworth, O. W., and Sposito, G., 2005. Siderophore-manganese (III) interactions. I. Air-oxidation of manganese(II) promoted by desferrioxamine B. *Environmental Science Technology*, **39**, 6037–6044.
- Essen, S. A., Bylund, D., Holmstrom, S. J. M., Moberg, M., and Lundstrom, U. S., 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *Biometals*, **19**, 269–282.
- Hantke, K., 2001. Iron and metal regulation in bacteria. *Current Opinion in Microbiology*, **4**, 172–177.
- John, S. G., Ruggiero, C. E., Hersman, L. E., Tung, C. S., and Neu, M. P., 2001. Siderophore mediated plutonium accumulation by *Microbacterium flavescens* (JG-9). *Environmental Science Technology*, **35**, 2942–2948.
- Kim, H. J., Graham, D. W., DiSpirito, A. A., Alterman, M. A., Galeva, N., Larive, C. K., Asunskis, D., and Sherwood, P. M. A., 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science*, **305**, 1612–1615.
- Kraemer, S. M., 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science*, **66**, 3–18.
- Kraemer, S. M., Cheah, S. F., Zapf, R., Xu, J. D., Raymond, K. N., and Sposito, G., 1999. Effect of hydroxamate siderophores on Fe release and Pb(II) adsorption by goethite. *Geochimica et Cosmochimica Acta*, **63**, 3003–3008.
- Kraemer, S. M., Butler, A., Borer, P., and Cervini-Silva, J., 2005. Siderophores and the dissolution of iron bearing minerals in marine systems. *Reviews in Mineralogy and Geochemistry*, **59**, 53–76.
- Kraemer, S. M., Crowley, D., and Kretzschmar, R., 2006. Siderophores in plant iron acquisition: geochemical aspects. *Advances in Agronomy*, **91**, 1–46.
- Liermann, L. J., Kalinowski, B. E., Brantley, S. L., and Ferry, J. G., 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta*, **64**, 587–602.
- Liu, X. W., and Millero, F. J., 2002. The solubility of iron in seawater. *Marine Chemistry*, **77**, 43–54.
- Martell, A. E., Smith, R. M., and Motekaitis, R. J., 2001. NIST critically selected stability constants of metal complexes database. Gaithersburg MD: NIST. http://www.nist.gov/srd/WebGuide/Critical/46_8.htm.
- Martinez, J. S., and Butler, A., 2007. Marine amphiphilic siderophores: marinobactin structure, uptake, and microbial partitioning. *Journal of Inorganic Biochemistry*, **101**, 1692–1698.
- Miethke, M., and Marahiel, M. A., 2007. Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews*, **71**, 413–451.
- Powell, P. E., Cline, G. R., Reid, C. P. P., and Szaniszlo, P. J., 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature*, **287**, 833–834.
- Reichard, P. U., Kraemer, S. M., Frazier, S. W., and Kretzschmar, R., 2005. Goethite dissolution in the presence of phytosiderophores: rates, mechanisms, and the synergistic effect of oxalate. *Plant and Soil*, **276**, 115–132.
- Reichard, P. U., Kretzschmar, R., and Kraemer, S. M., 2007. Dissolution mechanisms of goethite in the presence of siderophores and organic acids. *Geochimica et Cosmochimica Acta*, **71**, 5635–5650.
- Römheld, V., 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in gramineous species – an ecological approach. *Plant Soil*, **130**, 127–134.
- Rosenberg, D. R., and Maurice, P. A., 2003. Siderophore adsorption to and dissolution of kaolinite at pH 3 to 7 and 22 degrees C. *Geochimica et Cosmochimica Acta*, **67**, 223–229.
- Rue, E. L., and Bruland, K. W., 1995. Complexation of iron(III) by natural organic-ligands in the central North Pacific as determined by a new competitive ligand equilibration adsorptive cathodic stripping voltammetric method. *Marine Chemistry*, **50**, 117–138.
- Schaaf, G., Erenoglu, B., and von Wiren, N., 2004. Physiological and biochemical characterization of metal-phytosiderophore transport in graminaceous species. *Soil Science and Plant Nutrition*, **50**, 989–995.
- Takagi, S. I., 1976. Naturally occurring iron-chelating compounds in oat-root and rice-root washings. I. Activity measurement and preliminary characterization. *Soil Science and Plant Nutrition*, **22**, 423–433.
- Takagi, S., Nomoto, K., and Takemoto, T., 1984. Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *Journal Of Plant Nutrition*, **7**, 469–477.
- Völker, C., and Wolf-Gladrow, D. A., 1999. Physical limits on iron uptake mediated by siderophores or surface reductases. *Marine Chemistry*, **65**, 227–244.

Cross-references

Gallionella
 Leptothrix
 Metals, Acquisition by Marine Bacteria
 Microbial Biomineralization
 Microbial-Metal Binding

SILICA BIOMINERALIZATION, SPONGES

Hermann Ehrlich

Dresden University of Technology, Dresden, Germany

Synonyms

Biosilicification, Silication

Definition

Biosilicification: The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges,

molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants.

Biom mineralization and biosilicification

Introduction

The growth in geobiology and biogeochemistry has led to a number of new existing research areas where the distinctions between the biological, chemical, and earth science disciplines melt away (Weiner and Dove, 2003). Of the intriguing topics that are receiving renewed attention, the study of biomineral formation based on organic templates is one of the most fascinating topics today. During the processes of biomineralization, the organic material acts variously as nucleator, cooperative modifier, and matrix or mold for the mineral ions with respect to biominerals formation which are characterized by a hierarchical order (Bäuerlein, 2007).

Biominerals may be deposited within the organism, and within its immediate surroundings or environment, as a result of the metabolism of the living creature (Skinner, 2005). The physiological pathways by which organisms precipitate skeletal minerals and the forms and functions of the skeletons they fashion have been shaped by natural selection through geologic time, and all have constrained continuing evolution in skeleton forming clades (Knoll, 2003). The variety of biomineralizers recently reviewed by Ehrlich et al. (2008a) can best be expressed by the fact that, approximately 128,000 species of molluscs, about 800 species of corals, 15,000 species of sponges including 550 species of glass sponges, 700 species of calcareous green, red and brown algae, more than 300 species of deep-sea benthic foraminifera, and 200,000 diatom species (Mann and Droop, 1996) exist.

Precipitation of silica on organic templates could have been abiotic, in which organic matter merely serves as a template for nucleation, or biogenic, i.e., induced by biological activities or a combination of both (Mukhopadhyay et al., 2004). Silicon, in the form of silicic acid, is a fundamental nutrient for diatoms, silicoflagellates, radiolaria, and many sponges, all of which polymerize it to build skeletons of biogenic silica (Maldonado et al., 2005).

Sponges

Sponges (Porifera) are the most simple and ancient multicellular animals on earth and live attached to the seabed or another substratum. Sponges diverged from other animals earlier in evolutionary history than any other known animal group, extant or extinct (Reitner and Mehl, 1996), with the first sponge-related record in earth history found in 1.8 billion year old sediments (Nichols and Wörheide, 2005). The huge diversity with respect to their natural habitat is probably the reason for the estimated number of approximately 15,000 different sponge species (Hooper and van Soest, 2002). To support life, sponges pump huge amounts of seawater (170–72,000 × their own body volume per day) through their bodies (Simpson,

1984) and filtrate it to capture food particles, such as bacteria, micro algae, other unicellular organisms, or dead organic particles. The whole sponge body is designed for efficient filtration of the surrounding seawater, which is essential because of the low nutrient availability at the sea floor.

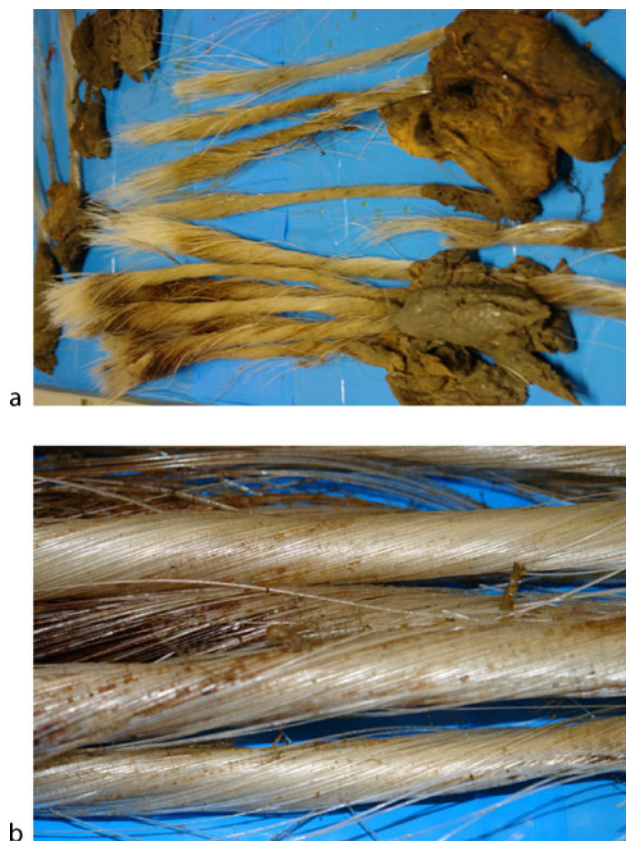
The phylum Porifera (sponges) is divided into three classes, Hexactinellida and Demospongiae that comprise a siliceous skeleton and the Calcarea with a calcareous skeletal network (Bergquist, 1978). Significant separation of the Calcarea from the Hexactinellida/Demospongiae (Silicispongea) clade is also implied by fundamental differences in morphology and development: unlike Silicispongea, calcareans lack morphologically distinct microscleres, and their calcitic spicules are secreted intercellularly within an organic sheath (vs. formation of silica spicules onto an intracellularly secreted axial organic filament in Silicispongea (Botting and Butterfield, 2005)). Sponges bear only a few different cell types of which the sclerocytes produce the spicules, i.e., siliceous structures, which are often needle-shaped. These spicules often form a distinct skeleton, but occasionally they are loosely distributed throughout the sponge body without identifiable order or they are lacking entirely. Size, type, shape, combination of spicules, and their skeletal arrangements are the fundamentals of current sponge systematics (Erpenbeck et al., 2006; Boury-Esnault and Rutzler, 1997).

Among the siliceous sponges most important are the monophyletic class Hexactinellida, and the polyphyletic “Lithistida,” which includes taxa belonging to different groups within the class Demospongiae. Both classes are characterized by rigid skeletons of fused spicules.

Among the class Hexactinellida, the order Hexactinosida (subclass Hexasterophora) (Schulze, 1886), known since the late Devonian (Rigby et al., 1981, 2001), developed a rigid and, to a certain extent, firm skeleton that consists of fused hexactins. This fused, three-dimensional, generally right-angled skeletal network stays intact even after the death of the sponge.

Hexactinellida

Hexactinellida Schmidt (Porifera) are deep-water marine sponges defined by their production of siliceous spicules of hexactinic, triaxonic (cubic) symmetry, or shapes clearly derived from such forms by reduction of primary rays or terminal branches added to the ends of primary rays. They lack calcareous minerals and sclerified organic spongin as skeletal components. Siliceous spicules may be entirely loose, or partially fused to form a rigid basal and choanosomal framework. Their living tissues are mainly syncytial, with distinctive porous plugs joining differentiated regions of the syncytium to each other or to discrete cellular components. Flagellated-collar units are anucleate. Hexactinellids are viviparous and, from detailed study of a single species, produce distinctive trichimella larvae. Two subclasses are recognized by different microsclere forms – amphidiscs and hexasters.



Silica Biomineralization, Sponges, Figure 1 Specimens of glass sponge *Hyalonema (Corynonema) populiferum* Schulze, 1899 recently collected in Pacific (Oregon, USA) (a) possess high flexible anchoring siliceous spicules (b).

Hexactinellids include more than 500 described species, 7% of all Porifera, distributed in five orders, 17 families, and 118 genera (Schulze 1886, 1904; Sollas, 1888, Okada, 1928; Reischwig, 1971, 2002; Lévi et al., 1989; Mehl, 1992; Leys, 2003).

The taxonomy of the class Hexactinellida is very incomplete in the sense that probably not even half of its species are known to science. This is obvious from the fact that many of the hexactinellids species collected during recent deep-sea expeditions are new. The spicules of most hexactinellids are larger (Figure 1) and more luxuriously architected than those in demosponges (Tabachnick, 2002).

Demospongiae

In demosponges (Demospongiae Sollas, 1885) we have much the largest group, with 95% of all extant sponges. They are also the most diverse group. Some are freshwater but predominantly they are marine species living from the intertidal to the deepest seas, with around 15 orders, 88 families, and about 500 valid genera (Hooper and van Soest, 2002). The skeleton is composed of monaxononic or

tetragononic siliceous spicules (never triaxononic) bound together with collagen-like protein spongin in discrete fibers or loosely aggregated and ubiquitous collagenous filaments forming the ground substance of the intercellular matrix.

Because the morphology of the spicules differs species-specifically, they are used as a major taxonomic character. However, very little is known about the molecular mechanism(s) that determine the position and arrangement of the spicules within the demosponge skeleton (Uriz et al., 2003; Uriz, 2006).

Lithistid demosponges

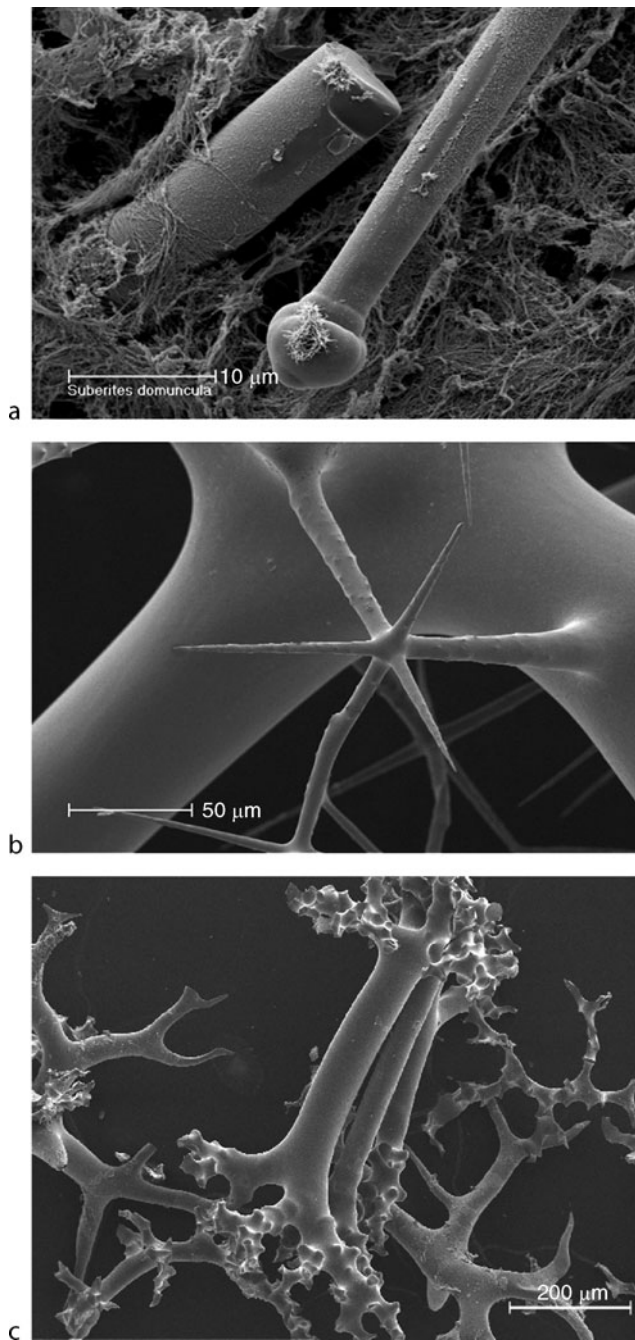
Like hexactinellid sponges, lithistid sponges also contain siliceous skeletons comprising spicules. These spicules (desmas) interlock, rendering the skeleton rigid and often stony (Pisera, 2000, 2003; Kelly, 2000, 2003). At present little is known about the deposition of silica within this taxonomic order.

Lithistid sponges are commonly found on tropical and temperate seamounts and continental margins down to depths of about 1,000 m (Kelly, 2000). Several Southwest Pacific species, form large cups, bowls, and plates that extend linearly from the sponge margin. Close inspection of the lamellae show irregular or concentric ridges, indicating that silica deposition is not continuous but rather variable in nature (Ellwood et al., 2007) presented detailed records of trace metals and carbon isotopes to understand siliceous spicule formation in the deep-sea lithistid sponge *Corallistes undulatus* Lévi and Lévi, 1983 (Demospongiae: Corallistidae). X-ray analysis of two longitudinal sections removed from the lamellae of the cup-shaped sponge revealed 144 and 137 light and dark density band-pairs, respectively, within the siliceous skeleton. Although there was some variability in the ^{32}Si data, the overall age established using these data indicated that the sponge was between 135- and 160-year old.

Forms of the silica deposition in sponges

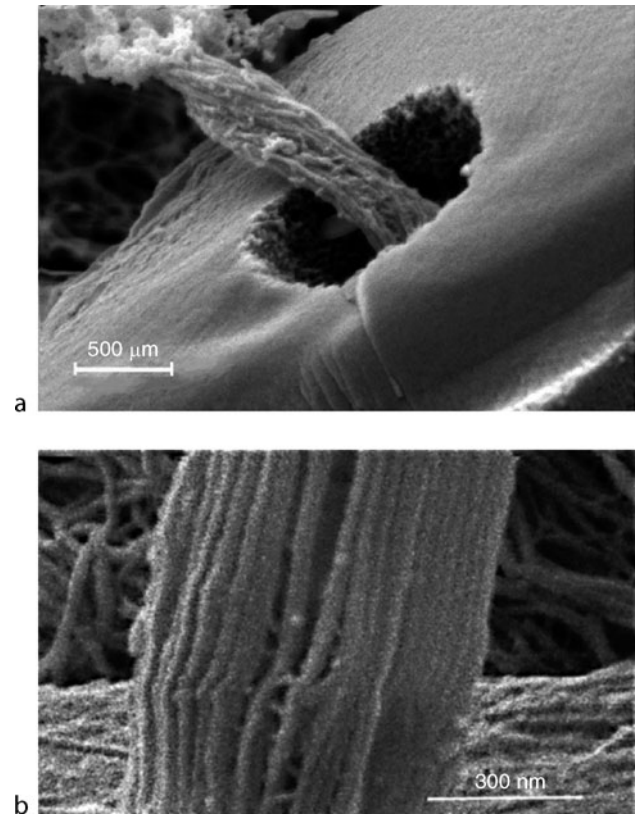
Spicules, skeletal frameworks and desmas are the main forms of the silica deposition in sponges as shown in Figure 2.

The history of the discoveries related to sponge spicules is well reviewed by Vosmaer and Wijsman (1905). According to these authors, after Schweigger in 1819 had demonstrated that the spicules of sponges in some cases do not consist of calcium carbonate, Grant in 1826 found them to contain silica, and Bowerbank in 1841 showed that, in addition to the silica some organic matter is present. Sollas in 1885 likewise finds that the sponge silica resembles opal. He states in general that the refractive index of sponge silica is that of opal or colloidal silica, and not of quartz. This kind of opal was proposed by Vosmaer and Wijsman to call *spicopal*, however, the organic matter of the spicule was called by F.E. Schulze *spiculine*. As to the structure of the spicules, Gray in 1835 had found them in *Hyalonema* glass sponge to



Silica Biomineralization, Sponges, Figure 2 SEM images of three main forms of the silica deposition in sponges: (a) spicules of demosponge *Suberites domuncula*; (b) skeletal silica-chitin-based framework of *Farrea occa* glass sponge (both images courtesy C. Ekkert), (c) siliceous desmas of *Neopelta* sp. lithistid demosponge. (Image courtesy A. Pisera.)

consist of layers, which became conspicuous by heating. These layers concentrically surround a “central canal,” which is filled out, as Kölliker in 1864 has shown, by an organic mass, the axial rod. Claus in 1868 found that the



Silica Biomineralization, Sponges, Silica Biomineralization, Sponges, Figure 3 SEM images of the axial rod (axial filament) of demosponge *S. domuncula* (a) show interconnections between individual proteinaceous nanofibrils (b) visible only at high magnification.

silica which directly surrounds this central rod, is homogenous. He called this homogenous cylinder the axial cylinder. The sponge spicule thus consists of a central organic axis, surrounded by concentric layers of opal, the outermost of which is invested in a spicule sheath of organic matter or rather of organic matter in intimate association with silica (Vosmaer and Wijsman, 1905) (Figure 3).

Using the freshwater sponge *Ephydatia fluviatilis*, Weissenfels and Landschoff (1977) and Weissenfels (1989) demonstrated that the formation of spicules starts in sclerocytes within a specific vesicle. After the production of an axial organic filament, silicon is deposited around it and the whole process of forming a spicule (190 µm in length and 6–8 µm in diameter) is completed after 40 h, at 21°C.

Siliceous sponge spicules have traditionally been separated into two categories termed, according to their size, megascleres and microscleres (Lévi, 1973). They are highly diverse in sponges and the selection pressures responsible are difficult to envisage. There are over 12 basic types of megasclere and 25 types of microsclere reported in Demospongiae, 20 basic types of megasclere, and 24 types of microsclere in

Hexactinellida, besides a long list of variations of the basic types (Lévi, 1973, 1993; Garrone et al., 1981; Simpson 1984, 1990; De Vos et al., 1991; Bavestrello et al., 1993; Boury-Esnault and Rutzler, 1997; Tabachnick and Reiswig, 2002).

Desmas differ from typical demosponge spicules: first they are joined by articulation; second, they always display irregularity and often complex morphology and sculpture. The most basic difference, perhaps, is in the fact that the crepis or axial filament is usually very short (or even invisible) and extends only a short distance from the spicule center. Growth ceases very rapidly, and an important part of a desma, including secondary branches as well as elements of sculptures, does not depend on the axial filament (Sollas, 1888; Lévi, 1991).

In contrast to the high diversity of spicules, there are relatively few basic types of skeletal framework in demosponges. Six elemental types of skeletons with intermediate forms can be differentiated: hymedesmoid, plumose, axial, radiate, reticulate, and arranged in strength confusion (Boury-Esnault and Rutzler, 1997)

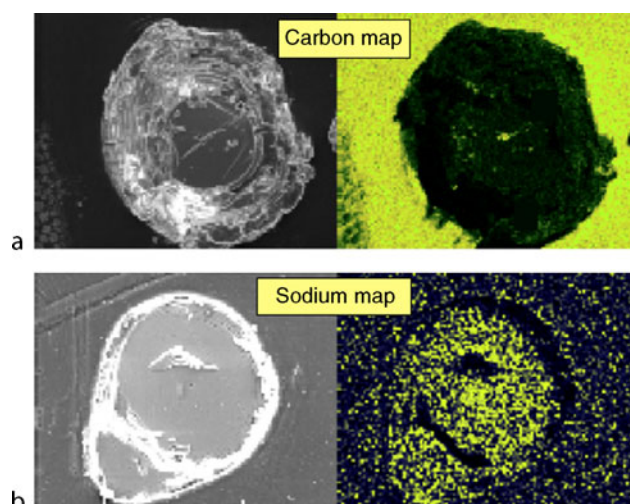
Chemistry of sponge silica

It is established that siliceous sponge spicules of both the Hexactinellida and Demospongiae consist of hydrated amorphous silica (Simpson, 1989; Uriz et al., 2003; Uriz, 2003, 2006). Chemically, they differ only slightly in their $\text{SiO}_2\text{:H}_2\text{O}$ proportions. Desma bearing demosponges ("Lithistida") have a ratio of 5:1 whereas Hexactinellida have 4:1 (Hartman, 1981). Holzhüter et al. (2005) reported that spicules of demosponge *Suberites domuncula* consist of more than 90% silicon and oxygen. They found that at the molecular level silica of sponge spicules has following amorphous structure: SiO_4 tetrahedra are connected to form polyhedra, which are comparable with those found in silica gels. These polyhedra form silica particles up to 3 nm in size which are interconnected forming a dense packing containing pores. However, recently Mugnaioli et al. (2009) reported for the first time crystalline nanorod-like structures which were localized within the organic axial filament of the primordial spicules of demosponge *S. domuncula*. These nanorods have a layered structure that matches smectitic phyllosilicates. It was suggested that this kind of crystalline structure acts as an inorganic template and gives shape and orientation to the newly forming spicule, although its function is admittedly not yet fully understood.

The major trace elements found in the spicules and siliceous sponge skeletons (i.e., S, Ca, K, Na, and Cl) are also the most common in seawater (Sandford, 2003). It was suggested (Aizenberg et al., 2004) that sodium can play an important role in physicochemical properties of glass sponge spicular formations (Figure 4).

Mechanisms of silicification in sponges

Silica deposition is a fundamental process in sponges. According to the modern point of view, two different

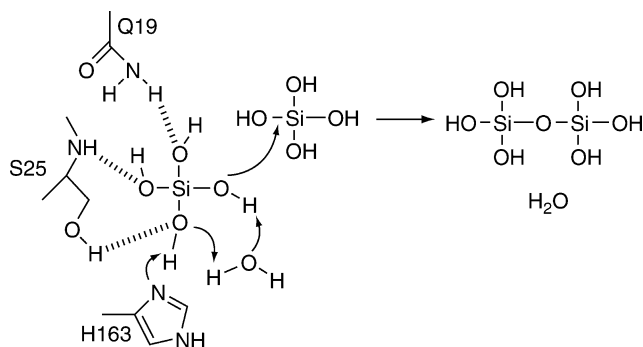


Silica Biomineralization, Sponges, Figure 4 SEM image and EDX carbon map (a) of microtome-cut spicule of *Euplectella aspergillum* glass sponge cross section highlights the presence of an organic filament. (b) EDX Na map indicates an enhanced concentration of Na ions within the spicule cross section, with the highest density of Na occurring in the 2- μm core. (Images courtesy J. Aizenberg.)

mechanisms of silicification in sponges are proposed: enzymatic (silicatein-based) and nonenzymatic, or self-assembling (chitin- and collagen-based).

Silicatein-based silicification

The group of Morse (Shimizu et al., 1998; Cha et al., 1999; Shimizu and Morse, 2000; Weaver and Morse, 2003; Weaver et al., 2003) discovered that organic filament in demosponge *Tethya aurantium* is composed of a cathepsin L related enzyme, termed silicatein. They cloned two of the proposed three isoforms of silicateins, the α - and β -forms, from this demosponge (Cha et al., 1999). In subsequent years these molecules were cloned also from other sponges, among them *S. domuncula* and *Lubomirskia baicalensis* (as reviewed in Müller, 2003; Müller et al., 2007). The spicules are produced in special cells, the sclerocytes; that formation starts intracellularly around a silicatein filament and is completed extracellularly by apposition of silicatein onto the growing spicules (Müller et al., 2005, 2006). Cathepsin L is an endopeptidase which cleaves peptide bonds with hydrophobic amino acid residues in P2 and P3 positions and occurs in lysosomes as well as extracellularly as secreted enzymes. The silicateins are distinguished from the cathepsins by the replacement of the first amino acid residue in the catalytic triad, cysteine by serine (Shimizu et al., 1998). It has been proposed that serine increases the nucleophilicity during the nucleophilic attack at the silicon atom. The polymerization-promoting activity of the silicateins has been shown to be catalytic and not stoichiometric. It is possible that the controlled punctuated secretion of low



Silica Biomineralization, Sponges, Figure 5 The chemical mechanism proposed by Fairhead et al. (2008) to operate for the polymerization of silicic acid using silicatein molecules. The authors proposed H163 deprotonates Si(OH)_4 ; the extensive water network could permit proton shuttling. They illustrated one possibility.

concentrations of monomeric silicateins, pulses in the transitory flux of silica-precursor molecules, oscillations in the pH, ionic, or other conditions of the condensation environment, or a combination of these factors may be responsible for the annular patterning and continued deposition of silica in spicule biosynthesis in vivo once the axial filament has become completely covered during the early stages of silica deposition around this proteinaceous core. Alternatively, the continued growth of silica may be a result of dangling bonds at the growth surface of the newly deposited silica (Saito et al., 1995).

There are two possible mechanisms for enzyme catalysis (Fairhead et al., 2008): (1) stabilize at the active site one molecule of deprotonated silicic acid (the nucleophile) which will then react with another molecule of silicic acid; or (2) stabilize a protonated silicic acid (the electrophile) which will then react with another molecule of silicic acid. In proposed mechanism (Figure 5) (Fairhead et al., 2008), the roles of C25S location and flanking mutations are simply to create a sufficiently sized pocket that will allow recognition of the tetrahedral Si(OH)_4 molecule in such a way that H163 location can deprotonate it. The deprotonated Si(OH)_4 protein complex can be thought of as a template for condensation reaction. In this proposal, there is no need to involve a high energy covalent intermediate. The presence of a specific Si(OH)_4 transporter in sponge suggests the true substrate in vivo is indeed silicic acid, not high energy silicon alkoxides. This being so, the simple acid–base activation mechanism proposed by authors seems a good model for the biological process.

According to Müller et al., formation of spicules is a biologically controlled extracellular process (Schröder et al., 2006; Müller et al., 2007). The following model of silicification in demosponges is derived from observations and propositions put forth by Morse's and Müller's groups (reviewed in Leys et al., 2007). Sclerocytes secrete axial filaments composed mainly of silicateins but other organic

molecules are included. Silicatein acts as a structural template for formation of the highly organized mesoporous axial filament. Silicate is used or is actively taken up into the sclerocyte and complexed with specific proteins to form an as yet unidentified organic-silica substrate. The organo-silica complex is transported across silicalemma to the "silica deposition space." Silica from organo-silica substrate is polymerized on the outer surface of the mesoporous axial filaments as nanospheres, perhaps at serine and histidine catalytic centre sites, and the complexing protein is released and possibly recycled to the sclerocyte cytoplasm.

Growth of the axial filament continues at spicule tips, providing primary patterning of the spicule, while at the same time transport and deposition of silica continue on lateral spicule surfaces between the tips. Once the first few layers of silica nanospheres encase the axial filament, enzymatic activity can no longer be expressed by silicateins of the axial filament. Continuing deposition of silica on the outer surface of spicules is facilitated and controlled by silicatein at active sites on the silicalemma, resulting in production of specific patterned morphologies of spicules. Several elements of this model remain unverified. Other unknowns include the roles of the minor non-silicatein organic compounds incorporated in axial filaments, whether silicateins associated with the silicalemma are the same forms as those in the axial filaments and how the final high-fidelity submicroscopic external patterning on spicule surfaces is genetically controlled. However, recently Kozhemyako et al. (2009) identified silicatein genes (*AcSilA* and *AcSilB*) in nonspicule-forming marine sponge *Acanthodendrilla* spp. The authors suggest that silicateins could participate also in the functions unrelated to spiculogenesis.

Chitin- and collagen-based silicification

Aminopolysaccharide chitin has a nanofibrous structure and the chain of pyranose ring arranges almost parallel to the (100) plane and extends along the fiber axis (Iijima and Moriwaki, 1990). The chitin molecule has C=O, O–H, and N–H groups and oxygen atoms, which have affinity to the calcium, phosphate, carbonate, and hydroxyl ions of corresponding calcium phases. However, the same functional groups possess affinity to silicate ions. Because there is a possibility that such an oriented organic matrix acts as a template, or an ordered structural framework, it was hypothesized the existence of naturally occurring silica-chitin composites (Ehrlich et al., 2007). Moreover, silicon was found associated with glycosaminoglycans bound as an ether or ester-like silicate with C–O–Si or C–O–Si–O–Si–O–C bonds, in amounts of one Si atom/130–280 repeating units of the organic (Schwartz, 1973). The role of chitin in biosilicification is still not completely understood. Although chitin is one of the most important biopolymers in nature, knowledge of its interaction with silicon in vivo was absent up to now. Only recently, Ehrlich et al., isolated and identified

chitin from skeletal formations of some marine glass sponges for the first time. The presence of chitin within the framework skeleton of *Farrea occa* (Ehrlich et al., 2007) and *Euplectella aspergillum* (Ehrlich and Worch, 2007a) as well as separate spicules *Rossella fibulata* (Ehrlich et al., 2008c) could also be revealed by gentle NaOH-based desilicification technique established by Ehrlich et al. (2006) and Ehrlich and Worch, (2007b). The structure of the chitin extracted from these sponges turned out to be similar to alpha-chitin from invertebrates.

It was suggested that silicate ions and silica oligomers preferentially interact with glycopyranose rings exposed at the chitin surface, presumably by polar and H-bonding interactions (Ehrlich et al., 2008b). On the basis of the obtained results, authors proposed a model for the nanostructure of the naturally occurring silica-chitin composite unit, including interaction between poly-N-acetylglucosamine fragment of the chitin nanofibril and silica nanoparticles, which can be seen in Figure 6.

Because chitin could play a crucial role also in biosilicification in fungi (Kolb et al., 2004), it was hypothesized (Ehrlich et al., 2007) that chitin molecules are probably part of a very old organic template system involved in a biosilicification phenomenon, which was established a long time before the origin of first metazoan (e.g., glass sponges).

Recently, Ehrlich et al. reported for the first time on example of *Hyalonema sieboldi* glass sponge that its spicules are a biocomposite containing a silicified collagen matrix, and that high collagen content is the origin of the unique mechanical flexibility of the spicules (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). Especially, interest is paid now to newly discovered highly hydroxylated collagen isolated from *H. sieboldi* spicules (Prof. M. Collins personal communication). Glass sponges are largely restricted to deep, cold-water (between 0 and 4°C) habitats (Tabachnick, 1991; Janussen et al., 2004; Dohrmann et al., 2008). Therefore, from the ecological point of view collagen- (as well as chitin-) based silicification which occurs in spicules and other skeletal formations of these sponges is an example of unique cold-water biomineralization. There is no evidence in the literature about silicatein activities at this temperature level. Skeletal biomineralization requires energy and so imposes a metabolic cost on skeleton-forming organisms (Knoll, 2003). The hypothesis is that probably some phosphate moieties and ATPase-based mechanisms are in some way involved in biosilicification at temperatures near zero (Ehrlich, 2009).

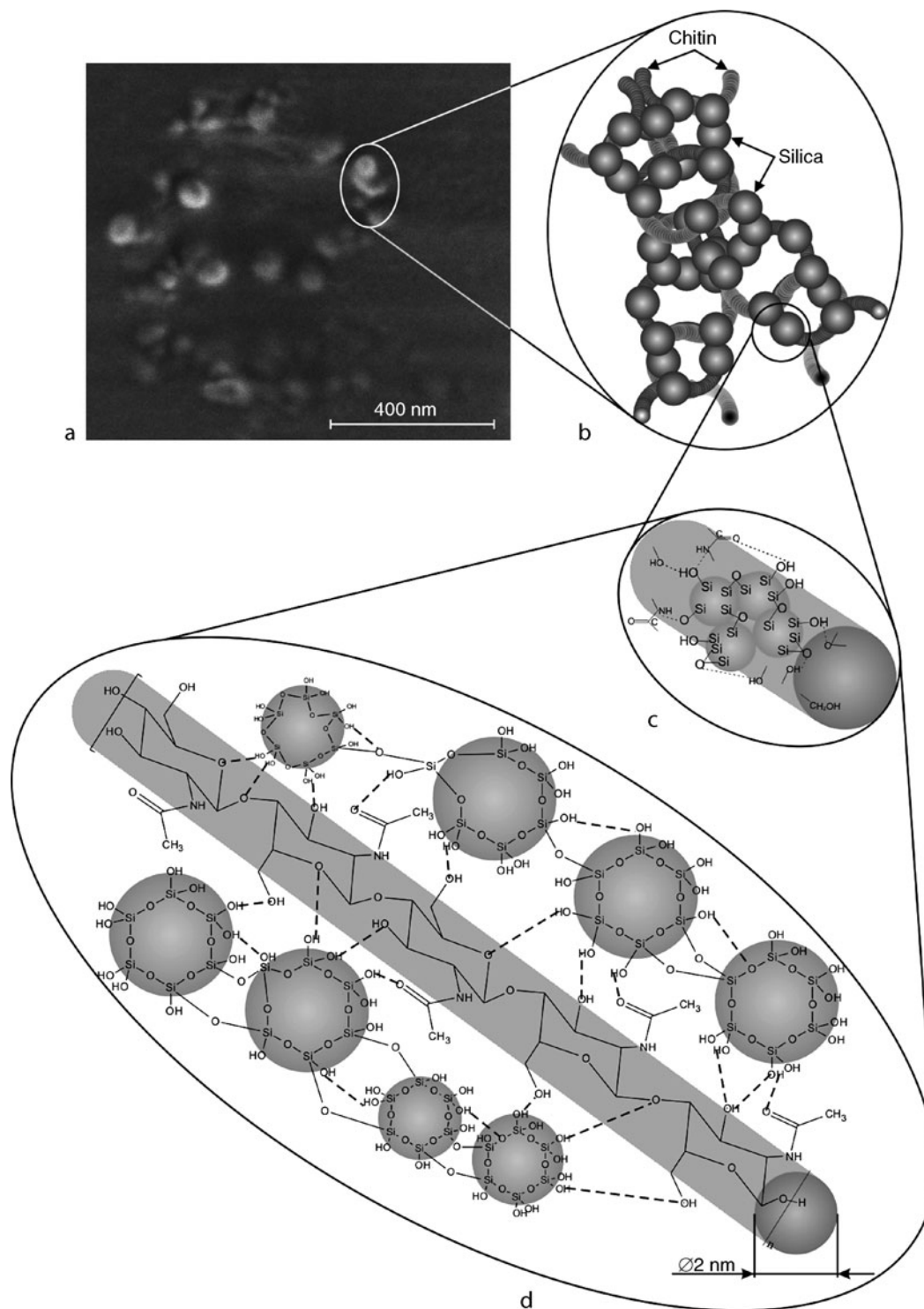
The procedure of alkali slow etching opens the possibility to observe the forms of collagen fibrils located within silica layers of sponge spicules and their distribution. For example, the results obtained by SEM observations of the desilicified spicular layers of *Monorhaphis chuni* provide strong evidence that collagen fibrils' orientation within spicules possesses twisted plywood architecture (Ehrlich et al., 2008b). Typical fibrillar formations were observed within the tubular silica structures of this sponge in all layers starting from the inner axial channel

containing axial filament (Figure 7c) up to the outermost surface layer of the spicules as shown in Figure 7b and Figure 7d. The fibrils in each cylinder form individual concentric 2D networks with the curvature of the corresponding silicate layers. These layers of about 1 µm in thickness are connected among each other by protein fibers (Figure 7a), which possess a characteristic nanofibrillar organization. Partially desilicified nanofibrillar organic matrix observed on the surface of silica-based inner layers of the demineralized spicule provides strong evidence that silica nanoparticles of diameter about 35 nm are localized on the surface of corresponding nanofibrils (Ehrlich et al., 2008b). This kind of silica nanodistribution is very similar to the silica distribution on the surface of collagen fibrils in the form of nanopearl necklets, firstly observed in the glass sponge *H. sieboldi* (Ehrlich et al., 2006; Ehrlich and Worch, 2007b). It was suggested that the nanomorphology of silica on proteinous structures described above could be determined as an example of biodirected epitaxial nanodistribution of the amorphous silica phase on oriented collagenous fibrillar templates (Ehrlich et al., 2008b). From this point of view, basal spicules of *Monorhaphis* sponges could be also defined as natural plywood-like silica-ceramics organized similarly to the crossed-lamellar layers of seashells. The authors suggested also that the matrix of the *M. chuni* anchoring spicule is silicified fibrillar collagen rather than collagen-containing silica which is the reason for their remarkable mechanical flexibility.

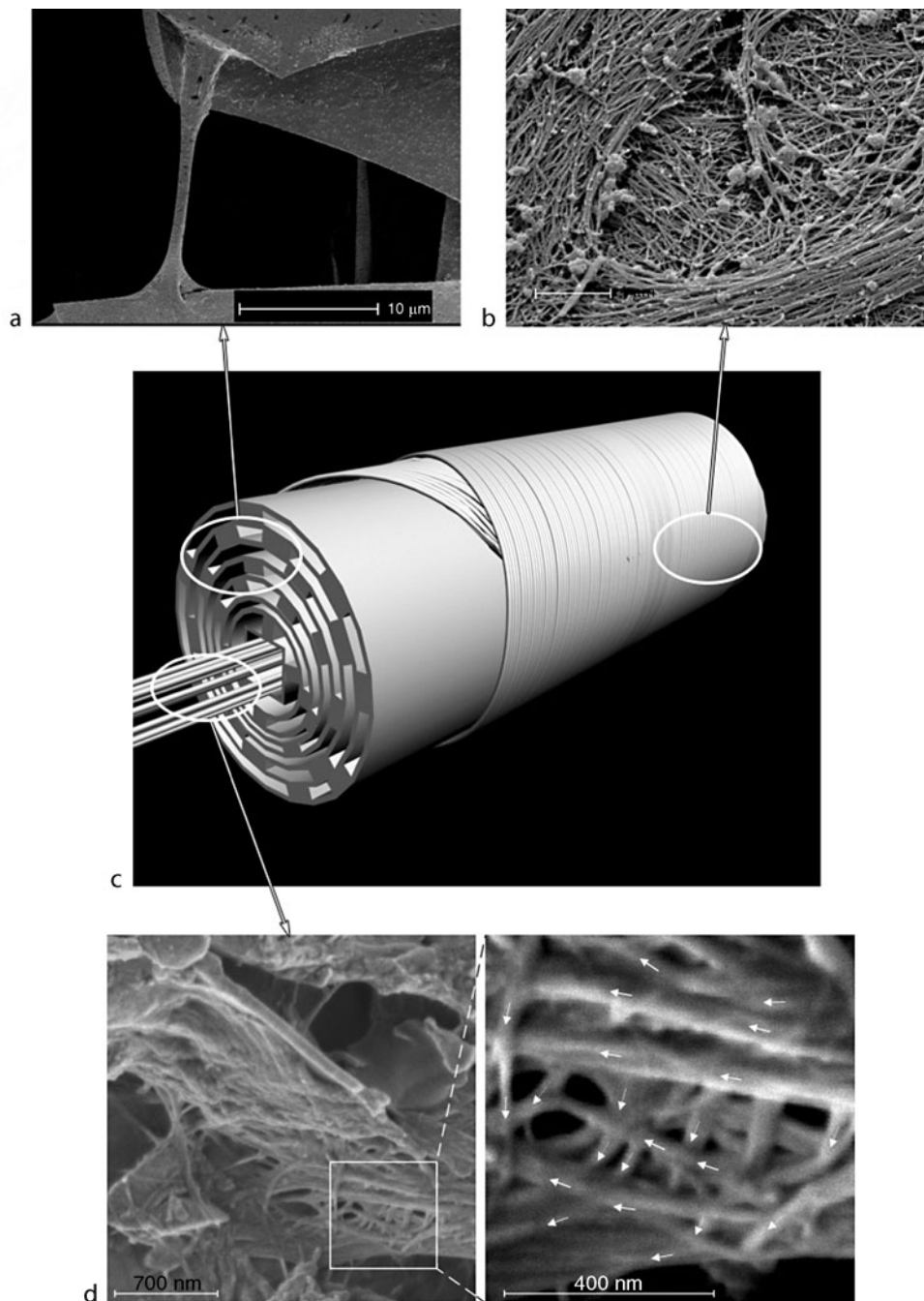
Biogeological aspects of silica deposition by sponges

Analysis of the biogeochemical cycle of silicon suggests that the biospheric environmental silicic acid concentration may have varied considerably during the time of life on Earth. Biochemical evolution today is proceeding within environments in which the silicic acid concentration is considerably lower than previous evolutionary periods (Exley, 1998).

Concomitant with the advent of dioxygen, about 2500 million years ago, and its subsequent gradual increase in atmospheric concentration from approximately 1% towards the level of 20% which is characteristic of today, there occurred an increasing number of organisms within which silicic acid was processed to silica. The most important of these, in the terms of their diversity, ubiquity (both freshwater and marine species) and biomass, were the diatoms, which occur however late in earth history (Mesozoic). The diatoms are characterized by a silica frustule which surrounds their cell wall. Interestingly that chitin-based organic networks are an integral part of cell wall biosilica from the diatom *Thalassiosira pseudonana* (Brunner et al., 2009). According to Maldonado et al. (2005), our current understanding of the silicon cycle in the ocean assumes that diatoms dominate not only the uptake of silicic acid, but also the production and



Silica Biomineralization, Sponges, Figure 6 Proposed model (adapted from Ehrlich et al., 2008c) of nanostructural organization of the naturally occurring silica-chitin composite unit (a) isolated from spicules of *Rossella fibulata* glass sponge. Silica nanoparticles tightly surround chitinous nanofibrils (b) Schematic view (c) shows a possible nanodistribution of silica on the surface of chitinous nanofibril. Image (d) represents the hypothetical scheme of interaction between silica and poly-N-acetylglucosamine-fragment of the chitin nanofibril and formation of the corresponding hydrogen bonds.



Silica Biomineralization, Sponges, Figure 7 Proposed model (c) of micro- and nanostructural organisation of the basal spicule of *Monorhaphis chuni* glass sponge with respect to organic matrix. Collagen nets, surrounding the spicules, showed a tight mat of nanofibrils (b, image courtesy C. Eckert). SEM image (a) shows a collagenous fibrillar matrix which could function as glue between concentric layers. Schematic image (c) represents the region of the axial canal and axial filament. The axial canal of *M. chuni* possesses a characteristic quadratic opening and contains oriented bundles of unsilicified collagenous nanofibrils. The base material of the walls of the axial canal and concentric layers distributed above it consists of silicified collagen fibrils with a twisted plywood orientation (d). This kind of fibrillar architecture could be responsible for the remarkable micromechanical properties of the spicule as a biocomposite made by silica and collagen.

recycling of biogenic silica, and that other organisms with siliceous skeletons, including sponges, radiolarians, and silicoflagellates, play a negligible role. The authors showed that the retention of Si by siliceous sponges in some sublittoral and bathyal environments is substantial and that sponge populations function as Si sinks. Therefore, sponges may affect Si cycling dynamics and Si availability for diatoms, particularly in Si-depleted environments. It was strongly suggested (Maldonado et al., 2005) that the role of sponges in the benthopelagic coupling of the Si cycle is significant. For example, Antarctic giant hexactinellids, such as *Rossella nuda* and *Scolymastra joubini*, which may be up to 2 m tall, 1.4 m in diameter, and up to 600 kg wet weight, containing up to 50 kg biogenic silica each (Maldonado et al., 2005). They are extremely slow-growing and seemingly reproduce only at long time intervals (Dayton et al., 1974) and become old, probably more than 1,500 years (Dayton, 1979; Gatti, 2002). While their living tissues represent only a modest biomass, the silicious spicules of hexactinellids become an important ecological factor. After the death of the sponges, the megascleres do not dissolve but accumulate on the bottom and over large areas to form spicule mats commonly about 50 cm thick but occasionally exceeding 2 m (Koltun, 1968; Dayton et al., 1974; Henrich et al., 1992). The mats structure the fauna living in and on them.

Marine sponges fractionate silicon isotopes during opal biomineralization to a greater degree than any other process on Earth thus far investigated (De La Rocha, 2003). This ability is in keeping with both their extremely negative silicon isotope composition and their inefficient uptake of silicic acid relative to other silicon-biomineralizing organisms.

Sponges seem to be important players in numerous geobiological processes (Reitner, 2004). For example, siliceous sponge reefs had a wide distribution in prehistoric times and once constructed the largest reefs known on earth, reaching an acme in the Upper Jurassic when a deep-water reef belt on the northern Tethys shelf existed that was 7,000-km long (Krautter et al., 2001). In present times hexactinellid sponges of the order Hexactinosida have constructed reefs at several localities off the coast of British Columbia, Canada. The reefs occur on relict glaciated seafloor areas with a low sedimentation rate and a high dissolved silica concentration in the Queen Charlotte Basin and in Georgia Basin (Conway et al., 2004, 2005) and the reefs represent stable communities that have been growing for up to 9,000 years. The main framebuilders include the hexactinosidan species *Aphrocallistes vastus*, *F. occa* and *Heterochone calyx* in Queen Charlotte Basin, and *A. vastus* and *H. calyx* in Georgia Basin (Conway et al., 2004). The framework of these reefs is constructed through several processes of framebuilding, and the reef matrix is derived from trapping of suspended sediments (Krautter et al., 2001). Similar framebuilding processes are thought to have contributed to the formation of the ancient reefs (Krautter

et al., 2006). Upwelling and downwelling oceanographic processes in a biologically productive coastal sea have contributed to the development of large reef complexes. The reefs form as bioherms (mounds) and biostromes (beds or sheets) that may rise up to 21 m above the seafloor and they cover around 1000 km² on the continental shelf. Individual reef complexes may cover areas of more than 300 km² and occur in 90–240 m depth range (Conway et al., 2001, 2005; Lehnert et al., 2005). Trapping fine-grained siliciclastic sediments in suspension in near-bottom currents is an important part of the mound-forming process, as it prevents the reef framework from collapsing under its own increasing weight.

Summary

Sponges are exclusively aquatic, sedentary, filter-feeding invertebrates, producing silica-made skeletons consisting of individualized elements (spicules) of lengths ranging from micrometers to centimeters, which can subsequently fuse or interlock with each other. The high diversity of spicule shapes and sizes in both fossil and living sponges has been repeatedly reported in literature. However, the mechanisms that determined such diversity remained elusive until recently. We are beginning to understand the mechanisms of spicule secretion and formation and the role of spicules and skeletal frameworks in the biogeology.

Molecular techniques and ecological experiments have demonstrated the genetic control of the process and the contribution of environmental factors to the expression of a sponge spicule, respectively. Silicatein proteins govern the enzymatic and structurally controlled synthesis of silica in marine demosponges. However, new biomineralization topics such as the role of chitin and/or collagen as self-assembling and nanofibrillar organized templates in sponge related biosilicification phenomena require further investigation.

Bibliography

- Aizenberg, J., Sundar, V. C., Yablon, A. D., Weaver, J. C., and Chen, G., 2004. Biological glass fibers: correlation between optical and structural properties. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 3358–3363.
- Bäuerlein, E., 2007. Handbook of biomineralization. In Bäuerlein, E., Behrens, P., and Epple, M., (eds.), *Growth and Form: What is the Aim of Biomineralization?* Weinheim: Wiley, pp. 1–20.
- Bavestrello, G., Bonito, M., and Sarà, M., 1993. Influence of depth on the size of sponge spicules. In Uriz, M. J., and Rützler, K. (eds.), *Recent Advances in Ecology and Systematics of Sponges*. Barcelona: Scientia Marina, Vol. 57(4), pp. 415–420.
- Bergquist, P. R., 1978. *Sponges*. London: Hutchinson & Co., Ltd.
- Botting, J., and Butterfield, N. J., 2005. Reconstructing early sponge relationships by using the Burgess Shale fossil *Eiffelia globosa*, Walcott. *Proceedings of National Academy of Sciences of the United States of America*, **102**, 1554–1559.
- Boury-Esnault, N., and Rützler, K., 1997. Thesaurus of sponge morphology. *Smithsonian Contributions to Zoology*, **596**, 1–55.
- Brunner, E., Richthammer, P., Ehrlich, H., Paasch, S., Simon, P., Ueberlein, S., and van Pee, K.-H., 2009. Chitin-based organic networks – an integral part of cell wall biosilica from the diatom

- Thalassiosira pseudonana. *Angewandte Chemie International Edition*, doi:10.1002/anie200.
- Cha, J. N., Shimizu, K., Zhou, Y., Christiansen, S. C., Chmelka, B. F., Stucky, G. D., and Morse, D. E., 1999. Silicate in filaments and subunits from a marine sponge direct the polymerization of silica and silicones in vitro. *Proceedings of National Academy of Sciences of the United States of America*, **96**, 361–365.
- Conway, K. W., Krautter, M., Barrie, J. V., and Neuweiler, M., 2001. Hexactinellid sponge reefs on the Canadian continental shelf: a unique 'living fossil'. *Geoscience, Canada*, **28**(2), 71–78.
- Conway, K. W., Barrie, J. V., and Krautter, M., 2004. Modern siliceous sponge reefs in a turbid, siliciclastic setting: Fraser River delta, British Columbia, Canada. *Neues Jahrbuch für Geologie und Paläontologie Monatshefte*, **6**, 335–350.
- Conway, K. W., Krautter, M., Barrie, J. V., Whitney, F., Thomson, R. E., Reisswig, H., Lehnert, H., Mungov, G., and Bertram, M., 2005. Sponge reefs in the Queen Charlotte Basin, Canada: controls on distribution, growth and development. In Freiwald, A., and Roberts, J. M. (eds.), *Cold Water Corals and Ecosystems*. Berlin: Springer, pp. 601–617.
- Dayton, P. K., 1979. Observations of growth, dispersal and population dynamics of some sponges in McMurdo Sound, Antarctica. In Lévi, C., and Boury-Esnault, N. (eds.), *Colloques internationaux du C.N.R.S. 291. Biologie des spongiaires*. Paris: Editions du Centre National de la Recherche Scientifique, pp. 271–282.
- Dayton, P. K., Robilliard, G. A., Paine, R. T., and Dayton, L. B., 1974. Biological accommodation in the benthic community at the McMurdo Sound, Antarctica. *Ecological Monographs*, **44**, 105–128.
- De La Rocha, C. L., 2003. Silicon isotope fractionation by marine sponges and the reconstruction of the silicon isotope composition of ancient deep water. *Geology*, **31**, 423–426.
- De Vos, L., Rützel, K., Boury-Esnault, N., Donadey, C., and Vacelet, J., 1991. *Atlas of Sponge Morphology*. Washington: Smithsonian Institution Press.
- Dohrmann, M., Janussen, D., Reitner, J., Collins, A., and Wörheide, G., 2008. Phylogeny and evolution of glass sponges (Porifera: Hexactinellida). *Systematic Biology*, **57**(3), 388–405.
- Ehrlich, H., 2010. Chitin and collagen as universal and alternative templates in biomineralization. *International Geology Review* (in press).
- Ehrlich, H., and Worch, H., 2007a. Collagen, a huge matrix in glass-sponge flexible spicules of the meter-long *Hyalonema sieboldi*. In Bäurlein, E. (ed.), *Handbook of Biomineralization. The Biology of Biominerals Structure Formation*, Weinheim: Wiley, Vol. 1, Chap. 2, pp. 23–41.
- Ehrlich, H., and Worch, H., 2007b. Sponges as natural composites: from biomimetic potential to development of new biomaterials. In Custodio, M. R., Lobo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera Research: Biodiversity, Innovation and Sustainability*, pp. 217–223.
- Ehrlich, H., Ereskovsky, A., Drozdov, A., Krylova, D., Hanke, T., Meissner, H., Heinemann, S., and Worch, H., 2006. A modern approach to demineralisation of spicules in the glass sponges (Hexactinellida: Porifera) for the purpose of extraction and examination of the protein matrix. *Russian Journal of Marine Biology*, **32**, 186–193.
- Ehrlich, H., Krautter, M., Hanke, T., Simon, P., Knieb, C., Heinemann, S., and Worch, H., 2007. First evidence of the presence of chitin in skeletons of marine sponges. Part II. Glass sponges (Hexactinellida: Porifera): *Journal of Experimental Zoology (Molecular Development and Evolution)*, **308B**, 473–483.
- Ehrlich, H., Kotsoulos, P. G., Demadis, K. D., and Pokrovsky, O., 2008a. Principles of demineralization: modern strategies for the isolation of organic frameworks. Part I. Common definitions and history. *Micron*, **39**, 1062–1091.
- Ehrlich, H., Heinemann, S., Heinemann, C., Simon, P., Bazhenov, V., Shapkin, N., Born, R., Tabachnick, K., Hanke, T., and Worch, H., 2008b. Nanostructural organization of naturally occurring composites. Part I. Silica-collagen-based biocomposites. *Journal of Nanomaterials* (published online, doi:10.1155/2008/623838).
- Ehrlich, H., Janussen, D., Simon, P., Heinemann, S., Bazhenov, V. V., Shapkin, N. P., Mertig, M., Erler, C., Born, R., Worch, H., and Hanke, T., 2008c. Nanostructural organisation of naturally occurring composites: Part II. Silica-chitin-based biocomposites, *Journal of Nanomaterials* (published online, Article ID 670235, 8 pages).
- Ellwood, M. J., Kelly, M., and de Forges, B. R., 2007. Silica banding in the deep-sea lithistid sponge *Corallistes undulatus*: investigating the potential influence of diet and environment on growth. *Limnology and Oceanography*, **52**(5), 1865–1873.
- Erpenbeck, D., Breeuwer, J. A. J., Parra-Velandia, F. J., and van Soest, R. W. M., 2006. Speculation with spiculation? – three independent gene fragments and biochemical characters versus morphology in demosponge higher classification. *Molecular Phylogenetics and Evolution*, **38**, 293–305.
- Exley, C., 1998. Silicon in life: a bioinorganic solution to bioorganic essentiality. *Journal of Inorganic Biochemistry*, **69**, 139–153.
- Fairhead, M., Johnson, K. A., Kowatz, T., McMahon, S. A., Carter, L. G., Oke, M., Liu, H., Naismith, J. H., and van der Walle, C. F., 2008. Crystal structure and silica condensing activities of silicatein a–cathepsin L chimeras. *Chemical Communications*, **21**, 1765–1767.
- Garrone, R., Simpson, T. L., and Pottu, J., 1981. Ultrastructure and deposition of silica in sponges. In Simpson, T. L., and Volcani, B. E. (eds.), *Silicon and Siliceous Structures in Biological Systems*. New York: Springer, pp. 495–525.
- Gatti, S., 2002. High Antarctic carbon and silicon cycling – how much do sponges contribute? VI International Sponge Conference. In *Book of Abstracts, Bollettino dei Musei Istituti Biologici*. Genoa: University of Genoa, Vol. 66–67, p. 76.
- Hartman, W. D., 1981. Form and distribution of silica in sponges. In Simpson, T. S., and Volcani, B. E. (eds.), *Silicon and Siliceous Structures in Biological Systems*. New York: Springer, pp. 453–493.
- Henrich, R., Hartmann, M., Reitner, J., Schäfer, P., Steinmetz, S., Freiwald, A., Dietrich, P., and Thiede, J., 1992. Facies belts, bio-coenoses, volcanic structures and associated sediments, of the arctic seamount Vesterisbanken (Central Greenland Sea). *Facies*, **27**, 71–104 Erlangen.
- Holzhüter, G., Lakshminarayanan, K., and Gerber, T., 2005. Silica structure in the spicules of the sponge *Suberites domuncula*. *Analytical and Bioanalytical Chemistry*, **382**, 1121–1126.
- Hooper, J. A., and van Soest, R. W. M., 2002. *Systema Porifera: A guide to the Classification of Sponges*. New York: Kluwer.
- Iijima, M., and Moriwaki, Y., 1990. Orientation of apatite and organic matrix in *Lingula unguis* shell. *Calcified Tissue International*, **47**, 237–242.
- Janussen, D., Tabachnick, K. R., and Tendal, O. S., 2004. Deep-sea Hexactinellida (Porifera) of the Weddell Sea. *Deep-Sea Research II*, **51**, 1857–1882.
- Kelly, M., 2000. Description of a new lithistid sponge from north-eastern New Zealand and consideration of the phylogenetic affinities of families Corallistidae and Neopeltidae. *Zoosystema*, **22**, 265–283.
- Kelly, M., 2003. Revision of the sponge genus *Pleroma* Sollas (Lithistida: Megamorina: Pleromidae) from New Zealand and New Caledonia, and description of a new species. *NZ Journal of Marine and Freshwater Research*, **37**, 113–127.

- Knoll, A., 2003. Biomineralization and evolutionary history. *Reviews in Mineralogy and Geochemistry*, **54**, 329–356.
- Kolb, V. M., Philip, A. I., and Perry, R. S., 2004. Testing the role of silicic acid and biochemical materials in the formation of rock coatings. In Hoover, R. B., Levin, G. V., and Rozanov, A.Y. (eds.), *Instruments, Methods, and Missions for Astrobiology VIII*. Bellingham: SPIE, pp. 116–125.
- Koltun, V. M., 1968. Spicules of sponges as an element of bottom sediments in the Antarctic. *SCAR Symp. Antarctic Oceanography*. Cambridge: Scott Polar Research Institute.
- Kozhemyako, V. B., Veremeichik, G. N., Shkryl, Y. N., Kovalchuk, S. N., Krasokhin, V. B., Rasskazov, V. A., Zhuravlev, Y. N., Bulgakov, V. P., and Kulchin, Y. N., 2009. Silicatein genes in spicule-forming and nonspicule-forming Pacific Demosponges. *Marine Biotechnology*, doi: 10.1007/s10126-009-9225-y.
- Krautter, M., Conway, K. W., Barrie, J. V., and Neuweiler, M., 2001. Discovery of a 'living dinosaur': globally unique modern hexactinellid sponge reefs off British Columbia, Canada. *Facies*, **44**, 265–282.
- Krautter, M., Conway, K. W., and Barrie, J. V., 2006. Recent hexactinosidan sponge reefs (silicate mounds) off British Columbia, Canada: frame-building processes. *Journal of Paleontology*, **80**(1), 38–48.
- Lehnert, H., Conway, K. W., Barrie, J. V., and Krautter, M., 2005. *Desmacella austini* sp. nov. from sponge reefs off the Pacific coast of Canada. *Contributions to Zoology*, **74**(3/4), 265–270.
- Lévi, C., 1973. Systématique de la classe des Demospongiaria (Demosponges). In Grassé, P. (ed.), *Spongiaires. Traité de Zoologie* 3(1). Paris: Masson, pp. 577–632.
- Lévi, C., 1991. Lithistid sponges from the Norfolk rise. Recent and Mesozoic genera. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 72–82.
- Lévi, C., 1993. Porifera Demospongiae: spongiaires bathyaux de Nouvelle-Calédonie, récoltés par le 'Jean Charcot' Campagne BIOCAL, 1985. In Crosnier, A. (ed.), *Résultats des Campagnes MUSORSTROM, II*. Paris: Mémoire du Muséum National d'Histoire Naturelle, pp. 9–87.
- Lévi, C., Barton, J. L., Guillemet, C., Le Bras, E., and Lehuède, P., 1989. A remarkably strong natural glassy rod: the anchoring spicule of the *Monoraphis* sponge. *Journal of Materials Science Letters*, **8**, 337–339.
- Leys, S. P., 2003. Comparative study of spiculogenesis in demosponge and hexactinellid larvae. *Microscopical Research and Technology*, **62**, 300–311.
- Leys, S. P., Mackie, G. O., and Reiswig, H. M., 2007. The biology of glass sponges. *Advances in Marine Biology*, **52**, 1–145.
- Maldonado, M., Carmona, M. C., Vela'squez, Z., Puig, A., Cruzado, A., Lo'pez, A., and Craig, M., 2005. Young siliceous sponges as a silicon sink: an overlooked aspect of benthopelagic coupling in the marine silicon cycle. *Limnology and Oceanography*, **50**(3), 799–809.
- Mann, D. G., and Droop, S. J. M., 1996. Biodiversity, biogeography and conservation of diatoms. *Hydrobiologia*, **336**, 19–32.
- Mehl, D., 1992. Die Entwicklung der Hexactinellida seit dem Mesozoikum: Paläobiologie, Phylogenie und Evolution-ökologie. *Berliner geowissenschaftliche Abhandlungen*, **E**, **2**, 1–164.
- Mugnaioli, E., Natalio, F., Schloßmacher, U., Wang, X., Müller, W. E. G., and Kolb, U., 2009. Crystalline nanorods as possible templates for the synthesis of amorphous biosilica during spicule formation in Demospongiae. *Chemistry and Biochemistry*, **10**, 683–689.
- Mukhopadhyay, J., Gutzmer, J., and Beukes, N. J., 2004. Organotemplate silica deposition in Neoproterozoic deep-marine environments: evidence from the Penganga Group, Adilabad, India. *Terra Nova*, **16**, 338–343.
- Müller, W. E. G., 2003. *Silicon Biomineralization: Biology-Biochemistry-Molecular Biology-Biotechnology*. Berlin: Springer.
- Müller, W. E. G., Krasko, A., Le Pennec, G., and Schröder, H. C., 2003a. Biochemistry and cell biology of silica formation in sponges. *Microscopical Research and Technology*, **62**, 368–377.
- Müller, W. E. G., Krasko, A., Le Pennec, G., Steffen, R., Wiens, M., Ammar, M. S. A., Müller, I. M., and Schröder, H. C., 2003b. Molecular mechanism of spicule formation in the demosponge *Suberites domuncula*: Silicatein – myotrophin – collagen. In Müller, W. E. G. (ed.), *Silicon Biomineralization*, Berlin: Springer, pp. 195–222.
- Müller, W. E. G., Rothenberger, M., Boreiko, A., Tremel, W., Reiber, A., and Schröder, H., 2005. Formation of siliceous spicules in the marine demosponge *Suberites domuncula*. *Cell and Tissue Research*, **321**, 285–297.
- Müller, W. E. G., Belikov, S. I., Tremel, W., Perry, C. C., Gieskes, W. W. C., Boreiko, A., and Schröder, H. C., 2006. Siliceous spicules in marine demosponges (example *Suberites domuncula*). *Micron*, **37**, 107–120.
- Müller, W. E. G., Boreiko, A., Wang, X., Belikov, S. I., Wiens, M., Grebenjuk, V. A., schloßmacher, U., and Schröder, H. C., 2007. Silicateins, the major biosilica forming enzymes present in demosponges: protein analysis and phylogenetic relationship. *Gene*, **395**, 62–71.
- Nichols, S., and Wörheide, G., 2005. Sponges: new views of old animals. *Intergrative and Comparative Biology*, **45**, 333–334.
- Okada, Y., 1928. On the development of a Hexactinellid sponge, *Farrea sollasi*. *Journal of the Faculty of Science, Imperial University of Tokyo, Sect. 4, Zoology*, **2**, 1–27.
- Pisera, A., 2000. New species of lithistid sponges from the Paleogene of the Ukraine. *Zoosystema*, **22**, 285–298.
- Pisera, A., 2003. Some aspects of silica deposition in lithistid demosponge desmas. *Microscopy Research and Technique*, **62**, 312–326.
- Reiswig, H. M., 1971. The axial symmetry of sponge spicules and its phylogenetic significance. *Cahiers de Biologie Marine*, **12**, 505–514.
- Reiswig, H. M., 2002. Class Hexactinellida Schmidt, 1870. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1201–1202.
- Reitner, J., 2004. Sponges – a geobiological approach. *Integrative and Comparative Biology* **43**(6), 989.
- Reitner, J., and Mehl, D., 1996. Monophyly of the Porifera. *Verhandlungen des naturwissenschaftlichen Vereins Hamburg*, **36**, 5–32.
- Rigby, J. K., Racki, G., and Wrzolek, T., 1981. Occurrence of dictyloid hexactinellid sponges in the Upper Devonian of the Holy Cross Mts. *Acta Geologica Polonica*, **31**, 163–168.
- Rigby, J. K., Pisera, A., Wrzolek, T., and Racki, G., 2001. Upper Devonian sponges from the Holy Cross Mountains, central Poland. *Palaeontology*, **44**, 447–488.
- Saito, Y., Isobe, T., and Senna, M., 1995. Incipient chemical reaction on the scratched silicon (1 1 1) surface with ethoxy and hydroxyl groups. *Journal of Solid State Chemistry*, **120**(1), 96–100.
- Sandford, F., 2003. Physical and chemical analysis of the siliceous skeletons in six sponges of the two groups (Demospongiae and Hexactinellida). *Microscopical Research and Technology*, **62**, 336–355.
- Schröder, H. C., Boreiko, A., Korzhev, M., Tahir, M. N., Tremel, W., Eckert, C., Ushijima, H., Müller, I. M., and Müller, W. E. G., 2006. Coexpression and functional interaction of silicatein with galectin: matrix-guided formation of siliceous

- spicules in the marine demosponge *Suberites domuncula*. *Journal of Biological Chemistry*, **281**(17), 12001–12009.
- Schulze, F. E., 1886. *Über den Bau und das System der Hexactinelliden*. Berlin: Royal Prussian Academy of Sciences, pp. 3–97.
- Schulze, F. E., 1904. Hexactinellida. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **4**, 1–266.
- Schwartz, K., 1973. A bound form of silicon in glucosaminoglycans and polyuronides. *Proceedings of National Academy of Sciences of the United States of America*, **70**, 1608–1612.
- Shimizu, K., and Morse, D. E., 2000. The biological and biomimetic synthesis of silica and other polysiloxanes. In Baeuerlein, E. (ed.), *Biomimetalization: From Biology to Biotechnology and Medical Application*. Wiley, Weinheim, pp. 207–219.
- Shimizu, K., Cha, J. N., Stucky, G. D., and Morse, D. E., 1998. Silicatein a: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6234–6238.
- Simpson, T. L., 1984. *The Cell Biology of Sponges*. New York: Springer, pp. 1–660.
- Simpson, T. L., 1989. Silicification processes in sponges: *Geodia asters* and the problem of morphogenesis of spicule shape. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomimetalization in Plants and Animals*. New York: Kluwer, pp. 125–136.
- Simpson, T. L., 1990. Recent data on pattern of silicification and the origin of monaxons from tetraxons. In Rützler, K. (ed.), *New Perspectives in Sponge Biology. 3rd International Conference on the Biology of Sponges*, 1985. Washington: Smithsonian Institution Press, pp. 264–272.
- Skinner, H. C. W., 2005. Biominerals. *Mineralogical Magazine*, **69**(5), 621–641.
- Sollas, W. J., 1888. Report on Tetractinellida collected by H.M.S. challenger during the years 1873–1876. *Report of the Scientific Results of the Voyage of the H.M.S. Challenger. Zoology*, **5**, 1–458.
- Tabachnick, K. R., 1991. Adaptation of the Hexactinellid sponges to deep-sea life. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 378–386.
- Tabachnick, K. R., 2002. Family Monorhaphididae Ijima, 1927. In Hooper, N. A. V., and Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1264–1266.
- Tabachnick, K. R., and Reisswig, H. M., 2002. Dictionary of Hexactinellida. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1224–1229.
- Uriz, M.-J., 2003. Biology of silica deposition of sponges. *Microscopical Research and Technology*, Wiley-Liss. California, **62**(4), 277–381.
- Uriz, M.-J., 2006. Mineral skeletogenesis in sponges. *Canadian Journal of Zoology*, **84**, 322–356.
- Uriz, M.-J., Turon, X., Becero, M. A., and Agell, G., 2003. Siliceous spicules and skeletal frameworks in sponges: origin, diversità, ultrastructural patterns, and biological functions. *Microscopical Research and Technology*, **62**, 279–299.
- Vosmaer, G. C. J., and Wijsman, H. P., 1905. On the structure of some Siliceous Spicules of Sponges. I. The styli of *Tethya lyncurium*. *Koninklijke Nederlandsche Akademie van Wetenschappen Proceedings*, **8**, 15–28.
- Weaver, J. C., and Morse, D. E., 2003. Molecular biology of demosponge axial filaments and their roles in biosilicification. *Microscopical Research and Technology*, **62**(4), 356–367.
- Weaver, J. C., Pietrasanta, I., Hedin, N., Chmelka, B. F., Hansma, P. K., and Morse, D. E., 2003. Nanostructural features of demosponge biosilica. *Journal of Structural Biology*, **144**, 271–281.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–27.
- Weissenfels, N., 1989. *Biologie und Mikroskopische Anatomie der Süßwasserschwämme (Spongillidae)*. Stuttgart: Fischer.
- Weissenfels, N., and Landschoff, H. W., 1977. Bau und Funktion des Süßwasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die Entwicklung der monaxialen SiO₂-Nadeln in Sandwich-Kulturen, *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenese der Tiere*, **98**, 355–371.

Cross-references

[Biogeochemical Cycles](#)
[Critical Intervals in Earth History](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Origins of the Metazoa](#)
[Reefs](#)
[Sponges \(Porifera\) and Sponge Microbes](#)

SILICOFLAGELLATES

Silicoflagellates are planktonic, microscopic algae with siliceous skeletons, characterized by their simple geometries and remarkable variability. Taxonomically, they belong to different classes of the Stramenopiles and possess complex plastids derived from secondary endosymbiosis (see entry [Symbiosis](#)). Silicoflagellate skeletons may have considerable potential as environmental indicators and are common in fossil deposits, dating back to the middle Cretaceous (ca. 120 Ma), with species diversity reaching a peak in the Miocene (ca. 23–25 Ma). Today, only a single genus *Dictyocha* with approximately five recognized species exists. For more details, please refer to entry ["Algae \(Eukaryotic\)"](#).

SINTER

Robin W. Renaut¹, Brian Jones²
¹University of Saskatchewan, Saskatoon, SK, Canada
²University of Alberta, Edmonton, Alberta, Canada

Synonyms

Silica sinter; Siliceous sinter

Definition

Sinter: A sedimentary rock primarily composed of silica that is precipitated from hot waters at the vents of high-temperature (high-enthalpy) hot springs and geysers, and from cooled waters on their surrounding discharge aprons.

Geyserite. A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some high-temperature springs.

Some banded and laminated types of nonmarine carbonates (calcite or aragonite), including spring travertines and speleothems, have also been termed “sinter” or “calc-sinter.” We suggest that the geological term “sinter” should be restricted to *siliceous* deposits precipitated from silica-rich waters discharged at hot springs and geysers.

Introduction

Sinters are deposits of silica precipitated by hot waters discharged at the vents of hot springs and geysers. Most sinters are precipitated as noncrystalline opal-A, but they change to quartz during diagenesis. They form almost exclusively in high-enthalpy geothermal fields where the underground reservoir temperatures exceed $\sim 220^{\circ}\text{C}$, and so are most common in volcanic and tectonically active regions. Most geysers discharge from vents lined with sinter. Weed (1889) was among the first to discuss the role of microorganisms in the formation of sinter. After his pioneering work, very little scientific study was done on these rocks until Allen (1934) described sinters at Yellowstone. Walter (1976) undertook petrographic study of sinters, including stromatolitic types. Rimstidt and Cole (1983) made one of the first detailed studies of the chemistry of sinter formation. Krumbein and Werner (1983), followed later by Cady and Farmer (1996) and Jones and Renaut (1996), described, illustrated, and interpreted silicified microbes in recent sinters. Since then, interest in these unusual rocks has increased significantly, and more than 140 studies of sinters have been published during the past decade. The possible role of bacteria in silica precipitation has been a focus of much interest. Many filamentous and coccoid microbes preserved in recent sinters resemble the microfossils found in Precambrian quartzose cherts.

Morphology of sinter deposits

Most sinter is gray to white in color, but the addition of trace elements can produce vividly colored deposits such as the orange sinters found around the edge of Champagne Pool, Waiotapu, New Zealand (Jones and Renaut, 2003, Figure 2b). The main types of sinter deposit are *mounds*, which form around the vents of many geysers, *pool-rim dams* that precipitate around the margins of hot-spring pools, and various types of *terraces* and *platforms* that form downslope from the vent(s) on the spring or geyser discharge apron. Sinter mounds (Figure 1a) develop where aqueous silica precipitates from fluids that overflow the margins of a geyser vent or small-diameter point-sourced spring, or from silica that precipitates from air-borne spray. Sinter mounds can be columnar, domal, or broadly conical with slopes that range from nearly vertical to low angled ($<10^{\circ}$). Mounds vary from decimeters to meters in height and up to several meters in basal diameter. Many factors control mound morphology including the

original shape and dimensions of the vent, the volume and type of discharge (i.e., geyser versus hot spring activity), the original slope, climate (e.g., predominant wind direction), and water chemistry (e.g., silica concentration). Mound morphology can vary with time as the sinter deposits build up or the pattern or volume of discharge changes.

Pool-rim dams (Figure 1b) form where silica precipitates around the edges of hot-spring pools and some (fountain-type) geysers. The discharged water either flows across the lowest parts of the dam or escapes through erosional notches. Nested concentric pool-rim dams, each with different elevations, may form when mean water level in the pool changes because of external (e.g., hydrostatic) controls.

Water discharged from hot springs either flows across the land surface forming a “discharge apron” or it may flow directly into the local (nonthermal) drainage system. Outflow waters that mix with river or lake waters can be diluted below saturation with respect to amorphous silica, so that any sinter deposits are restricted to the vent area. If undiluted, however, the thermal outflow will cool progressively downslope leading to more silica precipitation. Sinter terraces (Figure 1c–f) commonly form where silica-laden waters flow across slopes, precipitating sheets of silica, as they cool and (or) evaporate. Each terrace is composed of a rimstone dam and pool. In scale, they range from miniterraces a few centimeters across, to large terraces tens of meters across, with dams several meters high. Some terrace pools temporarily pond water, but much of the outflow crosses terrace surfaces as largely unconfined sheets a few millimeters deep. Where several active vents lie near each other, their terraces may merge to form extensive flat sinter platforms.

Silica precipitated from hot waters also forms many small features including oncoids and pisoids (geyser eggs), which typically accrete in shallow pools near geyser vents; silica stalactites, which grow below overhanging terraces; and many small-scale ornate forms of geyserite on proximal sinter terraces (Figure 1e and g). Stromatolites develop locally in ponds or spring pools in the outflow system, usually where water temperature is $<70^{\circ}\text{C}$.

The thickest, most extensive sinter deposits form in neutral to alkaline waters (pH 7–9.5). The sinters that form in acid to neutral waters (pH 2–7) tend to be thin (millimeter to decimeter rather than meter thick). They lack some of the complex ornamentation of sinters forming in more alkaline fluids (Figure 1h), but commonly show spicular and bladed morphologies, and tend to be glassier in appearance than sinters forming in alkaline waters.

Microorganisms and sinter

Petrographic and scanning electron microscope (SEM) studies of sinter commonly reveal abundant silicified remains of microbes including coccoid and filamentous bacteria, eukaryotic algae, diatoms, fungi, and

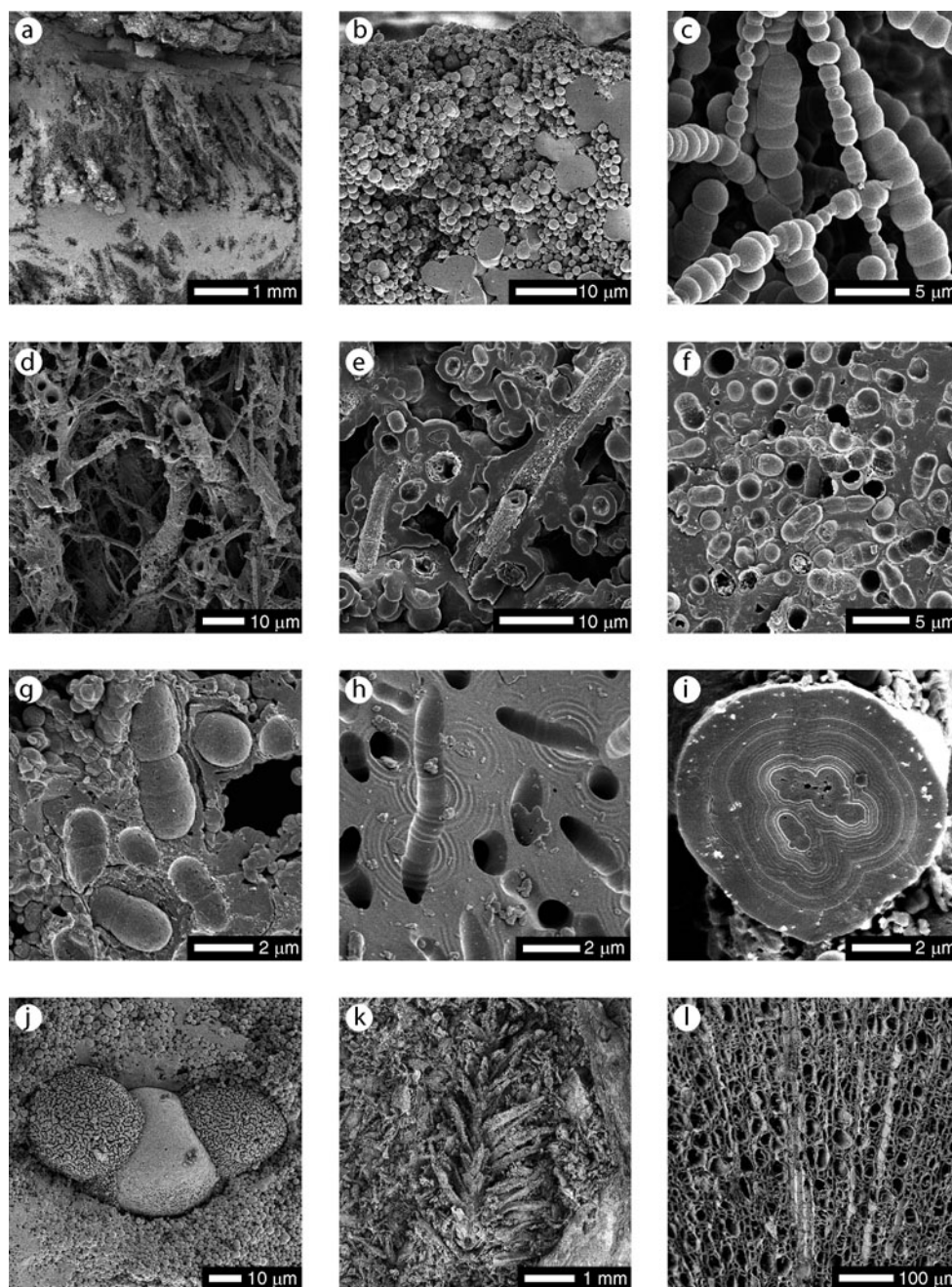


Sinter, Figure 1 Field photographs of sinter deposits. (a) Sinter mound of White Dome Geyser, Yellowstone National Park. (b) Pool-rim dam at Ngararatuatara hot spring, Whakarewarewa, New Zealand. (c) Rimstone dam at El Tatio geothermal field, Chile. Coated grains (oncoids) are strewn on the terrace at the base of the dam. (d) Sinter terraces covered by cyanobacterial mats on the discharge apron of Iodine Pool geyser, Waimangu, New Zealand. (e) Geyserite deposits around the vents of Anemone Geyser, Yellowstone National Park. (f) Sinter miniterraces on the surface of Primrose Terrace, Waiotapu, New Zealand. (g) Ornate geyserite deposits near a geyser vent at El Tatio geothermal field, Chile. (h) Thin platy sinter precipitated by acidic waters on the southern shore of Hot Pools Lake, Rotokawa, New Zealand. The sinter, which was probably precipitated by capillary processes, shows desiccation(?) cracks partly filled by silica cements.

extracellular polymeric substances (EPS) (Figure 2). Near spring vents and on many terraces where waters have cooled to $<70^{\circ}\text{C}$, microbial mats commonly cover the substrate and can become encrusted and (or) replaced by opaline silica. Bunches of current-aligned, partially silicified, filamentous cyanobacteria (stringers) are present in some channels. If hydrothermal discharge is ephemeral and the terrace surfaces dry out periodically, microbial mats may be absent. However, biofilms, some thinly

(millimeter thick) coated by opaline silica, and endoliths may still be present either at or just below the terrace surface, including sites very close to active vents.

Microbial fossils are preserved in most settings from vent to distal discharge apron. In waters $>75^{\circ}\text{C}$, nonphotosynthetic prokaryotes, including heterotrophic and chemolithotrophic bacteria and archaea, are present, although their silicified remains are often difficult to identify. Cyanobacteria and photosynthetic bacteria



Sinter, Figure 2 SEM photomicrographs of silicified biota in sinters from New Zealand (a–j) and Iceland (k, l). (a) Laminae formed of low-porosity opal-A alternating with laminae formed of (sub)erect filamentous microbes. (b) Opal-A spheres that formed in water column, settled to floor, and accumulated in pocket between erect microbial filaments. (c) Silicified microbes encased by opal-A spheres of variable diameters. (d) Large-diameter filamentous microbes (probably *Calothrix*) surrounded by small-diameter filaments (probably *Phormidium*). (e) Large-diameter filamentous microbes (probably *Calothrix*) and associated coccoid microbes (probably *Synechococcus*) encased by opal-A. (f) Dense accumulation of *Synechococcus* held in opal-A matrix. (g) Enlarged view of *Synechococcus* showing specimens in process of binary fusion. (h) Naturally etched surface showing microbes in groundmass formed of amalgamated opal-A spheres; etching has highlighted laminae in spheres showing their growth patterns. (i) Transverse cross section through silicified filamentous microbe – natural etching has highlighted the growth laminae in the opal-A. (j) Silicified pollen grain from a pine tree held in matrix of opal-A spheres. (k) Silicified bryophyte. (l) Transverse cross section through silicified twig showing perfect preservation of cells. Sample locations: a–c, h–j from discharge apron of Waikite Geyser, Whakarewarewa geothermal area, New Zealand; d–g from geyser system at Tokaanu, New Zealand; k and l from discharge apron of Geysir, Haukadalur, Iceland.

commonly inhabit waters from ~ 65 – 75°C (e.g., *Synechococcus*, *Chloroflexus*), with a wider range of cyanobacteria at temperatures $<65^{\circ}\text{C}$ (e.g., *Phormidium*, *Spirulina*, *Anabaena*, *Oscillatoria*, *Calothrix*, and others). Eukaryotic algae colonize substrates at temperatures $<60^{\circ}\text{C}$ and diatoms at $<45^{\circ}\text{C}$. Diatoms, fungi, and *Cyanidium* spp. are often the dominant fossil microbes in sinters precipitated from acidic fluids. Macrophytes, including grasses, wetland species, and trees and their root systems, may also be submerged by hot spring fluids and encrusted or replaced by amorphous silica.

Mineralogy and petrology

Most sinter is precipitated as noncrystalline opaline silica (opal-A: $\text{SiO}_2 \cdot n\text{H}_2\text{O}$) that can contain up to ~ 20 wt.% H_2O ; less commonly, chalcedony is a primary mineral. Although sinters precipitated from neutral and alkaline fluids are commonly monomineralic, silica precipitated from acidic fluids is commonly accompanied by kaolinite, ferric oxyhydroxides, alunite, jarosite, pyrite, and gypsum.

Sinters exhibit a wide range of textures and fabrics. Geyserites tend to be laminated, columnar, or stromatolitic, and generally have low primary porosity. Sintors that form distally from the vent are more diverse and include bedded and laminated facies that form on terraces, commonly with in situ silicified microbial mats, fenestral porosity, and intraclasts reworked from silicified mats upslope. Early cementation by opaline silica commonly masks the original fabrics, producing apparently massive sinter, but slight differences in the water content of the precipitated opal-A can help differentiate primary textures and cements (Jones and Renaut, 2004).

During diagenesis, the opal-A transforms to opal-CT, moganite, and ultimately quartz. The opal-A to opal-CT transformation can be rapid (Lynne et al., 2006; Jones and Renaut, 2007) and proceeds mainly by dissolution–reprecipitation reactions.

Origin of sinter

Silica solubility increases with increasing temperature, pH, and pressure, and can be influenced by other factors such as the presence of certain ions (e.g., Al, Fe) and salinity. Most silica precipitation at hot springs has been attributed to the cooling of thermal fluids that have equilibrated with respect to quartz, chalcedony, or volcanic glass at depth, and then rise rapidly to the surface, emerging as hot water supersaturated with respect to amorphous silica (White et al., 1956; Rimstidt and Cole, 1983; Fournier, 1985).

Much of the dissolved silica is present in its monomeric form (H_4SiO_4). As saturation increases, silica polymerization occurs through reactions that involve linear, cyclic, and three-dimensional forms of polymeric silica (Iler, 1979). In strongly supersaturated fluids, including some spring pools, nuclei grow by Ostwald ripening into nanospheres and microspheres (10 nm–1 μm) and may

form colloidal particle suspensions (sols). Continued growth of spheres can destabilize the sols and fine sediment settles from suspension. If the pH falls or the electrolyte concentrations increase (e.g., by evaporation), suspended particles may flocculate, forming aggregates that eventually sink to the substrate. In both cases, the silica sediment then undergoes cementation to form sinter. At sites of evaporative concentration, microspheres may coagulate to form gels. In contrast, at relatively low levels of supersaturation, dissolved silica precipitates mainly by heterogeneous nucleation upon existing substrates or suspended particles.

Evaporative concentration can induce silica precipitation at hot springs in semiarid and arid regions (e.g., Kenya Rift), although evaporation is usually less effective than cooling in humid environments. However, evaporation is an important mechanism on subaerial sites of periodic wetting including outflow terraces between geyser eruptions and sites receiving airborne spray. Seasonal freezing of geothermal outflow can also induce opal-A precipitation and agglomeration by “cryogelling,” which concentrates silica in residual fluid as ice is formed (Channing and Butler, 2007).

The pH and salinity also affect amorphous silica solubility. Nucleation and polymerization are inhibited in acidic waters because monomeric silica is slow to deprotonate (Fournier, 1985; Mountain et al., 2003), which explains the thin sinter development around acid hot springs. At $\text{pH} > \sim 9$, silica dissociates into other species, increasing the solubility. A rapid decrease in pH of a highly alkaline, silica-saturated thermal fluid might induce amorphous silica to precipitate on contact with neutral lake waters or groundwaters or if the fluid is acidified by microbes.

The waters that flow from most geysers and many hot springs in the geothermal fields of Iceland, New Zealand, Kamchatka, Chile, and Yellowstone, are strongly supersaturated with respect to amorphous silica so should precipitate silica abiotically upon rapid atmospheric cooling. Physical processes control the gross morphology of most sinter deposits, whereas chemical processes control the silica precipitation mechanisms. There has been much debate about whether or not microbes mediate silica precipitation and silicification, particularly in waters where homogeneous nucleation does not occur. Microbes can provide reactive surface ligands that adsorb aqueous silica from solution, and should therefore promote heterogeneous nucleation by reducing activation energy barriers. Three main mechanisms have been proposed: hydrogen bonding, cation bridging by metals (especially Fe), and electrostatic interactions (Phoenix et al., 2003; Lalonde et al., 2005; Konhauser, 2007, pp. 156–160). Although consensus has not yet been attained, most recent field studies (e.g., Jones et al., 2004) and laboratory experiments (e.g., Fein et al., 2002; Yee et al., 2003) suggest that the role of microbes in silica precipitation is largely incidental, especially in strongly supersaturated fluids. At lower levels of supersaturation, they may provide organic

substrates for heterogeneous nucleation. Bacteria (including cyanobacteria), however, control microfabric development in many types of sinter, over a wide range of scales and fluid temperatures (Jones and Renaut, 2003).

Fossil sinter

Fossil sinters, also termed paleosinters, are increasingly being recognized in the geological record. They commonly form “caprocks” to some epithermal mineral deposits (e.g., Guido et al., 2002) but are rarely a target of study. Two exceptions are the early Devonian Rhynie and Windyfield cherts of Aberdeenshire, Scotland (Trewin, 1994), and the Devonian–Carboniferous sinters of the Drummond Basin, Australia (Walter et al., 1996).

Conclusions

By mass, sinters are trivial compared to most sedimentary rocks, but their rarity belies their importance. Hot spring sinters form in one of the few environments where silica precipitation and silicification processes can be studied at the Earth’s surface, another environment being highly alkaline lakes. Consequently, they will remain important in studying how microbes and plants are silicified, both in modern environments and the geological record.

Bibliography

- Allen, E. T., 1934. The agency of algae in the deposition of travertine and silica from thermal waters. *American Journal of Science*, **27**, 373–389.
- Cady, S. L., and Farmer, J. D., 1996. Fossilization processes in siliceous thermal springs: trends in preservation along thermal gradients. In Bock, G. R., and Goode, J. A. (eds.), *Evolution of Hydrothermal Ecosystems on Earth (and Mars?)*. Ciba Foundation Symposium no. 202, Chichester, UK: Wiley, pp. 150–173.
- Channing, A., and Butler, I. B., 2007. Cryogenic opal-A deposition from Yellowstone hot springs. *Earth and Planetary Science Letters*, **257**, 121–131.
- Fein, J. B., Scott, S., and Rivera, N., 2002. The effect of Fe on Si adsorption by *Bacillus subtilis* cell walls: insights into the non-metabolic bacterial precipitation of silicate minerals. *Chemical Geology*, **182**, 265–273.
- Fournier, R. O., 1985. The behavior of silica in hydrothermal solutions. In Berger, B. R., and Bethke, P. M. (eds.), *Geology and Geochemistry of Epithermal Systems*, Reviews in Economic Geology. El Paso, TX: Society of Economic Geologists, Vol. 2, pp. 45–61.
- Guido, D., de Barrio, R., and Schalamuk, I., 2002. La Marciana Jurassic sinter—implications for exploration for epithermal precious-metal deposits in Deseado Massif, southern Patagonia, Argentina. *Applied Earth Science*, **111**, 106–113.
- Iler, R. K., 1979. *The Chemistry of Silica: Solubility, Polymerization, Colloid and Surface Properties, and Biochemistry*. New York: Wiley-Interscience.
- Jones, B., and Renaut, R. W., 1996. Influence of thermophilic bacteria on calcite and silica precipitation in hot springs with water temperatures above 90°C: evidence from Kenya and New Zealand. *Canadian Journal of Earth Sciences*, **33**, 72–83.
- Jones, B., and Renaut, R. W., 2003. Hot spring and geyser sinters: the integrated product of precipitation, replacement, and deposition. *Canadian Journal of Earth Sciences*, **40**, 1549–1569.

- Jones, B., and Renaut, R. W., 2004. Water content of opal-A: implications for the origin of laminae in geyserite and sinter. *Journal of Sedimentary Research*, **74**, 117–128.
- Jones, B., and Renaut, R. W., 2007. Microstructural changes accompanying the opal-A to opal-CT transition: new evidence from the siliceous sinters of Geysir, Haukadalur, Iceland. *Sedimentology*, **54**, 921–948.
- Jones, B., Konhauser, K. O., Renaut, R. W., and Wheeler, R., 2004. Microbial silicification in Iodine Pool, Waimangu geothermal area, North Island, New Zealand: implications for recognition and identification of ancient silicified microbes. *Journal of the Geological Society, London*, **161**, 983–993.
- Konhauser, K. O., 2007. *Introduction to Geomicrobiology*. Oxford: Blackwell.
- Konhauser, K. O., Phoenix, V. R., Bottrell, S. H., Adams, D. G., and Head, I. M., 2001. Microbial–silica interactions in Icelandic hot spring sinter: possible analogues for some Precambrian siliceous stromatolites. *Sedimentology*, **48**, 415–433.
- Krumbein, W. E., and Werner, D., 1983. The microbial silica cycle. In Krumbein, W. E. (ed.), *Microbial Geochemistry*. London: Blackwell, pp. 125–157.
- Lalonde, S. V., Konhauser, K. O., Reysenbach, A. L., and Ferris, F. G., 2005. The experimental silicification of Aquificales and their role in hot spring sinter formation. *Geobiology*, **3**, 41–52.
- Lynne, B. Y., Campbell, K. A., Perry, R. S., Moore, J., and Browne, P. R. L., 2006. Acceleration of sinter diagenesis in an active fumarole, Taupo Volcanic Zone, New Zealand. *Geology*, **34**, 749–752.
- Mountain, B. W., Benning, L. G., and Boerema, J., 2003. Experimental studies on New Zealand hot spring sinters: rates of growth and trace metal incorporation. *Canadian Journal of Earth Sciences*, **40**, 1643–1667.
- Phoenix, V. R., Konhauser, K. O., and Ferris, F. G., 2003. Experimental study of iron and silica immobilization by bacteria in mixed Fe–Si systems: implications for microbial silicification in hot springs. *Canadian Journal of Earth Sciences*, **40**, 1669–1678.
- Rimstidt, J. D., and Cole, D. R., 1983. Geothermal mineralization I: the mechanism of formation of the Beowawe, Nevada, siliceous sinter deposit. *American Journal of Science*, **283**, 861–875.
- Trewin, N. H., 1994. Depositional environments and preservation of biota in the Lower Devonian hot springs of Rhynie, Aberdeenshire, Scotland. *Philosophical Transactions of the Royal Society of Edinburgh, Earth Sciences*, **84**, 433–442.
- Walter, M. R., 1976. Geyserites of Yellowstone National Park: an example of abiogenic “stromatolites”. In Walter, M. R. (ed.), *Stromatolites*. Amsterdam: Elsevier, pp. 87–112.
- Walter, M. R., Des Marais, D., Farmer, J. D., and Hinman, N. W., 1996. Lithofacies and biofacies of mid-Paleozoic thermal spring deposits in the Drummond basin, Queensland, Australia. *Palaaios*, **11**, 497–518.
- Weed, W. H., 1889. Formation of travertine and siliceous sinter by the vegetation of hot springs. *US Geological Survey 9th Annual Report (for 1887–1888)*, pp. 613–676.
- White, D. E., Brannock, W. W., and Murata, K. J., 1956. Silica in hot-spring waters. *Geochimica et Cosmochimica Acta*, **10**, 27–59.
- Yee, N., Phoenix, V. R., Konhauser, K. O., Benning, L. G., and Ferris, F. G., 2003. The effect of cyanobacteria on silica precipitation at neutral pH: implications for bacterial silicification in geothermal hot springs. *Chemical Geology*, **199**, 83–90.

Cross-references

Cherts
 Hot Springs and Geysers
 Hydrothermal Environments, Fossil
 Hydrothermal Environments, Terrestrial
 Silica Biomineralization, Sponges

SKELETON

Biologically, a skeleton is a hard or rigid framework that could provide protection and support in many types of organisms. Most of skeletons are mineralized, but some may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, not including hydroskeletons. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites. Endoskeletons are internal hard parts (typical of many vertebrates). Most of endoskeletons are generally surrounded by skin and musculature. See entry "[Animal Skeletons, Advent](#)" for further reading.

SMALL SHELLY FOSSILS

Small shelly fossils (SSF, also "Small Shelly Fauna") is a non-taxonomic term, first used by Matthews and Missarzhevsky in the title of their review paper in 1975, to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites. See entries "[Animal Skeletons, Advent](#)" and "[Critical Intervals in Earth History](#)" for further reading.

SNOWBALL EARTH

Paul F. Hoffman
Harvard University, Cambridge, MA, USA

Synonyms

Cryochron; Global glaciation; Hard snowball; Ice-albedo catastrophe; Snowball (in geology); White Earth

Definition

Snowball Earth is a climate in which the oceans and most land areas are permanently covered by glacial ice. As less sunlight is absorbed by ice than by water or land, a critical area of ice will cause surface temperatures to fall below freezing everywhere, resulting in a snowball Earth. This arguably occurred near the beginning and end of the Proterozoic eon. Snowball Earths self-destruct after millions of years due to the buildup of atmospheric carbon dioxide of volcanic and metamorphic origin, which could not be converted into organic matter or limestone. Biological evolution is a postulated cause and consequence of snowball episodes.

History of the concept

Sedimentary deposits of glacial origin were first described from the late Proterozoic (Neoproterozoic) of Scotland in 1871 and from the early Proterozoic (Paleoproterozoic) of Canada in 1907. By 1940, it was apparent that the Neoproterozoic glaciation(s) had been more extreme than those of the Pleistocene and at least equal in severity to the late Paleozoic glaciations of Gondwanaland. In the 1960s, the occurrence of glacial deposits within thick successions of nearly pure carbonate strata suggested that Neoproterozoic ice sheets had entered what were normally the warmest parts of the globe. This was confirmed in the 1980–1990s by reliable paleomagnetic data showing that ice sheets had reached the ocean close to the paleoequator in the Neoproterozoic of Australia and Paleoproterozoic of South Africa (Evans, 2003). Moreover, evidence of marine geochemical anomalies concurrent with glaciation came increasingly to light in the form of sedimentary Fe_2O_3 and MnO_2 ore deposits, large excursions in stable carbon and sulfur isotopic compositions of minerals precipitated from seawater, and distinctive post-glacial "cap" carbonate sediments that rest directly on the terminal glacial deposit at all latitudes (Hoffman and Schrag, 2002). Neoproterozoic glacial deposits are now known from 22 different paleocontinents and microcontinents, and Paleoproterozoic deposits from 6.

The fraction of incoming solar radiation absorbed by ocean water under cloudless skies is 0.9, but for continuous ice it is only 0.4–0.6 and as low as 0.1 if the ice is covered by fresh snow. In the 1950–1960s, calculations by meteorologists of zonal average surface temperatures at different latitudes, assuming radiative energy balance and incorporating crude parameterizations of the radiative effects of clouds, meridional heat transport, and positive feedbacks due to the reflectivity (albedo) of ice, suggested that a lowering of solar luminosity by a few percent would result in a globe-encircling glaciation, or "white" Earth (North, 1990). If the luminosity is incrementally lowered, polar ice caps expand until their edges reach a critical latitude, beyond which ice advances rapidly to the equator without any additional luminosity reduction, or even with increased luminosity. This is due to runaway ice-albedo feedback – more ice reflects more radiation, creating more ice. (The same calculations indicated that a small increase in radiative forcing, due to anthropogenic CO_2 emissions for example, could cause a sudden loss of polar sea ice at a critical $p\text{CO}_2$ threshold.) These sensational findings greatly stimulated the nascent field of climate modeling, but proponents were puzzled how a "white" Earth disaster had been averted given that solar luminosity has increased by 25–30% over geological time (the so-called faint young Sun paradox). They assumed (incorrectly, as it turned out) that such an occurrence would extinguish all life – deep-sea chemoautotrophs and polar extremophiles were then unknown – and would be permanent because of the very large increase (~25%) in radiative forcing required to overwhelm a "white" Earth's high albedo.

As meteorologists assumed that a “white” Earth never occurred, they did not think to consult geologists, who remained oblivious to their findings.

In 1981, planetary scientists (Walker et al., 1981) proposed that Earth’s climate is modulated within the habitable range by a negative climate feedback resulting from the temperature-dependence of silicate-rock weathering. In the geochemical cycle of carbon, this is the means by which atmospheric CO_2 (dissolved in rainwater) is converted into bicarbonate ion (HCO_3^-), which dissolved in runoff makes its way to the ocean, where it is removed from circulation by the burial of carbonate sediments and organic matter. The feedback operates on a geological timescale ($\sim 10^6$ year) and causes the climate system to slowly seek whatever atmospheric CO_2 level, and hence climate, that is needed to balance the rate of CO_2 removal by silicate weathering with the rate of CO_2 emitted by volcanoes and metamorphic reactions. Walker et al. (1981) suggested that this feedback had prevented a “white” Earth disaster but noted that if one did occur, the consequent reduction of weathering rates would lead to an inexorable increase in atmospheric CO_2 as plate tectonics and therefore of CO_2 emissions would continue unabated. With the present emission rate and no CO_2 consumption, $p\text{CO}_2$ would increase to ~ 1.0 bar after 20 Myr. At some critical $p\text{CO}_2$, the “white” Earth would self-destruct violently due to ice-albedo and other positive feedbacks, leaving a transient aftermath in which high $p\text{CO}_2$, low surface albedo, and the recently deglaciated surface would conspire to cause intense weathering, exponentially lowering atmospheric $p\text{CO}_2$. The hysteresis loop in CO_2 versus planetary albedo means that a “white” Earth would be long-lived (10^6 – 10^7 year) but swift to melt (10^3 – 10^4 year), giving rise to a “greenhouse” transient (10^5 – 10^6 year). These are geologically testable predictions. But as the paleomagnetic evidence for global glaciation was not secure until 1986 (Evans, 2000), consideration of a self-reversing “white” Earth as a model for the geological record was delayed.

It was the paleomagnetist Joseph L. Kirschvink who first applied these concepts to low-latitude Neoproterozoic (Kirschvink, 1992) and Paleoproterozoic (Kirschvink et al., 2000) glaciations. He related the synglacial Fe_2O_3 and MnO_2 ore deposits to ocean stagnation and anoxia, caused by ice cover, which allowed the metals to be transported widely in solution in reduced form, and be precipitated upon deglaciation when the ocean was again ventilated. He predicted the global occurrence of sediments recording the abrupt climate switch at glacial terminations, later recognized as cap carbonates (Hoffman et al., 1998b). He speculated that an unusual preponderance of low-latitude continents (a situation that never occurred after 500 Ma) raised the planetary albedo to the critical threshold. He named the phenomenon “snowball” Earth as that is how the globe would appear from outer space. The name tacitly recognizes that the hydrological cycle, although greatly diminished on the cold solid

surface, would still be weakly maintained by sublimation and precipitation, not of snowflakes, but of tiny hexagonal snow prisms due to the global average surface temperature of around -50°C (Furukawa and Wettlaufer, 2007).

Number, age, and duration of snowballs

At least three snowball episodes are inferred from the geological record, two in the Cryogenian Period (~ 850 – 635 Ma) of the Neoproterozoic era ($1,000$ – 551 Ma), and one or more in the first half of the Paleoproterozoic era ($2,500$ – $1,600$ Ma). The 1.5×10^9 -year interval between older and younger snowballs is curiously devoid of glacial deposits. The timing of the youngest snowball is the best constrained and of the oldest the most poorly known. This is primarily a function of differences in their respective areas of preservation. Termination of the youngest snowball occurred in 635 Ma, coincident with the boundary between the Cryogenian and Ediacaran Periods (Knoll et al., 2006b). The age is based on ultra-precise (0.5 Myr uncertainty) U-Pb geochronology of primary zircons from volcanic ash-falls in Namibia and South China (Halverson, 2006). Sections in other regions are correlated with these based on synde glacial “cap” carbonates (see below), which are uniquely distinctive in many areas. The onset of the snowball is loosely constrained. It is younger than 654.5 ± 3.8 Ma in South China, based on ion-microprobe U-Pb geochronology (Zhang et al., 2008). The maximum duration of this snowball was 23 Myr.

The timing of the older Cryogenian snowball is weakly constrained (Halverson, 2006): its onset is younger than 726 ± 1 Ma in Oman and 725 ± 10 Ma in South China, and its termination occurred before 667 ± 5 Ma in the western USA. In Oman, strata within the glacial succession are no older than 713 ± 1 Ma. Interregional correlation of glacial strata is more uncertain than for the younger Cryogenian glaciation. There is fragmentary evidence for an earlier Cryogenian glaciation of presumed regional scale ~ 750 Ma in central and southern Africa, while a mid-Ediacaran glaciation of short duration occurred in eastern Newfoundland, Canada, at 582 Ma (Knoll et al., 2006b), with possible correlatives in western and northern Europe, central Asia, Western Australia, and southwestern Brazil.

Paleoproterozoic glacial deposits in the western Transvaal, South Africa, are interfingering with volcanic lavas erupted near sea level at $11 \pm 5^\circ$ paleolatitude (Evans et al., 1997) approximately 2.22 Ga (10^9 year before present) (Kopp et al., 2005). The duration of the low-latitude glaciation is unknown. Three distinct glaciations occurred between 2.45 and 2.22 Ga in the Huronian succession of Ontario, Canada, and correlative glacial deposits occurred in Wyoming (USA), Quebec (Canada) and possibly Finland (Ojakangas, 1988). The paleolatitudes of these deposits being unknown, they are assumed to represent regional-scale glaciations.

Paleogeographic reconstructions and paleoclimatic indicators in bounding strata

At the time of the Cryogenian snowballs, many continents were in low paleolatitudes and few if any were near the poles (Trindade and Macouin, 2007). This is consistent with cold climates because a preponderance of low-latitude continents maximizes the rate of atmospheric CO₂ consumption by silicate weathering. It was also a time of fragmentation and dispersal of the long-lived (1.0–0.8 Ga) supercontinent Rodinia (Li et al., 2008). Continental fragmentation also enhances silicate weathering by bringing land closer to the source of moisture. Cryogenian paleogeography contrasted with that of the late Paleozoic ice ages, when a supercontinent (Pangea) had just formed and the glaciated lands (Gondwanaland) lay in a polar region. Little is known of early Paleoproterozoic paleogeography, but there are suggestions that they accompanied the fragmentation of land-masses consolidated in the late Archean eon (2.7–2.5 Ga).

Cryogenian glacial deposits are found within thick successions of shallow-water carbonate rocks in Namibia, California, Arctic Alaska, and southern Mongolia (Halverson et al., 2005). This is paradoxical because non-skeletal carbonates form preferentially in the warmest parts of the surface ocean on account of their “reverse” solubility. Throughout Earth history, thick carbonate successions formed only at latitudes less than 35° (Ziegler et al., 1984; Opdyke and Wilkinson, 1990). Their range did not change between warmer and colder climates because their distribution depends on the relative not the absolute temperature. Thus the meridional temperature gradient has never been reversed due to high orbital obliquity (Evans, 2006), as has been proposed to account for low-latitude glaciation (Schmidt and Williams, 1995). If glaciers reached the ocean in the warmest areas of the world, then the colder areas must also have been frozen.

Sedimentology of the glaciogenic deposits

The sedimentology and inferentially the depositional processes of Proterozoic and Phanerozoic glacial deposits are quite similar (Boulton and Deynoux, 1981; Hambrey and Harland, 1981). Those more interested in the rocks themselves than in their distribution have a hard time believing that Proterozoic ice ages were different in character. They range from terrestrial lodgement, moraine and outwash (sandur) complexes to glaciomarine grounding-line wedges and fans, and proglacial suspension fallout with or without sediment gravity flows and ice-rafted debris (Kellerhals and Matter, 2003).

Shaped and striated clasts, subglacial pavements with grooves and chatter marks, and stratigraphic relations suggestive of ice streams (currents of fast-flowing ice within an ice sheet) all attest to dynamic glacial action (Hoffman, 2005). At first it was thought that dynamic ice sheets were inconsistent with a snowball because the frozen ocean could not resupply them with snow. However, climate models suggest that net sublimation of subtropical

sea-ice would supply ice sheets with up to centimeters of ice-equivalent annually, meaning that ice sheets would thicken sufficiently to generate dynamic ice streams within a few 10⁵ year, a fraction of the estimated snowballs duration (Donnadieu et al., 2003; Pollard and Kasting, 2004). Accordingly, the evidence for dynamic ice sheets is not incompatible with a frozen ocean.

On the Otavi carbonate platform of Namibia, the younger Cryogenian glaciation left a continuous grounding-line prism situated 500–1,000 m vertically below the rim of the platform (Hoffman, 2005). This is quite unlike any Quaternary ice grounding line in polar seas, which typically switched between the inner and outer shelf. The grounding-line prism in Namibia is directly overlain by a synde glacial “cap” dolostone (see below) deposited above prevailing wave-base, the only shallow-water deposit at such depth on the slope (Hoffman et al., 2007). This indicates glacioeustatic changes of >500 m, consistent with an average ice-sheet thickness of >1.1 km over all continents. An important implication of this finding is that shelves and platforms were above sea level during most of the glacial interval, and any glaciomarine deposits found there could only have been deposited when the deglaciation was well-advanced. The nature of such deposits has often been cited as inconsistent with snowball conditions (e.g., wave action, phototrophism, ice-rafting, etc.). This is true; by the time they were deposited, the snowball no longer existed.

Associated marine geochemical anomalies

Fe₂O₃- and MnO₂-ore formations

During the Fe-ore exploration boom following World War II, it was learned that the only “banded iron-formations” (BIF) younger than 1.9 Ga are intimately and exclusive associated with Cryogenian glacial deposits (Klein, 2005). Extensive ore-grade deposits of Fe₂O₃ (±MnO₂) were found in SE Australia, SW Brazil, NW Canada, and Namibia. The Australian and Canadian deposits are in the older Cryogenian assemblage, the Brazilian ones are in the younger(?) Cryogenian assemblage, and the Namibian deposits are in both(?). As only reduced Fe(II) is soluble in seawater, Martin (1964) attributed “this peculiar combination of sediments” to “oxygen deficiency in stagnating bottom waters caused by an ice cover.” Canfield and Raiswell (1999) pointed out that decreased continental runoff during a snowball would lower the influx of sulfate to the ocean, which after bacterial sulfate reduction, removes dissolved Fe(II) from seawater in the form of FeS₂ (pyrite). Lowering the influx of sulfate therefore raises the concentration of Fe(II). The large sea-level fall associated with a snowball also raises the Fe:S flux ratio in mid-ocean ridge hydrothermal vent fluids on account of the drop in hydrostatic pressure (Kump and Seyfried, 2005).

Kirschvink (1992) suggested that the dissolved Fe(II) was oxidized to insoluble Fe(III) at snowball terminations, consistent with the stratigraphic position of the Australian

BIF near the top of the glacial sequence. Elsewhere, BIF occurs as sporadic lenses within glaciomarine sequences and Fe oxidation may have been caused by discharging of oxic subglacial meltwater along the grounding lines of ice shelves.

The 2.22-Ga Makganyene glaciation in South Africa ended with outpourings of mafic lava and the deposition of major Fe- and Mn-ore deposits. The latter are the world's largest and imply the presence of abundant molecular O_2 (not required for Fe oxidation) ~ 0.22 Gyr after atmospheric O_2 first rose above 10^{-6} PAL (present atmospheric level) according to mass-independent sulfur isotope fractionation data (Farquhar et al., 2000). The youngest pre-Cryogenian BIFs are limited to collisional (foredeep) basins (1.89 Ga) around the Superior craton of North America, unrelated to glaciation.

"Cap" carbonates

"Cap" carbonates are highly continuous, meter- to decameter-thick layers of relatively pure carbonate lying sharply without significant hiatus on top of the last glacial deposits of a snowball (Hoffman and Schrag, 2002; Shields, 2005). They typically extend far beyond the actual glacial deposits at the same stratigraphic horizon.

The best developed cap carbonates are those associated with the youngest snowball deglaciation in 635 Ma: they are global in extent and sedimentologically unique in the entire stratigraphic column (Allen and Hoffman, 2005; Shields, 2005; Hoffman et al., 2007). They begin with a layer of pale gray, often pinkish, micro- and macropeloidal dolostone ($Ca_{0.5}Mg_{0.5}CO_3$). Its global average thickness is ~ 20 m and it was laid down diachronously, mainly above storm wave-base, during glacioeustatic flooding of continental margins and inland seas (Hoffman et al., 2007). Assuming that strong positive climate feedbacks (e.g., ice albedo, ice elevation, CO_2 degassing, gas-hydrate destabilization) drove snowball deglaciation on timescales < 10 kyr, cap dolostone accumulation rates were > 2 mm year $^{-1}$.

The dolostone contains anomalous sedimentary features such as giant wave ripples (Allen and Hoffman, 2005) and stromatolite biostromes containing strictly vertical tubes of internal sediment with meniscus-like laminae (Corsetti and Grotzinger, 2005). The top of the dolostone layer in some areas is marked by a continuous crust of primary seafloor $BaSO_4$ (barite) cement in the form of digitate rosettes (pseudostromatolites) with internal growth lamellae (Hoffman and Schrag, 2002). The barite crust and other redox-sensitive geochemical (Hurtgen et al., 2006) and biomolecular (Elie et al., 2007) proxies indicate that an oxic-anoxic interface within the water column intersected the seafloor at this horizon. In subsiding areas, the dolostone is overlain by deeper water shales or limestones, locally with layer-upon-layer of seafloor cements in the form of macroscopic crystal fans originally composed of aragonite, the orthorhombic polymorph of $CaCO_3$ (James et al., 2001). In

non-subsiding areas, the dolostone layer ends at a subaerial exposure surface heavily disrupted by products of phreatic and vadose diagenesis (Hoffman and Schrag, 2002; Shields et al., 2007).

The nature and extent of the 635-Ma cap carbonate indicates an ocean in which the mixed layer and thermocline waters were highly oversaturated with respect to $(CaMg)CO_3$ and $CaCO_3$ respectively. Sources of alkalinity responsible for the anomalous saturation state include the weathering of carbonate and silicate rock powder and debris exposed by deglaciation (Higgins and Schrag, 2003; Anderson, 2007), anaerobic oxidation of CH_4 released by melting of permafrost in organic-rich shelf sediments glacioeustatically exposed during the snowball (Kennedy et al., 2001; Jiang et al., 2003), and anaerobic respiration at depth of organic matter produced in surface waters newly exposed to sunlight (Font et al., 2006). It was suggested (Kennedy, 1996; Ridgwell et al., 2003) that the 635-Ma cap carbonate can be explained by analogy with the "coral reef hypothesis" of Quaternary glacial-interglacial cycles (Berger, 1982), in which alkalinity builds up in deep waters during glacial stages because of the loss of shelf area, where carbonate is normally sequestered without passage through corrosive deep waters. This mechanism presupposes continued carbonate production in open ocean surface waters during the glaciation, for which evidence is lacking.

The cap carbonate ending the older Cryogenian snowball is different in character but comparable in thickness to the one just described, as well as in its sharp basal contact. Lithologically, it is usually a dark, fetid, limestone or dolostone, locally rhodochrosite ($MnCO_3$), deposited below storm wave-base. Former aragonite seafloor cements are prominent in some areas, notably in eastern Brazil (Vieira et al., 2007). In Namibia, there are hundreds of meters of continuous sublittoral microbialaminates with distinctive roll-up structures (Hoffman et al., 1998a). Missing altogether is the transgressive cap dolostone characteristic of the 635-Ma deglaciation. Carbonate production evidently did not begin until after deglaciation was complete, suggesting a weaker alkalinity flux into the ocean or a lower saturation state when the deglaciation began.

Cap carbonates a few centimeters to decimeters thick occur locally above the mid-Ediacaran (582 Ma) and early Cryogenian (~ 750 Ma) glacial deposits, believed to represent regional-scale glaciations.

In the Paleoproterozoic, the middle Huronian glacial formation has an impure cap limestone up to 17 m thick, the only carbonate unit in a terrigenous clastic succession up to 12 km thick (Bekker et al., 2005). The low-latitude Makganyene glaciation (~ 2.22 Ga) in South Africa has a possible cap carbonate (Mooidrai Formation) known only from subsurface bore holes (Kirschvink et al., 2000).

Post-glacial isotopic anomalies

Cap carbonates are typically depleted in ^{13}C (i.e., $\delta^{13}C < 0.0\text{‰}$ PDB) and detailed studies of expanded

sections of the 635-Ma cap carbonate in different areas show large negative isotopic excursions (Halverson et al., 2005). Excursions are deviations over time in a positive (higher $\delta^{13}\text{C}$) or negative direction, followed by a return to previous values. The excursion begins $\sim 0\text{‰}$ at the base of the oldest (deepest) cap dolostone, descends through the cap dolostone to a low of -6‰ in the basal limestones (after a $\sim 1.5\text{‰}$ step-function across the dolostone–limestone transition), and then rises slowly back to $\sim 0\text{‰}$. Its stratigraphic thickness depends on the sedimentation rate: on the Otavi platform, Namibia, it is ~ 400 m thick but only ~ 50 m thick on the deeper slope sections (formerly the ice grounding-line zone). The origin of the excursion is disputed (e.g., Kennedy et al., 2001; Higgins and Schrag, 2003).

The same cap carbonate hosts negative excursions in $\delta^{11}\text{B}$, interpreted as an expression of greatly elevated $p\text{CO}_2$ (Kasemann et al., 2005), and $\delta^{34}\text{S}$ of carbonate-associated sulfate (CAS), interpreted as oxidized sulfidic snowball waters (Hurtgen et al., 2006). The negative $\delta^{34}\text{S}$ excursion is followed by a positive one with lows and highs of 15 and 45‰, respectively. The positive values (the highest in Earth history) are attributed by a rapid increase in isotopic fractionation between sedimentary sulfate and pyrite, without a compensatory decline in the fractional burial rate of pyrite (Halverson and Hurtgen, 2007).

The older Cryogenian cap carbonate preserves only the rising leg of the post-glacial isotopic excursions, reflecting stratigraphic truncation (non-deposition) during glacioeustatic flooding. Surface waters became strongly enriched ($\delta^{13}\text{C} > 4\text{‰}$) again soon after carbonate production resumed (Yoshioka et al., 2003). Paired negative and positive $\delta^{34}\text{S}_{\text{CAS}}$ excursions resemble those above the younger snowball but data are fewer (Hurtgen et al., 2002).

The two known Paleoproterozoic cap carbonates are also depleted in ^{13}C (Kirschvink et al., 2000; Bekker et al., 2005), but lack local isotopic context as they occur in clastic-dominated successions. A major positive $\delta^{13}\text{C}$ excursion (Lomagundi excursion) appears to begin before age-equivalents of the Makganyene glaciation in North America (Bekker et al., 2006).

Pre-glacial isotopic anomalies

Both Cryogenian snowballs were preceded by long ($>10^7$ year) intervals of consistent isotopic enrichment ($\delta^{13}\text{C} \geq 5\text{‰}$ PDB), followed by Myr-long excursions to much depleted values ($\delta^{13}\text{C} \leq -5\text{‰}$), variably truncated by subglacial erosion. There is no accompanying $\delta^{34}\text{S}$ excursion. The excursions largely predate sea-level falls due to ice-sheet growth. Schrag et al. (2002) suggest that the prolonged isotopic enrichment reflects high fractional organic burial due to rapid sediment burial and expanded suboxic zones on preponderantly low-latitude continental margins. They propose that this situation ultimately led to a slow rise in atmospheric CH_4 due to methanogenesis in

organic-rich sediments. The rise in CH_4 is held responsible for the negative $\delta^{13}\text{C}$ excursion (Halverson et al., 2002). Silicate weathering feedback would lower $p\text{CO}_2$ in response to a slow rise in CH_4 , causing the climate to become more unstable because of its greater sensitivity to CH_4 concentration and the absence of buffering of its concentration by the ocean. Greater stochastic variability in a cold climate increases the risk of exceeding the critical threshold for runaway ice-albedo feedback. Unusual stromatolite and oolite development during the pre-glacial negative $\delta^{13}\text{C}$ excursions could reflect a rise in pH caused by the proposed lowering of $p\text{CO}_2$. The methane substitution model (Schrag et al., 2002) may not be viable, however, because it depends on an unrealistic residence time for CH_4 in the Cryogenian atmosphere of $>10^5$ year. Unless their timing around 1 Myr before the onsets of both Cryogenian snowballs is a coincidence, or merely wrong, the negative $\delta^{13}\text{C}$ excursions should have some bearing on snowball causation.

Climate modeling

Climate models are used to simulate the conditions under which a snowball could initiate, develop over time, and deglaciate. Ideally, an atmospheric general circulation model (AGCM) would be coupled to a dynamic ocean model, a dynamic ice-sheet model, a dynamic sea-ice model, and an interactive model of the geochemical cycle of carbon. In reality, this is computationally unattainable in the foreseeable future. To begin with, full-blown AGCMs are typically run to equilibrium over ~ 100 model years, which are ~ 4 orders of magnitude less than the adjustment time for the geochemical carbon cycle. Therefore, atmospheric CO_2 concentrations are either prescribed at various levels, providing “snapshots” under arbitrary conditions, or else a greatly simplified AGCM must be employed.

Snowball climate models must be adjusted for ambient Proterozoic conditions. Solar luminosity was $\sim 7\%$ lower than present in the Cryogenian and 16–20% lower at 2.22 Ga (Crowley and North, 1991). The Earth’s rotation rate was $\sim 10\%$ higher in the Cryogenian (Williams, 2000) and faster by an unknown amount in the Paleoproterozoic. And Proterozoic continents had higher albedos in the absence of forest cover. These factors all tend to lower the global mean surface temperature and would have resulted in compensatory higher levels of atmospheric CO_2 (Walker et al., 1981).

Modeling suggests that while snowball initiation is resisted by ocean dynamics (Bendtsen, 2002; Poulsen and Jacob, 2004), it is assisted by atmospheric Hadley convection (Bendtsen, 2002), flowage of ice-shelf ice (Goodman and Pierrehumbert, 2003), and wind-driven sea ice (Lewis et al., 2007). Both the thickness of tropical sea ice and the CO_2 level required for deglaciation are highly sensitive to the prescribed ice albedos (Warren et al., 2002; Pollard and Kasting, 2005; Lewis et al., 2006; Le Hir et al., 2007). The role of clouds is complex

and is a major source of uncertainty in the models (Pierrehumbert, 2005).

Theories of causation

In addition to the methane substitution model described above, a number of other astronomical, oceanographic, geodynamic, and biological theories have been proposed to account for low latitude glaciation. Astronomical theories include solar variations (Harland, 1964), large orbital obliquity (Williams, 1975), ice-ring collapses (Sheldon, 1984), impact ejecta (Bendtsen and Bjerrum, 2002; Fawcett and Boslough, 2002), and passage through giant molecular clouds (Pavlov et al., 2005). The large obliquity hypothesis is the most testable and contested of these models (Néron de Surgy and Laskar, 1997; Williams et al., 1998; Pais et al., 1999; Maloof et al., 2002; Levrard and Laskar, 2003). Oceanographic theories include enhanced carbonate burial (Roberts, 1971), intermittent ocean stagnation (Kaufman et al., 1993; Grotzinger and Knoll, 1995), glacioeustatic hypsometry change (Ridgwell et al., 2003), and enhanced organic burial (Kaufman et al., 1997). Geodynamic theories involve continental distribution (Kirschvink, 1992; Schrag et al., 2002), supercontinent breakup (Donnadieu et al., 2004), flood-basalt weathering (Goddéris et al., 2003), and true polar wander (Li et al., 2004). Biological theories include the evolution of biocatalyzed weathering (Carver and Vardavas, 1994), methane destruction (Pavlov et al., 2000, 2003; Catling et al., 2001; Claire et al., 2006), methane substitution (Halverson et al., 2002; Schrag et al., 2002), and the evolution of oxygenic photosynthesis (Kopp et al., 2005). As the ultimate cause of the Quaternary ice ages remains obscure, it is not surprising that the “why” question for Proterozoic snowballs is undecided. Different snowball may have had different causes, but whatever their cause, it was an uncommon occurrence.

Snowballs and the history of oxygenation

There is increasing evidence for stepwise increases in environmental oxygenation near the beginning and end of the Proterozoic eon, roughly contemporaneous with the inferred snowballs. This is the empirical motivation for causative theories involving the oxidative destruction of reduced “greenhouse” gases (Pavlov et al., 2000, 2003; Catling et al., 2001; Kopp et al., 2005; Claire et al., 2006). To make a climatic impact, changes in oxygenation must occur rapidly ($<10^6$ year), or else silicate weathering feedback will cause compensatory adjustments in $p\text{CO}_2$, largely nullifying the effect on radiative forcing.

Mass-independent sulfur isotope fractionation data indicate that atmospheric O_2 concentrations remained below 10^{-6} PAL (present atmospheric level) until ~ 2.45 Ga, close to the first Huronian glaciation and ~ 230 Myr before the Makganyene snowball. The same data suggest the possibility that O_2 remained very low

(but $>10^{-6}$ PAL) during the intervening interval. However, deposition of the succeeding Kalahari MnO_2 -ore formation required at least transient O_2 concentrations >5 orders of magnitude higher than this, possibly caused by a photosynthetic “bloom” and resultant organic burial in the snowball aftermath (Kirschvink et al., 2000) or by H_2O_2 (hydrogen peroxide) released by the melting of snowball ice (Liang et al., 2006).

There is increasing evidence that the deep oceans remained anoxic, but sulfidic not ferrous, during most of the 1.5 Gyr ice-age gap between the Makganyene and the Cryogenian snowballs (Scott et al., 2008). Mass-dependent S-isotope data imply that seawater sulfate concentrations remained low (<4 mM) between the Cryogenian snowballs (Hurtgen et al., 2002), but rose rapidly to near modern (29 mM) levels in the early Ediacaran (Halverson and Hurtgen, 2007). This rise in marine sulfate, presumably caused by increased atmospheric $p\text{O}_2$, appears to follow rather than lead the end-Cryogenian snowball. However, the response time between oxygenation and methane destruction is much shorter than between oxygenation and growth of the marine sulfate reservoir, so an increase in oxygenation to trigger the snowball is not ruled out by these data.

Implications for evolutionary biology

Eukaryotic survival

The younger Phanerozoic glaciations had little impact on the course of biological evolution as measured by rates of origination or extinction of taxa: only the Hirnantian (latest Ordovician) glaciation ~ 445 Ma was accompanied by major mass extinctions. Snowballs, however, presented qualitatively different challenges to existing biota, if they actually occurred. For millions of years, mean annual surface temperatures at the equator would have resembled those of Antarctica today. Areas where sunlight and liquid water coexisted were severely limited, hot springs and zones of perpetual crack development where mobile sea ice encountered fixed rocky substrates. With permanent ice cover, seawater would be starved of oxygen, becoming anoxic due to sinks for oxygen in the ocean. It would become progressively more acidic due to submarine volcanic CO_2 emissions and air-sea gas exchange through cracks at rates lower than the oxygen consumption rate. The geochemical response of the ocean to these afflictions is complex, but seawater pH would probably be lowered by 2.0–2.5 pH units (Le Hir et al., 2008). Deep-sea vent biota would be adversely affected by reduced Eh and pH gradients between hydrothermal fluids and seawater. With low overall productivity, nutrient levels would be high, except for fixed nitrogen due to denitrification. Upon deglaciation, a “bloom” of primary producers would occur, contingent upon nitrate availability. The bloom might be delayed because meltwater injection and surface warming would combine to produce an unusually stable density stratification (Shields, 2005), temporarily suppressing nutrient-upwelling from depth. A 635-Ma

cap carbonate in southwestern Brazil preserves a molecular record of this bloom in the form of an unusual assemblage of biomarkers pointing to a low-diversity eukaryotic flora dominated by red algae and a prokaryotic biota rich in green sulfur bacteria (Elie et al., 2007). The latter imply anoxic waters at the base of the photic zone and the former are consistent with low levels of illumination as exists, for example, in a turbid water column.

No biologist doubts that prokaryotic life, bacteria and archaea, could survive a snowball cryochron and its aftermath. Some do question if eukaryotic algae could survive (but see Vincent et al., 2004). As several extant clades of eukaryotic algae evolved before the Cryogenian, their survival causes some to doubt the existence of snowballs (Knoll, 2003). This is the attraction of more benign but less physically plausible climate states like “slushball” Earth, wherein continents are glaciated at all latitudes (satisfying geological observations) but half of the ocean remains ice-free (Peltier et al., 2004). However, the slushball solution provides no satisfactory explanation for synglacial Fe- and Mn-ore deposits (Klein, 2005), for cap carbonates (Pollard and Kasting, 2005) or for the multimillion-year duration of Cryogenian glaciations inferred from stratigraphic subsidence analysis (Hoffman and Schrag, 2002).

The only fossil record that is sufficiently continuous to bear directly on the evolutionary impact of snowballs is the record of organic-walled, acid-resistant microfossils representing a variety of eukaryotic algae, protists, and possibly fungi (Knoll et al., 2006a). Their Proterozoic record is characterized by extremely low rates of turnover (extinctions and originations) of taxa, roughly 100× slower than in the Ediacaran and Phanerozoic, following the last snowball (Peterson et al., 2005). The sparser macrofossil record is also consistent with slow turnover. Taxonomic diversity drops precipitously during the interval that includes the Cryogenian snowballs (Knoll et al., 2006a), with the inevitable result that it is little studied by palynologists. There have been no systematic studies to determine if the interval of very low diversity actually begins at the first Cryogenian snowball. The microfossil record effectively starts only after the Paleoproterozoic snowball (Knoll, 2003).

Evolutionary innovation

Since the mid-twentieth century, geologists have speculated that Neoproterozoic glaciation and its aftermath was somehow responsible for the advent of multicellular animals, the first fossils of which were known to occur not too far above the glacial deposits in many areas (Harland and Rudwick, 1964). (Note this was not the advent of multicellularity *per se*, which occurred earlier in algae (Knoll, 2003).) In the 1990s, with improvements of radiometric dating, this connection appeared less likely: the canonical Cambrian “explosion” in diversity of animal taxa with hard parts did not occur until ~530–520 Ma and

the oldest macrofossils arguably attributable to metazoans, the enigmatic Ediacara biota, appear ~580 Ma. This is >100 Myr and 55 Myr, respectively, after the last snowball ended in 635 Ma. In contrast, molecular divergence in modern animals, calibrated by known branching points in the geological record (so-called molecular “clocks”), gave estimates of animal origins much older than the Cryogenian snowballs.

However, recent evidence from microfossils and molecular fossils appears to put the connection back in play once again. In South China, remarkable cysts enclosing metazoan diapause eggs and embryos extend down to strata deposited within ~3 Myr (632 Ma) of the last snowball termination in the same area (Yin et al., 2007). In Oman, molecular fossils (24-isopropylcholesterol) believed to be diagnostic of sponges are found down to and even within correlative glacial deposits (Love et al., 2009). In addition, new molecular “clock” analyses place the origin of eumetazoa more in line with the new fossil evidence (Peterson and Butterfield, 2005), while the molecular signature of animal radiation indicates a geologically instantaneous event (Rokas et al., 2005).

How might the environmental stress of a snowball and its aftermath have contributed to revolutionary biological innovation? One idea involves the dual role of heat-shock protein 90 (HSP90) in suppressing the expression of mutant signal transduction proteins and in promoting the proper folding of stress-damaged proteins (Baker, 2006). Under extreme temperatures, HSP90 is diverted from the former role in favor of the latter, allowing accumulated cryptic mutations to be phenotypically expressed and therefore subject to natural selection. Another possibility is that spatial isolation of biological refugia in a snowball favored the evolution of altruism, a basic requirement for intercellular cooperation, by promoting kin selection and eliminating non-altruistic invaders (Boyle et al., 2007).

The evolutionary impact of the Paleoproterozoic snowball(s) is even more conjectural as there is no detailed fossil evidence for synchronicity. Nevertheless, it has been suggested that horizontal gene transfer between taxa at levels higher than at the species level was triggered by environmental catastrophes, and that these gene exchanges were responsible for such macroevolutionary innovations of possible Paleoproterozoic age as thermophilic and photosynthetic bacteria, and the eukaryotic cell (Hartman, 2002). Molecular fossil evidence for an Archean (pre-snowball) origin for oxygenic photosynthesis and for eukaryotes has been presented (Brocks et al., 1999; Summons et al., 1999); however, more recent studies suggest that the “biomarkers” in question are less phylogenetically diagnostic than earlier believed (Fischer et al., 2005; Kopp et al., 2005; Rashby et al., 2007). Finally, the surprising finding that the highest 18S rRNA gene diversity in extant protists and the richest in ancestral lineages occurs in Arctic communities (Stoeck et al., 2007) suggests the possibility that snowballs figured prominently in their evolution.

Conclusion

Important new evidence for extreme atmospheric carbon dioxide concentrations during and after the Marinoan snowball Earth has recently been obtained from mass-independent oxygen isotope ratios in sulfate minerals (Bao et al., 2008, 2009).

Summary

Virtually all continents were covered by large ice sheets during two discrete intervals of the Cryogenian Period (between 725 and 635 Ma). The multimillion-year duration of the glacial intervals, the deposition of syn-glacial Fe₂O₃ and MnO₂ sedimentary ores in different areas and of post-glacial “cap” carbonates at all latitudes support the hypothesis that the world ocean was also ice-covered. These episodes represent a climate state unknown on Earth since 635 Ma, or in the 1.5 Gyr before 725 Ma. Climate models indicate that the pan-glacial climates, popularly known as snowball Earth, were the result of runaway ice-albedo feedback, and that they were reversed by infra-red radiative forcing due to the accumulation of atmospheric CO₂ of volcanic-metamorphic origin, which could not be removed because of the absence of liquid precipitation when surface temperatures were below freezing everywhere. New microfossil and molecular biomarker evidence opens up the possibility that the origin of multicellular animals was related to these climate shocks. Evidence for glaciation at low paleolatitudes around 2.22 Ga, also associated with sedimentary Fe and Mn ores, suggests the existence of snowball states possibly related to the initial rise of atmospheric O₂.

Bibliography

- Allen, P. A., and Hoffman, P. F., 2005. Extreme winds and waves in the aftermath of a Neoproterozoic glaciation. *Nature*, **433**, 123–127.
- Anderson, S. P., 2007. Biogeochemistry of glacial landscape systems. *Annual Reviews of Earth and Planetary Sciences*, **35**, 375–399.
- Baker, M. E., 2006. The genetic response to snowball Earth: role of HSP90 in the Cambrian explosion. *Geobiology*, **4**, 11–14.
- Bao, H., Lyons, J. R., and Zhou, C., 2008. Triple oxygen isotope evidence for elevated CO₂ levels after a Neoproterozoic glaciation. *Nature*, **452**, 504–506.
- Bao, H., Fairchild, I. J., Wynn, P. M., and Spoetl, C., 2009. Stretching the envelope of past surface environments: Neoproterozoic glacial lakes from Svalbard. *Science*, **323**, 119–122.
- Bekker, A., Kaufman, A. J., Karhu, J. A., and Eriksson, K. A., 2005. Evidence for Paleoproterozoic cap carbonates in North America. *Precambrian Research*, **137**, 167–206.
- Bekker, A., Karhu, J. A., and Kaufman, A. J., 2006. Carbon isotope record for the onset of the Lomagundi carbon isotope excursion in the Great Lakes area, North America. *Precambrian Research*, **148**, 145–180.
- Bendtsen, J., 2002. Climate sensitivity to changes in solar insolation in a simple coupled climate model. *Climate Dynamics*, **18**, 595–609.
- Bendtsen, J., and Bjerrum, C. J., 2002. Vulnerability of climate on Earth to sudden changes in insolation. *Geophysical Research Letters*, **29**, 10.1029/2002GL014829.
- Berger, W. H., 1982. Increase of carbon dioxide in the atmosphere during deglaciation: the coral reef hypothesis. *Naturwissenschaften*, **69**, 87–88.
- Boulton, G. S., and Deynoux, M., 1981. Sedimentation in glacial environments and the identification of tills and tillites in ancient sedimentary sequences. *Precambrian Research*, **15**, 397–422.
- Boyle, R. A., Lenton, T. M., and Williams, H. T. P., 2007. Neoproterozoic ‘snowball Earth’ glaciations and the evolution of altruism. *Geobiology*, **5**(4), 337–349.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of eukaryotes. *Science*, **285**, 1033–1026.
- Canfield, D. E., and Raiswell, R., 1999. The evolution of the sulfur cycle. *American Journal of Science*, **299**, 697–723.
- Carver, J. H., and Vardavas, I. M., 1994. Precambrian glaciations and the evolution of the atmosphere. *Annales Geophysicae*, **12**, 674–682.
- Catling, D. C., Zahnle, K. J., and McKay, C. P., 2001. Biogenic methane, hydrogen escape, and the irreversible oxidation of early Earth. *Science*, **293**, 839–843.
- Claire, M. W., Catling, D. C., and Zahnle, K. J., 2006. Biogeochemical modelling of the rise in atmospheric oxygen. *Geobiology*, **4**, 239–269.
- Corsetti, F. A., and Grotzinger, J. P., 2005. Origin and significance of tube structures in Neoproterozoic post-glacial cap carbonates: example from Noonday Dolomite, Death Valley, United States. *Palaos*, **20**, 348–363.
- Crowley, T. J., and North, G. R., 1991. *Paleoclimatology*. New York: Oxford University Press.
- Donnadieu, Y., Fluteau, F., Ramstein, G., Ritz, C., and Besse, J., 2003. Is there a conflict between the Neoproterozoic glacial deposits and the snowball Earth interpretation: an improved understanding with numerical modeling. *Earth and Planetary Science Letters*, **208**, 101–112.
- Donnadieu, Y., Goddérès, Y., Ramstein, G., Nédélec, A., and Meert, J., 2004. A ‘snowball Earth’ climate triggered by continental break-up through changes in runoff. *Nature*, **428**, 303–306.
- Elie, M., Nogueira, A. C. R., Nédélec, A., Trindade, R. I. F., and Kenig, F., 2007. Biodiversity collapse and red algal bloom in the aftermath of the Marinoan Snowball Earth. *Terra Nova*, **19**, 303–308.
- Evans, D. A. D., 2000. Stratigraphic, geochronological, and paleomagnetic constraints upon the Neoproterozoic climatic paradox. *American Journal of Science*, **300**, 347–433.
- Evans, D. A. D., 2003. A fundamental Precambrian–Phanerozoic shift in earth’s glacial style? *Tectonophysics*, **375**, 353–385.
- Evans, D. A. D., 2006. Proterozoic low orbital obliquity and axial-dipolar geomagnetic field from evaporite palaeolatitudes. *Nature*, **444**, 51–55.
- Evans, D. A., Beukes, N. J., and Kirschvink, J. L., 1997. Low-latitude glaciation in the Palaeoproterozoic era. *Nature*, **386**, 262–266.
- Farquhar, J., Bao, H., and Thieme, M., 2000. Atmospheric influence of Earth’s earliest sulfur cycle. *Science*, **289**, 756–758.
- Fawcett, P. J., and Boslough, M. B. E., 2002. Climatic effects of an impact-induced equatorial debris ring. *Journal of Geophysical Research*, **107**, 10.1029/2001JD001230.
- Fischer, W. W., Summons, R. E., and Pearson, A., 2005. Targeted genomic detection of biosynthetic pathways: anaerobic production of hopanoid biomarkers by a common sedimentary microbe. *Geobiology*, **3**, 33–40.
- Font, E., Nédélec, A., Trindade, R. I. F., Macouin, M., and Charrière, A., 2006. Chemostratigraphy of the Neoproterozoic Mirassol d’Oeste cap dolostones (Mato Grosso, Brazil): an alternative model for Marinoan cap dolostone formation. *Earth and Planetary Science Letters*, **250**, 89–103.

- Furukawa, Y., and Wettlaufer, J. S., 2007. Snow and ice crystals. *Physics Today*, **60**, 70–71.
- Godd  ris, Y., Donnadieu, Y., N  d  lec, A., Dupr  , B., Dessert, C., Grard, A., Ramstein, G., and Francois, L. M., 2003. The Sturtian ‘snowball’ glaciation: fire and ice. *Earth and Planetary Science Letters*, **211**, 1–12.
- Goodman, J., and Pierrehumbert, R. T., 2003. Glacial flow of floating marine ice in ‘Snowball Earth’. *Journal of Geophysical Research*, **108**, (C10): 10.1029/2002JC001471.
- Grotzinger, J. P., and Knoll, A. H., 1995. Anomalous carbonate precipitates: Is the Precambrian the key to the Permian? *Palaaios*, **10**, 578–596.
- Halverson, G. P., 2006. A Neoproterozoic chronology. In Xiao, S., and Kaufman, A. J. (eds.), *Neoproterozoic Geobiology and Paleobiology*. Dordrecht: Springer, pp. 231–271.
- Halverson, G. P., and Hurtgen, M. T., 2007. Ediacaran growth of the marine sulfate reservoir. *Earth and Planetary Science Letters*, **263**, 32–44.
- Halverson, G. P., Hoffman, P. F., Schrag, D. P., and Kaufman, J. A., 2002. A major perturbation of the carbon cycle before the Ghaub glaciation (Neoproterozoic) in Namibia: prelude to snowball Earth? *Geophysics, Geochemistry, Geosystems*, **3**, 10.1029/2001GC000244.
- Halverson, G. P., Hoffman, P. F., Schrag, D. P., Maloof, A. C., and Rice, A. H. N., 2005. Toward a Neoproterozoic composite carbon-isotope record. *Geological Society of America Bulletin*, **117**, 1181–1207.
- Hambrey, M. J., and Harland, W. B., 1981. *Earth’s Pre-Pleistocene Glacial Record*. London: Cambridge University Press.
- Harland, W. B., 1964. Evidence of late Precambrian glaciation and its significance. In Nairn, A. E. M. (ed.), *Problems in Palaeoclimatology*. London: Interscience, pp. 115–118.
- Harland, W. B., and Rudwick, M. J. S., 1964. The great infra-Cambrian ice age. *Scientific American*, **211**(2), 28–36.
- Hartman, H., 2002. Macroevolution, catastrophe and horizontal transfer. In Syvanen, M., and Kado, C. I. (eds.), *Horizontal Gene Transfer*, 2nd edn. San Diego: Academic, pp. 411–415.
- Higgins, J. A., and Schrag, D. P., 2003. Aftermath of a snowball Earth. *Geophysics, Geochemistry, Geosystems*, **4**, 10.1029/2002GC000403.
- Hoffman, P. F., 2005. On Cryogenian (Neoproterozoic) ice-sheet dynamics and the limitations of the glacial sedimentary record. *South African Journal of Geology*, **108**, 557–576.
- Hoffman, P. F., and Schrag, D. P., 2002. The snowball Earth hypothesis: testing the limits of global change. *Terra Nova*, **14**, 129–155.
- Hoffman, P. F., Kaufman, J. A., and Halverson, G. P., 1998a. Comings and goings of global glaciations on a Neoproterozoic carbonate platform in Namibia. *GSA Today*, **8**, 1–9.
- Hoffman, P. F., Kaufman, J. A., Halverson, G. P., and Schrag, D. P., 1998b. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Hoffman, P. F., Halverson, G. P., Domack, E. W., Husson, J. M., Higgins, J. A., and Schrag, D. P., 2007. Are basal Ediacaran (635 Ma) post-glacial ‘cap dolostones diachronous? *Earth and Planetary Science Letters*, **258**, 114–131.
- Hurtgen, M. T., Arthur, M. A., Suits, N. S., and Kaufman, A. J., 2002. The sulfur isotopic composition of Neoproterozoic seawater sulfate: implications for a snowball Earth? *Earth and Planetary Science Letters*, **203**, 413–429.
- Hurtgen, M. T., Halverson, G. P., Arthur, M. A., and Hoffman, P. F., 2006. Sulfur cycling in the aftermath of a 635-Ma snowball glaciation: evidence for a syn-glacial sulfidic deep ocean. *Earth and Planetary Science Letters*, **245**, 551–570.
- James, N. P., Narbonne, G. M., and Kyser, T. K., 2001. Late Neoproterozoic cap carbonates: Mackenzie Mountains, northwestern Canada: precipitation and global glacial meltdown. *Canadian Journal of Earth Sciences*, **38**, 1229–1262.
- Jiang, G., Kennedy, M. J., and Christie-Blick, N., 2003. Stable isotopic evidence for methane seeps in Neoproterozoic postglacial cap carbonates. *Nature*, **426**, 822–826.
- Kasemann, S. A., Hawkesworth, C. J., Prave, A. R., Fallick, A. E., and Pearson, P. N., 2005. Boron and calcium isotope composition in Neoproterozoic carbonate rocks from Namibia: evidence for extreme environmental change. *Earth and Planetary Science Letters*, **231**, 73–86.
- Kaufman, A. J., Jacobsen, S. B., and Knoll, A. H., 1993. The Vendian record of Sr and C isotopic variations in seawater: implications for tectonic and paleoclimate. *Earth and Planetary Science Letters*, **120**, 409–430.
- Kaufman, A. J., Knoll, A. H., and Narbonne, G. M., 1997. Isotopes, ice ages, and terminal Proterozoic earth history. *Proceedings of the National Academy of Sciences (USA)*, **94**, 6600–6605.
- Kellerhals, P., and Matter, A., 2003. Facies analysis of a glaciomarine sequence, the Neoproterozoic Mirbat Sandstone Formation, Sultanate of Oman. *Eclogae Geologicae Helveticae*, **96**, 49–70.
- Kennedy, M. J., 1996. Stratigraphy, sedimentology, and isotopic geochemistry of Australian Neoproterozoic postglacial cap dolostones: deglaciation, $\delta^{13}\text{C}$ excursions, and carbonate precipitation. *Journal of Sedimentary Research*, **66**, 1050–1064.
- Kennedy, M. J., Christie-Blick, N., and Sohl, L. E., 2001. Are Proterozoic cap carbonates and isotopic excursions a record of gas hydrate destabilization following Earth’s coldest intervals? *Geology*, **29**, 443–446.
- Kirschvink, J. L., 1992. Late Proterozoic low-latitude glaciation: the snowball Earth. In Schopf, J. W., and Klein, C. (eds.), *The Proterozoic Biosphere*. Cambridge: Cambridge University Press, pp. 51–52.
- Kirschvink, J. L., Gaidos, E. J., Bertani, L. E., Beukes, N. J., Gutschmer, J., Maepa, L. N., and Steinberger, R. E., 2000. Paleoproterozoic snowball Earth: extreme climatic and geochemical global change and its biological consequences. *Proceedings of the National Academy of Sciences (USA)*, **97**, 1400–1405.
- Klein, C., 2005. Some Precambrian banded iron-formations (BIFs) from around the world: their age, geologic setting, mineralogy, metamorphism, geochemistry, and origin. *American Mineralogist*, **90**, 1473–1499.
- Knoll, A. H., 2003. *Life on a Young Planet*. Princeton: Princeton University Press.
- Knoll, A. H., Javaux, E. J., Hewitt, D., and Cohen, P., 2006a. Eukaryotic organisms in Proterozoic oceans. *Philosophical Transactions of the Royal Society, London, Series B*, **361**, 1023–1038.
- Knoll, A. H., Walter, M. R., Narbonne, G. M., and Christie-Blick, N., 2006b. The Ediacaran Period: a new addition to the geologic time scale. *Lethaia*, **39**, 13–30.
- Kopp, R. E., Kirschvink, J. L., Hilburn, I. A., and Nash, C. Z., 2005. The Paleoproterozoic snowball Earth: a climate disaster triggered by the evolution of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences (USA)*, **102**, 11131–11136.
- Kump, L. R., and Seyfried, W. E. Jr., 2005. Hydrothermal Fe fluxes during the Precambrian: effect of low oceanic sulfate concentrations and low hydrostatic pressure on the composition of black smokers. *Earth and Planetary Science Letters*, **235**, 654–662.
- Le Hir, G., Ramstein, G., Donnadieu, Y., and God  ris, Y., 2008. Scenario for the evolution of atmospheric $p\text{CO}_2$ during a snowball Earth. *Geology*, **36**, 47–50.
- Le Hir, G., Ramstein, G., Donnadieu, Y., and Pierrehumbert, R. T., 2007. Investigating plausible mechanisms to trigger a deglaciation from a hard snowball Earth. *Comptes Rendus Geoscience*, **339**, 274–287.

- Lévraud, B., and Laskar, J., 2003. Climate friction and the Earth's obliquity. *Geophysical Journal International*, **154**, 970–990.
- Lewis, J. P., Weaver, A. J., and Eby, M., 2006. Deglaciating the snowball Earth: sensitivity to surface albedo. *Geophysical Research Letters*, **33**, L23604, doi: 10.1029/2006GL027774.
- Lewis, J. P., Weaver, A. J., and Eby, M., 2007. Snowball versus slushball Earth: dynamic versus nondynamic sea ice? *Journal of Geophysical Research*, **112**, C11014, doi: 10.1029/2006JC004037.
- Li, Z. X., Evans, D. A. D., and Zhang, S., 2004. A 90° spin on Rodinia: possible causal links between the Neoproterozoic supercontinent, superplume, true polar wander and low-latitude glaciation. *Earth and Planetary Science Letters*, **220**, 409–421.
- Li, Z. X., Bogdanova, S. V., Collins, A. S., Davidson, A., De Waele, B., Ernst, R. E., Fitzsimons, I. C. W., Fuck, R. A., Gladkochub, D. P., Jacobs, J., Karlstrom, K. E., Lu, S., Natapov, L. M., Pease, V., Pisarevsky, S. A., Thrane, K., and Vernikovsky, V., 2008. Assembly, configuration, and break-up history of Rodinia: a synthesis. *Precambrian Research*, **160**, 179–210.
- Liang, M. C., Hartman, H., Kopp, R. E., Kirschvink, J. L., and Yung, Y. L., 2006. Production of hydrogen peroxide in the atmosphere of the Snowball Earth and the origin of oxygenic photosynthesis. *Proceedings of the National Academy of Sciences (USA)*, **103**(50), 18896–18899.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–722.
- Mallof, A. C., Kellogg, J. B., and Anders, A. M., 2002. Neoproterozoic sand wedges: crack formation in frozen soils under diurnal forcing during a snowball Earth. *Earth and Planetary Science Letters*, **204**, 1–15.
- Martin, H., 1964. Observations concerning the problem of the late Precambrian glacial deposits in South West Africa (in German with English summary). *Geologische Rundschau*, **54**, 115–127.
- Néron de Surgy, O., and Laskar, J., 1997. On the long term evolution of the spin of the Earth. *Astronomy and Astrophysics*, **318**, 975–989.
- North, G. R., 1990. Multiple solutions in energy balance climate models. *Palaeogeography, Palaeoclimatology, Palaeoecology Global and Planetary Change Section*, **82**, 225–235.
- Ojakangas, R. W., 1988. Glaciation: an uncommon “mega-event as a key to intracontinental and intercontinental correlation of early Proterozoic basin fill, North America and Baltic cratons. In Kleinspehn, K. L., and Paola, C. (eds.), *New Perspectives in Basin Analysis*. New York: Springer, pp. 431–444.
- Opdyke, B. N., and Wilkinson, B. H., 1990. Paleolatitude distribution of Phanerozoic marine ooids and cements. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **78**, 135–148.
- Pais, M. A., Le Mouél, J. L., Lambeck, K., and Poirier, J. P., 1999. Late Precambrian paradoxical glaciation and obliquity of the Earth – a discussion of dynamical constraints. *Earth and Planetary Science Letters*, **174**, 155–171.
- Pavlov, A. A., Hurtgen, M. T., Kasting, J. F., and Arthur, M. A., 2003. Methane-rich Proterozoic atmosphere? *Geology*, **31**, 87–90.
- Pavlov, A. A., Kasting, J. F., Brown, L. L., Rages, K. A., and Freedman, R., 2000. Greenhouse warming by CH₄ in the atmosphere of early Earth. *Journal of Geophysical Research*, **105**, 11,981–11,990.
- Pavlov, A. A., Toon, O. B., Pavlov, A. K., Bally, J., and Pollard, D., 2005. Passing through a giant molecular cloud: “Snowball” glaciations produced by interstellar dust. *Geophysical Research Letters*, **32**, L03705, 10.1029/2004GL021890.
- Peltier, W. R., Tarasov, L., Vettoretti, G., and Solheim, L. P., 2004. Climate dynamics in deep time: modeling the “snowball bifurcation” and assessing the plausibility of its occurrence. In Jenkins, G. S., McMenamin, M. A. S., McKey, C. P., and Sohl, L. (eds.), *The Extreme Proterozoic: Geology, Geochemistry, and Climate*. Geophysical Monograph. Washington: American Geophysical Union, Vol. 146, pp. 107–124.
- Peterson, K. J., and Butterfield, N. J., 2005. Origin of the Eumetazoa: testing ecological predictions of molecular clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences (USA)*, **102**, 9547–9552.
- Peterson, K. J., McPeck, M. A., and Evans, D. A. D., 2005. Tempo and mode of early animal evolution: inferences from rocks, Hox, and molecular clocks. *Paleobiology*, **31**, 36–55.
- Pierrehumbert, R. T., 2005. Climate dynamics of a hard snowball Earth. *Journal of Geophysical Research*, **110**, D01111, 10.1029/2004JD005162.
- Pollard, D., and Kasting, J. F., 2004. Climate-ice sheet simulations of Neoproterozoic glaciation before and after collapse to Snowball Earth. In Jenkins, G. S., McMenamin, M. A. S., McKey, C. P., and Sohl, L. (eds.), *The Extreme Proterozoic: Geology, Geochemistry, and Climate*. Geophysical Monograph. Washington: American Geophysical Union, Vol. 146, pp. 91–105.
- Pollard, D., and Kasting, J. F., 2005. Snowball Earth: a thin-ice solution with flowing glaciers. *Journal of Geophysical Research*, **110**, C07010: 10.1029/2004JC002525.
- Poulsen, C., and Jacob, R., 2004. Factors that inhibit snowball Earth simulation. *Paleoceanography*, **19**, PA4021, doi: 10.1029/2004PA001056.
- Rashby, S. E., Sessions, A. L., Summons, R. E., and Newman, D. K., 2007. Biosynthesis of 2-methylbacteriohopanepolyols by an anoxygenic phototroph. *Proceedings of the National Academy of Sciences (USA)*, **104**, 15099–15104.
- Ridgwell, A. J., Kennedy, M. J., and Caldeira, K., 2003. Carbonate deposition, climate stability, and Neoproterozoic ice ages. *Science*, **302**, 859–862.
- Roberts, J. D., 1971. Late Precambrian glaciation: an anti-greenhouse effect? *Nature*, **234**, 216.
- Rokas, A., Krüger, D., and Carroll, S. B., 2005. Animal evolution and the molecular signature of radiations compressed in time. *Science*, **310**, 1933–1938.
- Schmidt, P. W., and Williams, G. E., 1995. The Neoproterozoic climatic paradox: equatorial paleolatitude for Marinoan glaciation near sea level in South Australia. *Earth and Planetary Science Letters*, **134**, 107–124.
- Schrag, D. P., Berner, R. A., Hoffman, P. F., and Halverson, G. P., 2002. On the initiation of a snowball Earth. *Geophysics, Geochemistry, Geosystems*, **3**, 10.1029/2001GC000219.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X., and Anbar, A. D., 2008. Tracing stepwise oxygenation of the Proterozoic biosphere. *Nature*, **452**, 456–460.
- Sheldon, R. P., 1984. Ice-ring origin of the Earth's atmosphere and hydrosphere and Late Proterozoic–Cambrian hypothesis. *Geological Survey of India Special Publication*, **17**, 17–21.
- Shields, G. A., 2005. Neoproterozoic cap carbonates: a critical appraisal of existing models and the plumeworld hypothesis. *Terra Nova*, **17**, 299–310.
- Shields, G. A., Deynoux, M., Strauss, H., Paquet, H., and Nahon, D., 2007. Barite-bearing cap dolostone of the Taoudéni Basin, northwest Africa: sedimentary and isotopic evidence for methane seepage after a Neoproterozoic glaciation. *Precambrian Research*, **154**, 209–235.
- Stoeck, T., Kasper, J., Bunge, J., Leslin, C., Ilyin, V., and Epstein, S., 2007. Protistan diversity in the Arctic: a case of palaeoclimate shaping of modern biodiversity? *PloS ONE*, **2**(8), e728. doi: 10.1371/journal.pone.0000728.

- Summons, R. E., Jahnke, L. L., Hope, J. M., and Logan, G. A., 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. *Nature*, **400**, 554–557.
- Trindade, R. I. F., and Macouin, M., 2007. Paleolatitude of glacial deposits and paleogeography of Neoproterozoic ice ages. *Comptes Rendus Geoscience*, **339**, 200–211.
- Vieira, L. C., Trindade, R. I. F., Nogueira, A. C. R., and Ader, M., 2007. Identification of a Sturtian cap carbonate in the Neoproterozoic Sete Lagoas carbonate platform, Bambuí Group, Brazil. *Comptes Rendus Geoscience*, **339**, 240–258.
- Vincent, W. F., Mueller, D. R., and Bonilla, S., 2004. Ecosystems on ice: the microbial ecology of Markham Ice Shelf in the high Arctic. *Cryobiology*, **48**, 103–112.
- Walker, J. C. G., Hays, P. B., and Kasting, J. F., 1981. A negative feedback mechanism for the long-term stabilization of Earth's surface temperature. *Journal of Geophysical Research*, **86** (C10), 9776–9782.
- Warren, S. G., Brandt, R. E., Grenfell, T. C., and McKay, C. P., 2002. Snowball Earth: ice thickness on the tropical ocean. *Journal of Geophysical Research*, **107**(C10), 10.1029/2001JC001123.
- Williams, G. E., 1975. Late Precambrian glacial climate and the Earth's obliquity. *Geological Magazine*, **112**, 441–544.
- Williams, G. E., 2000. Geological constraints on the Precambrian history of Earth's rotation and the Moon's orbit. *Reviews of Geophysics*, **38**(1), 37–59.
- Williams, D. M., Kasting, J. F., and Frakes, L. A., 1998. Low-latitude glaciation and rapid changes in the Earth's obliquity explained by obliquity–oblateness feedback. *Nature*, **396**, 453–455.
- Yin, L., Zhu, M., Knoll, A. H., Yuan, X., Zhang, J., and Hu, J., 2007. Doushantuo embryos preserved inside diapause egg cysts. *Nature*, **446**, 661–663.
- Yoshioka, H., Asahara, Y., Tojo, B., and Kawakami, S., 2003. Systematic variations in C, O, and Sr isotopes and elemental concentrations in Neoproterozoic carbonates in Namibia: implications for a glacial to interglacial transition. *Precambrian Research*, **124**, 69–85.
- Zhang, S., Jiang, G., and Han, Y., 2008. The age of the Nantuo Formation and Nantuo glaciation in South China. *Terra Nova*, **20**, 289–294.
- Ziegler, A. M., Hulver, M. L., Lottes, A. L., and Schmachtenberg, W. F., 1984. Uniformitarianism and paleoclimates: inferences from the distribution of carbonate rocks. In Brenchley, P. J. (ed.), *Fossils and Climate*. New York: Wiley, pp. 3–25.

Cross-references

Algae (Eukaryotic)
 Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Bacteria
 Banded Iron Formations
 Biomarkers (Molecular Fossils)
 Breakup of Rodinia
 Cap Carbonate
 Carbon Cycle
 Carbonate Environments
 Critical Intervals in Earth History
 Dolomite, Microbial
 Endosymbiosis
 Extreme Environments
 Hydrothermal Environments, Marine
 Iron Sulfide Formation
 Isotopes (Methods)
 Lateral Gene Transfer
 Methane Oxidation (Aerobic)
 Microbialites, Modern
 Origins of the Metazoa
 Stromatolites

SODA LAKES

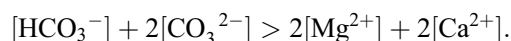
Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The term “soda lake” designates a class of lakes with waters showing an excess of the total alkalinity ($TA \cong [HCO_3^-] + 2[CO_3^{2-}]$, i.e., the sum of the charges of the bicarbonate ion plus carbonate ion) over the charges of the alkaline earth ions magnesium and calcium:



When such water is evaporated, the high $[CO_3^{2-}]$ concentrations will cause a rise in pH and eventually, Na carbonates precipitate. These are soda (or natron, $Na_2CO_3 \cdot 10H_2O$, monoclinic), trona ($Na_2CO_3 \cdot NaHCO_3 \cdot 2H_2O$, monoclinic), and thermonatrite ($Na_2CO_3 \cdot H_2O$, orthorhombic). They are commercially used in the glass-, textile-, paint-, soap- and metal-industry, mined worldwide (Garret, 1992).

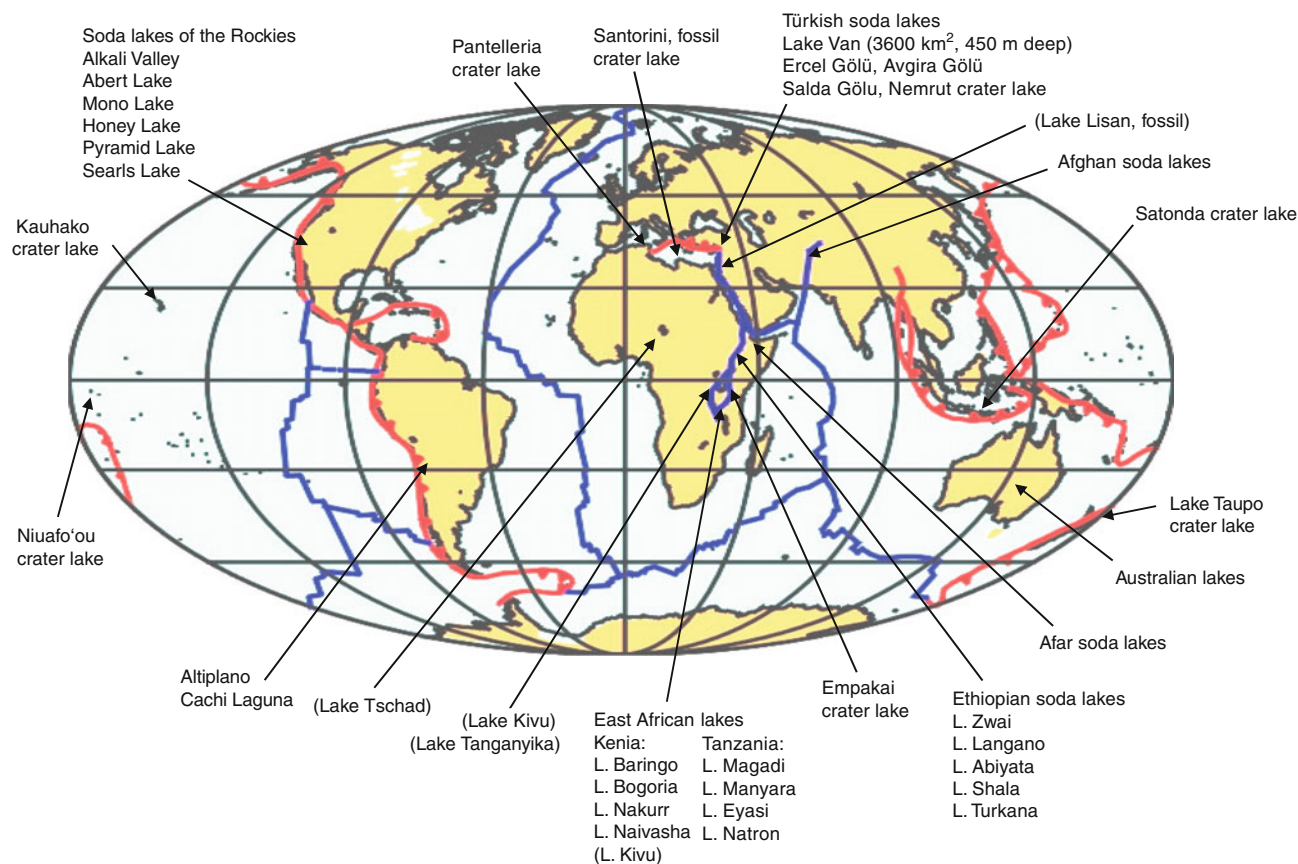
Occurrence

Soda lakes occur worldwide, albeit in very specific zones: they seem to be associated with active tectonic and volcanic zones (Figure 1). Most of the famous soda lakes occur in East Africa along the East African rift (e.g., Kraml and Bull, 1998/1999). Here, the succession of soda lakes left deeply buried soda deposits that also serve as the source of natro-carbonatite lavas (Ol Doinyo Lengai Stratovolcano, Tanzania). Another well-known group of alkaline lakes, some fossil such as Searles Lake, occur in the western USA. Less well known are lakes on the Altiplano, or in Australia, Afghanistan, Turkey, and other places around the world. So far no comprehensive list of soda lakes has apparently been compiled.

Setting

However, there seem to be two different types of lakes that acquire soda chemistry: most of the larger lakes are depressions in endorheic regions closed toward exorheic runoff by tectonic movements or by volcanic dams. The ratio between the tributary area and lake surface area is large. The largest soda lake on the Earth is Lake Van (Van Gölü) in eastern Anatolia (e.g., Kempe et al., 1991). Table 1 gives its morphometric data; it is (because Lake Aral dried up) by volume the third largest closed basin lake on Earth. Lake Van occupies the former headwaters of the Euphrates before it was dammed by an eruption of the Nemrut volcano.

The other class of soda lakes occur in volcanic craters or calderas. They typically do not have any surface tributaries and their ratio between recharge area and lake surface area is small. We have discovered the alkaline nature of several crater lakes, including Satonda Crater,



Soda Lakes, Figure 1 The global distribution of some of the major soda lakes or soda lake groups and their relation to margins of tectonic plates.

Soda Lakes, Table 1 Lake Van morphometric data

Total basin area	16,096 km ²
Total tributary area	12,574 km ²
Lake surface	3,522 km ²
Lake volume	576 km ³
Lake maximum depth	451 m
Annual water exchange	3.78 km ³
Altitude of lake level	1,648 m a.s.l.

Source: Kempe and Kazmierczak (2003); Compiled from Reimer (1995)

Indonesia (Kempe and Kazmierczak, 1993), Niuafo'ou Caldera Lake, Tonga (Kazmierczak and Kempe, 2006), and Kauhako Crater Lake, Molokai, Hawaii (unpublished data and Donachie et al., 2004). Other soda lakes occupy the Empakai Crater in Kenya, the crater in Pantelleria, and possibly a score of other craters that have not been investigated as yet. Even Santorini, Greece, must have had an alkaline crater lake for some period prior to the famous Minoan eruption that blew out the stromatolites of that lake.

Chemistry

Soda lakes can reach pH in excess of 10.5 and alkalinities of >150 meq/l. Some of the most alkaline water bodies are Lake Magadi (Eugster, 1986) in Kenya and Mono Lake in California (e.g., Bischoff et al., 1993). Lake Van has a pH of 9.7 and an alkalinity of 151 meq/l (Kempe et al., 1991; Reimer et al., 2009). These are examples of highly alkaline lakes. But there are soda lakes “in the making,” i.e., lakes that obey the basic condition of $TA > (Mg + Ca)$ but have not acquired higher salinities. One of them is the crater lake of the Nemrut Volcano itself that has dammed Lake Van. In Table 2 the water of Lake Van is compared with that of the Nemrut Lake.

The Table lists the main parameters that are used to characterize natural waters, all in all the two lakes are not very different in their respective surface and bottom samples, i.e., they are relatively well mixed. First of all the conductivity gives a rough measure of the amount of total salts dissolved, which is in seawater about 35 g/l, in Lake Van it is about 22 g/l, and in Nemrut Lake only about 440 mg/l, i.e., Nemrut Lake is what would be classified as a “fresh water” lake. But this is only one of the criteria: when calculating the soda lake criterion

(i.e., $TA/(Mg + Ca)$) we find 16.65 and 16.85 for Lake Van (0 and 440 m, respectively), 3.11 and 3.18 for Lake Nemrut (10 and 150 m, respectively) and 0.018 for seawater. This ratio tells us that Lake Van is a typical soda lake with >16 times as much alkalinity as alkaline earth ions, but that also Lake Nemrut is a soda lake with >3 times higher alkalinity than alkaline earth ions; whereas seawater has much more Mg and Ca than dissolved carbonates (i.e., >54 times). We also see that the chemical

composition of both lakes do not differ much with depth, apart from the pH in Nemrut Crater that drops considerably. Lake Van has a very high pH as expected from its high alkalinity. But Nemrut crater lake has – at the surface – the same slightly alkaline pH as seawater, not identifying it (yet) as a soda lake. When from these data (and temperature not given here) the CO_2 pressure of the water (PCO_2) is calculated (using PHREEQE; Parkhurst et al., 1990) and compared to the PCO_2 of the atmosphere of

Soda Lakes, Table 2 Chemical composition of Lake Van, the largest contemporary soda lake, compared to Nemrut Crater Lake, a “soda lake in the making” and to modern seawater

Lake/Depth	pH	PCO_2 ppmv	Alkal. meq/l	Cl meq/l	SO_4 meq/l	Na meq/l	Mg meq/ l	Ca meq/l	Cond $\mu S/cm$	SI_{Cc}	SI_{Ara}	SI_{Dol}
Seawater at surface	8.24	363	2.33	546	56.6	468	106.2	20.6	ca. 48,000	0.59	0.44	1.96
Van 3 m	9.74	309	151.2	160	48.9	338	8.870	0.210	26,000	1.14	0.99	4.09
Van 440 m	9.88	257	155.6	166	50.9	348	9.06	0.174	26,700	1.06	0.91	3.81
Nemrut 10 m	8.27	1,240	4.61	0.43	0.22	3.67	0.52	0.96	456	0.37	0.22	0.43
Nemrut 150 m	6.89	28,600	4.77	0.45	0.25	3.80	0.53	0.97	469	–1.11	–1.27	–2.59
Same degassed	8.73	380	4.77	0.45	0.25	3.80	0.53	0.97	–	1.30	1.15	2.25

Source: From Kempe and Kazmierczak (2003); data see Reimer (1995); (Reimer et al., 2009)

Soda Lakes, Table 3 List of modern soda lakes with $CaCO_3$ precipitation in comparison with modern oceanic conditions

Site	Minerals dominating	Saturation index	Kind of $CaCO_3$ precipitate	Source
North Sea 1986	Calcite	0.375–0.904 Cc mean of 122 samples: 0.567	Biomineralization of coccolithophorids	Pegler and Kempe, 1988
Bahamas banks	Aragonite	0.47–0.6 Ara	Extremely low rates (if any) of aragonite (not the reason of whittings)	Morse et al., 2002
Satonda crater lake	Aragonite, Calcite	0.98 Cc, 0.84 Ara	Biomineralization by red algae and cyanobacterial sheaths	Kempe and Kazmierczak, 1993; Kazmierczak et al., 2004
Lake Van	Aragonite, Calcite	1.14 at 0 m to 1.06 at 440 m Cc, 0.99–0.91 Ara 4.09–3.81 Dol	Permineralization of cyanobacterial sheaths, inorganic chemical gardens, river plume whiting, summer whittings	Kempe et al., 1991; Reimer, 1995; Kazmierczak et al., 2004; Reimer et al., 2008
Lake Tanganyika	High-Mg Calcite	0.96 Cc	Five zones of massive thrombolites down to 50 m	Kempe and Kazmierczak, 1990 and citations therein
Andros Island	Low-Mg Calcite	Upon degassing and evaporation 1.14 Cc	Inland lakes with weakly calcified cyanobacterial and algal mats	Same as above
Walker Lake	Low-Mg Calcite	1.11 Cc, 0.95 Ara, 3.90 Dol	Small cabbage-like stromatolites up to 4 cm high, built by filamentous green alga and oscillatorian and coccoidal cyanobacteria.	Same as above; Kempe and Kazmierczak, 1997
Mono Lake Figure 2 top	Aragonite (Ikaite)	1.24 Cc, 1.09 Ara, 3.74 Dol	Large tufa columns at former groundwater outlets	Kempe and Kazmierczak, 1997; Bischoff et al., 1993
Pyramid Lake Figure 2 bottom	Calcite	1.36 Cc, 1.21 Ara, 3.89 Dol	Huge towers and mounds at former groundwater outlets	Benson, 1994; Arp et al., 1999
Niuafou, crater lake	Aragonite	0.65 Cc, 0.50 Ara, 2.65 Dol	Head-like stromatolites built by coccoid and filamentous cyanobacteria	Kazmierczak and Kempe, 2006
Kauhako crater lake	Calcite	0.97 Cc, 0.82 Ara	Calcified sheaths of filamentous cyanobacteria	Kempe, unpublished

Cc = calcite; Ara = aragonite; Dol = dolomite

380 ppm, then we find that the crater lake has a much higher PCO_2 than the other samples, indicating that it receives volcanic CO_2 from subsurface springs, while Lake Van does not.

PHREEQE also allows for calculating the state of saturation of water with respect to minerals. The saturation index (SI) is defined as the logarithm of the ion activity product divided by the solubility constant of a given mineral (here, for calcite as an example):

$$SI_{\text{calcite}} = \log([Ca^{2+}] * [CO_3^{2-}] / K_{\text{calcite}}).$$

The SI yields negative values if water is undersaturated, zero if saturated, and positive values if supersaturated. The results (Table 2) show that all samples, with the exception of the deep Nemrut sample, are oversaturated with respect to calcite, aragonite, and dolomite. But when the deep Nemrut sample is numerically degassed, it will also show high carbonate supersaturation (last line in Table 2). The main difference between Lake Van and seawater is that the carbonate supersaturation (which is >10 times its saturation) of Lake Van allows for in situ precipitation of aragonite, while in seawater, even though supersaturated, no spontaneous precipitation occurs. Rather, the supersaturation of seawater is kept low by the multitude of enzymatically biomineralizing organisms that are generally absent in soda lakes due to the high alkalinity.

Soda lake microbialites

Those of the mature soda lakes that reach high supersaturation values (i.e., above $\approx SI\ 0.8$, Kempe and Kazmierczak, 1990) produce cyanobacterial microbialites. Table 3 lists

a few of them for which sufficient geochemical data are available to calculate saturation. The water analysis available for Andros Island originally do not show supersaturation, but the permineralizing cyanobacterial mats grow in ephemeral very shallow nonmarine inland lakes that apparently sustain growth only after an extensive evaporative phase. In case of one of the Niuafu'ou caldera lakes (Vai Lahi), the current water composition also does not sustain in situ permineralization, and the stromatolites discovered there stopped growing about 200 years ago. Overall, soda lakes represent the best sites to study modern stromatolites, their structure, microbial ecosystem, and growth rates. Many of them have shown to produce structures strikingly similar to those of Precambrian microbialites.

Genesis of soda lakes

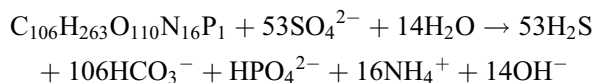
These data presented here and in the cited papers show that soda chemistry can arise in various climatic, geologic, and morphologic settings. We find water bodies that are soda lakes in the making that will eventually acquire mature soda chemistry, such as Nemrut Crater Lake, a lake that originated only a few hundred years ago after the last eruption of the Nemrut caldera. Other crater lakes, such as Lake Taupo in New Zealand, will never reach this state, since they have an outlet. Astonishing is also the fact that Lake Kivu and Lake Tanganyika (both with an outlet) have soda lake characteristics (Soda Lake Index for Tanganyika: 1.5) and (at least in Lake Tanganyika) grow microbialites.

It has long been stated that the type of chemistry is dependent on the proportions of the ions in the waters



Soda Lakes, Figure 2 Calcareous tufa towers in Mono Lake (*top*) and Pyramid Lake (*bottom*). These towers originally formed under water at inlets of groundwater.

delivered to it (Garrels and Mackenzie, 1967). Eugster and Hardie (1978) constructed flow diagrams that show the various possibilities of brine evolution. In case of soda lakes, the criterion is the ratio of TA/(Mg + Ca). From the distributions of the soda lakes along the plate boundaries it appears that this ratio most likely arises in areas with fresh volcanic rocks. Here, biogenic CO₂ and/or (see Lake Nemrut) volcanic CO₂ can react with fresh silicates, mobilizing Na, K, Mg, and Ca, in the necessary proportions. Ongoing weathering within the crater lakes and their hydrothermal system (as in the case of Niuafu'ou), or continuing evaporation in the terminal lakes will then lead to mature soda lake chemistry and to a CaCO₃ supersaturation of around SI = 1 that will sustain microbialite growth. However, this is only a very broad picture. When looking at Satonda and Kauhako crater lakes, we find involvement of a second process that causes the alkalinity to rise. This is sulfate reduction. Both lakes are marine-derived lakes, i.e., are filled originally with sulfate-rich seawater. Microbial disintegration of organic matter must replace the charge of the sulfate ion with that of bicarbonate ions according to



This pathway of alkalinity production in anaerobic water bodies was coined “alkalinity pump” (Kempe, 1990; Kempe and Kazmierczak, 1994), supplementing the shift toward a soda chemistry, where sulfate is available.

Summary

Soda lake waters show an excess of the total alkalinity over the charges of the alkaline earth ions magnesium and calcium ($[\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] > 2[\text{Mg}^{2+}] + 2[\text{Ca}^{2+}]$). They are characterized by high pH values (increasing with evaporation) and, eventually, precipitation of Na carbonates. Mature soda lakes that reach high supersaturation values with respect to calcite, aragonite, and dolomite (above $\approx$ SI 0.8) may produce cyanobacterial microbialites. The ubiquitous soda lakes in areas with fresh volcanic rocks and high weathering potential and the microbialites found therein gave rise to the *Soda Ocean Hypothesis* (see separate entry), stating that the early ocean, by following simple geological rules, should have been highly alkaline, possibly similar to present Lake Van.

Bibliography

- Arp, G., Thiel, V., Reimer, A., Michaelis, W., and Reitner, J., 1999. Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA. *Sedimentary Geology*, **126**, 159–196.
- Benson, L. V., 1994. Carbonate deposition, Pyramid Lake subbasin, Nevada: I. Sequence of formation and elevational distribution of carbonate deposits (tufas). *Palaeogeography, Palaeoecology, Palaeoclimatology*, **109**, 55–87.
- Bischoff, J. L., Stine, S., Rosenbauer, R. J., Fitzpatrick, J. A., and Stafford, T. W., 1993. Ikaite precipitation by mixing of shoreline springs and lake water, Mono Lake, California, USA. *Geochimica et Cosmochimica Acta*, **57**, 3855–3865.
- Donachie, S. P., Hou, S., Lee, K. S., Riley, C. W., Pikina, A., Kempe, S., Gregory, T. G., Bossuyt, A., Boerema, J., Liu, J., Freitas, T. A., Malahoff, A., and Alam, M., 2004. The Hawaiian Archipelago: a microbial diversity hotspot. *Microbial Ecology*, **48**, 509–520.
- Eugster, H. P., 1986. Lake Magadi, Kenya: a model for rift valley hydrochemistry and sedimentation? In Frostick, L. E. et al., (eds.), *Sedimentation in the African Rifts*. London: Geological Society, Special Publications 25, pp. 177–189.
- Eugster, H. P., and Hardie, L. A., 1978. Lake Magadi, Kenya: a model for rift valley hydrochemistry and sedimentation? In Lerman, A. (ed.), *Physics and Chemistry of Lakes*, New York: Springer, pp. 237–293.
- Garrels, R. M., and Mackenzie, F. T., 1967. Origin of the chemical composition of some springs and lakes. In equilibrium concepts of natural water systems. *American Chemical Society Advances in Chemistry*, **67**, 222–242.
- Garret, D. E., 1992. *Natural Soda Ash, Occurrences, Processing, and Use*. New York: Van Nostrand Reinhold.
- Kazmierczak, J., and Kempe, S., 2006. Modern analogues of Precambrian stromatolites from caldera lakes of Niuafu'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kazmierczak, J., Kempe, S., and Altermann, W., 2004. Microbial origin of Precambrian carbonates: lessons from modern analogues. In Eriksson, P., Altermann, W., Nelson, D., Mueller, W., and Catuneanu, O. (eds.), *The Precambrian Earth: Tempos and Events*. Amsterdam: Elsevier, pp. 545–564.
- Kempe, S., 1990. Alkalinity: the link between anaerobic basins and shallow water carbonates? *Naturwissenschaften*, **77**, 426–427.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In Ittekkot, V. A., Kempe, S., Michaelis, W., and Spitz, A. (eds.), *Facets of Modern Biogeochemistry*, (Festschrift for E.T. Degens). Berlin: Springer-Verlag, pp. 255–278.
- Kempe, S., and Kazmierczak, J., 1993. Satonda Crater Lake, Indonesia: Hydrogeochemistry and biocarbonates. *Facies*, **28**, 1–32.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Bulletin de l'Institut océanographique, Monaco, no. spec. 13, 61–117.
- Kempe, S., and Kazmierczak, J., 1997. A terrestrial model for an alkaline martian hydrosphere. *Planetary and Space Science*, **45**, 1493–1499.
- Kempe, S., and Kazmierczak, J., 2003. Modern soda lakes: model environments for an early alkaline ocean. In Müller, T., and Müller, H. (eds.), *Modelling in Natural Sciences; Design, Validation and Case Studies*. Berlin: Springer-Verlag, pp. 309–322.
- Kempe, S., Kazmierczak, J., Landmann, G., Konuk, T., Reimer, A., and Lipp, A., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kraml, M., and Bull, A., 1998/1999. Sodaseen im Ostafrikanischen Graben – ihre Entstehung und Bedeutung. *Berichte der Naturforschenden Gesellschaft zu Freiburg i. Br.*, **88/89**, 85–118.
- Morse, J. W., Gledhill, W. K., and Millero, F. J., 2002. CaCO₃ precipitation kinetics in waters from the Grand Bahama Bank: implications for the relationship between hydrochemistry and whittings. *Abstracts of the 6th International Symposium on the Geochemistry of Earth's Surface*, May 20–24, 2002, Honolulu, Hawaii, pp. 138–140.

- Parkhurst, D. L., Thorstenson, D. C., and Plummer, L. N., 1990. PHREEQE – A computer program for geochemical calculation. (Conversion and upgrade of the prime version of PHREEQE to IBM PC-compatible systems by Tirisanni, J. V., Glynn, P. D.,) US Geological Survey and Water Research Reports 80–96, 197 pp.
- Pegler, K., and Kempe, S., 1988. The carbonate system of the North Sea: determination of alkalinity and TCO_2 and calculation of PCO_2 and Sical (Spring 1986). In Kempe, S., Liebezeit, G., Dethlefsen, V., and Harms, U. (eds.), *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications to Fisheries Biology*. Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, SCOPE/UNEP Sonderband, **65**, pp. 35–87.
- Reimer, A., 1995. *Hydrochemie und Geochemie der Sedimente und Porenwasser des hochalkalinen Van Sees in der Osttürkei*, Faculty of Geosciences. Unpublished PhD Theses, University of Hamburg, 136 pp.
- Reimer, A., Landmann, G., and Kempe, S., 2009. Lake Van, Eastern Anatolia, hydrochemistry and history. *Aquatic Geochemistry*, **15**, 195–222.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Ocean Hypothesis
 Tufa, Freshwater

SODA OCEAN HYPOTHESIS

Stephan Kempe¹, Jozef Kazmierczak²

¹University of Technology, Darmstadt, Germany

²Polish Academy of Sciences, Warszawa, Poland

Definition

The “soda ocean hypothesis” (SOH) stands for the concept of an early (i.e., in essence Precambrian) alkaline or even highly “alkaline ocean,” in analogy to the chemistry of the present-day “soda lakes.”

Soda ocean hypothesis (SOH)

The SOH has been advanced in biology (e.g., Snyder and Fox, 1975) for biochemical reasons before it was developed in earth sciences for geochemical reasons (Kempe and Degens, 1985; Kempe et al., 1989; Kempe and Kazmierczak, 1994).

In *biology*, the SOH rests on the observations that certain reactions considered essential for biogenesis would be favored by alkaline conditions (e.g., Abelson, 1966). One of those is the experimental observation that peptide bonds are more stable in alkaline than in acidic environments (e.g., Dose and Rauchfuss, 1972).

In *earth sciences*, the SOH rests on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes. These arguments are in short:

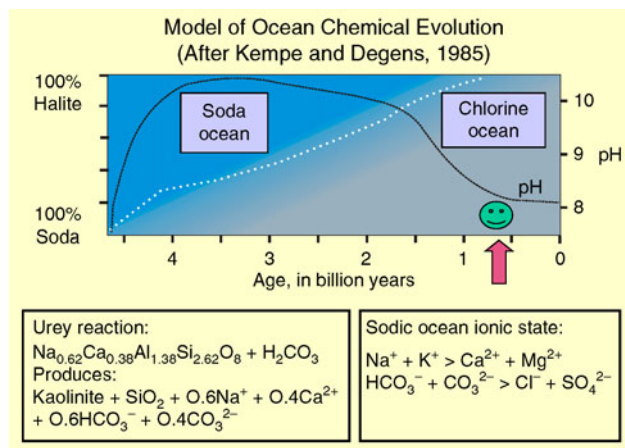
1. A CO_2 -rich atmosphere in the presence of water would react vigorously with the highly meta-stable, widespread, fine-grained glassy silicates from impacts and volcanic eruptions as would be present during the period of the terminal cataclysm in the Hadean. Carbonic acid weathering of silicates (“Urey Reaction”) will liberate cations in equivalence to the consumed carbonic acid, producing bicarbonate and carbonate as anions.
2. Continental silicate weathering today (aided by high soil PCO_2 due to biological activity; $\text{PCO}_2 = \text{CO}_2$ Pressure) consumes annually $0.15 \cdot 10^9 \text{ t C}$ (Hartmann et al., 2009); thus today’s volume of atmospheric CO_2 would be consumed in about 5,000 years by the Urey Reaction. This rate suggests that a high PCO_2 atmosphere is not stable on geological timescales.
3. Other primordial acids, such as HCl , have much less mass and therefore the primordial ocean must have had TA (total alkalinity $\equiv [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] > [\text{Cl}^-]$).
4. Sulfur-based acids were not available in larger quantities since not enough free oxygen was in the system to allow formation of either sulfurous or sulfuric acids.
5. The entire mass of carbon available in crustal compartments (i.e., $65.5 \cdot 10^{21} \text{ gC}$) as carbonates and organic carbon today could in principle have been dissolved as alkali carbonates in the oceans - as long as H_2O and CO_2 were degassed at similar rates - because this would amount to $48.5 \text{ gC/kg H}_2\text{O}$ while Na_2CO_3 is solvable at $53 \text{ gC/kg H}_2\text{O}$ (Kempe and Degens, 1985).
6. Observations of modern soda lakes show that their tributaries have inputs of $[\text{Na}^+] + [\text{K}^+] > [\text{Cl}^-] + [\text{SO}_4^{2-}]$ and $[\text{Ca}^{2+}] + [\text{Mg}^{2+}] < [\text{TA}]$ (Garrels and Mackenzie, 1967). When evaporated these waters will become alkaline because Ca and Mg carbonates (and sulfates in modern settings) will reach saturation first and Na and K carbonates will stay in solution causing increasingly higher alkalinity and pH. Soda lakes (i.e., lakes with an excess in Na-carbonates) almost exclusively occur today in or near volcanic regions. The early ocean (as well as possible primordial oceans on other planets such as Mars; Kempe and Kazmierczak, 1997) could have reacted the same way, storing CO_2 as TA as quickly as CO_2 was degassed from the mantle or deposited on Earth by comets.

These arguments are based on simple acid–base reactions but have several *derivatives*:

1. The atmosphere could not have had a high enough PCO_2 to counterbalance the lower insolation of the “faint early sun”.
2. In alkaline waters SiO_2 can be kept in solution in high concentrations (at least in the primordial seas where

- silica-sequestering organisms such as sponges, diatoms, silicoflagellates, and radiolarians were absent).
3. The higher the TA becomes, the lower the concentration of the free Ca-ion will become because of the fact that the IAP (the ion activity product) governing CaCO_3 solubility, i.e., $\text{IAP} = [\text{Ca}^{2+}] \cdot [\text{CO}_3^{2-}]$ is dominated by the carbonate ion.
 4. In the absence of biologically controlled biomineralization (as was typical for the entire Precambrian until, at the onset of the Cambrian, enzymatic biocalcification evolved) the ocean must have been of a very high supersaturation, since the spontaneous precipitation of CaCO_3 -minerals is kinetically inhibited in natural settings. Saturation is most commonly expressed as the saturation index (SI), i.e., the quotient between the IAP and the solubility product of the mineral (K_{Mineral}). $\text{SI} = \log \text{IAP}/K_{\text{Mineral}}$. A survey of nonenzymatic carbonate CaCO_3 -precipitating environments (Kempe and Kazmierczak, 1990; Kazmierczak and Kempe, 2006) shows that these environments have an SI of +0.8 to +1 (i.e., a 10-times supersaturation) while the modern ocean has a much lower supersaturation (North Sea $\text{SI}_{\text{calcite}}$ average summer 1986 = 0.567 ± 0.096 ; Pegler and Kempe, 1988) because it is controlled by enzymatic reactions and not by inorganic precipitation. Dolomite can be supersaturated to $\text{SI} = 4$, i.e., 10,000 times its solubility product in soda lakes (e.g., Kempe and Kazmierczak, 2007). This fact, i.e., the higher carbonate mineral supersaturation of the primordial ocean is today accepted widely (e.g., Grotzinger, 1990; Arp et al., 2001).

Because the soda ocean has vanished and the present day halite ocean prevails, there must have been a transition between the two (Figure 1). This transition must have been driven by a process with a long time constant. This process



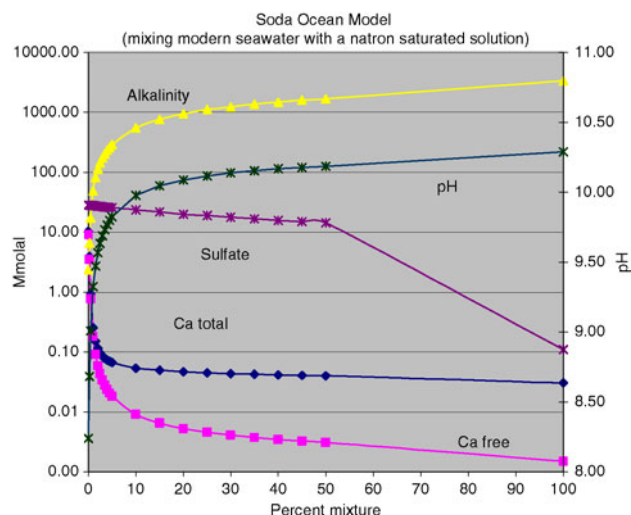
Soda Ocean Hypothesis, Figure 1 Simple representation of the timing of the rise and fall of the soda ocean due to the Urey reaction. The happy face marks the onset of multicellularity triggered by a massive increase of the free calcium ion towards the end of the Precambrian.

is thought to be the subduction of seawater with marine crust and sediments in subduction zones and the consecutive formation of continental crust. About 1 km^3 of seawater is subducted per year today. Any compound dissolved in the ocean has therefore a half-life of about 1 billion years, depending on the rate of subduction. Thus the soda ocean could in fact have prevailed for several billion years before being gradually diminished. According to the soda ocean model (Kempe and Degens, 1985), Na and K would have been transferred into feldspars of continental granodiorites and the carbon would have been deposited as CaCO_3 and kerogen on the continents where they are stored for longer times than deep sea deposits that are lost to subduction within less than 100 million years. The chloride subducted would be recycled as volcanic HCl, in a cycle quicker than continental weathering.

Consequences for biogenesis

The alkaline model has also a series of consequence for *biogenesis* and the evolution of life in the first 3.5 billion years of Earth history.

1. Thermodynamic calculations show that in highly alkaline solutions (up to the saturation of Na_2CO_3), the free $[\text{Ca}^{2+}]$ can be as low as $4 \cdot 10^{-7} \text{ mol}$ (Kazmierczak and Kempe, 2004), while, due to ion-pairs, total Ca is significantly higher. In the model calculation shown in Figure 2 seawater (i.e., a solution with high chloride and sulfate) was mixed with the natron-saturated solution based on the dilute water from Nemrut Crater Lake (-10 m), a “soda lake in the making” (see entry “*Soda Lakes*” Table 3) that leads to a free $[\text{Ca}^{2+}]$ of $1.5 \cdot 10^{-6} \text{ mol}$. Very low environmental Ca^{2+} concentrations are favorable for biogenesis because cells have to maintain a $[\text{Ca}^{2+}] < 10^{-6} \text{ mol}$ in their cytosol (e.g., Kretsinger, 1983; Carafoli, 1987). Otherwise, the proteins would be denaturalized and lose their ability to function. Thus the formation of life in an acidic and consequentially high $[\text{Ca}^{2+}]$ environment is even more difficult to understand.
2. The only macroscopic fossils throughout the Precambrian (with the exception of the Ediacara fauna and some problematic biostructures) are microbialites (stromatolites, thrombolites), biosedimentary structures produced by colonies of microorganisms. The majority of researchers accept that they have formed by the permineralization of the EPS of biofilms dominated by cyanobacteria (e.g., Riding, 2000; Altermann et al., 2006). This form of CaCO_3 -precipitation is nonenzymatic and the cyanobacteria do not instrumentalize these precipitates for functions. The high CaCO_3 -supersaturation caused by the alkaline marine condition is thus an environment in which these microbialites could have thrived easily. Today soda-lake microbialites are formed by the same processes (e.g., in Lake Van, Kempe et al., 1991; López-García et al., 2005, in Mono Lake, and in Niufo’ou caldera lakes, Kazmierczak and Kempe, 2006) while today’s



Soda Ocean Hypothesis, Figure 2 PHREEQUE mixing experiment of modern seawater (*left*) with the natron saturated Nemrut Crater Lake water (see Table 2 in entry “*Soda Lakes*”) (*right*), illustrating the possible transition of an alkaline ocean (*right*) to one dominated by halite and gypsum (*left*). After linear mixing of constituents, the solutions are adjusted to have a calcite saturation index of +1 and a $p\text{CO}_2$ of 3.5 (roughly the present atmospheric PCO_2). Note that the sharp drop in Ca concentration is accompanied by only a slight increase in alkalinity. Note also that the sulfate concentration would describe an exponential curve between 50 and 100% if subsamples were calculated for that section of the mixing experiment.

marine stromatolites are apparently growing predominantly by trapping and binding of allogene particles (e.g., Ginsburg, 1991).

- For three billion years cyanobacteria seem to be among the primary photosynthetic organisms. Most modern cyanobacteria are alkalophiles, suggesting that their evolution proceeded in alkaline environments.
- The development of eukaryotic organisms in the late Palaeoproterozoic may have also been triggered by the early ocean conditions: The gradual increase in the $[\text{Ca}^{2+}]$ ambient concentration due to the slow transition between the soda and halite dominated oceans, could have caused the geochemical stress that might have influenced the evolution of a cell nucleus by protecting the genetic material against the disruptive Ca^{2+} -influx.
- And even the onset of multicellular organisms may have been triggered by the increasing $[\text{Ca}^{2+}]$. Experiments with sponges suggest that adhesion of cells can be triggered by an increase in $[\text{Ca}^{2+}]$ in the environment (e.g., Kretsinger, 1977). Thus the appearance of faunas in Ediacara times may be the indication that the soda ocean was near its demise.
- The biomineralization that appears in many different taxa at the turn of the Precambrian/Cambrian is, in view of the SOH, a detoxification reaction to the

rapidly rising $[\text{Ca}^{2+}]$ when the ocean, as TA decreases (e.g., from 10.3 to 2.38 meq/l as in present seawater), goes through a sort of titration endpoint triggering a >10-fold increase in $[\text{Ca}^{2+}]$ (Kempe and Kazmierczak, 1994). Measurements of Ca in fluid inclusions seem to give this facet of the SOH credibility (Brennan et al., 2004; Petrychenko et al., 2005).

Counterarguments

Some arguments against the SOH were brought forward. These, however, do not really disprove the SOH:

- Models of early Earth often prescribe a long-lasting high PCO_2 -atmosphere to keep the oceans from freezing. However, it may also be argued that the lack of free oxygen in the oceans and atmosphere could have allowed for higher methane concentrations that prevented permanent freezing of Earth.
- Möller and Bau (1993), Bau and Möller (1994) state that the water of Lake Van has a positive cerium anomaly but Precambrian carbonates (or BIFs) do not. However, these authors compare water with sediments. Measurements showed that Lake Van sediments do not exhibit the same anomaly as the water body (own data, unpublished).

Outlook

The *acceptance of the SOH* in the literature is mixed. Some authors agree with it in essence (Arp et al., 2001), others accept certain aspects (such as the high CaCO_3 -supersaturation; Grotzinger and Kasting, 1993; Riding, 2000; or Mg/Ca ratios; Ries et al., 2008), and some doubt its relevance and follow own modeling lines (e.g., Morse and Mackenzie, 1998). Many papers deal with questions of the rising oxygen and sulfate, redox-reactions or certain sedimentary problems like BIF-formation or microbialite (stromatolite) structures without recurring to the overall chemical evolution of the ocean. Although the majority of researchers tend to ignore the SOH, it found entrance into several textbooks of general geology (Degens, 1989; Einsele, 1992; Bahlburg and Bretkreuz, 1998). Digital modeling appears to be not of much help - apart from stating thermodynamic conditions correctly - since the results depend on the adopted boundary conditions of the Hadean and Archean environment, which are as yet not well defined. For example, when prescribing certain PCO_2 values to avoid freezing of oceans because of the faint early sun, then no highly alkaline ocean can evolve. Future research will have to show which one of the many models explains the past of our planet and its geological and biological evolution best.

Summary

Resting on elemental mass balances, thermodynamic and kinetic arguments, and the analogy to modern soda lakes, the soda ocean hypothesis (SOH) states that the Precambrian ocean was alkaline, with a total alkalinity $> \text{Ca} + \text{Mg}$

and a high supersaturation of carbonate minerals (SI ca. 1). The soda ocean was lost slowly by subduction of seawater and replaced by the present halite-dominated ocean toward the end of the Precambrian. This facilitated the development of multicellularity and biomineralization as detoxification reactions of life against the rising Ca-concentrations. The SOH is also consistent with the widespread occurrence of stromatolites and cherts in the Precambrian.

Bibliography

- Abelson, P. H., 1966. Chemical events on the primitive Earth. *Proceedings of the National Academy of Sciences USA*, **55**, 1365–1372.
- Altermann, W., Kazmierczak, J., Oren, A., and Wright, D. T., 2006. Cyanobacterial calcification and its rock-building potential during 3.5 billion years of earth history. *Geobiology*, **4**, 147–166.
- Arp, G., Reimer, A., and Reitner, J., 2001. Photosynthesis-induced biofilm calcification and calcium concentrations in Phanerozoic oceans. *Science*, **292**, 1701–1704.
- Bahlburg, G., and Breitzkreuz, C., 1998. *Grundlagen der Geologie*. Stuttgart: F. Enke.
- Bau, M., and Möller, P., 1994. Präkambrische chemisch-sedimentäre Mineralisationen. *Geowissenschaften*, **12**, 333–336.
- Brennan, S. T., Lowenstein, T. K., and Horita, J., 2004. Seawater chemistry and the advent of biocalcification. *Geology*, **32**, 473–476.
- Carafoli, E., 1987. Intracellular calcium homeostasis. *Annual Review of Biochemistry*, **56**, 395–433.
- Degens, E. T., 1989. *Perspectives on Biogeochemistry*. Berlin: Springer-Verlag.
- Dose, K., and Rauffuss, H., 1972. On the electrophoretic behavior of thermal polymers of amino acids. In Rohlfsing, D. L., Oparin, A. I. (eds.), *Molecular Evolution: Prebiological and Biological*. New York: Plenum Press, pp. 1–199.
- Einsele, G., 1992. *Sedimentary Basins*. Berlin: Springer-Verlag.
- Garrels, R. M., and Mackenzie, F. T., 1967. Origin of the chemical composition of some springs and lakes. In *Equilibrium Concepts of Natural Water Systems. Advances in Chemistry*, 67. American Chemical Society, pp. 222–242.
- Ginsburg, R. N., 1991. Controversies about stromatolites: Vices and virtues. In Müller, D. W., McKenzie, J. A., and Weissert, H. (eds.), *Controversies in Modern Geology*. London: Academic Press, pp. 25–36.
- Grotzinger, J. P., 1990. Geochemical model for Proterozoic stromatolite decline. In Knoll, A. H., and Ostrom, J. H. (eds.), *Proterozoic Evolution and Environments*, American Journal of Science (P. E. Cloud Special Volume), 290-A, pp. 80–104.
- Grotzinger, J. P., and Kasting, J. F., 1993. New constraints on Precambrian ocean composition. *Journal of Geology*, **101**, 235–243.
- Hartmann, J., Kempe, S., Dürr, H. H., and Jansen, N., 2009. Global CO₂-consumption by chemical weathering: What is the contribution of highly active weathering regions? *Global and Planetary Change*, **69**, 185–194.
- Kazmierczak, J., and Kempe, S., 2004. Calcium build-up in the Precambrian sea: A major promoter in the evolution of eukaryotic life. In Seckbach, J. (ed.), *Origins, Evolution and Biodiversity of Microbial Life*. Dordrecht: Kluwer, pp. 329–345.
- Kazmierczak, J., and Kempe, S., 2006. Modern analogues of Precambrian stromatolites from caldera lakes of Niuafo'ou Island, Tonga. *Naturwissenschaften*, **93**, 119–126.
- Kempe, S., and Degens, E. T., 1985. An early soda ocean? *Chemical Geology*, **53**, 95–108.
- Kempe, S., and Kazmierczak, J., 1990. Calcium carbonate supersaturation and the formation of in situ calcified stromatolites. In Ittekkot, V. A., Kempe, S., Michaelis, W., and Spitz, A. (eds.), *Facets of Modern Biogeochemistry* Festschrift for E.T. Degens on occasion of his 60th birthday, Berlin: Springer-Verlag, pp. 255–278.
- Kempe, S., and Kazmierczak, J., 1994. The role of alkalinity in the evolution of ocean chemistry, organization of living systems and biocalcification processes. In Doumenge, F. (ed.), *Past and Present Biomineralization Processes. Considerations about the Carbonate Cycle*. Monaco: Bulletin de l'Institut océanographique, no. spec. 13, pp. 61–117.
- Kempe, S., and Kazmierczak, J., 1997. A terrestrial model for an alkaline martian hydrosphere. *Planetary and Space Science*, **45**, 1493–1499.
- Kempe, S., and Kazmierczak, J., 2003. Modern soda lakes: Model environments for an early alkaline ocean. In Müller, T., and Müller, H. (eds.), *Modelling in Natural Sciences; Design, Validation and Case Studies*. Berlin: Springer-Verlag, pp. 309–322.
- Kempe, S., and Kazmierczak, J., 2007. Hydrochemical key to the genesis of calcareous non-laminated and laminated cyanobacterial microbialites. In Seckbach, J. (ed.), *Algae and Cyanobacteria in Extreme Environments*. Berlin: Springer-Verlag, pp. 241–264.
- Kempe, S., and Pegler, K., 1991. Sinks and sources of CO₂ in coastal seas: the North Sea. *Tellus*, **43B**, 224–235.
- Kempe, S., Kazmierczak, J., and Degens, E. T., 1989. The soda ocean concept and its bearing on biotic and crustal evolution. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomineralization in Plants and Animals* Proceedings of the 5th International Symposium Biomineralization, Arlington, Texas, May, 1986, New York: Plenum Press, pp. 29–43.
- Kempe, S., Kazmierczak, J., Landmann, G., Konuk, T., Reimer, A., and Lipp, A., 1991. Largest known microbialites discovered in Lake Van, Turkey. *Nature*, **349**, 605–608.
- Kretsinger, R. H., 1977. Evolution of the informational role of calcium in eukaryotes. In Wasserman, R. H., Corradino, R. A., Kretsinger, R. H., MacLennan, D. H., and Siegel, F. L. (eds.), *Calcium Binding Proteins and Calcium Function*. New York: North Holland Publishing, pp. 63–7.
- Kretsinger, R. H., 1983. A comparison of the roles of calcium in biomineralization and in cytosolic signaling. In Westbroek, P., and De Jong, E. W. (eds.), *Biomineralization and Biological Metal Accumulation*. Dordrecht: D. Reidel Publishing Co., pp. 123–131.
- López-García, P., Kazmierczak, J., Benzerara, K., Kempe, S., Guyot, F., and Moreira, D., 2005. Bacterial diversity and carbonate precipitation in the microbialites of the highly alkaline Lake Van, Turkey. *Extremophiles*, **9**, 263–274.
- Möller, P., and Bau, M., 1993. Rare-earth patterns with positive cerium anomaly in alkaline waters from Lake Van, Turkey. *Earth and Planetary Science Letters*, **117**, 671–676.
- Morse, J. W., and Mackenzie, F. T., 1998. Hadean ocean carbonate geochemistry. *Aquatic Geochemistry*, **4**, 301–319.
- Pegler, K., and Kempe, S., 1988. The carbonate system of the North Sea: Determination of alkalinity and TCO₂ and calculation of PCO₂ and SICal (Spring 1986). In Kempe, S., Liebezeit, G., Dethlefsen, V., and Harms, U. (eds.), *Biogeochemistry and Distribution of Suspended Matter in the North Sea and Implications to Fisheries Biology*, Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg, SCOPE/UNEP Sonderband, **65**, pp. 35–87.
- Petrychenko, O. Y., Peryt, T. M., and Chechel, E. I., 2005. Early Cambrian water chemistry from fluid inclusions in halite from Siberian evaporates. *Chemical Geology*, **219**, 149–161.

- Riding, R., 2000. Microbial carbonates: the geological record of calcified bacterial-algal mats and biofilms. *Sedimentology*, **47**, 179–214.
- Ries, J. B., Anderson, M. A., and Hill, R. T., 2008. Seawater Mg/Ca controls polymorph mineralogy of microbial CaCO_3 : A potential for calcite-aragonite seas in Precambrian time. *Geobiology*, **6**, 106–119.
- Snyder, W. D., and Fox, S. W., 1975. A model for the origin of stable protocells in a primitive alkaline ocean. *Biosystems*, **7**, 222–229.

Cross-references

Alkalinity
 Biofilms
 Calcite Precipitation, Microbially Induced
 Carbonates
 Cyanobacteria
 Divalent Earth Alkaline Cations in Seawater
 Dolomite, Microbial
 Microbial Mats
 Microbialites, Modern
 Soda Lakes
 Tufa, Freshwater

SOILS

Erika Kothe
 Friedrich Schiller University Jena, Jena, Germany

Synonyms

Pedosphere

Definition

Soil: refers to the product of mineral weathering and secondary mineral formation, (microbial) mineralization, humus formation, and the resulting element mobilization/immobilization in the upper Earth crust in a pedogenetic process involving chemical, physical, and biological activities.

Introduction

Soil is the basis of terrestrial life, particularly for agriculture, forestry, and generally land-use by man (Driessen et al., 2001). It is also the largest terrestrial ecosystem dominated by high numbers of microorganisms and soil-living animals as well as the root systems of plants. The biota within this ecosystem, not only interact but also actively shape their environment (Fiedler et al., 2002). This can be easily seen with root systems or earthworm/mole tunnels, but the metabolic activities of microorganisms by far exceed these visible alterations. Microbes are essential for the decomposition of plant litter followed by humification. The pedogenesis differs with respect to base rock material, climatic conditions, and other factors leading to soil series of different soil types. Root exudates as well as microbiological acidification and release of

chelating agents take part in mobilization and immobilization processes of mineral elements, essential or ecotoxically relevant alike, and thus are major players in import into food chains, and input into water paths or as volatiles into the atmosphere.

Soil function

The soils of the world can be characterized from a more structural or a more functional point of view (FAO-UNESCO, 2002), and the geobiologically important functions involving metabolically active soil dwellers are characterized with reference to microbiology, botany and agriculture, and zoology.

Soil function is essential for plant nutrition. The capacity to solubilize nutrients like P and minerals including K, Na, Mg, Ca, and trace elements, as well as availability of S or N are largely the product of microbial mobilization from minerals, microbial degradation of organic matter, or fixation from air (e.g., for nitrogen). Soil mineralogy largely determines the buffering capacity for minerals adsorbed to different fractions (Jones and Bassington, 1998). The sustainability of microbial activity is connected to water availability for growth, and water storage and availability are also major functions for plant growth. Detrimental processes of soil degeneration like soil acidification, desertification, clay mineral destruction, or decrease of organic contents are global problems mankind experiences which will have a large impact on socioeconomic scales.

Agriculture and nutrition in modern systems are coupled to the use of fertilizers and plant protection chemicals which can constitute a threat to the water pathways, if management of agricultural use is not performed according to the laws and provisions on soil and water protection, good agricultural practice, or integrated farming, and monitoring has to be included to ensure best practice. Soil density increases due to the use of heavy machinery which leads to soil degradation in high-yield agricultural systems the world over.

In order to assess possible problems with land-use and soil degeneration, soil science has provided a very good reference system to classify and monitor soils based on soil mechanics, soil physics, soil genesis, soil composition, and soil functions reviewed in different contexts below (IUSS Working Group WRB, 2006). As soil can be regarded as a major sink for carbon depending on land-use, this might become more important as climate change discussions are gaining impact. Generally, soils and larger views on landscape should be regarded as eminent players in global cycles (Meir et al., 2006).

Soil texture

The soil texture can be divided in the proportion of sand (S: 63 μm –2 mm), silt (U: 2–63 μm), and clay (T: smaller than 2 μm) after removing skeleton particles (larger than 2 mm). While sand soils have more than 70 volume % of sand, clay soil is mainly composed of clay (>50%) with

only small portions of sand (<20%). Most soils in humic conditions are sandy loam (with at least 1/3 sand, 1/3 silt, and up to 1/3 loam) or sandy silt soils (with at least 1/3 sand, at least 1/3 silt, and lower amounts of loam).

These differences in texture are also reflected in mineral composition of the respective soils. While sand soil contains 80% of quartz, this mineral constitutes only 25% of clay soil. In contrast, 50% clay minerals (mostly illit and vermiculite, as well as smaller portions of montmorillonite and kaolinit) are present in clay soil while sand soil contains only 5% of clay minerals. As clay minerals show the highest buffering potential for water and mineral elements, like alkali and earth alkali elements, soil types with higher clay mineral contents show high sorption capacities. Sandy loam/silt shows the highest fertility because of a harmonized ration between water storage and sorption capacities.

Water-retention potentials are of major importance for soil functioning. While sand soil has a water capacity of only 10 volume %, 2/3 of that water (7 volume %) is bio-available. Loam has 45 volume % water capacity of which, however, only a small proportion, usually less than 12 volume %, is available to plant roots. Sand soil has the highest aeration with 30 volume % of gas-filled pores. This is dependent on the pore sizes. Pores that are smaller than 10 μm retain water. Large pores of over 50 μm diameter, in contrast, are air-filled and contain only adsorption water. Capillary water in unsaturated soil is found in pores of 5–10 μm diameter. This is important for the soil function with respect to plant growth. As plant root hairs can just about enter pores of 5–10 μm , most water transport in soil is not simply taken from water-filled pores, but has to be extracted with sucking tension. The investigation of in situ soil water, however, remains a challenge (Weihermüller et al., 2007).

Soil type

Soil types are distinguished depending on the development of soil (pedogenesis) over space and time. The formation of a soil type generally takes a long time, upward of 1000 years. The soil type also is dependent on the climatic conditions under which the pedogenesis has taken place. A typical example of this is the formation of laterite soils in tropical areas. Such soil types show strong coloration, with iron-rich red soil types dominating in most areas. This is due to mobilization and wash-out of elements released from minerals and retaining of insoluble iron(hydroxy)oxides.

The function of mineralic, climatic, and biological conditions over time leads to formation of soils defined by different horizons. These can extend over cm up to 100 m and more, depending on the conditions experienced.

Horizons and soil profiles

A visual inspection of a soil profile already leads to immediate distinction of several layers in a typical soil. In an autochthonous soil formation, the lowest typical layer

above groundwater is the C horizon, the base rock on which the soil develops. It presents the basis for the elemental composition of the soil developing on its surface.

The B horizon (subsoil) is the next layer, clearly dominated by weathering products. Immigration of clay and sesquioxides (Fe and Al-oxihydroxides) may be found to dominate such B horizons which is specified in B_v (weathering dominated), B_t (loam accumulation), and B_s (sesquioxides) subclassification.

The uppermost layer is the A horizon (topsoil) which is rich in humus resulting from degradation of leaf litter and often shows a darker color due to organic contents enriched in this horizon. The uppermost layer of humus also is specifically termed A_o horizon. In the A horizon, microbial populations are largest and metabolic activities of soil microorganisms can be shown easily by soil respiration analyses. The A horizon also is the site of dominant plant root penetration which again stabilizes and supports the soil structure as well as the microorganisms present. Again, this horizon can be subdivided into layers A_h or A_1 (uppermost layer rich in humus), A_p (altered upper layer due to ploughing) and A_e or A_2 (subsequent layer influenced by wash-out processes). A layer of litter above the topsoil horizon is formed by decaying plant material. Depending on soil type, not all horizons have to be present.

Soil biology

The animals found in soil, or edaphone, are earthworms, nematodes, insects, specifically collembolids, and moles, to name the most prominent. Their function in soil is especially to mix the soil, to draw leaves under the soil surface which accelerates decay because of higher humidity and better accessibility for microbes, and to form passages for water interflow allowing rain to enter a soil more easily.

Plant roots stabilize soil and protect from wind and water erosion. Decaying plant roots leave passages also allowing for preferential water flow. And living roots contain special tissues for aeration and therefore improve oxygen availability for microorganisms in soil. At the same time, up to 30% of plant net photosynthesis products can be excreted by the roots in the root exudates. These consist mainly of organic acids, like acetate, oxalate, malate, and citrate. These are able to feed microbes in the rhizosphere and also chelate iron and supply the plant with iron. On the other hand, these organic acids, as well as sugars and amino acids form root exudates, provide nutrition for soil microorganisms which multiply in the vicinity of roots. This constitutes the rhizosphere, the specific area found around a plant root (Watt et al., 2006). More directly associated with the plant root are the rhizoplane bacteria which colonize the surface of the plant root. Symbiotic root associations include the nitrogen-fixing rhizobial symbioses with leguminaceous plants, and the mycorrhizal mutual symbioses between fungi and plant roots. It has been stated that microbial populations structure the plant biodiversity seen above-ground by such soil-derived associations (van der Heijden et al., 2007).

The soil surface, if no plants are present, is often covered by a crust consisting of a biofilm with cyanobacteria, fungi, bacteria, and lichens. This crust will lead to covering of bare soils which will then be prone to succession with mosses and plants.

Soil microbiology

Soils are the major place of element turnover and biogeochemical cycling in terrestrial ecosystems (Crawford et al., 2005). The degradation of plant litter, decay of leaves, carcasses, feces, and wood rotting are maintained by soil microorganisms with subsequent processes of humification. In addition, denitrification, nitrogen fixation, and element mobilization are performed by the metabolic activities of soil microorganisms. The formation of humus and the binding of elements with humic and fulvic acids drive soil development, and its function for the soil ecosystem cannot be overestimated.

Populations of soil microorganisms contain eukaryotes, bacteria, and archaea. Depending on the metabolic functions prevailing, the soil generated at the site will be dominated by formation of humic and fulvic acids, like in moorlands, by high mobilization of cations, and the formation of dissolved organic matter (DOC) in luvisols and podzols (in woodlands) or tchernozem (in agricultural land and grasslands). To have an idea of the scale, in 1 hectare soil it has been calculated that 8 tons of microorganisms are present, while the edaphon makes only for 2 tons of biomass. With the smaller size of microbes, this makes for very high numbers of soil microorganisms consisting of eukaryotic, bacterial, and archaeal cells.

The eukaryotic population is dominated by fungi with minor populations of ciliates, flagellates, algae including oomycetes and amoebae including myxomycetes. Of special importance are the mycorrhizal fungi forming a mutual symbiosis with plant roots thereby influencing not only plant physiology, but also soil as the hyphae extending into soil can add to soil structure and texture (Rillig and Mummey, 2006).

The archaea found in soil include especially the anaerobic methanogens which can lead to methane exhalation. However, methane oxidation performed by other groups of related archaea, already sequester a large portion of methane in soils. Methane exhalation is more often seen from swamps and flooded areas.

The major taxa of soil bacteria are the Gram-negative proteobacteria, the cytophaga, and Gram-positive bacilli and actinobacteria. Microbial interactions are a driving force for the community structure (Wardle, 2006). Per gram dry weight of soil, usually 10^7 – 10^9 bacteria are found. However, the bacterial population so far mainly had been analyzed by plating techniques, which will allow only those to be found that are growing under the applied laboratory conditions. Estimates of direct counts have indicated higher numbers with only 1–10% of bacteria being cultivable. Modern techniques relying not on cultivation but on DNA extraction from soil with subsequent

amplification and cloning of 16S rDNA sequences give a better insight in microbial soil populations. New taxa occur by these analyses and the phylogenetic associations observed from the obtained 16S rDNA sequences show that entire branches in the phylogenetic tree of bacteria can be described. It will remain a task to now find possibilities to cultivate these organisms in order to evaluate their physiological properties. Only such new investigations will allow to assess the role of new classes of bacteria in soil function and specific pathways for microbial degradation and soil formation will be uncovered as such investigations and modeling making use of the results will progress (O'Donnell et al., 2007).

Humification

Soil organic matter is the basis for humification (Kögel-Knabner et al., 1990). The characteristics of soil organic matter have been analyzed in some detail (Schaumann, 2006). Humus (Stevenson, 1994) provides essential functions in soils. The formation of humic acids and fulvic acids is essential for A horizon development, and especially the A_h horizon of tchernozem is rich in humus. Both humic acids (brown and grey) and shorter-lived fulvic acids have been described as recalcitrant matter with turnover times of thousands of years. These figures are re-evaluated now as it becomes clear that microbial metabolism uses these recalcitrant substrates and incorporates the carbon moieties into their cell mass. However, it is clear that the humic and fulvic acids constitute a major portion of soil matter and that their specific function in water retention and mineral element adsorption contributes a great deal to soil fertility.

Humus constituents also are important for (temporal) fixation of minerals and organic exudates. Their release will be controlled by the microbiota prevalent in the environment (Watteau and Berthelin, 1994).

Summary

Soil is the basis of terrestrial life and both evolves from biological activities and in turn allows and shapes terrestrial ecosystems. Soil types can be distinguished with different compositions, functionalities, and fertilities for production in agronomy and forestry, or water quality. The development of a soil type, such as tchernozem, rendzina, and podzol (and in tropic conditions, latozol) is dependent on base rock, climate, physical and chemical weathering, and formation of humic substances which takes thousands of years. The pedogenesis, in addition, is a result of root functions and plant litter degradation. The resulting soil can be characterized in vertical direction by soil horizons, which are distinguished from the A horizon with high humus contents, over a B horizon with lower humus contents, to the C horizon mainly consisting of base rock material. Soil series attempts to combine soil types and soil structure to reveal parameters for soil function.

Bibliography

- Crawford, J. W., Harris, J. A., Ritz, K., and Young, J. M., 2005. Towards an evolutionary ecology of life in soil. *Trends in Ecology and Evolution*, **20**, 81–87.
- Driessen, P., Deckers, J., Spaargaren, O., and Nachtergaele, F., 2001. *Lecture Notes on the Major Soils of the World*. World Soil Resources Reports 94, Rome: FAO.
- FAO-UNESCO, 2002. *Digital Soil Map of the World and Derived Soil Properties*. Land and Water Digital Media Series No. 1 Rev. 1. Rome: FAO.
- Fiedler, S. H., Jungkunst, P. F., Kleber, M., Sommer, M., Jahn, R., and Stahr, K., 2002. Linking soil classification and soil dynamics – pedological and ecological perspectives. *Journal of Plant Nutrition and Soil Science*, **165**, 517–529.
- IUSS Working Group WRB, 2006. *World Reference Base for Soil Resources: Atlas*. Leuven, The Netherlands: ISRIC-FAO-ISSS-Acco, p. 63.
- Jones, D. L., and Bassington, D. S., 1998. Sorption of organic acids in acid soils and its implication in the rhizosphere. *European Journal of Soil Science*, **49**, 447–455.
- Kögel-Knabner, I., Hatcher, P. G., and Zech, W., 1990. *Decomposition and Humification Processes in Forest Soils: Implications from Structural Characterization of Forest Soil Organic Matter*. Kyoto, Japan: Trans. 14th Int. Congr. Soil Sci., pp. 218–223.
- Meir, P., Cox, P., and Grace, J., 2006. The influence of terrestrial ecosystems on climate. *Trends in Ecology and Evolution*, **21**, 254–260.
- O'Donnell, A. G., Young, J. M., Rushton, S. P., Shirley, M. D., and Crawford, J. W., 2007. Visualization, modeling and prediction in soil microbiology. *Nature Reviews Microbiology*, **5**, 689–699.
- Rillig, M. C., and Mummey, D. L., 2006. Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.
- Schaumann, G. E., 2006. Soil organic matter beyond molecular structure, part I: macromolecular and supramolecular characteristics. *Journal of Plant Nutrition and Soil Science*, **169**, 145–156.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: Wiley.
- van der Heijden, M. G., Bardgett, R. D., and van Straalen, N. M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.
- Wardle, D. A., 2006. The influence of biotic interactions on soil biodiversity. *Ecology Letters*, **9**, 870–886.
- Watt, M., Silk, W. K., and Passioura, J. B., 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of Botany*, **97**, 839–855.
- Watteau, F., and Berthelin, J., 1994. Microbial dissolution of iron and aluminum from soil minerals: efficiency and specificity of hydroxamate siderophores compared to aliphatic acids. *European Journal of Soil Biology*, **30**, 1–9.
- Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., and Pütz, T., 2007. In situ soil water extraction: a review. *Journal Of Environmental Quality*, **36**, 1735–1748.

Cross-references

Bacterial Clay Autigenesis
 Biogeochemical Cycles
 Carbon Cycle
 Denitrification
 Microbial Degradation
 Microbial-Metal Binding
 Mycorrhizae
 Nitrogen Fixation
 Siderophores

SPECIES (MICROBIAL)

Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “*Microbial Communities, Structure, and Function*” for further reading.

SPELEOTHEMS

Roman Aubrecht
 Comenius University, Bratislava, Slovakia

Synonyms

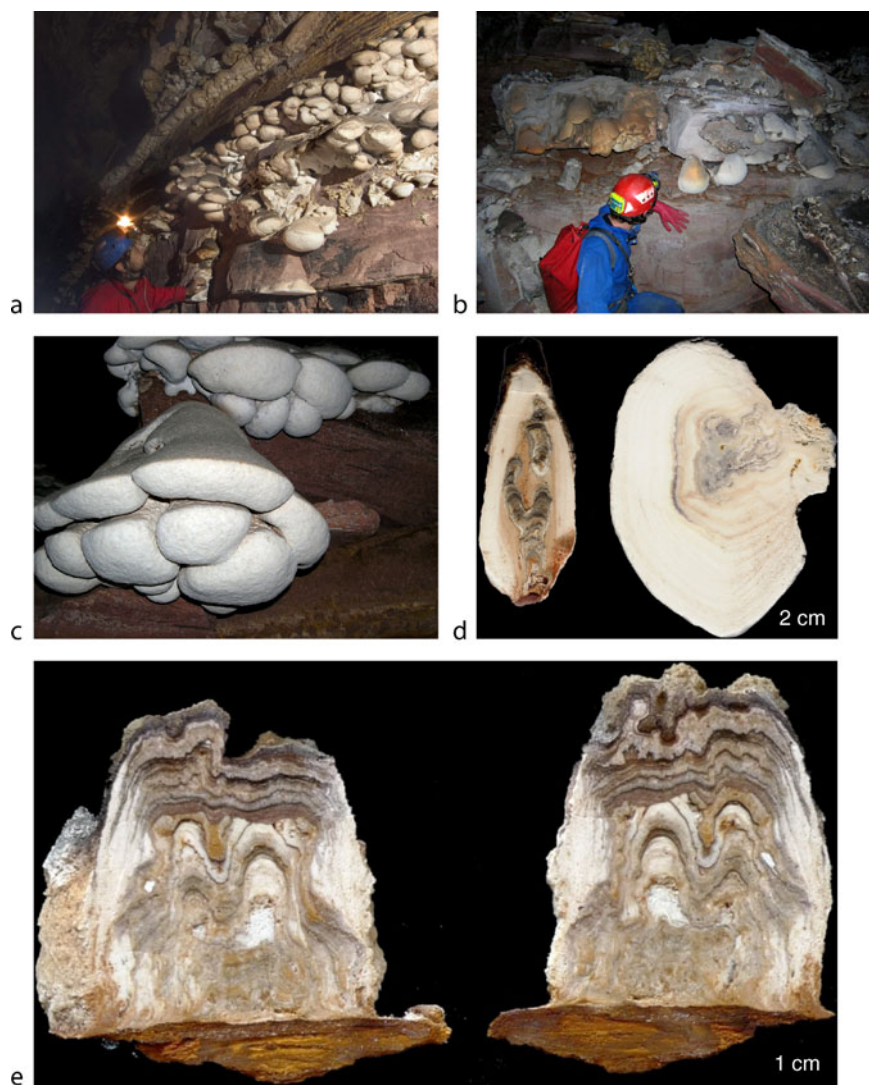
Cave deposits; Cave sinters

Definition

The word “speleothem” is from Greek and means “cave deposit.” According to its origin, this term can be used for any cave deposits, but it is used mostly for secondary mineral deposits formed (precipitated) in caves. In this entry, speleothem is understood as constructively formed, new cave material. Materials originated by destructive phenomena, such as erosion, weathering, etching, biocorrosion, or bioerosion are not subjects of this entry.

Biogenic and abiogenic speleothems

Most of the speleothems originate inorganically by precipitation of various minerals from solutions. The minerals are dissolved by undersaturated fluids and precipitate when the solutions become supersaturated, e.g., by evaporation, outgassing, or pH changes. Mineralogical compositions of speleothems vary depending on the environment in which the caves are formed. The most common speleothem-forming mineral is calcite (as most of the caves are formed of limestones), forming well-known sinter forms, such as dripstones (stalactites, stalagmites) and flowstones. Locally, mostly in hydrothermally influenced cavern systems, speleothems may be formed by its rhombic modification – aragonite. Gypsum and other sulfate minerals commonly form speleothems in gypsum karst but are widespread also in other types of caves. Speleothems formed in silicate rocks (e.g., sandstones, quartzites, granites, etc., [Figure 1](#)) are relatively rare, composed mostly of various forms of silica (from opal-A to microquartz depending on its recrystallization). Other speleothems can be represented by ferroan and manganese oxides and hydroxides forming cave flowstones.



Speleothems, Figure 1 Examples of microbial speleothems – cave stromatolites encrusted by silica in the sandstone cave Cueva Charles Brewer, Chimantá Plateau, Venezuela. (a–c) Mushroom-like stromatolites growing on the cave walls. (d) Slabs of stalactitic and mushroom-like stromatolites. Columnal stromatolites (*dark*) are formed by filamentous microbes; *white*, chalk-like parts represent stromatolites formed by tiny spherical microbial colonies. (e) Other shape of stromatolites with prevailing laminae formed by filamentous microbes.

Overall inventory of cave minerals is very large (for a summary see Hill and Forti, 1986).

The formation of some speleothems is influenced by organisms. The organisms can be passively covered (encrusted) by speleothems or they can directly biologically mediate their formation. In some cases it is not easy to distinguish between the active and passive role of the organisms. Biological mediation of mineral precipitation usually involves biomineralization (biomineralization (general aspects); microbial biomineralization; silica biomineralization (sponges)) and/or organomineralization. Biomineralization means direct precipitation of mineral skeleton (either internal or external) or encrustation by the organism itself (*syn vivo*). Organomineralization in

which the precipitation of minerals is mediated by organic matters produced by the organisms and by changing the ambient conditions (e.g., pH, enrichment or depletion of the solution by metabolism, etc.). They can occur either *syn vivo* (when the organisms are alive – they influence the ambient environment actively) or *postmortem* (after the organisms are dead – the ambient environment is influenced by the decay products of the organisms).

Organisms which mediate speleothem formation

The speleothem-forming organisms are mostly microorganisms (Archaea, Bacteria including Cyanobacteria, and Algae); more evolved multicellular organisms are

mostly represented by Fungi, mosses and lichens. Also roots of higher plants, penetrating to caves can form speleothems. All the mentioned organisms form speleothems from the autochthonous cave material (mostly taken from solutions). Excrements of animals (e.g., bats) can turn to well-known cave deposit – guano. Unlike the previously mentioned speleothems, guano and its minerals (rich group of phosphates, nitrates, and organic minerals) are completely allochthonous in caves, introduced originally from outside.

Microbes

Microbes inhabiting caves are mostly chemolithoautotrophic or chemoheterotrophic, i.e., their nutrition is not dependent on sunlight (for review see Konhauser, 2007). The exception is the so-called lampenflora, represented by phototrophic cyanobacteria and other algae which live on the lightened places in show caves. Phototrophic microorganisms normally inhabit the entrance areas and the number of microorganisms declines rapidly toward the twilight areas. Unicellular algae – diatoms – were found to contribute to the formation of speleothems, which occurred close to the entrance (Kashima et al., 1987; Kashima and Ogawa, 1995). Some cyanobacteria, although originally phototrophic, can adapt themselves to darkness and change their life mode to heterotrophic. For instance, the genera *Fisherella* and *Calothrix* were found to be able to change their mode of life to slow heterotrophic in complete darkness (Whitton, 1987). Some cyanobacteria were found to inhabit dark cave environment e.g., *Geitleria calcarea* and *Scytonema julianum* (Friedman, 1955; Bourrelly and Depuy, 1973) and contribute to speleothem formation. *Nostoc* is also easily adaptable to a heterotrophic mode of life, and is a common symbiont (endobiont) in lichens and higher plants. In addition to photosynthesis, *Nostoc* is able to perform nitrogen fixation (symbiosis of higher plants with *Nostoc* provides them with nitrate). *Nostoc* has also been reported from caves in Israel, growing in various zones, from well illuminated to complete darkness (Vinogradova et al., 1998). Moreover, *Nostoc* is also supposed to form large opal stromatolites in the Venezuelan sandstone caves (Figure 1; Aubrecht et al., 2008).

The most common in cave environments are chemolithoautotrophic and chemoheterotrophic microorganisms. Similar to other microorganisms, they use CO₂ as their carbon source but their source of energy comes from different chemical reactions. Chemoheterotrophs utilize preformed organic compounds, whereas chemolithoautotrophs utilize mostly inorganic compounds (Konhauser, 2007). These organisms and processes they utilize are numerous but only some of them are important speleothem-forming factors. Sulfur-oxidizing bacteria, such as *Beggiatoa* or *Thiothrix*, are responsible for deposition of elemental sulfur and sulfates in caves. They are particularly important in the caves with sulfur-rich waters, e.g., Parker Cave, Kentucky (Barton

et al., 2001). Bacteria also mediate formation of iron oxide and manganese oxide flowstones (Gradziński et al., 1995). Finally, they may also mediate formation of the carbonate speleothems, which are the most widespread speleothems in the world (Taboroši, 2006).

Fungi

Fungi are ubiquitous in all caves, being mostly chemoheterotrophs. They not only inhabit the caves but they actively contribute to the formation of some carbonate speleothems, such as a particular, soft form of sinter – moonmilk (Gradziński and Szulc, 1997; Gradziński et al., 1997).

Mosses

Mosses (often together with lichens) occur mostly in the near-entrance areas and are mostly passively encrusted by minerals (mostly calcite), thus forming moss tufas (Taboroši, 2006).

Plant roots

In shallow-placed galleries, plant roots sometimes penetrate from the surface. They are often mineralized, forming the so called rootsicles (Taboroši, 2006). It is not yet known whether they represent passive encrustations, or the roots actively mediate the precipitation. This is also true for other plant (and animal) remnants which are locally transported to the caves from the surface.

Marine organisms forming speleothems

Marine organisms represent a special chapter in the genesis of organic-mediated speleothems. Marine water, bringing nutrition to submarine caves, enables many organisms to live in this cryptic environment. Sessile organisms overgrowing the cave walls easily turn into speleothems. Very common are cryptic stromatolites (Reitner, 1993) formed by nonphotosynthetic organisms or serpulid buildups (Taboroši, 2006; Schlögl et al., 2008) formed by these filter feeders in submarine caves.

Methods of recognition of the speleothem-forming microorganisms

Cave-dwelling microorganisms, including those which contribute to speleothem formation, are considered to be extremophiles. That means they are organisms living in and adapted to environments which are not prolific for many organisms and which lack many aspects necessary for life; in caves, it is mainly the lack of sunlight and generally oligotrophic character of the environment. Adaptation of these organisms to the cave environment makes them uneasy to be determined by classical microbiological methods (Barton, 2006). For instance, for the complete identification of cyanobacteria, about 37 characteristics are needed, e.g., cell morphology, ultrastructure, morphology of colonies or filaments, genetic characteristics (DNA), cultivation conditions, and life conditions (Castenholz, 2001). Cultivation was so far the most

important tool in microbiology. This tool was developed mostly for medical purposes and was optimized for the research of microbes inhabiting animal bodies. However, only a small portion of free-living microbes is cultivable from environmental samples, including caves. Therefore, new culture-independent *in situ* methods have been developed for the detection of microbial groups in natural microbial assemblages, based on the molecular analysis of their DNA (Barton, 2006). Among these methods, one of the most important is *in situ* hybridization (FISH). Biomarker analysis may also be a suitable tool for the analysis of speleothems, in which only organic matter remained after dead, decayed organisms.

Conclusion

Speleothems are secondary mineral deposits formed in caves. They can form either inorganically or originate biologically. The inorganic formation pathway is based on physical precipitation from solutions after they become supersaturated. Biologically mediated precipitation is influenced by organic matter produced either by living or dead organisms and does not necessarily involve supersaturated solutions. This kind of precipitation acts via biomineralization or organomineralization, and may result in various sorts of minerals, such as calcite, elementary sulfur, sulfates, iron and manganese oxides, etc. The main biological protagonists forming speleothems are microbes. They are mostly chemolithoautotrophic and chemoheterotrophic microorganisms, or phototrophic organisms that changed their life modes and adapted themselves to the cave environment. Normally, the phototrophs are active only in near-entrance parts of caves. Other organisms forming speleothems are mosses, lichens, higher plants, or animals. These are however, mostly passively encrusted by minerals; their potential for active biological mediation to form speleothems is still the matter of research.

Bibliography

- Aubrecht, R., Brewer-Carías, Ch., Šmída, B., Audy, M., and Kováčik, L., 2008. Anatomy of biologically mediated opal speleothems in the world's largest sandstone cave Cueva Charles Brewer, Chimantá Plateau, Venezuela. *Sedimentary Geology*, **203**(3–4), 181–195.
- Barton, H. A., 2006. Introduction to cave microbiology: a review for the non-specialists. *Journal of Cave and Karst Studies*, **68**(2), 43–64.
- Barton, H. A., Spear, J. R., and Pace, N. R., 2001. Microbial life in the underworld: biogenicity in secondary mineral formations. *Geomicrobiology Journal*, **18**, 359–368.
- Bourrelly, P., and Depuy, P., 1973. Quelques stations françaises de *Geitleria calcarea*, Cyanophycée cavernicole. *Schweizerische Zeitschrift für Hydrologie*, **35**, 136–140.
- Castenholz, R. W., 2001. Phylum BX. Cyanobacteria. Oxygenic photosynthetic bacteria. In Boone, D. R., and Castenholz, R. W. (eds.), *Bergey's Manual of Systematic Bacteriology*, The Archaea and the deeply branching and phototrophic Bacteria, 2nd edn. New York: Springer, Vol. 1, pp. 473–599.
- Friedman, I., 1955. *Geitleria calcarea* n.gen. et n.sp., a new atmophytic lime-encrusting blue-green alga. *Botaniska Notiser*, **108**, 439–445.
- Gradziński, M., and Szulc, J., 1997. Recent microbial speleothems ("moonmilk" deposits). 3rd IFAA Regional Symposium & IGCP 380 International Meeting Guidebook, Cracow, Sept. 14–20, 1997, pp. 38–39.
- Gradziński, M., Banaś, M., and Uchman, A., 1995. Biogenic origin of manganese flowstones from Jaskinia Czarna Cave, Tatra Mts., Western Carpathians. *Annales Societatis Geologorum Poloniae*, **65**, 19–27.
- Gradziński, M., Szulc, J., and Smyk, B., 1997. Microbial agents of moonmilk calcification. In Jeannin, P.-Y. (ed.), *Proceedings of the 12th International Congress of Speleology*. Basel, Switzerland: IUS, Vol. 1, pp. 275–278.
- Hill, C. A., and Forti, P., 1986. *Cave Minerals of the World*. Huntsville: National Speleological Society, 238 pp.
- Kashima, N., and Ogawa, T., 1995. A note on biogenic effect of coralloid speleothems in Round Mountain Lava Cave, Oregon, U.S. *A. Journal of Speleological Society of Japan*, **19**, 8–12.
- Kashima, N., Teruo, I., and Kinoshita, N., 1987. Diatom, contributors of coralloid speleothems, from Togawa-Sakaidani-do Cave in Miyasaka prefecture, Central Kyushu, Japan. *International Journal of Speleology*, **16**, 95–100.
- Konhauser, K., 2007. *Introduction to Geomicrobiology*. Malden: Blackwell Publishing, 425 pp.
- Reitner, J., 1993. Modern cryptic microbialite/metazoan facies from Lizard Island (Great Barrier Reef, Australia). Formation and concepts. *Facies*, **29**, 3–40.
- Schlögl, J., Michalik, J., Zágorský, K., and Atrops, F., 2008. Early Tithonian serpulid-dominated cavity-dwelling fauna, and the recruitment pattern of the serpulid larvae. *Journal of Paleontology*, **82**(2), 382–392.
- Taboroši, D., 2006. Biologically influenced carbonate speleothems. In Harmon, R. S., and Wicks, C. M. (eds.), *Perspectives on Karst Geomorphology, Hydrology and Geochemistry*. GSA Special Paper 404, pp. 307–317.
- Vinogradova, O. N., Kovalenko, O. V., Wasser, S. P., Nevo, E., and Weinstein-Evron, M., 1998. Species diversity gradient to darkness stress in blue-green algae/cyanobacteria: a microscale test in a prehistoric cave, Mount Carmel, Israel. *Israel Journal of Plant Sciences*, **48**, 229–238.
- Whitton, B. A., 1987. The biology of *Rivulariaceae*. In Fay, P., and van Baalen, C. (eds.), *The Cyanobacteria – A Comparative Review*. Amsterdam: Elsevier, pp. 513–534.

Cross-references

Algae (Eukaryotic)
 Archaea
 Bacteria
 Beggiatoa
 Biomarkers (Organic, Compound-Specific Isotopes)
 Calcite Precipitation, Microbially Induced
 Chemolithotrophy
 Cyanobacteria
 Diatoms
 Extreme Environments
 Fungi and Lichens
 Karst Ecosystems
 Microbial Biomineralization
 Microbial Ecology of Submarine Caves
 Microbialites, Modern
 Moonmilk
 Nitrogen Fixation
 Organomineralization
 Sediment Diagenesis – Biologically Controlled

Silica Biomineralization, Sponges
Sinter
Stromatolites
Sulfate-Reducing Bacteria

SPONGES (PORIFERA) AND SPONGE MICROBES

Friederike Hoffmann¹, Marie-Lise Schläppy²

¹Sars International Centre for Marine Molecular Biology, Bergen, Norway

²Max Planck Institute for Marine Microbiology, Bremen, Germany

Synonyms

Sponge-associated bacteria; Sponge-associated microbes; Sponge-associated microbiota; Sponge bacteria; Sponge-microbe systems; Sponge symbionts

Definition

Sponges are sessile multicellular aquatic animals (Lat. Porifera = bearing pores) whose bodies have a typical body plan which allows water to pass through the animals. Sponges are characterized by a skeleton made of calcareous, siliceous spicules or horny fibers.

Sponge microbes: Bacteria and archaea that live in close and permanent association with sponges, usually in the mesohyl between sponge cells.

Introduction

Sponges are sedentary benthic organisms found in shallow waters on tropical coral reefs to the Arctic and the deep sea. Some species, however, are restricted to freshwater ecosystems. Sponges are characterized by a typical body plan built around a system of water canals and chambers (Figure 1a). Within the chambers, flagellated cells called choanocytes produce a water current that enters the sponge body through surface pores (ostia) and leaves through larger openings called oscula. Hence, sponges are filter feeders and incorporate particles from the water as energy and nutrient source. An external cell layer (pinacoderm) encloses the sponge mesohyl, a glycosidic matrix containing several cell types which perform a variety of functions. Sponge cells show a low degree of specialization and a high degree of independence so that the sponge body in some respect resembles a protozoan colony. However, sponges are, without any doubt, placed as true members of the Metazoan (Ax, 1995; Müller, 1998a, b).

Sponges have been grouped into three classes: Calcarea, Hexactinellida, and Demospongiae. Calcarea are characterized by the presence of calcareous spicules. The upgrade of this class to the phylum level has recently been proposed by Borchiellini et al. (2001). Hexactinellida are characterized by siliceous spicules of hexactine structure and syncytial tissue organization.

Demospongiae, the most numerous and diverse class, is a nonmonophyletic group (Boury-Esnault, 2006). They generally have a mineral skeleton made of siliceous spicules, but several lineages, like the common bath sponge, have no mineral skeleton but a network of fibers instead. Modern sponge phylogeny is now based on a combination of molecular methods and sponge morphological features, but sponge spicules are still important features for species determination and identification of sponges in the fossil record.

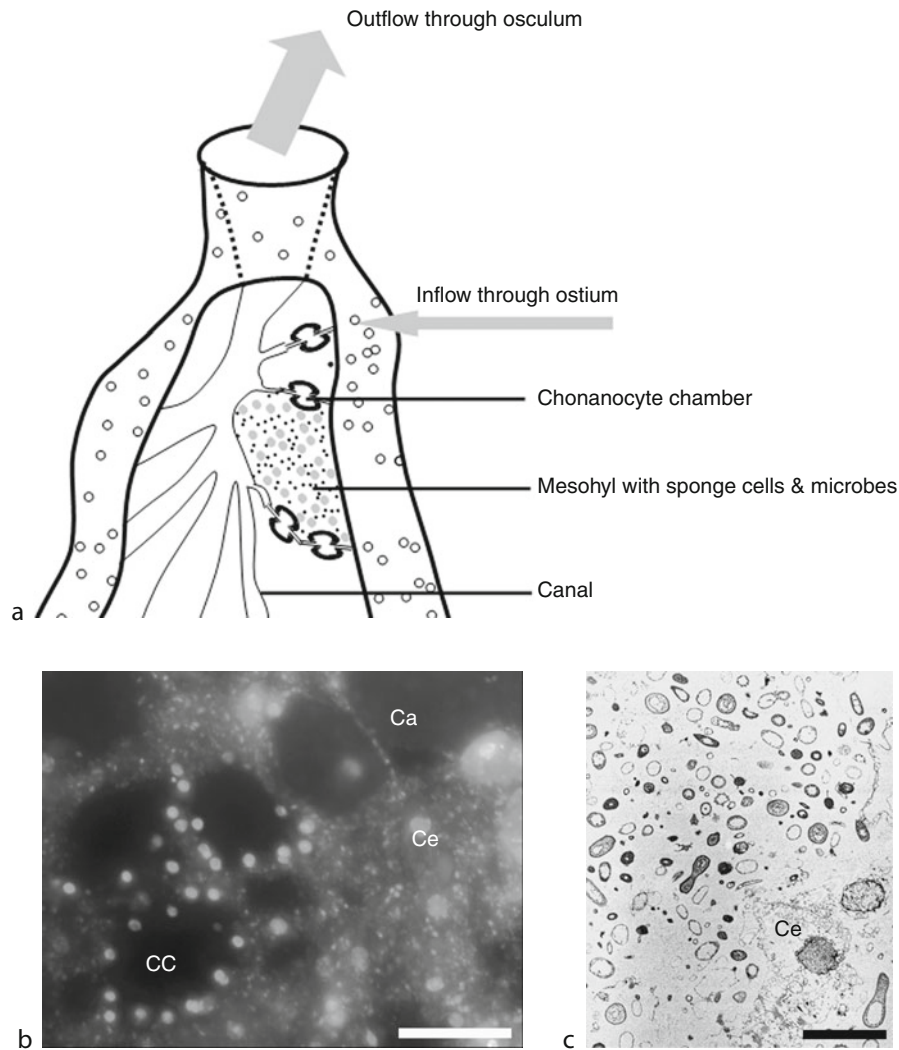
Sponges form one of the deepest radiations of the Metazoa and can be regarded as one of the oldest animal phylum still alive. The spicule record for sponges starts in the late Proterozoic (Reitner and Wörheide, 2002), while chemofossil records even indicate the presence of sponges – or their direct ancestors – already in the early Proterozoic: specific C₃₀ steranes (24-isopropylcholestanes), which are unambiguous biomarkers for demosponges, were found in 1,800 Ma old stromatolites (McCaffrey et al., 1994; Moldowan et al., 1994).

Choanoflagellates, a monophyletic group of protists which can show a colonial lifestyle, are the closest sister group of the Metazoa (Medina et al., 2001) and show a morphological resemblance to the flagellated choanocytes of sponges. It is, therefore, widely agreed that the “urmetazoa,” the last common ancestors of all Metazoa, would have many traits found in sponges (see review in Gaidos et al., 2007).

Numerous sponge species host vast amounts of microbes in their mesohyl matrix (Figure 1b and c) or sometimes in specialized cells, while others contain only few or no microorganisms at all. The former have been termed bacteriosponges (Reiswig, 1981) or high-microbial-abundance sponges (Hentschel et al., 2003) while the latter are referred to as “low-microbial-abundance sponges.” Bacterial population densities in bacteriosponges may reach 10⁸–10¹⁰ microbes per gram of sponge wet weight, while those in low-microbial-abundance sponges are within the range of natural seawater.

The first studies exploring the microbial community of sponges were based on electron microscopy and isolation of microbes and yielded invaluable information about the microorganism morphotypes and their specific location within the sponge (e.g., Wilkinson, 1978). Microbes were found to exist extra- and intra-cellularly (e.g., Vacelet and Donadey, 1977) and even inside the nucleus of sponge cells (Vacelet, 1970). More recently, the advent of molecular techniques have enabled a detailed phylogenetic description of the microorganisms associated with sponges (Hentschel et al., 2006), whereas environmental genomics have given insights into their metabolic and physiological properties (Grozdanov and Hentschel, 2007).

Phylogenetically complex, yet highly sponge-specific microbial communities were identified in numerous species over large geographical ranges (Hentschel et al., 2002). Sequences representing 16 bacterial phyla and both major archaeal lineages have been recovered to date (see



Sponges (Porifera) and Sponge Microbes, Figure 1 The sponge–microbe system (a) schematic representation of a sponge with microbes in the mesohyl (b) fluorescence micrograph of a DAPI stained tissue section of the sponge *Geodia barretti*, CC = choanocyte chamber, Ca = canal, Ce = sponge cells. Large DAPI signals represent nuclei of sponge cells, while small signals represent sponge microbes. Scale bar = 20 μ m (c) transmission electron microscope image of the sponge *G. barretti* showing sponge cells (lower right corner) and numerous, morphologically different sponge microbes. Scale bar = 5 μ m.

review by Taylor et al., 2007; Hentschel et al., 2003, 2006). One of these phyla, the Poribacteria, represents a new bacterial phylum, which is related to the Planctomycetales and has, so far, only been found in sponges (Fieseler et al., 2004, 2006). The other microbial phyla found in sponges are also found in other environments; however, many sponge microbes seem to form, within these phyla, monophyletic, sponge-specific 16S rRNA sequence clusters, which are absent in seawater (Taylor et al., 2007; Hentschel et al., 2003, 2006).

These recent review articles provide an excellent summary of our current knowledge about the diversity and phylogenetic affiliation of sponge microbes, and this topic will therefore not be addressed here in more detail. This article, in contrast, aims to focus on the geobiological

aspects of the sponge–microbe system: the question of a long-standing relationship between sponges and microbes; the consequences of fluctuating oxygen concentrations in sponge tissue for the sponge–microbe system; and the nature and possible function of microbial processes within sponges.

Sponge microbes: secret passengers through evolutionary history?

Did sponges and their associated microbes have an ancient partnership which remained unchanged since the origin of sponges (Precambrian), or is it a more recent but stable association which pertains to date? In an extensive review of this topic, Taylor et al. (2007) propose three scenarios which may explain the occurrence of sponge-specific

microbes. Firstly, it can be the product of a symbiosis of ancient origin perpetuated through the vertical (i.e., from adult to embryo) transmission of sponge-specific microorganisms. Secondly, the association can be the product of environmental acquisition through specific (immune system recognition, presence of favorable substrate, or bacterial protective capsule) or unspecific enrichment (rare sea-water microbes get trapped, accumulated and become thus detectable in the sponge). Finally, a combination of vertical and environmental transmissions is also possible.

The strongest evidence for a long-standing, close symbiotic relationship between sponges and some microorganisms comes from the demonstration of coevolution by comparative phylogenetic studies of sponges and their associated microbes. Erpenbeck et al. (2002) used a mitochondrial marker, cytochrome oxidase subunit 1 (CO1) and showed that the derived phylogenetic trees of four out of six symbionts were largely congruent with a tree containing sequences from the corresponding host sponges. This suggests that cospeciation had occurred (Erpenbeck et al., 2002). A subsequent study indicated a high degree of host specificity between the filamentous cyanobacterium *Oscillatoria spongelliae* and various dictyoceratid sponges (Thacker and Starnes, 2003). When comparing the phylogeny of 23 geographically, morphologically, and environmentally distant sponge species (based on their 28S rDNA gene sequences) and their associated archaea (based on 16S rDNA), a genus-specific association of these sponges with Crenarchaeota of the Marine Group I was apparent (Holmes and Blanch, 2007). These results suggest close coevolution and thus, a long-term symbiosis between these archaea and their sponge hosts. Coevolution requires that the host and symbiont maintain close association over evolutionary time. Vertical transmission, the mechanism through which coevolution could occur, has been shown for numerous sponge species belonging to all three classes of Porifera (Ereskovsky et al., 2005; Sharp et al., 2007; Schmitt et al., 2007; and references therein), including in species with highly varied reproductive strategies.

A pitfall for these comparative phylogenetic studies is that the phylogeny of sponges themselves is not yet fully resolved. This is particularly true for the Demosponges where phylogeny from the subclass to the family level is still in a state of flux (Boury-Esnault, 2006). Accordingly, our understanding of symbiont evolution in sponges will continue to develop only in parallel with improvement of our knowledge about host phylogeny. A recently initiated CO1 sequencing project for taxonomically diverse sponges (www.spongebarcoding.org) may provide data to achieve this goal in the future.

Further support for a possible ancient origin of the sponge-microbe system comes from biomarker studies. Various fatty acids of probable microbial origin occur in a wide range of sponges, irrespective of host phylogeny or geographic location (Thiel et al., 1999, 2002). The apparent absence of some of these biomarkers from

marine sediments and seawater led to the suggestion that the fatty acids and their microbial producers have been present in the sponges since ancient times.

The final type of evidence for an ancient and close association between sponges and microorganisms comes from the fossil record. Reef mounds constructed by siliceous sponges and cyanobacterial mats in the early Cambrian show that sponges and microbes closely coexisted hundreds of millions of years ago (Brunton and Dixon, 1994). Whether these microbes actually lived within the sponge tissue, as modern sponge microbes do, remains, at this stage of our knowledge, unclear.

In other words, Precambrian microorganism acquisition which started as a mere hypothesis is gaining support through studies of phylogeny, biomarkers, and fossil record. Thus, there is increasing evidence for a long-standing coevolution between sponges and certain microbial lineages, which may even date back to the roots of sponge evolution in the anaerobic, microbe-dominated world of the Proterozoic. There is evidence that a close association between sponges and bacteria may even have provided some of the genetic source material for Metazoan evolution (Lakshminarayan et al., 2004).

The alternative scenario, environmental acquisition, can, however, not be ruled out for certain sponge microbes or, in some cases, may be the more likely explanation. In this scenario, it is assumed that sponge-specific microbes are in fact also present in seawater but that our techniques fail to detect them because of their rarity. In the sponge, specific enrichment of those rare microorganisms could occur as a result of a selection through the sponge immune system (Müller and Müller, 2003) or because those microbes which possess a capsule are protected from sponge cells phagocytosis and would increase in number within the sponge body, thus becoming detectable. Unspecific enrichment is also possible and could happen through the ability of sponges to filter large amounts of water through their body. The likelihood of rare microbes being detected by our current techniques is higher in a sponge than in seawater as they might accumulate and be present in high enough numbers to be found.

The third scenario proposed by Taylor et al. (2007) is a combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) which seems the most likely scenario to explain microbial diversity that we find in sponges today (Hentschel et al., 2002).

An anaerobic world in sponges

Until recently, sponge metabolism was viewed as being based on aerobic respiration, similarly to all Metazoa. Oxygen is usually supplied in excess to the sponge body through the water current created by the choanocytes (flagellated cells) (Reiswig, 1974). The remarkable ability of sponges to pump large amounts of water through their bodies has led to the assumption that permanent oxygen saturation exists within the sponge body. However, the

presence of anaerobic microbes in some sponge species hinted toward the presence of spatial or temporal anoxic niches (e.g., Webster et al., 2001; Hoffmann et al., 2005a; Schläppy et al., 2010a).

Gatti et al. (2002) and Schönberg et al. (2004) were the first to use microsensors to investigate the oxygen distribution in the tissue of living sponges at a microscale. Gatti et al. (2002) used oxygen micro-optodes on sponges' primorpha (multicellular aggregates composed of previously dissociated single sponge cells) and described oxygen deficiency in *Suberites domuncula*. Clark-type oxygen microelectrodes have since been used to explore the fine-scale oxygen dynamics in several sponge species from the Mediterranean and the North Atlantic. Anoxic regions have been detected in the cold-water sponge *Geodia barretti* (Hoffmann et al., 2005a) and in its explants (Hoffmann et al., 2005b), as well as in the Mediterranean sponges *Dysidea avara* (Schläppy et al., 2007) and *Chondrosia reniformis* (Figure 2) kept in aquaria or cultivation tanks with recirculating seawater.

Similarly, fluctuating oxygen concentrations leading to anoxia lasting up to 1 h were also found in *Dysidea avara* in the field when measured with an oxygen microelectrode applied by SCUBA diving (Schläppy et al., 2010a). A positive correlation between pumping activity and oxygen content was described in the tissue and in the exhaled water of *Dysidea avara* (Schläppy et al., 2007) and *Aplysina aerophoba* (Hoffmann et al., 2008). When the sponge ventilated, its body was oxygen saturated. Figure 2a shows a typical profile of a pumping sponge, with oxygen saturated water above the sponge surface and near-saturated water within the sponge tissue (compare also Hoffmann et al., 2007; Schläppy et al., 2007).

Oxygen concentrations decreased dramatically when pumping activity ceased. After 15 min without ventilation, the entire sponge body was anoxic with the exception of a 1 mm surface layer where oxygen penetrated due to molecular diffusion over the sponge surface (Hoffmann et al., 2008).

Oxygen profiles are typically diffusive over the surface and into the tissue of a nonpumping sponge (Figure 2b) (compare also Hoffmann et al., 2005b, 2008; Schläppy et al., 2007, 2010a). In situ, many sponge species reduce or even stop their pumping activity for several hours at irregular intervals (Reiswig, 1971; Vogel, 1977; Pile et al., 1997). It remains unclear whether the interruption of ventilation is caused by external events such as, for example, a high sediment load in the water (Gerodette and Flechsig, 1979; Leys et al., 1999), decrease in salinity (Fell et al., 1989), or by an intrinsic rhythm specific to a species (see Reiswig, 1971). In nonpumping sponges, molecular diffusion across a diffusive boundary layer at the sponge surface is the only source of oxygen.

The consequences of these findings are twofold. Firstly, all sponge cells and microbes which lay as deeper as 1 mm below the sponge surface need to be able to survive without direct and regular access to oxygen. Since a lack of ventilation causes tissue anoxia, the parts of the sponge

body which are not affected by surface diffusion must be alternately exposed to oxic and anoxic conditions. Secondly, by modulating the presence or absence and the magnitude of water flow through their bodies sponges have the possibility of actively switching between aerobic and anaerobic metabolism. It follows that sponge-associated microorganisms must be able to tolerate both situations and their metabolism may be activated or inactivated depending on the oxygen concentration in the sponge. This means that anaerobic microbial processes, such as sulfate reduction, for example, may take place (Hoffmann et al., 2005a; but see Schläppy et al., 2010b).

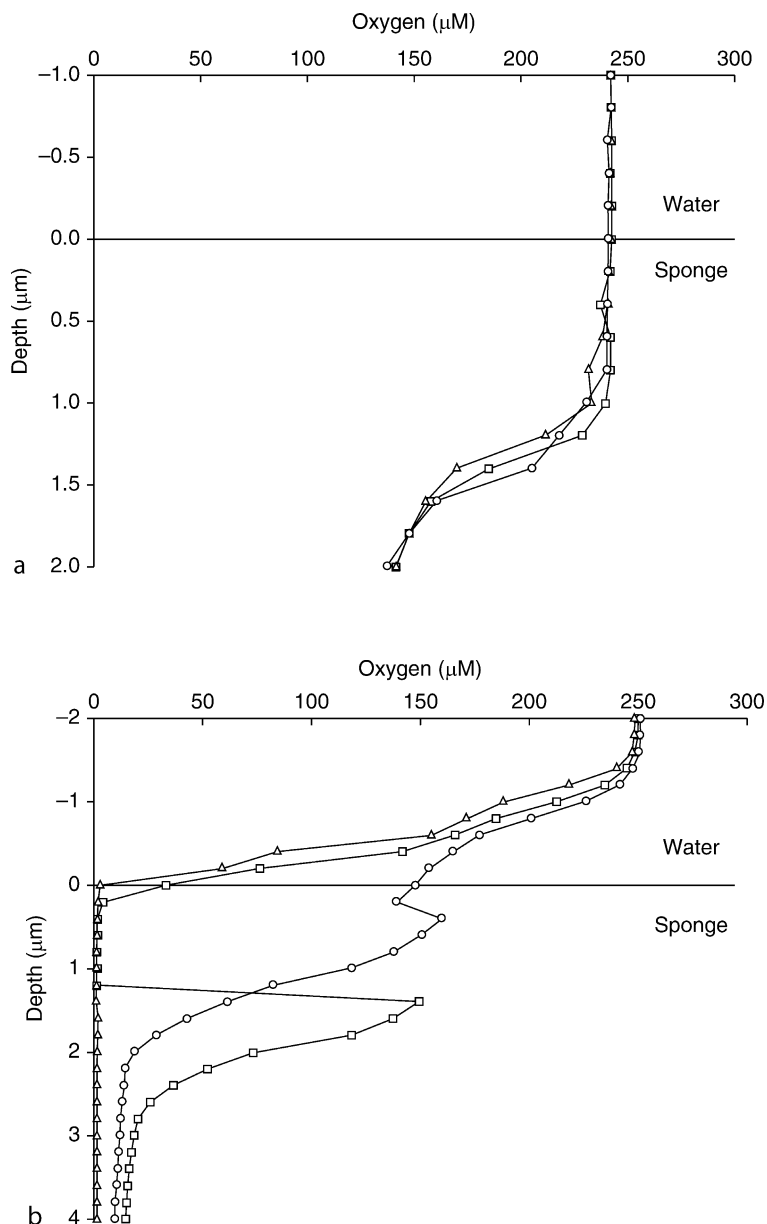
Another possible consequence of varying oxygen concentrations in sponge tissue is reported by Müller et al. (2004a, b). Using *Suberites domuncula* and its alphaproteobacterial symbiont (SB2) as a model system, they showed that the expression of genes which were essential for metabolic interaction of microbe and host was maximal under aerated conditions. Coupled with the observed loss of SB2 cells from the sponge surface under low-oxygen conditions, it was concluded that the oxygen level is responsible for regulating the bacterial fauna in *S. domuncula*. Whether this type of mechanism is equally important in other sponge-microbe systems remains to be investigated.

Tissue anoxia in sponges may act as a controlling factor toward the number of anaerobic sponge-associated microbes and act as a regulator of the nature and extent of their metabolic activity. Alternatively, it may act as a mechanism through which the sponge can kill undesirable seawater microbes. Regardless of whether anoxia in sponges is intentional or inevitable, many sponges can survive long periods of tissue anoxia and either tolerate or even foster anaerobic microbes and corresponding anaerobic microbial activity within their bodies. A close association with a diverse microbial community including (facultative) anaerobic or microaerophilic microorganisms may have been particularly advantageous at a time when fluctuating oxygen conditions were present on the Earth.

Interactions between sponges and sponge microbes

While our knowledge about phylogeny and metabolic capacities of the sponge microbes continuously increases, the role that microbes play for the sponge host is still a matter of debate. Microbes undoubtedly benefit from the nutrient-rich and protected environment of the sponge mesohyl, but the benefit to the sponge host remains unclear.

Sponge microbes may consume carbon sources which are not accessible to the sponge and may be subsequently ingested by the sponge cells. This process is commonly known as "microbial farming" and is well described (Wilkinson and Garrone, 1980; Ilan and Abelson, 1995; Vacelet et al., 1996 and own observation). Autotrophic



Sponges (Porifera) and Sponge Microbes, Figure 2 Oxygen profiles above and within *Chondrosia reniformis* showing (a) a pumping specimen with oxygen-saturated water above the sponge surface and near-saturated water within the sponge (b) a nonpumping specimen with oxygen-saturated water up to 1.5 cm above the sponge surface, a gradual decrease in the diffusive boundary layer and low oxygen concentration approaching anoxia from 2.5 cm into the sponge body. Note the different Y axes.

symbionts transform inorganic carbon (DIC) into organic carbon, which may then be transferred to the host. Energy transfer between symbiont and sponge is best described for phototrophic symbionts (e.g., cyanobacteria: Wilkinson, 1983). Chemolithotrophic processes, such as nitrification, are similar in principle. The energy which is needed for carbon fixation, however, is gained by a chemical process, the aerobic oxidation of ammonium to nitrite first and then of nitrite to nitrate. The presence of nitrifying microbes as well as the transformation of

ammonium to nitrite and nitrate have been demonstrated in many sponge species (e.g., Diaz and Ward, 1997; Diaz et al., 2004; Bayer et al., 2007; Schläppy et al., 2010b). In addition to carbon fixation, these microbes efficiently remove the sponge waste product ammonium and the toxic nitrite. Heterotrophic microbes consume simple compounds (dissolved organic carbon, DOC), while sponge cells prefer small particles such as pelagic bacteria or phytoplankton (Willenz, 1980; Pile et al., 1996; Witte et al., 1997; Ribes et al., 1999). Some sponges, however,

have been identified as important DOC sinks (Yahel et al., 2003; de Goeij et al., 2008). Interestingly, these sponges host high amount of associated bacteria. This leads to the conclusion that DOC is consumed by the sponge microbes, which in turn may be consumed by the sponge cells. A diverse community of associated microorganisms with high metabolic diversity is able to metabolize a wide array of simple molecules and to transfer them into biomass as a food resource for the sponge.

The second possibility for a mutual interaction between sponges and microbes lies within the production of secondary metabolites. Sponges produce a large variety of bioactive compounds (Blunt et al., 2003; Paul and Puglisi, 2004), which act as defenses against surface fouling, infection, or predation. Sponge microbes, which are obviously not harmed by the antimicrobial metabolites, are in fact often the actual producers of these compounds (Hildebrand et al., 2004; Piel, 2004, 2006). In addition to protecting the sponge, secondary metabolites act as a controlling factor in the host–microbe interaction.

At least some groups of sponge microbes are clearly beneficial to the sponge host, while others may be commensals.

Summary

Sponges are amongst the most ancient Metazoans on the Earth and are distributed over a wide range of aquatic ecosystems. Phylogenetically complex, yet highly sponge-specific microbial communities live in close association with numerous sponge species. A combination of both vertical and horizontal symbiont transmissions (parental and environmental acquisition, respectively) seems the most likely scenario to explain microbial diversity that we find in sponges today. Tissue anoxia is a feature found in several sponges species and is related to sponge pumping activity. Sponge microbes with autotrophic, heterotrophic, aerobic, or anaerobic metabolisms may contribute to the nutrition of the host. Other interactions between sponges and sponge microbes involve the synthesis of secondary metabolites. Due to modulation of internal oxygen concentration and the production of secondary metabolites, sponges are able to control their microbial communities. While our knowledge about the phylogeny of sponge microbes is increasing rapidly, many questions concerning the activity of sponge microbes and their interaction with the host sponge still remain unanswered. Future challenges will involve linking microbial phylogeny to microbial function on a microscale within the sponge tissue, and to examine the complex interactions within the sponge–microbe system from the origin of Metazoan life until today.

Bibliography

Ax, P., 1995. *Das System der Metazoa I*. Stuttgart: Fischer.
 Bayer, K., Schmitt, S., and Hentschel, U., 2007. Microbial nitrification in Mediterranean sponges: possible involvement of ammonium-oxidizing betaproteobacteria. In Custódio, M., Lôbo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera*

Research: Biodiversity, Innovation, Sustainability: Série Livros. Museu Nacional, Rio de Janeiro, pp. 165–171.
 Blunt, J. W., Copp, B. R., Munro, H. G. M., Northcote, P. T., and Prinsep, M. R., 2003. Marine natural products. *Natural Product Report*, **20**, 1–48.
 Borchellini, C., Manuel, M., Alivon, E., Boury-Esnault, N., Vacelet, J., and Le Parco, Y., 2001. Sponge paraphyly and the origin of metazoa. *Journal of Evolutionary Biology*, **14**, 171–179.
 Boury-Esnault, N., 2006. Systematic and evolution of demospongiae. *Canadian Journal of Zoology/Revue Canadienne de Zoologie*, **84**, 205–224.
 Brunton, F. R., and Dixon, O. A., 1994. Siliceous sponge-microbe biotic associations and their recurrence through the phanerozoic as reef mound constructors. *Palaios*, **9**, 370–387.
 de Goeij, J. M., Moodley, L., Houtekamer, M., Carballeira, N. M., and van Duyl, F. C., 2008. Tracing ¹³C-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca Caerulea*: evidence for Dom-feeding. *Limnology & Oceanography*, **53**, 1376–1386.
 Diaz, M. C., and Ward, B. B., 1997. Sponge-mediated nitrification in tropical benthic communities. *Marine Ecology Progress Series*, **156**, 97–107.
 Diaz, M. C., Akob, D., and Cary, C. S., 2004. Denaturing gradient gel electrophoresis of nitrifying microbes associated with tropical sponges. *Bollettino dei Musei e degli Istituti Biologici dell'Università di Genova*, **68**, 279–289.
 Ereskovsky, A. V., Gonobobleva, E., and Vishnyakov, A., 2005. Morphological evidence for vertical transmission of symbiotic bacteria in the viviparous sponge *Halisarca Dujardini* Johnston (porifera, demospongiae, halisarcida). *Marine Biology*, **146**, 869–875.
 Erpenbeck, D., Breeuwer, A. J., van der Velde, H. C., and Van Soest, R. W. M., 2002. Unravelling host and symbiont phylogenies of halichondrid sponges (Demospongiae, Porifera) using a mitochondrial marker. *Marine Biology*, **141**, 377–386.
 Fell, P. E., Knight, P.-A., and Rieders, W., 1989. Low-salinity tolerance of and salinity-induced dormancy in the estuarine sponge *Microciona Prolifera* (Ellis & Solander) under long-term laboratory culture. *Journal of Experimental Marine Biology and Ecology*, **133**, 195–211.
 Fieseler, L., Horn, M., Wagner, M., and Hentschel, U., 2004. Discovery of the novel candidate phylum “Poribacteria” in marine sponges. *Applied & Environmental Microbiology*, **70**, 3724–3732.
 Fieseler, L., Quaiser, A., Schleper, C., and Hentschel, U., 2006. Analysis of the first genome fragment from the marine sponge-associated, novel candidate phylum poribacteria by environmental genomics. *Environmental Microbiology*, **8**, 612–624.
 Gaidos, E., Dubuc, T., Dunford, M., McAndrew, P., Padilla-Gamino, J., Studer, B., Weersing, K., and Stanley, S., 2007. The precambrian emergence of animal life: a geobiological perspective. *Geobiology*, **5**, 351–373.
 Gatti, S., Brey, T., Müller, W. E. G., Heilmayer, O., and Holst, G., 2002. Oxygen microoptodes: a new tool for oxygen measurements in aquatic animal ecology. *Marine Biology*, **140**, 1075–1085.
 Gerodette, T., and Flechsig, A. O., 1979. Sediment-induced reduction in the pumping rate of the tropical sponge *Verongia Lacunosa*. *Marine Biology*, **55**, 103–110.
 Grozdanov, L., and Hentschel, U., 2007. An environmental genomics perspective on the diversity and function of marine sponge-associated microbiota. *Current Opinion in Microbiology*, **10**, 215–220.
 Hentschel, U., Hopke, J., Horn, M., Friedrich, A. B., Wagner, M., Hacker, J., and Moore, B. S., 2002. Molecular evidence for a uniform microbial community in sponges from different oceans. *Applied and Environmental Microbiology*, **68**, 4431–4440.

- Hentschel, U., Fieseler, L., Wehrh, M., Gernert, C., Steinert, M., Hacker, J., and Horn, M., 2003. Microbial diversity of marine sponges. In Müller, W. E. G. (ed.), *Marine Molecular Biotechnology*. Berlin: Springer, pp. 59–88.
- Hentschel, U., Usher, K. M., and Taylor, M. W., 2006. Marine sponges as microbial fermenters. *FEMS Microbiology Ecology*, **55**, 167–177.
- Hildebrand, M., Waggoner, L. E., Lim, G. E., Shar, K. H., Ridley, C. P., and Haygood, M. G., 2004. Approaches to identify, clone, and express symbiont bioactive metabolite genes. *Natural Product Reports*, **21**, 122–142.
- Hoffmann, F., Larsen, O., Thiel, V., Rapp, H. T., Pape, T., Michaelis, W., and Reitner, J., 2005a. An anaerobic world in sponges. *Geomicrobiology Journal*, **22**, 1–10.
- Hoffmann, F., Larsen, O., Rapp, H. T., and Osinga, R., 2005b. Oxygen dynamics in choanosomal sponge explants. *Marine Biology Research*, **1**, 160–163.
- Hoffmann, F., Sauter, E., Sachs, O., Røy, H., and Klages, M., 2007. Oxygen distribution in *Tentorium Semisuberites* and in its habitat in the Arctic deep sea. In Custódio, M., Lôbo-Hajdu, G., Hajdu, E., and Muricy, G. (eds.), *Porifera Research: Biodiversity, Innovation, Sustainability: Série Livros*. Museu Nacional, Rio de Janeiro, pp. 379–382.
- Hoffmann, F., Røy, H., Bayer, K., Hentschel, U., Pfannkuchen, M., Brümmer, F., and de Beer, D., 2008. Oxygen dynamics and transport in the Mediterranean sponge *Aplysina Aerophoba*. *Marine Biology*, **153**, 1257–1264.
- Holmes, B., and Blanch, H., 2007. Genus-specific associations of marine sponges with Group I Crenarchaeota. *Marine Biology*, **150**, 759–772.
- Ilan, M., and Abelson, A., 1995. The life of a sponge in a Sandy Lagoon. *Biological Bulletin*, **189**, 363–369.
- Lakshminarayan, M. I., Aravind, L., Coon, S. L., Klein, D. C., and Koonin, E. V., 2004. Evolution of cell-cell signaling in animals: Did late horizontal gene transfer from bacteria have a role? *Trends in Genetics*, **20**, 292–299.
- Leys, S. P., Mackie, G. O., and Meech, R. W., 1999. Impulse conduction in a sponge. *Journal of Experimental Biology*, **202**, 1139–1150.
- McCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Paleoenvironmental implications of Novel C30 steranes in precambrian to cenozoic age petroleum and Bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Medina, M., Collins, A. G., Silberman, J. D., and Sogin, M. L., 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *PNAS*, **98**, 9707–9712.
- Moldowan, J. M., Dahl, J., Jacobsen, S. R., Huizinga, B. J., McCaffrey, M. A., and Summons, R. E., 1994. Molecular fossil evidence for late proterozoic – early paleozoic environments. *Terra Nova Abstracts*, **14**, 4.
- Müller, W. E. G., 1998a. *Molecular Evolution: Towards the Origin of Metazoa*. Berlin: Springer.
- Müller, W. E. G., 1998b. Origin of metazoa: sponges as living fossils. *Naturwissenschaften*, **85**, 11–25.
- Müller, W. E. G., and Müller, I. M., 2003. Origin of the metazoan immune system: identification of the molecules and their functions in sponges. *Integrative & Comparative Biology*, **43**, 281–292.
- Müller, W. E. G., Grebenjuk, V. A., Thakur, N. L., Thakur, A. N., Batel, R., Krasko, A., Müller, I. M., and Breter, H. J., 2004a. Oxygen-controlled bacterial growth in the sponge *Suberites Domuncula*: toward a molecular understanding of the symbiotic relationships between sponge and bacteria. *Applied and Environmental Microbiology*, **70**, 2332–2341.
- Müller, W. E. G., Perovic, S., Schroder, H. C., and Breter, H. J., 2004b. Oxygen as a morphogenic factor in sponges: expression of a tyrosinase gene in the sponge suberites *Domuncula*. *Micron*, **35**, 87–88.
- Paul, V. J., and Puglisi, M. P., 2004. Chemical mediation of interactions among marine organisms. *Natural Product Reports*, **21**, 189–209.
- Piel, J., 2004. Metabolites from symbiotic bacteria. *Natural Product Reports*, **21**, 519–538.
- Piel, J., 2006. Bacterial symbionts: prospects for the sustainable production of invertebrate-derived pharmaceuticals. *Current Medicinal Chemistry*, **13**, 39–50.
- Pile, A. J., Patterson, M. R., and Witman, J. D., 1996. In situ grazing on plankton < 10 µM by the boreal sponge *Mycale Lingua*. *Marine Ecology Progress Series*, **141**, 95–102.
- Pile, A. J., Patterson, M. R., Savarese, M., Chernykh, V. I., and Fialkov, V. A., 1997. Trophic effects of sponge feeding within lake Baikal's littoral zone. 1. In situ pumping rate. *Limnology and Oceanography*, **42**, 171–178.
- Reiswig, H. M., 1971. In situ pumping activities of tropical demospongiae. *Marine Biology*, **9**, 38–50.
- Reiswig, H. M., 1974. Water transport, respiration and energetics of three tropical marine sponges. *Journal of Experimental Marine Biology and Ecology*, **14**, 231–249.
- Reiswig, H. M., 1981. Partial carbon and energy budgets of the bacteriosponge *Verongia Fistularis* (Porifera: Demospongiae) in Barbados. *Marine Ecology Progress Series*, **2**, 273–293.
- Reitner, J., and Wörheide, G., 2002. Non-lithistid fossil demospongiae – origins of their palaeobiodiversity and highlights in history of preservation. In Hooper, J. N. A., and Soest, R. W. M. v. (eds.), *Systema Porifera*. New York: Kluwer, pp. 52–68.
- Ribes, M., Coma, R., and Gili, J. M., 1999. Natural diet and grazing rate of the temperate sponge *Dysidea avara* (Demospongiae, Dendroceratida) throughout an annual cycle. *Marine Ecology Progress Series*, **176**, 179–190.
- Schläppy, M.-L., Hoffmann, F., Røy, H., Wijffels, R. H., Mendola, D., Sidri, M., and de Beer, D., 2007. Oxygen dynamics and flow patterns of *Dysidea avara* (Porifera, Demospongiae). *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1677–1682.
- Schläppy, M.-L., Schöttner, S. I., Lavik, G., Kuypers, M. M. M., de Beer, D., and Hoffmann, F., 2010a. Evidence of nitrification and denitrification in high and low microbial abundance sponges. *Marine Biology*, **157**, 593–602, doi:10.1007/s00227-009-1344-5.
- Schläppy, M.-L., Weber, M., Mendola, D., Hoffmann, F., and de Beer, D., 2010b. Heterogeneous oxygenation resulting from active and passive flow in two Mediterranean sponges, *Dysidea avara* and *Chondrosia reniformis*. *Limnology and Oceanography*, **55**(3), 1289–1300. doi:10.4319/lo.2010.55.3.1289.
- Schmitt, S., Weisz, J., Lindquist, N., and Hentschel, U., 2007. Vertical transmission of a phylogenetically complex microbial consortium in the viviparous sponge *Ircinia felix*. *Applied and Environmental Microbiology*, **73**, 2067–2078.
- Schönberg, C. H. L., Hoffmann, F., and Gatti, S., 2004. Using microsenors to measure sponge physiology. *Bollettino dei Musei e degli Istituti Biologici dell' Università di Genova*, **68**, 593–604.
- Sharp, K., Eam, B., Faulkner, D., and Haygood, M., 2007. Vertical transmission of diverse microbes in the tropical sponge *Corticium* Sp. *Applied and Environmental Microbiology*, **73**, 622–629.
- Taylor, M. W., Radax, R., Steger, D., and Wagner, M., 2007. Sponge-associated microorganisms: evolution, ecology, and

- biotechnological potential. *Microbiology and Molecular Biology Reviews*, **71**, 259–347.
- Thacker, R. W., and Starnes, S., 2003. Host specificity of the symbiotic cyanobacterium *Oscillatoria Spongelia* in marine sponges, *Dysidea spp.* *Marine Biology*, **142**, 643–648.
- Thiel, V., Jenisch, A., Wörheide, G., Löwenberg, A., Reitner, J., and Michaelis, W., 1999. Mid-chain branched alkanolic acids from “living fossil” demosponges: a link to ancient sedimentary lipids? *Organic Geochemistry*, **30**, 1–14.
- Thiel, V., Blumenberg, M., Hefter, J., Pape, T., Pomponi, S., Reed, J., Reitner, J., Wörheide, G., and Michaelis, W., 2002. A chemical view of the most ancient metazoa – biomarker chemotaxonomy of Hexactinellid sponges. *Naturwissenschaften*, **89**, 60–66.
- Vacelet, J., 1970. Description De Cellules à Bactéries Intranucléaires Chez Des Eponges *Verongia*. *Journal de Microscopie*, **9**, 333–346.
- Vacelet, J., and Donadey, C., 1977. Electron microscope study of the association between some sponges and bacteria. *Journal of Experimental Marine Biology and Ecology*, **30**, 301–314.
- Vacelet, J., Fiala-Médioni, A., Fisher, C. R., and Boury-Esnault, N., 1996. Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous Cladorhizid sponge. *Marine Ecology Progress Series*, **145**, 77–85.
- Vogel, S., 1977. Current-induced flow through living sponges in nature. *Proceedings of the National Academy of Science USA*, **74**, 2069–2071.
- Webster, N. S., Wilson, K. J., Blackall, L. L., and Hill, R. T., 2001. Phylogenetic diversity of bacteria associated with the marine sponge *Rhopaloeides odorabile*. *Applied and Environmental Microbiology*, **67**, 434–444.
- Wilkinson, C. R., 1978. Microbial association in sponges. II. Numerical analysis of sponge and water bacterial populations. *Marine Biology*, **49**, 169–176.
- Wilkinson, C. R., 1983. Net primary productivity in coral reef sponges. *Science*, **219**, 410–412.
- Wilkinson, C. R., and Garrone, R., 1980. Nutrition of marine sponges. Involvement of symbiotic bacteria in the uptake of dissolved carbon. In Smith, D. C., and Tiffon, Y. (eds.), *Nutrition in Lower Metazoa*. Oxford: Pergamon Press, pp. 157–161.
- Willenz, P., 1980. Kinetic and morphological aspects of particle ingestion by the freshwater sponge *Ephydatia Fluviatilis* L. In Smith, D. C., and Tiffon, Y. (eds.), *Nutrition in Lower Metazoa*. Oxford: Pergamon Press, pp. 163–178.
- Witte, U., Brattegard, T., Graf, G., and Springer, B., 1997. Particle capture and deposition by deep-sea sponges from the Norwegian-Greenland Sea. *Marine Ecology Progress Series*, **154**, 241–252.
- Yahel, G., Sharp, J. H., Marie, D., Hase, C., and Genin, A., 2003. In situ feeding and element removal in the symbiont-bearing sponge *Theonella swinhoei*: bulk DOC is the major source for carbon. *Limnology & Oceanography*, **48**, 141–149.

Cross-references

[Aerobic Metabolism](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Early Earth](#)
[Early Precambrian Eukaryotes](#)
[Histology](#)
[Microbial Ecology of Submarine Caves](#)
[Microsensors for Sediments, Microbial Mats, and Biofilms](#)
[Origins of the Metazoa](#)
[Symbiosis](#)

STROMATACTIS

Roman Aubrecht

Comenius University, Bratislava, Slovakia

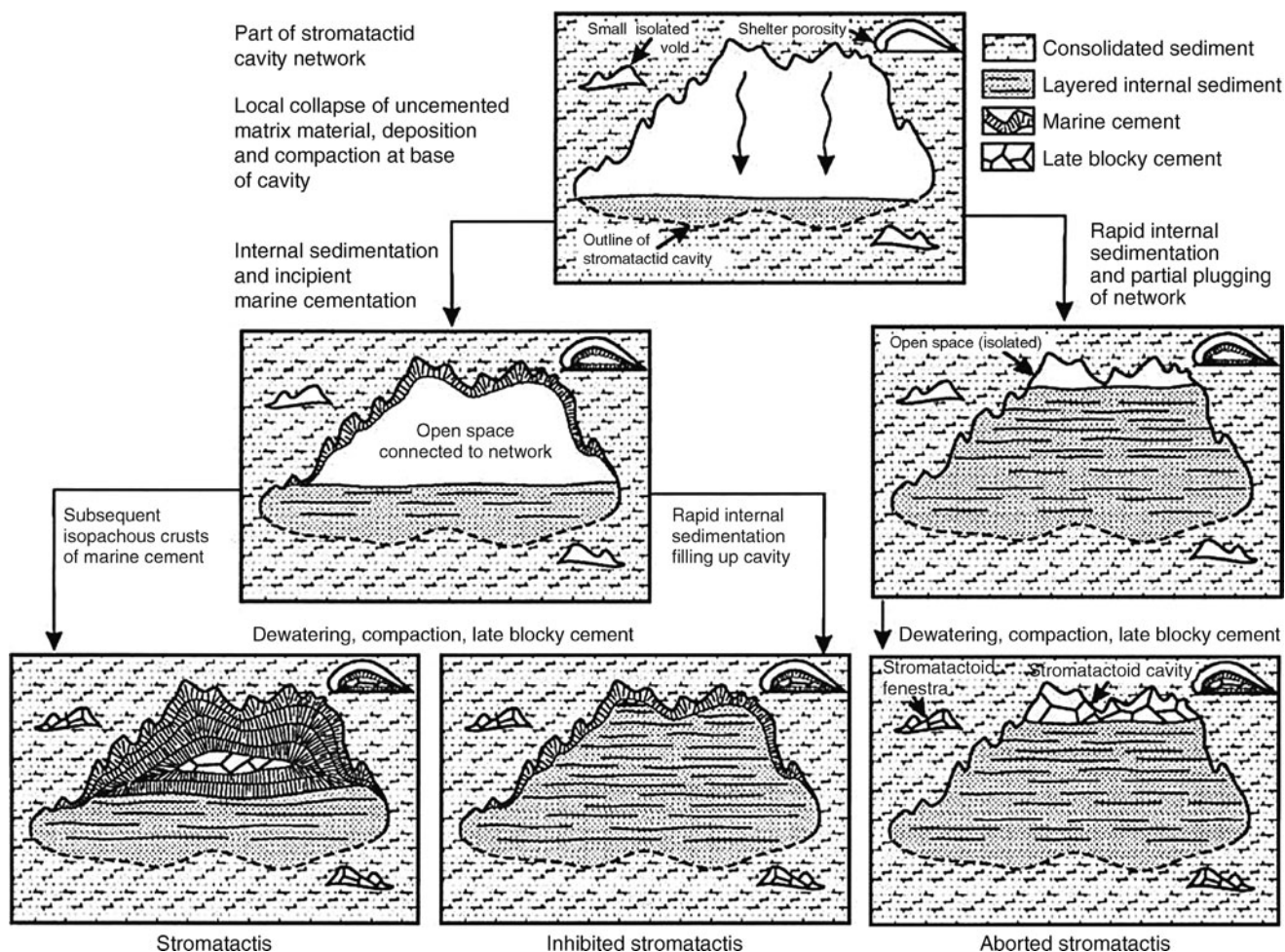
Definition

Stromatactis is defined as a mass of spar (with partial substitution of internal sediment) which has smooth base, digitate roof, occurs in swarms and has reticulate distribution (Bathurst, 1982). Neuweiler et al. (2001) distinguished the terms “stromatactis,” “inhibited stromatactis,” and “aborted stromatactis” (Figure 1). Stromatactis originated when early internal sedimentation occurred in the open space of the cavity network followed by centripetal cementation by early marine cements in residual cavity space. Aborted stromatactis resulted from internal sediment filling of the entire or near-entire original cavity network. Inhibited stromatactis refers to incipient marine cementation of the stromatactid cavity followed by entire or near-entire internal sediment filling.

Stromatactis often (but not always) occur in mud-mounds (see entry [Mud Mounds](#)). Spar bodies similar to stromatactis but not fitting to all the points of this definition are termed in literature as “stromatactoid” or “stromatactis-like” bodies. Similar to stromatactis but smaller are fenestral pores occurring in intertidal limestones. Similar to limestones with stromatactis structures are “zebra limestones” in which the spar bodies are flat, straight, elongated, and the individual spar layers are not always interconnected.

Evolution of the term stromatactis

The term stromatactis was first used by Dupont (1881, 1882). Until present, there is still no agreement in the way of its origin. There are two main groups of opinions about the origin of stromatactis: abiogenic and biogenic. Most researchers agree that stromatactis originally represented cavities which were later filled with internal sediment and sparry calcite. The suggested abiogenic origins for stromatactis included internal erosion and reworking of small cavities (e.g., Kukal, 1971; Wallace, 1987; Bridges and Chapman, 1988; Matyszkiewicz, 1993, 1997), dewatering or escape of fluids (Heckel, 1972; Desbordes and Maurin, 1974; Bernet-Rollande et al., 1981), slumps (Schwarzacher, 1961), and freshwater karstification (Dunham, 1969). Most recent ideas involve frozen clathrate hydrates in the calcareous mud, after which the stromatactis cavities remained (Krause, 2001) or the cavities are interpreted as a result of sedimentation of stirred polydisperse sediment (Hladil, 2005; Hladil et al., 2006; Hladil et al., 2007). Some researchers supposed that stromatactis did not represent cavities and originated either by neomorphism or recrystallization of the calcareous mud (Black, 1952; Orme and Brown, 1963; Ross et al., 1975) or by dynamic metamorphism (Logan and Semeniuk, 1976). Among the opinions



Stromatactis, Figure 1 Origin of stromatactis, inhibited stromatactis and aborted stromatactis (After Neuweiler et al., 2001).

Stromatactis originated when early internal sedimentation occurred in the open space of the cavity network followed by centripetal cementation by early marine cements in residual cavity space. Aborted stromatactis resulted from internal sediment filling of the entire or near-entire original cavity network. Inhibited stromatactis refers to incipient marine cementation of the stromatactid cavity followed by entire or near-entire internal sediment filling.

favoring biogenic origin, the most widespread interpretation is that they are cavities which remained after decomposition of an unknown soft-bodied organism or by neomorphism of carbonate-secreting organism. The suggested organisms include stromatoporoids (Dupont, 1881, 1882; Lowenstam, 1950; Carozzi and Zadnik, 1959), bryozoans (Textoris and Carozzi, 1964), algae (Philcox, 1963; Textoris, 1966; Coron and Textoris, 1974), stromatolites (Cross and Klosterman, 1981), microbial colonies (Tsien, 1985), and burrowing activity of crustaceans (Shinn, 1968). The organisms that are most frequently considered to be involved in stromatactis formation are sponges. The sponge theory was first suggested by Bourque and Gignac (1983), followed by Bourque and Boulvain (1993), Neuweiler et al. (2001), and Delecat and Reitner (2005). Aubrecht et al. (2009) provided evidence that sponges were indeed involved in stromatactis

formation within Middle Jurassic mud-mound deposits. Some authors suggested an opinion that a combination of several processes played role in the onset of stromatactis, such as microbial binding of the sediment and excavating of the unbound mud (Bathurst, 1982; Pratt, 1982) or a succession of sponges and microbial colonies (Flajs and Hüssner, 1993; Flajs et al., 1996).

Stratigraphic distribution of stromatactis

Stromatactis cavities are time-dependent structures (Bosence and Bridges, 1995; Flajs and Hüssner, 1993; Neuweiler et al., 2001). The age of stromatactis cavities ranges from the Neoproterozoic (Pratt, 1995) and Cambrian (James and Gravestock, 1990), through a maximum in Devonian and Carboniferous, up to the late Jurassic (Matyszkiewicz, 1993, 1997; Jansa et al., 1989;

Pratt, 1995; Aubrecht et al., 2002). Some younger occurrences of problematic stromatactis-like cavities from the Aptian–Albian of northern Spain were reported by Pascal and Przybyla (1989), Pratt (1995), and Neuweiler et al. (1999). Even younger stromatactis-like cavities occur in Lower Albian mud-mounds of the Pyrenees (Canerot, 2001), in Turonian mud-mounds of Tunisia (Camoin and Maurin, 1988), in late Cretaceous methane-seep mounds of Tepee Buttes, USA (Kauffman et al., 1996), and in Holocene lithoherms found in the Straits of Florida (Neumann et al., 1977).

Neuweiler et al. (2001) ascribed the stratigraphic dependance and rare occurrences of stromatactis in Meso–Cenozoic sediments to the taphonomy of younger sponge taxa which was different from the Paleozoic. The post-Paleozoic taxa were prone to more rapid decay resulting in common cavity collapse and sediment filling (inhibited and aborted stromatactis).

Conclusions

Stromatactis is a mass of spar with smooth base, digitate roof, occurring in swarms, and having reticulate distribution. Various forms of stromatactis were distinguished: stromatactis, inhibited stromatactis, and aborted stromatactis, where the latter two refer to stromatactis cavities partly or completely filled by internal sediment.

There is still no agreement on how stromatactis originated. There are two main groups of opinions: abiogenic and biogenic. The theories on an abiogenic origin involved internal erosion and reworking of small cavities, dewatering or escape of fluids, slumps, dynamic metamorphism, fresh-water karstification, neomorphism, or recrystallization of the calcareous mud, frozen clathrate hydrates after which the stromatactis cavities remained, or the cavities were interpreted as a result of sedimentation of stirred polydisperse sediment. The biogenicity theories preferred the opinion that the stromatactis cavities remained after decomposition of an unknown soft-bodied organism including various hypothetical representatives, such as stromatoporoids, bryozoans, algae, stromatolites, microbial colonies, and burrowing activity of crustaceans. The most favorable are sponges for which direct evidence was found.

Stromatactis is time-dependent. Its age ranges from the Neoproterozoic and Cambrian, through a maximum in Devonian and Carboniferous, up to the late Jurassic. Some but problematic younger occurrences were found in Cretaceous and Quaternary (lithoherms in the Florida Straits). The stratigraphic dependance and rare occurrences of stromatactis in Meso–Cenozoic sediments are considered to result from the taphonomy of younger, post-Paleozoic sponge taxa.

Bibliography

Aubrecht, R., Krobicki, M., Wierzbowski, A., Matyja, A., and Schlögl, J., 2002. Jurassic stromatactis mud-mounds in the Pieniny Klippen Belt (Western Carpathians) – petrography and stratigraphy. In Bucur, I. I., and Filipescu, S. (eds.), *Research*

- Advances in Calcareous Algae and Microbial Carbonates. Proceedings of the 4th IFAA Regional Meeting Cluj-Napoca*, August 29–September 5, 2001. Cluj University Press, pp. 1–16.
- Aubrecht, R., Schlögl, J., Krobicki, M., Wierzbowski, H., Matyja, B. A., and Wierzbowski, A., 2009. Middle Jurassic stromatactis mud-mounds in the Pieniny Klippen Belt (Carpathians) – a possible clue to the origin of stromatactis. *Sedimentary Geology*, **213**(3–4), 97–112.
- Bathurst, R. G. C., 1982. Genesis of stromatactis cavities between submarine crusts in paleozoic carbonate mud buildups. *Journal of the Geological Society, London*, **139**, 165–181.
- Bernet-Rollande, M. C., Maurin, A. F., and Monty, C. L. V., 1981. De la bactérie au réservoir carbonaté. *Pétrole et Techniques*, **283**, 96–98.
- Black, W. W., 1952. The origin of the supposed tufa bands in Carboniferous reef limestones. *Geological Magazine*, **89**, 195–200.
- Bosence, D. W. J., and Bridges, P. H., 1995. A review of the origin and evolution of carbonate mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-Mounds: Their Origin and Evolution*. IAS special publication 23, pp. 11–48.
- Bourque, P. A., and Gignac, H., 1983. Sponge constructed stromatactis mud-mounds. Silurian of Gaspé, Québec. *Journal of Sedimentary Petrology*, **53**, 521–532.
- Bourque, P. A., and Boulvain, F., 1993. A model for the origin and petrogenesis of the red stromatactis limestone of Paleozoic carbonate mounds. *Journal of Sedimentary Petrology*, **63**, 607–619.
- Bridges, P. H., and Chapman, A. J., 1988. The anatomy of a deep water mud-mound complex to the southwest of the Dinantian platform in Derbyshire, UK. *Sedimentology*, **35**, 139–162.
- Camoin, G., and Maurin, A.-F., 1988. Rôles des micro-organismes (bactéries, cyanobactéries) dans la genèse des “Mud Mounds”. Exemples du Turonien des Jebels Biréno et Mrhila (Tunisie). *Comptes Rendus de l'Académie des sciences Paris*, **307**Sér. II, 401–407.
- Canerot, J., 2001. Cretaceous mud-mounds from the Western Pyrenees and the South-Aquitaine Basin (France). Stratigraphic and geodynamic settings; petroleum trap properties. *Géologie Méditerranée*, **28**(1–2), 33–36.
- Carozzi, A. V., and Zadnik, V. E., 1959. Microfacies of Wabash reef, Wabash, Indiana. *Journal of Sedimentary Petrology*, **29**, 164–171.
- Coron, C. R., and Textoris, D. A., 1974. Non-calcareous algae in Silurian carbonate mud mound, Indiana. *Journal of Sedimentary Petrology*, **44**, 1248–1250.
- Cross, T. A., and Klosterman, M. J., 1981. Primary submarine cements and neomorphic spar in a stromatolitic-bound phylloid algal bioherm, Laborcita Formation (Wolfcampian), Sacramento Mountains, New Mexico, U.S.A. In Monty, C. L. V. (ed.), *Phanerozoic Stromatolites*. Berlin: Springer, pp. 60–73.
- Delecat, S., and Reitner, J., 2005. Sponge communities from the Lower Liassic of Adnet (Northern Calcareous Alps, Austria). *Facies*, **51**, 385–404.
- Desbordes, B., and Maurin, A. F., 1974. Trois exemples d'études du Frasnien de l'Alberta, Canada. *Notes et Mémoires de la Compagnie Française des Pétroles (Paris)*, **11**, 293–336.
- Dunham, R. J., 1969. Early vadose silt in Townsend mound (reef), New Mexico. In Friedman, G. M. (ed.), *Depositional Environments in Carbonate Rocks - A Symposium*. SEPM special publication, Vol. 14, pp. 139–181.
- Dupont, E., 1881. Sur l'origine des calcaires dévoniens de la Belgique. *Bulletin de Musée royal d'Histoire naturelle de Belgique*, **1**, 264–280.
- Dupont, E., 1882. Les Iles coralliennes de Roly et de Phillippeville. *Bulletin de l'Académie Royal de Belgique*, sér.3, **2**, 89–160.

- Flajs, G., and Hüssner, H., 1993. A microbial model for the Lower Devonian stromatolite mud mounds of the Montagne Noire (France). *Facies*, **29**, 179–194.
- Flajs, G., Hüssner, H., and Vigener, M., 1996. Stromatolite mud mounds in the Upper Emsian of the Montagne Noire (France): formation and diagenesis of stromatolite structures. In Reitner, J., Neuweiler, F., and Gunkel, F. (eds.), *Global and Regional Controls on Biogenic Sedimentation. I. Reef Evolution*. Research reports. *Gött. Arb. Geol. Paläont.*, **Sb2**, 345–348.
- Heckel, P. H., 1972. Possible inorganic origin for stromatolite in calcilutite mounds in the Tully Limestone, Devonian of New York. *Journal of Sedimentary Petrology*, **42**, 7–18.
- Hladil, J., 2005. The formation of stromatolite-type fenestral structures during the sedimentation of experimental slurries – a possible clue to a 120-year-old puzzle about stromatolite. *Bulletin of Geosciences*, **80**, 193–211.
- Hladil, J., Růžicka, M. C., and Koptíková, L., 2006. Stromatolite cavities in sediments and the role of coarse-grained accessories. *Bulletin of Geosciences*, **81**, 123–146.
- Hladil, J., Koptíková, L., Růžicka, M., and Kulaviak, L., 2007. Experimental effects of surfactants on the production of stromatolite-shaped cavities in artificial carbonate sediments. *Bulletin of Geosciences*, **82**, 37–50.
- James, N. P., and Gravestock, D., 1990. Lower Cambrian shelf and shelf margin buildups, Flinders Ranges, South Australia. *Sedimentology*, **37**, 455–480.
- Jansa, L. F., Pratt, B. R., and Dromart, G., 1989. Deep water thrombolite mounds from the Upper Jurassic of offshore Nova Scotia. In Geldsetzer, H. H., James, N. P., and Tebutt, G. E. (eds.), *Reefs, Canada and Adjacent Areas*. *Canadian Society of Petroleum Geologists*, **13**, 725–735.
- Kauffman, E. G., Arthur, M. A., Howe, B., and Scholle, P. A., 1996. Widespread venting of methane-rich fluids in Late Cretaceous (Campanian) submarine springs (Tepee Buttes), Western Interior seaway, USA. *Geology*, **24**(9), 799–802.
- Krause, F. F., 2001. Genesis and geometry of the Meiklejohn Peak lime mud-mound, Bare Mountain Quadrangle, Nevada, USA. *Sedimentary Geology*, **145**, 189–213.
- Kukal, Z., 1971. Open-space structures in the Devonian limestones of the Barrandian (Central Bohemia). *Časopis pro mineralogii a geologii*, **16**, 345–362.
- Logan, B. W., and Semeniuk, V., 1976. Dynamic metamorphism; processes and products in Devonian carbonate rocks, Canning Basin, Western Australia. *Geological Society of Australia Special Publication*, **6**, 1–138.
- Lowenstam, H. A., 1950. Niagaran reefs of the Great Lakes area. *Journal of Geology*, **58**, 430–487.
- Matyszkiewicz, J., 1993. Genesis of stromatolite in an Upper Jurassic carbonate buildup (Mlynka, Cracow region, Southern Poland): internal reworking and erosion of organic growth cavities. *Facies*, **28**, 87–96.
- Matyszkiewicz, J., 1997. Stromatolite cavities and stromatolite-like cavities in the Upper Jurassic carbonate buildups at Mlynka and Zabierzów (Oxfordian, southern Poland). *Annales, Société Géologique de Pologne*, **67**, 45–55.
- Neumann, A. C., Kofoed, J. W., and Keller, G. H., 1977. Lithohermes in the straits of Florida. *Geology*, **5**, 4–10.
- Neuweiler, F., Bourque, P.-A., and Boulvain, F., 2001. Why is stromatolite so rare in Mesozoic carbonate mud mounds? *Terra Nova*, **13**, 338–346.
- Neuweiler, F., Gautret, P., Thiel, V., Langes, R., Michaelis, W., and Reitner, J., 1999. Petrology of Lower Cretaceous carbonate mud mounds (Albian, N. Spain): insights into organomineralic deposits of the geological record. *Sedimentology*, **46**, 837–859.
- Orme, G. R., and Brown, W. W. M., 1963. Diagenetic fabrics in the Avonian Limestones of Derbyshire and North Wales. *Proceedings of the Yorkshire Geological Society*, **34**, 51–66.
- Pascal, A., and Przybyla, A., 1989. Processus biosédimentaires et diagénétiques précoces dans les mud-mounds (Thrombolite-mounds) urgoniens d'Espagne du Nord (Aptien-Albien) et leur signification. *Géologie Méditerranée*, **16**(2–3), 171–183.
- Philcox, M. E., 1963. Banded calcite mudstone in the Lower Carboniferous “reef” knolls of the Dublin Basin, Ireland. *Journal of Sedimentary Petrology*, **33**, 904–913.
- Pratt, B. R., 1982. Stromatolite framework of carbonate mud-mounds. *Journal of Sedimentary Petrology*, **52**, 1203–1227.
- Pratt, B. R., 1995. The origin, biota and evolution of deep-water mud-mounds. In Monty, C. L. V., Bosence, D. W. J., Bridges, P. H., and Pratt, B. R. (eds.), *Carbonate Mud-Mounds: Their Origin and Evolution*. IAS special publication 23, pp. 49–123.
- Ross, R. J. Jr., Jaanusson, V., and Friedman, I., 1975. Lithology and origin of Middle Ordovician calcareous mudmounds at Meiklejohn Peak, southern Nevada. *U.S.G.S. Professional Paper*, **871**, 1–48.
- Schwarzacher, W., 1961. Petrology and structure of some Lower Carboniferous reefs in northwestern Ireland. *AAPG Bulletin*, **46**, 1481–1503.
- Shinn, E. A., 1968. Burrowing in Recent lime sediments of Florida and the Bahamas. *Journal of Paleontology*, **42**, 879–894.
- Textoris, D. A., 1966. Algal cap for a Niagaran (Silurian) carbonate mud mound of Indiana. *Journal of Sedimentary Petrology*, **36**, 455–461.
- Textoris, D. A., and Carozzi, A. V., 1964. Petrography and evolution of Niagaran (Silurian) reefs, Indiana. *AAPG Bulletin*, **48**, 397–426.
- Tsien, H. H., 1985. Origin of Stromatolite - a replacement of colonial microbial accretion. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 274–289.
- Wallace, M. W., 1987. The role of internal erosion and sedimentation in the formation of stromatolite mudstones and associated lithologies. *Journal of Sedimentary Petrology*, **57**, 695–700.

Cross-references

[Calcite Precipitation, Microbially Induced Carbonates Mud Mounds](#)
[Origins of the Metazoa Reefs](#)
[Sediment Diagenesis – Biologically Controlled Sponges \(Porifera\) and Sponge Microbes](#)
[Waulsortian Mud Mounds](#)

STROMATOLITES

Stromatolites are laminated benthic microbial deposits (Riding, 1991).

Bibliography

Riding, R., 1991. Classification of microbial carbonates. In Riding, R. (ed.), *Calcareous algae and stromatolites*. Berlin: Springer-Verlag, pp. 21–51.

Cross-references

[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thombolites](#)

SUBSURFACE FILAMENTOUS FABRICS

Beda A. Hofmann

Natural History Museum Bern, Bern, Switzerland

Synonyms

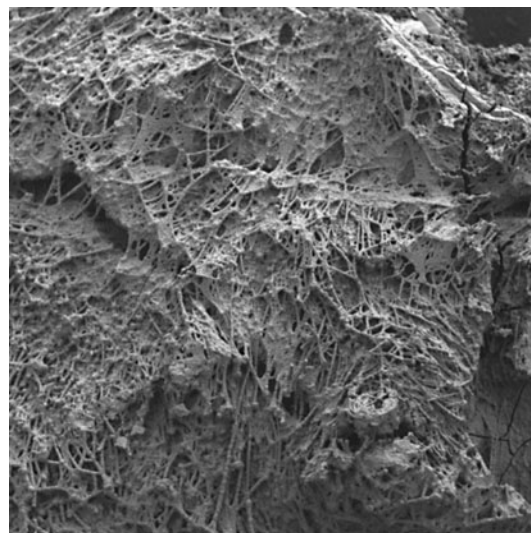
Moss agate p.p.; SFF; Stalactitic minerals p.p.

Definition

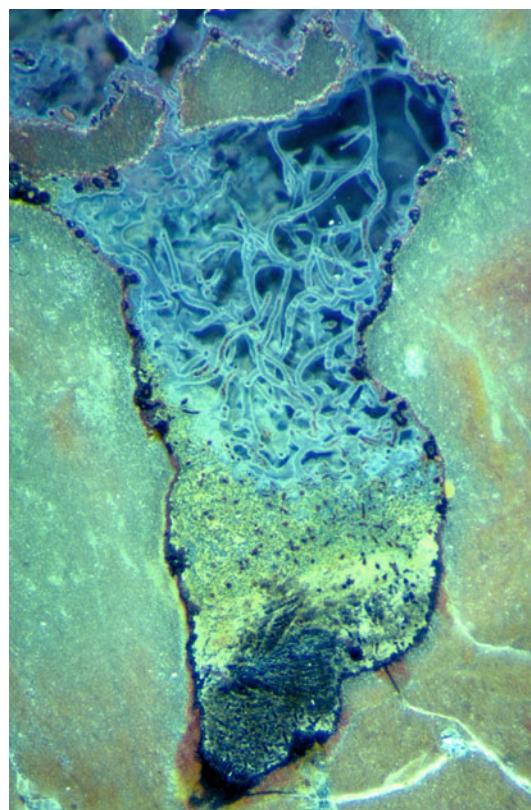
Subsurface filamentous fabrics (SFF, [Figures 1–5](#)) result from the encrustation of microbial filaments with minerals in subsurface geological environments, followed by oxidation of organic matter ([Hofmann, 2007](#); [Hofmann and Farmer, 2000](#); [Hofmann et al., 2008](#)). Encrustations of fibrous minerals may yield similar fabrics, but a biogenic origin is indicated by evidence for high flexibility, and a low variability of the smallest diameter of the filamentous forms, typically 1–3 μm . SFF textures vary from delicate mineral webs to massive rocks containing enclosed filaments, depending on the degree of mineral precipitation after initial filament encrustation in voids. SFF are most commonly found in voids in volcanic rocks and in the oxidation zone of ore deposits rich in reduced elements (S, Fe), but also occur in paleokarst environments ([Baele, 1998](#); [Feldmann et al., 1997](#); [Kretzschmar, 1982](#)), Mississippi Valley-type base metal deposits ([Hofmann et al., 2008](#)), cavities in fossils ([Weitschat, 1986](#)), mineral veins ([Hofmann, 1989](#); [Reitner, 2004](#); [Trewin and Knoll, 1999](#)), and cavities in impact rocks ([Hofmann et al., 2008](#)). Many of these occurrences show low-temperature hydrothermal influences. Historically, SFF have aroused the interest of researchers because of their often conspicuous nature. Examples of long-known forms are particularly the so-called moss agates, for which various interpretations have



Subsurface Filamentous Fabrics, Figure 1 Characteristic example of pseudostalactitic SFF. Encrustation of mat-like, vertically draped fabric by goethite. From the oxidation zone of a sulfide deposit, Kintore open cut, Broken Hill, New South Wales, Australia. Scale in cm.



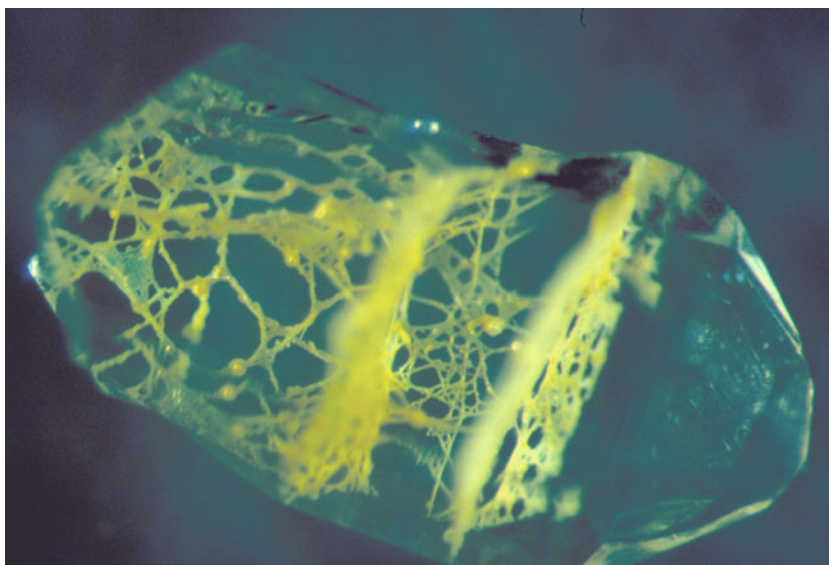
Subsurface Filamentous Fabrics, Figure 2 SEM image of goethite-encrusted microbial filaments from the oxidation zone (in contact with glacial till) of a pyrite-rich small orebody, Lengenbach, Binntal, Switzerland. Image width: 2.9 mm.



Subsurface Filamentous Fabrics, Figure 3 Image of filamentous forms enclosed within chalcedony in a fracture within a lithophysa in Tertiary rhyolite, Priday Ranch, Oregon, USA. Image width: 4 mm.



Subsurface Filamentous Fabrics, Figure 4 Filaments encrusted by iron hydroxides, associated to numerous folded mats, and enclosed by chalcedony. Host rocks are basaltic volcanics of Jurassic/Cretaceous age. This assemblage is known as moss agate. Image width: 18 mm. Arts Bogd Uul, Mongolia.



Subsurface Filamentous Fabrics, Figure 5 Filaments encrusted by iron hydroxides, enclosed in a single quartz crystal. Paleokarst within Devonian limestones (mineralization probably of Tertiary age), Hohenlimburg, Germany. Image width: 2.8 mm.

been brought forward (Bowerbank, 1842; Daubenton, 1782; Razumovsky, 1835). Similarities do exist with some types of dendrites (present in some moss agates) and speleothems. Many examples of “stalactitic” minerals (e.g., goethite, chalcedony, chrysocolla) were formed around filamentous centers with diameters in the order of microns, much too small for true speleothems the size of which is governed by the diameter of a water droplet (Hill

and Forti, 1997). Features discriminating SFF from similar fabrics are (Hofmann et al., 2008) (a) median width of filamentous substrate of $<5\ \mu\text{m}$ with narrow, near-normal distribution; (b) evidence of bending (e.g., U-loops) and aggregation to mat-like (biofilms), stromatolite-like, or rope-like fabrics; (c) filamentous cores show a high degree of irregular bending ($>0.5^\circ/\mu\text{m}$); (d) filamentous cores show many direction changes ($>10/\text{mm}$);

(e) association of SFF with or occurrence within SFF of other morphological bioforms such as fossil *Gallionella* or *Metallogenium*-like structures; (f) presence of vertically oriented elements (may resemble stalactites) resulting from mineral encrustation of filaments, filament strands, or mats while they are still flexible; and (g) inferred environmental conditions compatible with microbial growth, such as an abundant energy source and temperatures between ambient and $<120^{\circ}\text{C}$.

Types of organisms/substrates involved: The micro-morphology of SFF indicates that only filamentous micro-organisms are involved in their formation. Filamentous substrates of microbiological origin may include archaea, bacteria, and fungi. Some fabrics formed by encrustation of mineral fibers (these may be dissolved later) may show similar morphologies, but can usually be discriminated by evidence for characteristics typical of mineral fibers: Radial growth, variable fiber thickness, straight development, brittle behavior, and remnants of mineral fibers.

Conclusions

SFF represent a common, but often overlooked, type of biosignature from subsurface environments. SFF often display macroscopically visible fabrics that are easily recognizable and may be regarded as subsurface microbialites and the most conspicuous expression of the deep biosphere, representing in some ways an analog to stromatolites from surface environments. The very common occurrence in a wide variety of different rock types demonstrates that SFF are a common expression of subsurface life, wherever filamentous forms generate a large amount of biomass in relatively short time, allowing mineralization before organic filaments are destroyed. Organisms involved were chemolithotrophs (based on oxidation of Fe, S), but may also include heterotrophs living on chemolithotrophic primary producers. The formation in subsurface environments implies that no photosynthesizing organisms are involved. SFF-like features represent a target in the search for past life on Mars (Hofmann, 2007), as they might potentially be found in nearly all rock types.

Bibliography

- Baele, J. M., 1998. Reliques silicifiées et minéralisées de paléokarsts post-varisques sur le Dévonien en Belgique méridionale (Entre-Sambre-et-Meuse). *Annales de la Société Géologique du Nord*, **6**(2), 127–133.
- Bowerbank, J. S., 1842. On the spongy origin of moss agates and other siliceous bodies. *AMNH*, **10**, 9–18, 84–91.
- Daubenton, L. J. M., 1782. Sur les causes qui produisent trois sortes d'herborisations dans les pierres. *Mémoires de l'Académie Royale des Sciences*, 667–673.
- Feldmann, M., Neher, J., Jung, W., and Graf, F., 1997. Fungal quartz weathering and iron crystallite formation in an Alpine environment, Piz Alv, Switzerland. *Eclogae Geologicae Helvetiae*, **90**, 541–556.
- Hill, C., and Forti, P., 1997. *Cave Minerals of the World*. Huntsville: National Speleological Society.
- Hofmann, B., 1989. Genese, Alteration und rezentess Fließ-System der Uranlagerstätte Krunkelbach (Menzenschwand, Südschwarzwald). *Nagra Technical Report* 88–30, Nagra, Baden.
- Hofmann, B. A., 2007. Morphological biosignatures from subsurface environments: recognition on planetary missions. *Space Science Reviews*, DOI 10.1007/s11214-007-9147-9.
- Hofmann, B. A., and Farmer, J. D., 2000. Filamentous fabrics in low-temperature mineral assemblages: are they fossil biomarkers? implications for the search for a subsurface fossil record on the early Earth and Mars. *Planetary and Space Science*, **48**, 1077–1086.
- Hofmann, B. A., Farmer, J. D., von Blanckenburg, F., and Fallick, A. E., 2008. Subsurface filamentous fabrics: an evaluation of possible modes of origins based on morphological and geochemical criteria, with implications for exoplaeontology. *Astrobiology*, **8**, 87–117.
- Kretzschmar, M., 1982. Fossile Pilze in Eisen-Stromatolithen von Warstein (Rheinisches Schiefergebirge). *Facies*, **7**, 237–260.
- Razumovsky, G., 1835. Les agates mousseuses. *Bulletin de la société géologique de France*, **6**, 165–168.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie*. Göttingen: Universitätsdruckerei Göttingen.
- Trewin, N. H., and Knoll, A. H., 1999. Preservation of Devonian chemotrophic filamentous bacteria in calcite veins. *Palaos*, **14**, 288–294.
- Weitschat, W., 1986. Phosphatisierte Ammonoideen aus der Mittleren Trias von Central-Spitzbergen. *Mitteilungen aus dem Geologisch-Paläontologischen Institut der Universität Hamburg*, **61**, 249–279.

Cross-references

[Biofilms and Fossilization](#)
[Biosignatures in Rocks](#)
[Chemolithotrophy](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Fe\(II\)-Oxidizing Prokaryotes](#)
[Gallionella](#)
[Hydrothermal Environments, Marine](#)
[Hydrothermal Environments, Terrestrial](#)
[Karst Ecosystems](#)
[Microbialites, Stromatolites, and Thrombolites](#)

SULFATE-REDUCING BACTERIA

Heribert Cypionka
 University of Oldenburg, Oldenburg, Germany

Synonyms

Dissimilatory sulfate reducers; Sulfate-reducing prokaryotes

Definition

Sulfate reducers are prokaryotic microorganisms (Archaea or Bacteria) that use sulfate as electron acceptor of an anaerobic respiration process.

Introduction

Sulfur is one of the essential elements for life. It makes about 1% of the dry mass of living organisms and is present in two amino acids, iron sulfur clusters, and some coenzymes. Several organisms can use sulfate as sulfur source and carry out assimilatory sulfate reduction, which yields sulfide groups of amino acids. In contrast, dissimilatory sulfate reducers release large amounts of hydrogen sulfide (H_2S and HS^-) as product of their energy metabolism and cause the characteristic smell of rotten eggs. The process is carried out by a heterogeneous group of bacteria and archaea that occur in anoxic environments with temperatures up to 105°C (Widdel, 1988; Castro et al., 2000). Due to the high sulfate concentration of seawater (28 mM), sulfate reduction is especially important in marine sediments but does also occur in many other anoxic environments, e.g., lake sediments, animal guts, etc.

Evolution

The capacity of dissimilatory sulfate reduction appears to be very ancient. Its antiquity can be derived from phylogenetic analyses as well as from the geologic record (Canfield and Raiswell, 1999; Shen and Buick, 2004). Near the root of the phylogenetic tree are numerous Archaea or Bacteria metabolizing sulfur species including prokaryotes living from dissimilatory elemental sulfur reduction, dissimilatory sulfate reduction, and anoxygenic photosynthesis. As in a mixture of different isotopes of the same element lighter species are (mass-dependent) preferably metabolized by enzymes, isotope analysis of sediments can give strong evidence for their biogenic origin. The sulfur isotope distribution (^{32}S , ^{34}S , and ^{36}S) in microscopic pyrites in the ~ 3.47 billion years (Ga) old North Pole barite deposit of northwestern Australia provides the oldest evidence of microbial sulfate reduction and the earliest indication of a specific microbial metabolism. Furthermore, the observation of non-mass-dependent sulfur isotope ratios in sedimentary rocks more than 2.45 Ga old and the disappearance of this signal in younger sediments is taken as one of the strongest lines of evidence for the transition from an anoxic to an oxic atmosphere around 2.45 Ga ago (Farquhar et al., 2007).

Phylogeny

More than 120 species of sulfate-reducing prokaryotes (SRP), belonging to four bacterial and two archaeal phyla are known today (Thauer et al., 2007). The largest group are (1) mesophilic delta-proteobacteria (e.g., *Desulfovibrio*, *Desulfobacter*, *Desulfobulbus*), the other groups are (2) thermophilic Gram-negative bacteria (*Thermodesulfovibrio*), (3) *Desulfobium*, a recently established thermophilic genus not closely related to other bacteria, (4) Gram-positive bacteria with the spore-forming genus *Desulfotomaculum*, (5) Euryarcheota with the hyperthermophile *Archaeoglobus*, and (6) Crenarcheota with the hyperthermophile *Caldivirga* (Itoh et al., 1999).

Physiological groups

The most important substrates utilized by SRP are fermentation products as H_2 , alcohols, and organic acids like acetate, propionate, and butyrate. While most sulfate reducers have a limited spectrum of substrates that support growth, new isolates have been obtained that metabolize a wide variety of compounds including hexadecane, toluene, and several types of substituted aromatics (Hansen, 1994). Physiologically, sulfate reducers can be separated in two groups: Representatives of the first group generally grow fast and oxidize organic substrates incompletely to acetate, while members of the second group, grow more slowly, but oxidize their substrates completely to CO_2 . An important electron donor oxidized with sulfate as terminal electron acceptor is methane. However, so far, it has not been clarified whether sulfate reducers can oxidize methane directly or if they receive reducing equivalents in another form from an archaeal partner.

Pathway of sulfate reduction

Sulfate reduction is a rather complicated process (Figure 1). First, the double-charged anion has to be taken up by specific transport systems. Then ATP is hydrolyzed to activate sulfate to adenosine phosphosulfate (APS). This is now reduced to (bi-)sulfite, which is reduced to sulfide by sulfite reductase. APS reductase and sulfite reductase are the key enzymes. The genes coding for them (*apr* and *dsr*) are often used in molecular studies to analyze phylogeny or community structures of sulfate-reducing SRP.

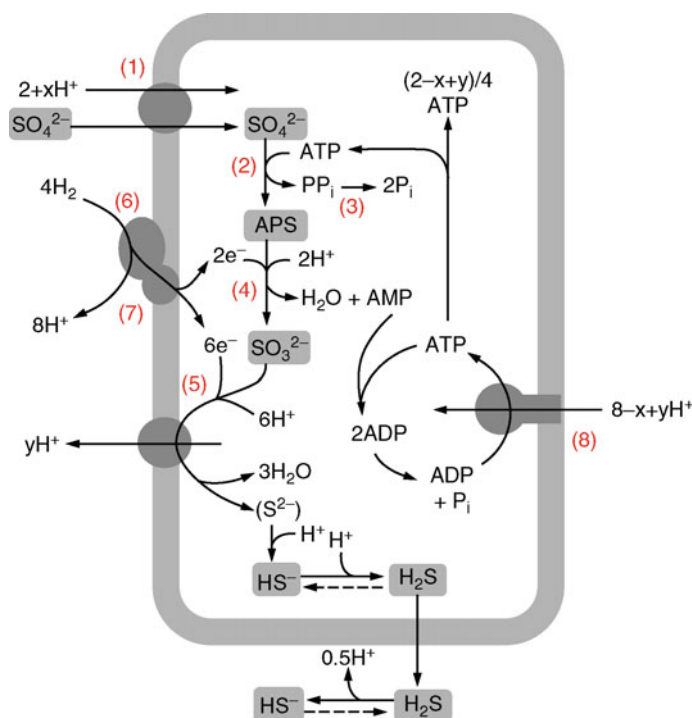
Reduction of sulfate with H_2 has a ΔG_0 of -151 kJ/mol of sulfate, which is about 1/6 of the value possible with oxygen as electron acceptor. Accordingly, there is not more than 1 ATP conserved per sulfate reduced.

Alternative electron acceptors

Most sulfate reducers can utilize other sulfur compounds than sulfate, often even with a better growth yield. Sulfite is an intermediate of sulfate reduction, but does not require an energy-dependent activation. Thiosulfate can be transformed to sulfide and sulfite in a single-step catalyzed thiosulfate reductase. Some sulfate reducers can also reduce elemental sulfur. Several sulfate reducers can utilize partially reduced compounds in the absence of an electron donor: Thiosulfate, sulfite, and elemental sulfur can be disproportionated to sulfate and sulfide, and the resulting (low amount of) free energy can be used for energy conservation (Bak and Cypionka, 1987).

Furthermore, some SRP can reduce nitrate or nitrite to ammonia, some can reduce metal ions as Fe^{3+} , Mn^{4+} or even U^{6+} (Lovley, 1993), some can reduce CO_2 to acetate (homoacetogenesis), and many can grow by fermentation of simple organic molecules like pyruvate.

Several SRB have been found to reduce molecular oxygen at high rates. The process is coupled to ATP conservation. However, it appears to be a defense mechanism. Sustainable growth of sulfate reducers with O_2 as electron acceptor has never been observed (Cypionka, 2000).



Sulfate-Reducing Bacteria, Figure 1 Steps of dissimilatory sulfate reduction and chemiosmotic energy conservation with H_2 as electron donor. (1) Uptake by symport with protons, (2) ATP sulfurylase, (3) pyrophosphatase, (4) APS reductase, (5) sulfite reductase, (6) hydrogenase, (7) cytochrome c, (8) ATP synthase (Cypionka, 2006).

Summary

Sulfate-reducing prokaryotes reduce sulfate to sulfide in an anaerobic respiration process. Sulfate reduction is ancient and found in Bacteria and Archaea. The main electron donors used by sulfate reducers are fermentation products. Sulfate reduction yields little energy and proceeds via several steps. Sulfate reducers are anaerobes, although some of them are able to reduce oxygen not coupled to growth. Alternatively, other sulfur compounds, nitrate or nitrite, and some oxidized metal ions can be used by some strains.

Bibliography

- Bak, F., and Cypionka, H., 1987. A novel type of energy metabolism involving fermentation of inorganic sulphur compounds. *Nature*, **326**, 891–892.
- Canfield, D. E., and Raiswell, R., 1999. The Evolution of the Sulfur Cycle. *American Journal of Science*, **299**, 697–723.
- Castro, H. F., Williams, N. H., and Ogram, A., 2000. Phylogeny of sulfate-reducing bacteria. *FEMS Microbiology Ecology*, **31**, 1–9.
- Cypionka, H., 2000. Oxygen respiration by *Desulfovibrio* species. *Annual Review of Microbiology*, **54**, 827–848.
- Cypionka, H., 2006. *Grundlagen der Microbiologie*. Heidelberg: Springer-Verlag.
- Farquhar, J., Peters, M., Johnston, D. T., Strauss, H., Masterson, A., Wiechert, U., and Kaufman, A. J., 2007. Isotopic evidence for Mesoarchaeal anoxia and changing atmospheric sulphur chemistry. *Nature*, **449**, 706–710.
- Hansen, T. A., 1994. Metabolism of sulfate-reducing prokaryotes. *Antonie van Leeuwenhoek*, **66**, 165–185.
- Itoh, T., Suzuki, K., Sanchez, P. C., and Nakase, T., 1999. *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *International Journal of Systematic Bacteriology*, **49**, 1157–1163.
- Lovley, D. R., 1993. Dissimilatory metal reduction. *Annual Review of Microbiology*, **47**, 263–290.
- Shen, Y., and Buick, R., 2004. The antiquity of microbial sulfate reduction. *Earth-Science Reviews*, **64**, 243–272.
- Thauer, R. K., Stackebrandt, E., and Hamilton, W. A., 2007. Energy metabolism and phylogenetic diversity of sulphate-reducing bacteria. In Barton, L. L., and Hamilton, W. A. (eds.), *Sulphate-Reducing Bacteria*. Environmental and Engineered Systems. Cambridge: Cambridge University Press, pp. 1–37.
- Widdel, F., 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In Zehnder, A. J. B. (ed.), *Biology of Anaerobic Microorganisms*. New York: John Wiley & Sons, pp. 469–585.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Deep Biosphere of Sediments](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Sulfur Cycle](#)
[Sulfur Isotopes](#)

SULFIDE MINERAL OXIDATION

D. Kirk Nordstrom

U.S. Geological Survey, Boulder, CO, USA

Synonyms

Sulfide-mineral weathering; Sulfide-ore oxidation

Definition

Sulfide mineral. A metal-sulfide compound, such as pyrite (FeS_2), which forms at high temperature ($>50^\circ\text{C}$) in well-crystallized veins or masses and at low temperatures ($<50^\circ\text{C}$) in poorly crystalline and fine-grained particles.

Oxidation. The chemical process of reacting with oxygen. More generally, the chemical process of removing electrons from an atom or group of atoms.

Introduction

Metal-sulfide minerals are valuable as ores for metals that have a wide variety of uses from jewelry to components in vehicles and electronic equipment. They are found primarily in hydrothermal mineral deposits that occur in numerous geologic environments. The most common sulfide mineral is pyrite; other important sulfide ore minerals include chalcopyrite (copper ore), molybdenite (molybdenum ore), sphalerite (zinc ore), galena (lead ore), and cinnabar (mercury ore). When these minerals are exposed to weathering at the Earth's surface, either through natural processes or as a result of mining activities, they react with oxygen in air in the presence of water to form drainage that is frequently acidic and detrimental to the environment because of the high metal concentrations. The general term for this water is acid-rock drainage. When it originates from mining activities, it is called acid-mine drainage (Nordstrom and Alpers, 1999). Acid-rock drainage is produced by numerous chemical and microbiological processes within a complex hydrogeological environment. Extensive research has been employed to understand these processes, so that mine-site remediation can be more cost-effective and long-lasting. Weathering of sulfide minerals leads to natural enrichment in copper, gold, and silver in a process known as supergene enrichment. Copper dissolves in the infiltrating water and precipitates when it reaches unweathered sulfides downgradient, enriching the original sulfides. Gold and silver are highly insoluble and are enriched in the residual iron oxide cap or gossan that forms above the unweathered ore deposit. Sulfide-mineral oxidation from both mining and natural weathering is a major source of both dissolved sulfate and dissolved metals in natural waters.

Metal extraction of primary ores and of waste piles from previous mining activities is also accomplished by hydrometallurgical techniques that have optimized sulfide-mineral oxidation by increasing the temperature of leaching, adding catalytic reagents, and bioleaching by

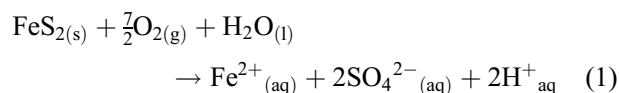
adding microorganisms that are specially suited for increasing the rate of sulfide-mineral oxidation.

Chemolithotrophy

Acid-mine drainage has been known since the early days of mining by ancient civilizations and was documented by early Greek and Roman writers (Nordstrom, 2009). Oxidation of sulfide minerals by microorganisms, however, has a more recent history. Winogradsky (1888) recognized that microbes could actually derive metabolic energy from the oxidation of inorganic compounds. These microbes gain energy from the oxidation (or reduction) of inorganic compounds and acquire their carbon needs from the carbon dioxide in the air (autotrophy). Hence, many of them are chemolithoautotrophs or chemoautotrophs for short. During the first quarter of the twentieth century, it was suspected that microbes affected the oxidation of sulfide minerals, but it was not until Rudolfs and Helbronner (1922) demonstrated that microbes could oxidize zinc sulfide, and Colmer and Hinkle (1947) isolated *Thiobacillus ferrooxidans*, now *Acidithiobacillus ferrooxidans*, from acid-mine drainage that microbes were recognized to be important catalysts in the production of acid-mine drainage from sulfide-mineral oxidation.

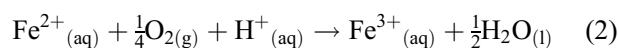
Biogeochemistry

The overall reaction of a metal sulfide like pyrite with air and water can be represented by the equation:

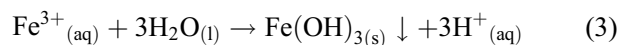


Pyrite + oxygen + water $\rightarrow$ acid ferrous sulfate solution.

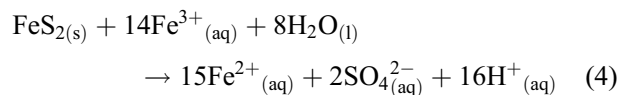
The acidity originates from the protons needed to balance out some of the loss of electrons from iron and sulfur oxidation. The dissolved ferrous iron further oxidizes to ferric iron:



Dissolved ferric iron easily hydrolyzes and precipitates as a colloidal phase, usually a mixture of fine-grained minerals, represented by either ferric hydroxide or commonly called hydrous ferric oxide (HFO):



Dissolved ferric iron is the oxidant that directly attacks the pyrite surface so that the more correct way to write the reaction would be:



Because dissolved ferrous iron oxidizes (Equation 2) much more slowly than the oxidation of pyrite by dissolved ferric iron (Equation 4), the oxidation of pyrite

would become negligible were it not for chemolithoautotrophic microorganisms. Many types of bacteria and archaea have been found to gain energy from the oxidation of ferrous iron and reduced sulfur in acidic environments (Norris, 1990; Nordstrom and Southam, 1997; Bond et al., 2000; Ehrlich, 2002). Some bacteria, such as *Acidithiobacillus ferrooxidans*, are capable of oxidizing both ferrous iron and reduced sulfur compounds (hydrogen sulfide, elemental sulfur, thiosulfate, and tetrathionate). Other bacteria, such as *Leptospirillum ferrooxidans*, are only able to oxidize ferrous iron and some bacteria, such as *Acidithiobacillus thiooxidans*, are only capable of oxidizing reduced sulfur compounds. Iron-oxidizing archaea include *Acidianus brierleyi*, *Sulfolobus acidocaldarius* (Nordstrom and Southam, 1997), *Ferroplasma acidiphilum* (Golyshina et al., 2000), and *Ferroplasma acidarmanus* (Edwards et al., 2000). Although *Acidithiobacillus ferrooxidans* has been the subject of more studies, *Leptospirillum ferrooxidans* seems to be the more common species in acid-mine drainage (Rawlings et al., 1999; Bond et al., 2000).

The geochemistry of sulfide-mineral oxidation and its effect on surface-water quality can vary substantially depending on the composition and grain size of the sulfide minerals. Common sulfide minerals are usually either disulfides like pyrite (FeS_2) and molybdenite (MoS_2) or monosulfides like sphalerite (ZnS) and galena (PbS). Molybdenite is hydrophobic and rather insoluble so that it oxidizes and weathers very slowly whereas sphalerite is more soluble and readily reacts with acid water to produce

hydrogen-sulfide gas. The grain size of these minerals can greatly affect their oxidation and dissolution rate. The smaller the grain size, the faster they react. Hence, fine-grained crystals of pyrite can react more quickly than large crystals of sphalerite even though sphalerite would dissolve more quickly if it were the same grain size as pyrite. Marcasite has the same composition as pyrite but it reacts more rapidly because it has a different crystal structure that is more amenable to oxidative weathering.

As acid-mine drainage mixes with surface waters, the dissolved ferrous iron oxidizes and precipitates to hydrous ferric oxide (HFO) which is an excellent sorbent for trace metals. Consequently, some of the dissolved copper, lead, zinc, arsenic, and other metals will be sorbed onto colloidal particles of HFO and decrease the potential toxicity, one of several processes known as natural attenuation. This process is enhanced by the growth of masses of bacteria and algae which also sorb some of the metals, removing them from solution and decreasing the concentration transported further downstream.

Environmental consequences of sulfide mineral oxidation

Acid-mine drainage from sulfide-mineral oxidation usually discharges into streams, rivers, and lakes (Figure 1). The pH values of acid-mine drainage range from 1 to 4 although values as low as -3.5 have been measured (Nordstrom et al., 2000). The high concentrations of metals such as copper, chromium, zinc, lead, cadmium,



Sulfide Mineral Oxidation, Figure 1 Effluent from the Tinto and Santa Rosa mines flowing into the Villar River, Spain. (Photograph courtesy of Manuel Caraballo Monge, Barcelona, Spain.)

mercury, manganese, iron, and aluminum found in acid-mine drainage is too toxic for invertebrates, amphibians, fish, and other forms of aquatic or terrestrial life. Only bacteria, algae, and fungi seem to survive when the pH is about 3 or less and the concentration of metals is a few hundred parts per billion or higher. Occasionally cyanide is also released into surface waters from gold processing with harmful effects. Releases of acid-mine drainage have caused the loss of enormous quantities of fish as well as harming livestock, mammalian wildlife, and crops. Two of the largest reported spills destroyed temporarily the biological habitat of two separate river systems. One spill was from the Aznalcollar impoundment in southern Spain in April, 1998, discharging 6 million cubic meters of acid water and pyritic fines into the Guadiamar River and ruining thousands of hectares of farmland. The other spill occurred in January, 2000, releasing a hundred thousand cubic meters of cyanide and heavy metals from a 4 km impoundment at the Aurul gold extraction plant near Baia Mare in northwestern Romania. The cyanide-rich water moved down to the Lapus River, then to the Tisza River, and finally to the Danube River all the way to the Black Sea. Smaller spills from the same region occurred again over the next 2 months. Approximately, 1,200 t of fish were killed from this spill, bird life was affected, thousands of fishermen were out of work, and water supplies for several towns and rural communities were badly contaminated.

Treatment technology

Innumerable ideas have been suggested for alleviating the harmful effects of sulfide-mineral oxidation and acid-mine drainage production. These suggestions include passive wetlands treatment, bioreactors, electrochemical treatment, exchange resins, reverse osmosis, and the use of various biosolids. However, the most commonly used, safe, and cost-effective treatments today are lime–lime-stone neutralization and precipitation of the metal-rich sludge in settling ponds combined with water-management techniques. An important engineering aspect is the construction of containment structures for hazardous materials that can withstand intense rainstorms and floods and that will prevent releases into surface-water and groundwater supplies.

Conclusion

Sulfide-mineral oxidation is a complex hydrobiogeochemical process involving the oxidation of metal-sulfide minerals, catalyzed by numerous bacterial and archaeal microbial species, and producing acid-rock drainage. Mining activities enhance the rate of this process, causing the production of acidic metal-rich drainage that make receiving streams, lakes, and rivers unfit for general usage and injurious to aquatic and terrestrial biota. Major spills of mine wastes into receiving waters have severely damaged valuable aquatic resources,

farmland, community water supplies, and the economic stability of those who depend on farming and fishing. Much effort has gone into the remediation of old inactive mines and current mining activities to improve water quality for beneficial uses but the scale and complexity of the problem continues to challenge engineers and regulators.

Bibliography

- Bond, P. L., Druschel, G. K., and Banfield, J. F., 2000. Comparison of acid mine drainage microbial communities in physically and geochemically distinct ecosystems. *Applied and Environmental Microbiology*, **66**, 4962–4971.
- Colmer, A. R., and Hinkle, M. E., 1947. The role of microorganisms in acid mine drainage. *Science*, **106**, 253–256.
- Edwards, K. J., Bond, P. L., Gihring, T. M., and Banfield, J. F., 2000. An archaeal Fe-oxidizing extreme acidophile important in acid mine drainage. *Science*, **287**, 1796–1799.
- Ehrlich, H. L., 2002. *Geomicrobiology*, 4th edn. New York: Marcel Dekker.
- Golyshina, O. V., Pivovarova, T. A., Karavaiko, G. I., Kondrat'eva, T. F., Moore, R. B., Abraham, W. R., Lunsdorf, H., Timmis, K. N., Yakimov, M. M., and Golyshin, P. N., 2000. *Ferroplasma acidiphilum*, gen. nov., sp. Nov., an acidophilic, autotrophic, ferrous-Fe-oxidizing, cell-wall-lacking, mesophilic member of the Ferroplasmaceae fam., comprising a distinct lineage of the Archaea. *International Journal of Systematic and Evolutionary Biology*, **50**, 997–1006.
- Nordstrom, D. K., 2009. Acid rock drainage and climate change. *Journal of Geochemical Exploration*, **100**, 97–104.
- Nordstrom, D. K., and Alpers, C. N., 1999. Geochemistry of acid mine waters. In Plumlee, G. S., and Logsdon, M. J. (eds.), *The Environmental Geochemistry of Mineral Deposits. Part A. Processes, Methods and Health Issues*, Reviews in Economic Geology, Littleton, CO: Society of Economic Geology, Vol. 6A, pp. 133–160.
- Nordstrom, D. K., and Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between Microbes and Minerals*, Reviews in Mineralogy 35. Washington, DC: Mineralogical Society of America, pp. 361–390.
- Nordstrom, D. K., Alpers, C. N., Ptacek, C. J., and Blowes, D. W., 2000. Negative pH and extremely acid waters from Iron Mountain, California. *Environmental Science and Technology*, **34**, 254–258.
- Norris, P. R., 1990. Acidophilic bacteria and their activity in sulfide mineral oxidation. In Ehrlich, H. L., and Brierley, C. L. (eds.), *Microbial Mineral Recovery*. New York: McGraw-Hill, pp. 3–27.
- Rawlings, D. E., Tributsch, H., and Hansford, G. S., 1999. Reasons why 'Leptospirillum'-like species rather than *Thiobacillus ferrooxidans* are the dominant Fe-oxidizing bacteria in many commercial processes for biooxidation of pyrite and related ores. *Microbiology*, **145**, 5–13.
- Rudolfs, A., and Helbronner, A., 1922. Oxidation of zinc sulfide by microorganisms. *Soil Science*, **14**, 459–464.
- Winogradsky, S. N., 1888. Über Eisenbakterien. *Botanische Zeitung*, **46**, 261–276.

Cross-references

[Acid Rock Drainage](#)
[Heavy Metals](#)
[Ores, Microbial Precipitation and Oxidation](#)
[Pyrite Oxidation](#)

SULFUR CYCLE

Michael E. Böttcher

Leibniz Institute for Baltic Sea Research, Warnemünde, Germany

Synonyms

Biogeochemical sulfur cycle; Sulfate reduction and sulfide oxidation; Transformation of sulfur compounds

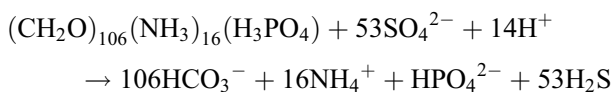
Definition

Sulfur. Chemical element that is one of the constituents of the Earth's compartments and living organisms. While the average sulfur content of the whole Earth is about 2%, the crust contains only about 0.07% sulfur (Brimblecombe, 2003).

Sulfur cycle. Biogeochemical system of biotic and abiotic transformations of inorganic and organic sulfur-bearing components, in and between, the lithosphere, hydrosphere, atmosphere, and biosphere. Sulfur initially enters the biogeochemical cycle via volcanic activity and continental erosion. Today, the modern sulfur cycle is influenced by human activity. The Earth sulfur reservoir is assumed to have been essentially constant through time (Garrels and Lerman, 1984).

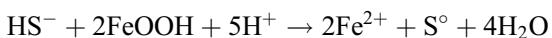
Assimilative sulfate reduction. Sulfate (SO_4^{2-}) is reduced to organic R-SH groups by plants, algae, and fungi.

Dissimilative sulfur and sulfate reduction. Hydrogen sulfide (H_2S) is produced from elemental sulfur (S^0) or dissolved sulfate by sulfur- and sulfate-reducing microorganisms, respectively. For sulfate reduction coupled to the oxidation of marine organic matter or methane, the overall reactions are:

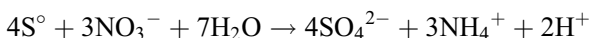


Desulfuration. Hydrogen sulfide is produced from sulfur-containing organic molecules.

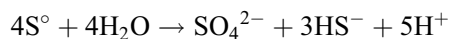
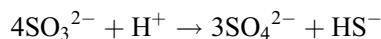
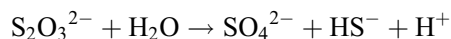
Oxidation of hydrogen sulfide. Production of sulfur intermediates, elemental sulfur, thiosulfate ($\text{S}_2\text{O}_3^{2-}$), sulfite (SO_3^{2-}), and/or sulfate by bacterial or abiotic oxidation (Jørgensen and Nelson, 2004). Possible reactions are:



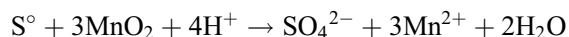
Oxidation of sulfur. Sulfate is generated by the oxidation of elemental sulfur. A possible reaction is (gradient bacterium *Thioploca*):



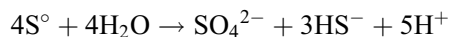
Disproportionation of sulfur intermediates. Sulfate and hydrogen sulfide are produced by the low-temperature bacterial metabolism of sulfur intermediates. The stoichiometries of the reactions are (Finster, 2008):



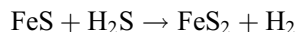
In the presence of oxy(hydroxi)des of iron(III) or manganese (IV) the following stoichiometries are obtained (Böttcher and Thamdrup, 2001; Finster, 2008):



Hydrolysis of elemental sulfur. Sulfate and hydrogen sulfide can be produced by the abiotic hydrolysis of elemental sulfur at high temperatures (Smith, 2000), according to:



Formation of iron disulfide (pyrite). The low-temperature formation of iron disulfide typically takes place via the polysulfide ($\text{S}^{\circ*}$) or hydrogen sulfide pathway by the reaction with the precursor iron monosulfide (Rickard and Luther III, 2007):



The degree of pyritization (DOP) indicates the relative amount of reactive sedimentary iron that has already been reacted to pyrite.

The global sulfur cycle

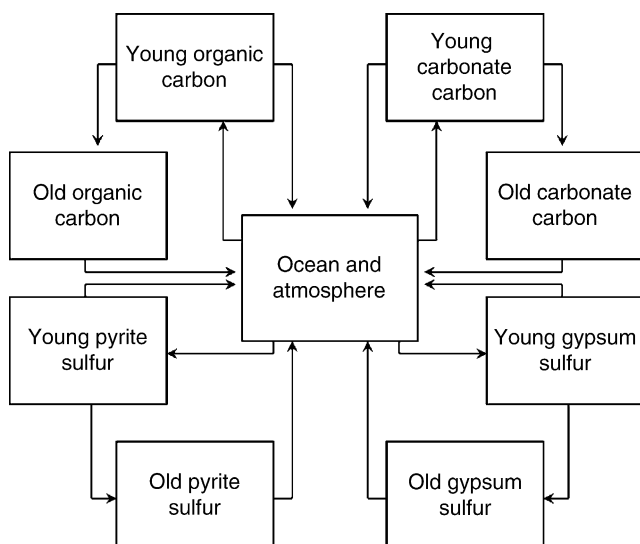
The global reduced and oxidized reservoirs of sulfur, carbon, oxygen, and selected metals are coupled on different scales via redox-driven chemical, biological, and geological processes (Figures 1–4). Parts of this cycling, as the reaction between sulfuric acid (that later became popular in industrialized times as “acid rain”) and carbonate rocks, were already described in the nineteenth century by Johann Wolfgang von Goethe (Figure 1) in his famous novel “Elective affinities” (German: “Wahlverwandschaften”; published in 1809).

On Earth, pure sulfur occurs in elemental form as for example in volcanic regions, and the quantitative use of sulfur-bearing compounds by man has been used as a proxy for economic wealth (Brimblecombe, 2003). Iron sulfide sulfur occurs in almost all types of rocks and sediments and is even found in extraterrestrial meteorites. In sediments that are not under the influence of hydrothermal activity, the occurrence of sulfide sulfur is indicative for the activity of sulfate-reducing bacteria

"... Thus what we call limestone is a more or less pure calcareous earth in combination with a delicate acid, which is familiar to us in the form of a gas. Now, if we place a piece of this stone in diluted sulphuric acid, this will place a possession of the lime, and appear with it in the form of gypsum, the gaseous acid at the same time going off in vapour. Here is a case of separation; a combination arises, and we believe ourselves now justified in applying to it the words 'Elective Affinity;' it really looks as if one relation had been deliberately chosen in preference to another...."

Johann Wolfgang von Goethe 'Elective Affinity'
(*German: Wahlverwandtschaften*)

Sulfur Cycle, Figure 1 Coupling of the sulfur and carbon cycles according to Johann Wolfgang von Goethe. (Translation from Niles, 1872.)



Sulfur Cycle, Figure 2 Mass balance model of coupling between the young and old global carbon and sulfur reservoirs. (Redrawn from Berner, 1987, as a modification of the model by Garrels and Lerman, 1984.)

(e.g., Jørgensen and Kasten, 2006). Whereas sulfur is assimilated by living organisms where it is bound to, for instance, amino acids, most of the organic sulfur found in sediments is derived from early diagenetic reaction of organic matter with reduced and intermediate sulfur species (Sinninghe Damsté and de Leeuw, 1990). The decomposition of organic matter in the presence of sulfate is catalyzed by microorganisms that gain energy from the dissimilatory reduction of sulfate (e.g., *Desulfovibrio* and *Desulfobacter*; Rabus et al., 2001). Microbial oxidation of sulfide (e.g., by green and purple sulfur bacteria; gradient bacteria as *Beggiatoa* or *Thioploca*; Jørgensen

and Nelson, 2004), disproportionation (e.g., *Desulfovibrio*, *Desulfocapsa*, Finster, 2008), or further oxidation of sulfur intermediates (e.g., *Thiobacillus*) can take place aerobically or anaerobically. In the aquatic low-temperature environment, dissolved and metal-bound sulfide may also be abiotically oxidized, e.g., by manganese dioxide (Jørgensen and Nelson, 2004). The lowest oxidation state of sulfur is $-II$ (e.g., in hydrogen sulfide). In its highest oxidation state $+VI$ (e.g., in sulfate), sulfur is bound to oxygen and found as (biogenic or abiotic) barite or evaporitic sulfate minerals (e.g., gypsum or anhydrite).

Sulfur in the Atmosphere

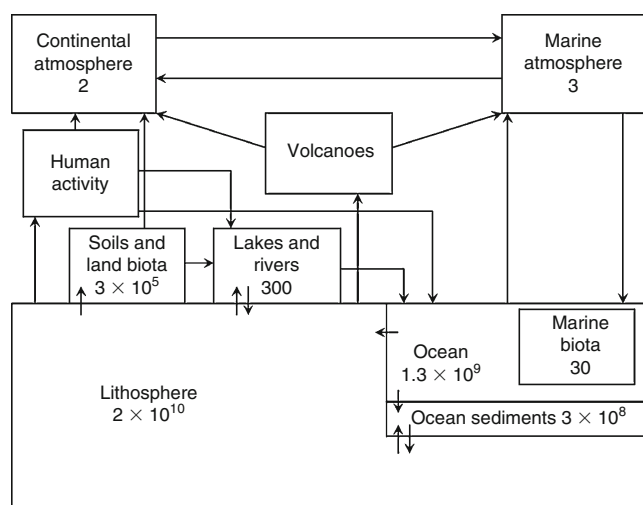
The first atmospheric sulfur components on Earth (mainly SO_2 and H_2S) were derived from volcanic activity and may have led to the first acid rain on Earth (Farquhar et al., 2000). Volcanoes still contribute to the atmospheric sulfur budget, besides emissions from the oceans (e.g., dimethyl sulfide (DMS), carbon disulfide), coastal and wetland areas. Most of the different components temporarily end as sulfate. The anthropogenic combustion of sulfur-bearing fossil fuels in the past century not only produced CO_2 , but also led to the formation of sulfuric acid ("acid rain"), which partly led to increased rock weathering that again linked the carbon and sulfur cycles (Figure 1). In the ongoing discussion of dealing with climate change, the observations from the eruption of Mount Pinatubo are taken as an event-type base for geo-engineering concepts to decrease the global surface temperature by the artificial release of sulfur components into the stratosphere (Crutzen, 2006).

Sulfur in surface and groundwaters, and freshwater sediments

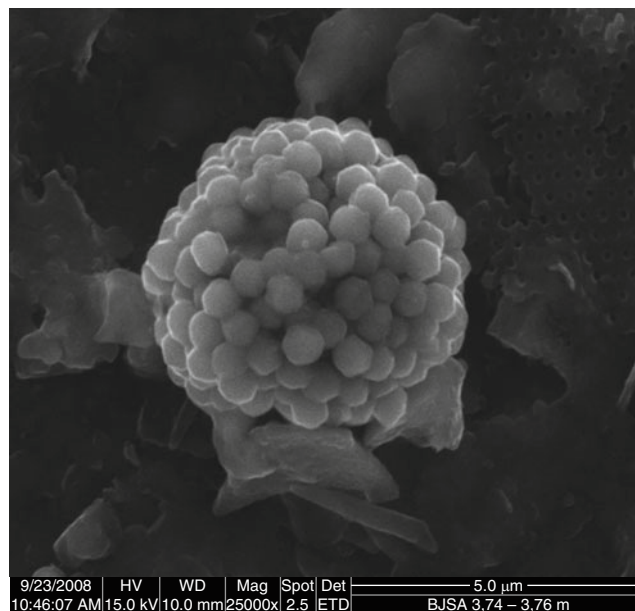
Rainwater sulfate, sulfur dioxide, and organic sulfur enter the water-unsaturated soil zone, where further

transformations take place. Geological contributions from soil and aquifer minerals vary strongly and may include gypsum dissolution and metal sulfide oxidation. Near-surface oxidation of pyrite from coal-mining activities leads today to the anthropogenically induced phenomenon of “acid mine drainage” (Holmer and Storkholm, 2001). Sulfur cycling in freshwater lake sediments associated with organic matter diagenesis is controlled by a number of factors, including temperature, light conditions, the availability of sulfate, organic matter, electron acceptors and nutrients, and macro faunal abundance (Holmer and Storkholm, 2001). Groundwater may play an important role in influencing sulfur cycling, for instance, via the

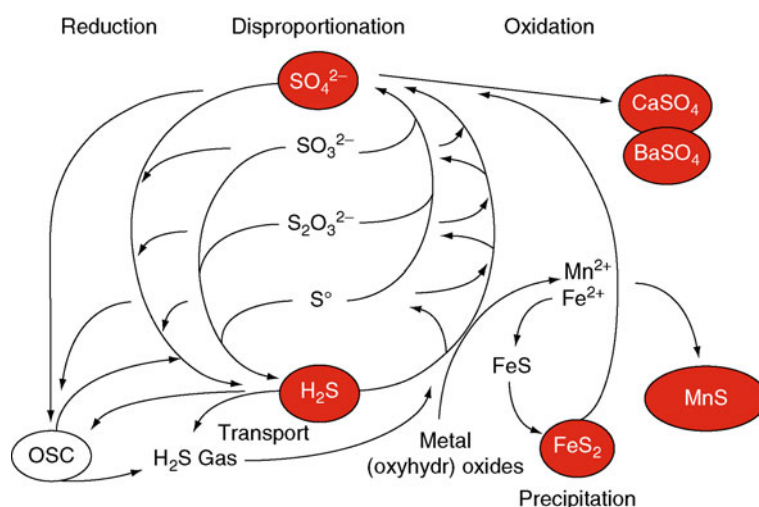
supply of evaporite-derived sulfate (Swiss Lago di Cadagno, as an example) and rock weathering (Böttcher, 1999). Sulfur cycling via organic sulfur compounds (Figure 4) plays an important role in freshwater sediments and soils (Mitchell et al., 1992; Holmer and Storkholm, 2001). Finally, sulfate may reach the ocean where further processing takes place (Figure 4).



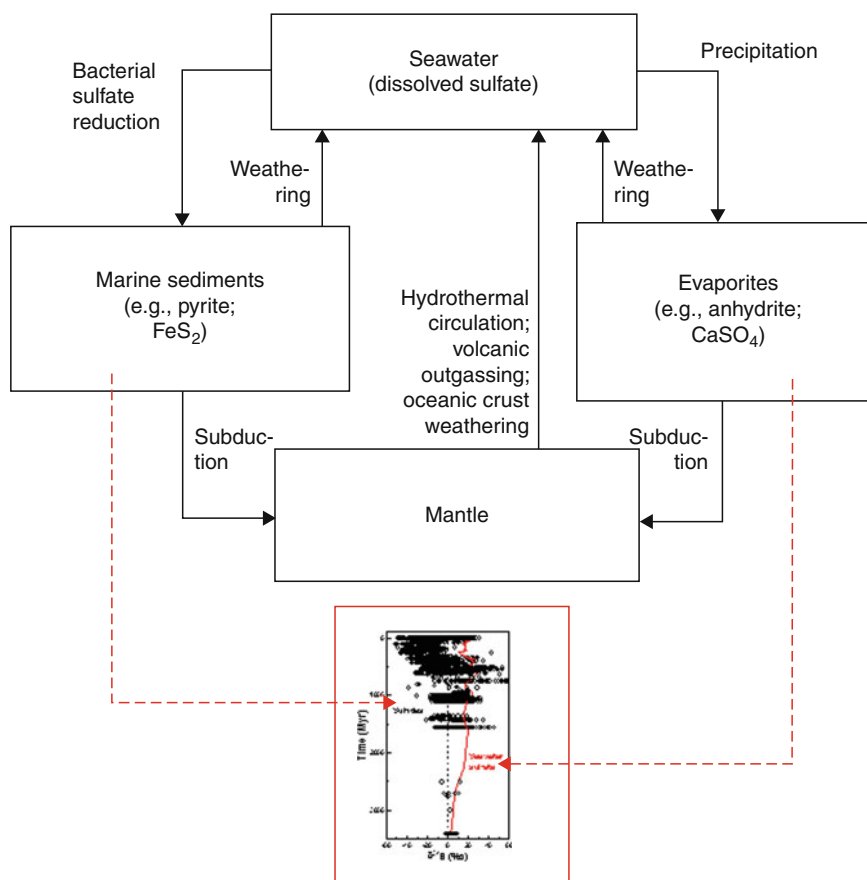
Sulfur Cycle, Figure 3 Major reservoirs and burdens of sulfur in Tg. (Modified after Brimblecombe, 2003.)



Sulfur Cycle, Figure 5 Framboidal iron disulfide (pyrite) as indicator for the activity of sulfate-reducing bacteria in sediments. (Example from marine intertidal North Sea sediments; Böttcher and Bahlo, unpublished scanning electron microscope picture, 2008.)



Sulfur Cycle, Figure 4 Simplified sedimentary sulfur cycle and its link to selected metals (neglecting, e.g., transformation of polysulfides, thionates, thiols, and methane). OSC: Organic sulfur compounds.



Sulfur Cycle, Figure 6 *Upper part:* Box model for the global sulfur cycle (after: Canfield, 2004). *Lower part:* Variation of the stable sulfur isotope composition of sedimentary sulfides and coexisting seawater sulfate (from evaporites and barite). (After data from Canfield and Teske, 1996, with additions by Böttcher, unpublished data, 1998–2000.)

Sulfur in seawater and marine sediments

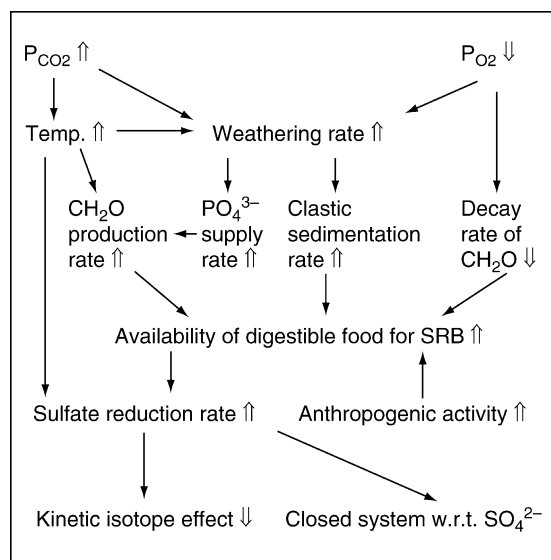
With a residence time of at least 8 million years, dissolved sulfate in seawater is well-mixed and reflects changes in the fluxes between and sizes of the major reservoirs (Figures 2 and 6). Whereas at mid-oceanic ridges, chemical sulfate reduction occurs in heated seawater that circulates through the Earth's crust, the low temperature dissimilatory sulfate reduction is linked to the enzymatic activity of sulfate-reducing bacteria which requires the abundance of metabolizable dissolved organic carbon compounds or methane (Jørgensen and Kasten, 2006). The anaerobic oxidation of methane associated with microbial reduction of sulfate is an important trap to prevent methane from escaping into the water column (Jørgensen and Kasten, 2006). In continental margin sediments, microbial sulfate reduction accounts for up to more than 50% of the organic matter oxidation (Skyring, 1987; Jørgensen and Kasten, 2006). Sulfate reduction may also take place in anoxic water columns (e.g., Black Sea). Most of the biogenic sulfide is reoxidized at the benthic or pelagic redoxclines by microbial or chemical processes (Jørgensen and Nelson, 2004) leading to the

metastable formation of intermediate sulfur species, or finally sulfate. The sulfur intermediates can be further oxidized, reduced, or disproportionated (Figure 4). Only a small portion of sulfide is finally buried as iron sulfides (essentially pyrite; Figure 5) or organic sulfur (Figures 2 and 4).

A number of external factors may directly or indirectly influence the rates of microbial sulfate reduction and sulfide burial efficiency, on different scales that can be reflected by the contents of sedimentary sulfur and the "reservoir" effect reflected in overall isotope discrimination (Figure 7).

Geochemical tracers for sulfur cycling: Stable sulfur and oxygen isotopes

Fractionation of stable sulfur (in dissolved and solid phase sulfur) and oxygen isotopes (in sulfate and sulfate-bearing minerals) takes place via biological and abiotic transformations and leads to isotope signatures that can be used to identify sources and biological, chemical, and physical processes involved (Canfield and Raiswell, 1999; Böttcher, 2010). Oxygen isotope fractionation can be used



Sulfur Cycle, Figure 7 Compilation of potential factors influencing availability of oxidizable organic matter available to sulfate-reducing bacteria and consequences for sulfate reduction rates and associated kinetic and reservoir size-induced sulfur isotope effect. (Modified after Ohmoto, 1992.)

to trace sulfate sources in aerobic environments (Clark and Fritz, 1997). The composition of dissolved sulfate and sedimentary diagenetic barite after modification by microbial sulfate, on the other hand, is finally governed by intracellular oxygen isotope exchange toward equilibration with surrounding water (Wortmann et al., 2007). The fossil sedimentary isotope record of pyrite (Figure 6) and anhydrite/barite/carbonate-bound sulfur, reflects the coupled changes in the atmospheric composition, the biogeochemistry of the sedimentary sulfur cycle and the fluxes between the different sulfur-bearing reservoirs (Figures 1–4 and 6) (e.g., Berner, 1987; Canfield and Teske, 1996; Canfield and Raiswell, 1999; Canfield, 2004).

Conclusion

The sulfur cycle is of general importance due to its involvement in the anaerobic mineralization of organic matter, and therefore, tight coupling to the carbon cycle. Its geochemical signature in sediments provides hints for identification of key processes associated with the evolution of life and the atmosphere on Earth. Some of the many open questions pertaining to the (bio)geochemistry and geomicrobiology of sulfur are (1) the importance of microbial versus chemical sulfide oxidation, (2) the quantitative importance of metastable intermediate and reduced sulfur species in the low-temperature sulfur cycle (Rickard and Morse, 2005; Finster, 2008), (3) the quantitative role of sulfur cycling for the deep biosphere (Jørgensen and D'Hondt, 2006), and (4) the role of metal

sulfides in extreme environments on the pre-biotic Earth for the later development of the precursors of life (Rickard and Luther III, 2007).

Bibliography

- Berner, R. A., 1987. Models for carbon and sulfur cycles and atmospheric oxygen: application to Paleozoic geologic history. *American Journal of Sciences*, **287**, 177.
- Böttcher, M. E., 1999. The stable isotope geochemistry of the sulfur and carbon cycles in a modern karst environment. *Isotopes in Environment and Health Studies*, **35**, 39.
- Böttcher, M. E., 2010. Isotopes (sulfur). In Reiter, J., and Thiel, V. (eds.), *Encyclopaedia of Geobiology*. Berlin: Springer, in press.
- Böttcher, M. E., and Thamdrup, B., 2001. Anaerobic oxidation and stable isotope fractionation associated with bacterial sulfur disproportionation in the presence of MnO₂. *Geochimica et Cosmochimica Acta*, **65**, 1573.
- Brimblecombe, P., 2003. The global sulfur cycle. In Schlesinger, W. (ed.), *Treatise on Geochemistry, Biogeochemistry*. Amsterdam: Elsevier, Vol. 8, pp. 645.
- Canfield, D. E., 2004. The evolution of the Earth surface sulfur cycle. *American Journal of Sciences*, **304**, 839.
- Canfield, D. E., and Raiswell, R., 1999. The evolution of the sulfur cycle. *American Journal of Science*, **290**, 697.
- Canfield, D. E., and Teske, A., 1996. Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulfur isotope studies. *Nature*, **328**, 127.
- Clark, I. D., and Fritz, P., 1997. *Environmental Isotopes in Hydrogeology*. Boca Raton: Lewis Publishers.
- Crutzen, P. J., 2006. Albedo enhancement by stratospheric sulfur injections: a contribution to resolve a policy dilemma? *Climate Change*, **77**, 211.
- Farquhar, J., Bao, H., and Thiemens, M., 2000. Atmospheric influence of Earth's earliest sulphur cycle. *Science*, **4**, 756.
- Finster, K., 2008. Microbiological disproportionation of inorganic sulfur compounds. *Journal of Sulfur Chemistry*, **29**, 281.
- Garrels, R. M., and Lerman, A., 1984. Coupling of the sedimentary sulfur and carbon cycles – an improved model. *American Journal of Sciences*, **284**, 989.
- Holmer, M., and Storkholm, P., 2001. Sulfate reduction and sulphur cycling in lake sediments: a review. *Freshwater Biology*, **46**, 431.
- Jørgensen, B. B., and D'Hondt, S., 2006. A starving majority deep beneath the seafloor. *Science*, **314**, 932.
- Jørgensen, B. B., and Kasten, S., 2006. Sulfur cycling and methane oxidation. In Schulz, H. D., and Zabel, M. (eds.), *Marine Geochemistry*. Berlin: Springer, p. 271.
- Jørgensen, B. B., and Nelson, D. C., 2004. Sulfide oxidation in marine sediments: geochemistry meets microbiology. In Ahmend, J. P., Edwards, K. J., and Lyons, T. W. (eds.), *Sulfur Biogeochemistry – Past and Present*. Geological Society of America Special Paper, p. 379.
- Mitchell, M. J., David, M. B., and Harrison, R. B., 1992. Sulphur dynamics of forest ecosystems. In Howarth, R. W., Stewart, J. W. B., and Ivanov, M. V. (eds.), *Sulphur Cycling on the Continents: Wetlands, Terrestrial Ecosystems and Associated Water Bodies*. John Wiley & Sons, New York, p. 215.
- Niles, D. W., 1872. *Goethe's Elective Affinities (with an introduction by Victoria C. Woodhall)*. Boston: D. W. Niles, p. 41.
- Ohmoto, H., 1992. Biogeochemistry of sulfur and the mechanisms of sulfide-sulfate mineralization in Archean oceans. In Schidlowski, M., Golubic, S., Kimberley, M. M., McKirdy, D. M., and Trudinger, P. A. (eds.), *Early Organic Evolution: Implications for Mineral & Energy Resources*, Berlin: Springer, p. 378.

- Rabus, R., Hansen, T., and Widdel, F., 2001. Dissimilatory sulfate- and sulfur-reducing prokaryotes. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E. (eds.) *The Prokaryotes: An evolving electronic resource for the microbiological community*. Heidelberg, Springer Science Online. Available from: www.prokaryotes.com.
- Rickard, D., and Luther III, G. W., 2007. Chemistry of iron sulfides. *Chemical Reviews*, **107**, 514.
- Rickard, D., and Morse, J. W., 2005. Acid volatile sulfide (AVS). *Marine Chemistry*, **97**, 141.
- Sinninghe Damsté, J. S., and de Leeuw, J. W., 1990. Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. *Organic Geochemistry*, **16**, 1077.
- Skyring, G. W., 1987. Sulfate reduction in coastal ecosystems. *Geomicrobiology Journal*, **5**, 295.
- Smith, J. W., 2000. Isotopic fractionations accompanying sulfur hydrolysis. *Geochemical Journal*, **34**, 95.
- Wortmann, U. G., Chernyavsky, B., Bernasconi, S. M., Brunner, B., Böttcher, M. E., and Swart, P. K., 2007. Oxygen isotope biogeochemistry of pore water sulfate in the deep biosphere: dominance of isotope exchange reactions with ambient water during microbial sulfate reduction (ODP Site 1130). *Geochimica et Cosmochimica Acta*, **71**, 4221.

Cross-references

[Anaerobic Transformation Processes, Microbiology](#)
[Biogeochemical Cycles](#)
[Sulfur Isotopes](#)

SULFUR ISOTOPES

Michael E. Böttcher
 Leibniz Institute for Baltic Sea Research, Warnemünde,
 Germany

Synonyms

Radioactive sulfur isotopes; Stable sulfur isotopes

Definition

Sulfur. A chemical element that is one of the constituents of living organisms and of the Earth.

Isotopes. Isotopes are different nuclear forms of the same element. For a given element, a constant number of protons but different numbers of neutrons in the nucleus correspond to different isotopes. Sulfur of standard atomic mass 32.065 u has 18 isotopes, most of which are not stable and undergo radioactive decay.

Stable sulfur isotopes. Stable isotopes are isotopes that do not decay within an experimentally observable time frame. Stable isotopes have essentially the same chemical characteristics and, therefore, their behavior is chemically almost identical. The different masses lead to isotope fractionation in chemical, physical, and especially biological reactions. Four stable isotopes occur in nature: ^{32}S (95.02%), ^{33}S (0.75%), ^{34}S (4.21%), and ^{36}S (0.02%) (Hoefs, 2008). Typically, the ratio of the two most

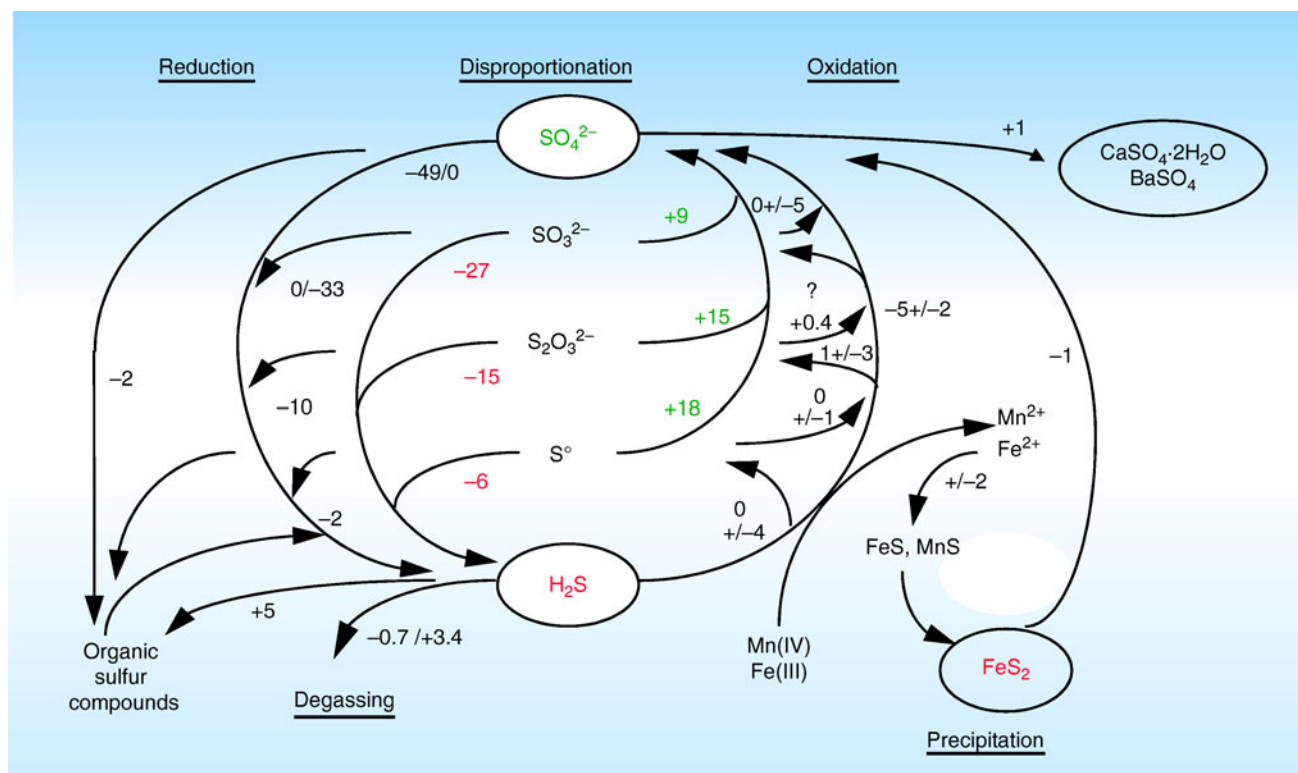
abundant sulfur isotopes is measured and presented in the conventional δ notation as opposed to the Vienna-Canyon-Diablo-Troilite (V-CDT) standard ($\delta^{34}\text{S}$ values per mil versus the V-CDT standard). In aerobic surface environments, the sulfur isotopic composition of sulfate is often used for source identification (e.g., anthropogenic versus geogenic sulfate in rain, river, and ground waters (e.g., Clark and Fritz, 1997). Only recently has the fractionation of the minor isotopes ^{33}S and ^{36}S also been introduced into sulfur isotope biogeochemistry (e.g., Farquhar et al., 2003).

Radioactive sulfur isotopes. Sulfur has 14 unstable isotopes. Radioactive sulfur with mass 35 is formed in the atmosphere from cosmic ray spallation of argon with mass 40 and has a half-life of 87 days. Therefore, it is used in hydrological studies of biologically less active surface environments. ^{35}S -labeled sulfur compounds are used in experiments to determine microbial and chemical sulfur turnover, upon microbial dissimilatory sulfate reduction, for instance (e.g., Kallmeyer et al., 2004). The other radioactive sulfur isotopes are all short-lived.

Fractionation of stable sulfur isotopes

Sulfur isotopes are fractionated upon microbial and abiotic sulfur transformations and the isotope signal may be preserved in sedimentary sulfide- and sulfate-bearing solids, organic matter, and to a minor extent, elemental sulfur (e.g., Canfield, 2001; Amend et al., 2004). The majority of geochemical and microbial studies on sulfur isotopes have so far focused on the fractionation of ^{32}S to ^{34}S . The most important isotope discrimination step occurs during dissimilatory reduction of dissolved sulfate within the bacterial cell. The overall change in the valence state of sulfur by pure cultures leads to hydrogen sulfide that is enriched in the lighter isotope by up to about 50 per mil when compared to sulfate (Figure 1). The magnitude of isotope discrimination depends on conditions such as cellular sulfate reduction rate, substrate and sulfate concentrations, and the organisms (e.g., Kaplan and Rittenberg, 1964; Chambers and Trudinger, 1979; Bolliger et al., 2001; Canfield, 2001; Amend et al., 2004). A maximum fractionation upon sulfate reduction of up to about 70 per mil is obtained from pore water modeling in natural environments (e.g., Wortmann et al., 2001), which is close to the value predicted for thermodynamic equilibrium conditions. Further partial or complete oxidation of hydrogen sulfide is associated with only small isotope discrimination, but may lead to the formation of (metastable) sulfur intermediates. The bacterial disproportionation of these compounds (e.g., elemental sulfur, thiosulfate, sulfite) yields hydrogen sulfide that is further enriched in the lighter isotope compared with the original sulfide (Figure 1).

This may contribute to the magnitude of isotope partitioning found in shallow natural environments and the fossil record (Canfield, 2001).



Sulfur Isotopes, Figure 1 Synthesis of low temperature experimental studies on microbial and abiotic S-34/S-32 fractionations upon sulfur transformations and transport (Amrani and Aizenshtat, 2004; Böttcher and Piel, 2008; Böttcher et al., 1998; Bolliger et al., 2001; Canfield et al., 1998; Chambers and Trudinger, 1979; Cypionka et al., 1998; Fry, 2006; Habicht et al., 1998; Kaplan and Rittenberg, 1964).

Conclusions

Stable sulfur isotope partitioning preserved in the sedimentary record may serve as a proxy for microbial sulfur cycling (e.g., Amend et al., 2004; Canfield, 2001). One of the many open questions within the biogeochemistry of stable sulfur isotopes regards the conditions leading to the sulfur isotope extremes in sediments, which differ from experimental results with modern bacterial cultures. New insights are expected to derive from experiments carried out under boundary conditions that more closely approach natural environments, the consideration of new cultures, and the application of multi-isotope approaches including analysis of the minor sulfur isotopes (e.g., Farquhar et al., 2003) and sulfur-bond oxygen (e.g., Brunner et al., 2005).

Bibliography

- Amend, J. P., Edwards, K. J., and Lyons, T. W. (eds.), 2004. *Sulfur Biogeochemistry - Past and Present*. Boulder: Geological Society of America.
- Amrani, A., and Aizenshtat, Z., 2004. Mechanisms of sulfur introduction chemically controlled: $\delta^{34}\text{S}$ imprint. *Organic Geochemistry*, **35**, 1319.
- Bolliger, C., Schroth, M. H., Bernasconi, S. M., Kleikemper, J., and Zeyer, J., 2001. Sulfur isotope fractionation during microbial

sulfate reduction by toluene-degrading bacteria. *Geochimica et Cosmochimica Acta*, **65**, 3289.

- Böttcher, M. E., and Piel, C., 2008. Kinetic S-34/S-32 fractionation during degassing and hydroxylation of hydrogen sulfide, and protonation of bisulfide. *Geophysical Research Abstracts*, **10**, 232.
- Böttcher, M. E., Smock, A., and Cypionka, H., 1998. Sulfur isotope fractionation during experimental precipitation of iron(II) and manganese(II) sulfide at room temperature. *Chemical Geology*, **146**, 127.
- Brunner, B., Bernasconi, S. M., Kleikemper, J., and Schroth, M. H., 2005. A model for oxygen and sulfur isotope fractionation in sulfate during bacterial sulfate reduction processes. *Geochimica et Cosmochimica Acta*, **69**, 4773.
- Canfield, D. E., 2001. Biogeochemistry of sulfur isotopes. In Valley, J. W., and Cole, D. R. (eds.), *Reviews in Mineralogy and Geochemistry*, The Mineralogical Society of America, 607.
- Canfield, D. E., Thamdrup, B., and Fleischer, S., 1998. Isotope fractionation and sulfur metabolism by pure and enrichment cultures of elemental sulfur disproportionating bacteria. *Limnology and Oceanography*, **43**, 253–264.
- Chambers, L. A., and Trudinger, P. A., 1979. Microbiological fractionation of stable sulfur isotopes: a review and critique. *Geomicrobiology Journal*, **1**, 249.
- Clark, I. D., and Fritz, P., 1997. *Environmental Isotopes in Hydrogeology*. Boca Raton: Lewis.
- Cypionka, H., Smock, A. M., and Böttcher, M. E., 1998. A combined pathway of sulfur compound disproportionation in

- Desulfovibrio desulfuricans*. *FEMS Microbiology Letters*, **166**, 181.
- Farquhar, J., Johnston, D. T., Wing, B. A., Habicht, K. S., Canfield, D. E., Airieau, S., and Thieme, M. H., 2003. Multiple sulphur isotopic interpretations of biosynthetic pathways: implications for biological signatures in the sulphur isotope record. *Geobiology*, **1**, 27.
- Fry, B., 2006. *Stable Isotope Ecology*. Berlin: Springer.
- Habicht, K. S., Canfield, D. E., and Rethmeier, J., 1998. Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite. *Geochimica et Cosmochimica Acta*, **62**, 2585.
- Hoefs, J., 2008. *Stable Isotope Geochemistry*, 6th edn. Berlin: Springer.
- Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., and Jørgensen, B. B., 2004. A cold chromium distillation procedure for radiolabelled sulfide applied to sulfate reduction measurements. *Limnology and Oceanography: Methods*, **2**, 171.
- Kaplan, I. R., and Rittenberg, S. C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology*, **34**, 195.
- Wortmann, U. G., Bernasconi, S. M., and Böttcher, M. E., 2001. Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step bacterial sulfate reduction. *Geology*, **29**, 647.

Cross-references

[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Sulfur Cycle](#)

SYMBIOSIS

Sharmishtha Dattagupta¹, Frank Zielinski²

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany

Definition

A close, prolonged physical and/or metabolic association between two or more distinct organisms.

Introduction

Most living organisms interact intimately with one or more symbiotic partners that are often vital for their health and survival. Symbiotic organisms are important ecosystem engineers (e.g., corals); they significantly impact biogeochemical cycles (e.g., legumes), and they contribute substantially to geological processes such as rock weathering (e.g., lichens and mycorrhizal fungi). Moreover, symbioses have played a crucial role in the origin and diversification of eukaryotic life.

Important terms and concepts

Symbioses are categorized as being mutualisms, where both organisms involved benefit, commensalisms, where

one organism benefits and the other remains unaffected, or parasitisms, where one organism benefits but the other is harmed. When metabolite exchange occurs between symbiotic organisms, it is called syntrophy. Syntrophic interactions among microorganisms can drive otherwise thermodynamically unfavorable reactions forward due to the removal of metabolic end products.

In symbiosis terminology, the smaller symbiotic partner (often a microbe) is referred to as the “symbiont,” whereas the larger partner is called the “host.” In ectosymbioses, the symbiont is located on the external surface of the body of its host, whereas in endosymbioses, the symbiont lives inside the body of its host, either within its cells or in extracellular spaces. Symbioses vary from being *obligate*, where the interaction is critical for the survival of at least one organism involved, or *facultative*, where the organisms involved can also survive independently of each other. The method by which the host acquires its symbionts is called the mode of transmission. In *vertical transmission*, symbionts are inherited from the previous generation, as they are passed from the parent to the egg or embryo. In *horizontal transmission*, symbionts are acquired anew in each generation either from co-occurring hosts or from the environment.

History

Symbiosis is derived from the Greek words, *syn* (together) and *bio* (life). German biologist Anton de Bary is usually credited with coining the term in the mid-nineteenth century, though an equally important contribution was made by his contemporary, Albert Bernhard Frank (who instead used the term *symbiotism*) (Sapp, 2004). De Bary introduced the term to discuss lichens, which he discovered were composites of algae and fungi. Frank, on the other hand, reported on the intimate association between fungi and roots of forest trees, which he named “mykorrhiza” (fungus root). De Bary defined symbiosis as the phenomenon in which “unlike organisms live together” and explicitly included parasitism in his definition (Kutschera and Niklas, 2005). Therefore, the original definition of symbiosis was meant to include the entire gamut of close and long-term associations between two distinct organisms, irrespective of the benefit or harm caused by the interaction. The historical definition of symbiosis was eventually replaced in many modern textbooks with a more restricted definition that included only mutually beneficial associations, making the meaning of symbiosis synonymous with “mutualism” (Wilkinson, 2001). Unfortunately, research on symbioses has been fraught with confusion and controversies, in part due to the two coexisting definitions of the term (Smith, 2001; Sapp, 2004).

Conceptually, the original definition of symbiosis makes more sense in light of the fact that “benefit” is difficult to quantify in studies of symbioses (Smith, 2001). Cost-benefit ratios of symbioses can change with environmental conditions. For example, symbiotic mycorrhizal

fungi benefit their host plants through nutrient uptake, but represent a metabolic cost to the plant under high nutrient conditions (Johnson et al., 1997). Moreover, the molecular mechanisms of communication underlying mutualistic and parasitic interactions are rather similar (Hentschel et al., 2000), and the nature of the interaction can switch during the course of evolution (Sachs and Simms, 2006). Thus, a natural continuum exists among mutualism, commensalism, and parasitism, and arguments about restricting the definition of symbiosis only serve to obscure a true understanding of the phenomenon.

Symbiosis and evolution

Symbioses can induce diverse physiological, morphological, and developmental modifications and thereby strongly influence the evolution of the organisms involved (Sapp, 2004). Microbial symbionts often confer novel metabolic capabilities to their hosts, allowing them to occupy new ecological niches. They can even alter the reproductive behavior of their hosts, and induce speciation through reproductive isolation of host populations (Engelstädter and Hurst, 2009). Conversely, the mode of transmission has a strong impact on symbiont evolution. Many vertically transmitted symbionts have severely reduced genomes, some of them retaining only limited genes corresponding to biosynthetic pathways for nutrients they provide to their host (Moran et al., 2008; McCutcheon, 2010).

Moreover, it is now commonly accepted that the rise of eukaryotes was intimately tied to symbiotic interactions. According to the “endosymbiosis hypothesis” popularized by Lynn Margulis (Margulis, 1970), mitochondria and plastids of present-day eukaryotes evolved from bacterial endosymbionts. Although the nature of the original host cell is still debated, molecular analyses have revealed that present-day mitochondria share a common ancestor with extant alpha-proteobacteria, while chloroplasts evolved from an ancient cyanobacterium (Kutschera and Niklas, 2005).

Symbiosis and its relevance to geobiology

Symbiosis research is inherently interdisciplinary in nature (Sapp, 2004), and in many cases lies at the interface between geology and biology. A few examples are given below to underscore the importance that symbioses hold for geobiologists.

Mineral weathering by symbiotic organisms

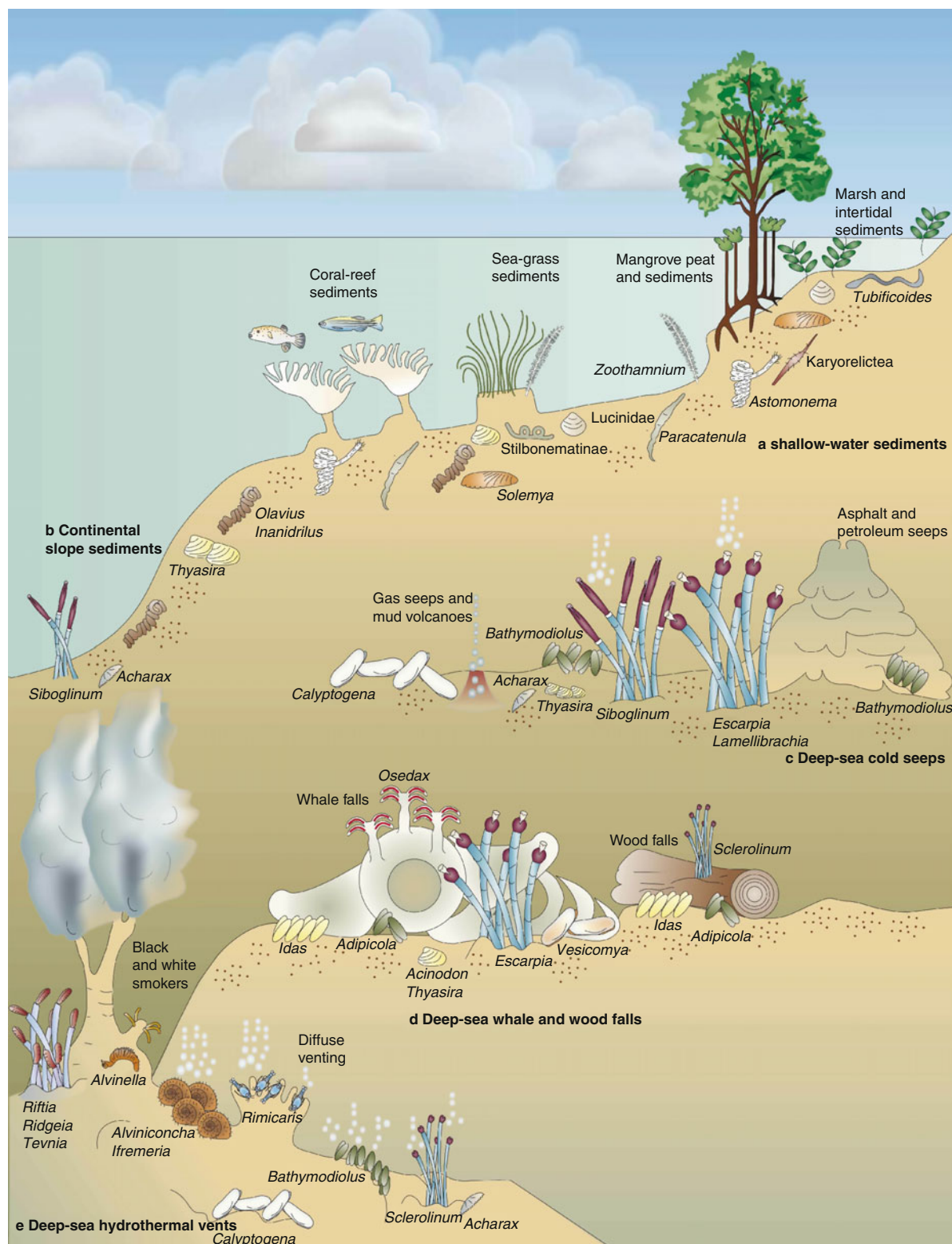
Bare rock surfaces are harsh environments for biological organisms, as they experience intense solar radiation, and strong fluctuations in temperature and humidity. Hence, cooperation rather than competition is favored, and many mutualistic or commensalistic interactions are observed in communities colonizing rock surfaces (Gorbushina, 2007). Perhaps the most well known of rock weathering agents are lichens (Fungi and Lichens). Lichens are usually considered to be symbiotic

associations of fungi and photosynthetic algae and/or cyanobacteria, but in reality they comprise complex communities of several types of microbes (Banfield et al., 1999). Lichens contribute to rock weathering through both physical and chemical processes (Chen et al., 2000). The fungal partners produce hyphae (long, branching filamentous cells) that penetrate minerals, and in combination with freeze-thaw cycles cause mineral breakdown. In addition, lichens dissolve minerals by exuding organic acids, the production of which is enhanced by symbiotic bacteria (Uroz et al., 2009). Lichens have colonized terrestrial habitats since the early Devonian (Jahren et al., 2003), and are widely distributed, occurring even in the southernmost parts of Antarctica (Chen and Blume, 2002). They can colonize and weather the same location on a rock for decades (Uroz et al., 2009), and therefore play a profound role in rock weathering.

In soil environments, mycorrhizal fungi dominate mineral weathering processes. Mycorrhizae (from the Greek *mycos* = fungus, and *rhiza* = root), which are plant-fungal associations, are the most widespread type of terrestrial symbiosis (Smith and Read, 2008). They are broadly of two types: ectomycorrhizal fungi (EMF), in which the fungal partner remains outside the plant cells, and endomycorrhiza (which includes arbuscular mycorrhizal fungi; AMF), in which part of the fungal hyphae penetrates inside the cell. EMF aggressively weather minerals by acidifying the surrounding soil microenvironment, and by exuding organic molecules that chelate metal ions (Taylor et al., 2009). They are referred to as “rock-eating fungi” as they not only dissolve surfaces of minerals but also form tunnels inside mineral grains (van Schöll et al., 2008). On the other hand, AMF indirectly enhance weathering by their plant hosts by supporting increased plant biomass and more extensive rooting systems (Taylor et al., 2009).

The role of symbiotic organisms in biogeochemical cycles

Symbiotic organisms influence biogeochemical cycles of global importance. Methane, an important green house gas, is produced in copious amounts by methanogenic symbionts of ruminants and insects (Thauer et al., 2008; Brune, 2010). In turn, Anaerobic Oxidation of Methane (AOM) in marine sediments, mediated by microbial consortia comprising intimate syntrophic associations between archaea and eubacteria, is a major biological sink of methane (Hoffmeister and Martin, 2003). AOM consortia are responsible for building massive carbonate reefs in present-day anaerobic environments such as the Black Sea and were likely important players in carbon cycling during anaerobic periods of Earth’s history (Michaelis et al., 2002). Carbonate accretion in the ocean is an important part of the global carbon cycle (Ridgwell and Zeebe, 2005), and can be enhanced by reef-building corals that depend on nutrients from their endosymbiotic dinoflagellates to maintain high calcification rates (Hoegh-Guldberg



Symbiosis, Figure 1 A wide variety of animals with chemosynthetic symbioses occur in diverse marine habitats. (From Dubilier et al., 2008, reprinted with permission from Macmillan Publishers Ltd.)

et al., 2007). Carbon cycling in Earth's history was also strongly influenced by mycorrhizal symbioses, which were intimately linked to the advent of land plants over 400 million years ago, an event that caused dramatic declines in atmospheric carbon dioxide levels (Taylor et al., 2009). Mycorrhizal symbioses also impact the global nitrogen cycle, but not as directly as nitrogen-fixing plant symbionts (Jackson et al., 2008; see entry "*Algae (Eukaryotic)*"). Bacteria belonging to the *Rhizobium* genera are often symbionts of leguminous plants, and can convert atmospheric nitrogen into ammonia, a process that has wide impact on the environment and on agriculture (Dixon and Kahn, 2004).

Geochemical influence on distribution of symbiotic organisms

Symbiotic organisms impact geological processes, and conversely, environmental geochemistry can influence the distribution and abundance of symbiotic fauna. Prime examples are chemosynthetic symbioses, which involve chemolithoautotrophic or methanotrophic microbes and invertebrate hosts (reviewed in Stewart et al., 2005; Cavanaugh et al., 2006; Dubilier et al., 2008; Petersen and Dubilier, 2009). Chemosynthetic microbes use chemical energy derived from reduced compounds such as sulfide and methane to fix inorganic carbon. The first case of a chemosynthetic symbiosis involving the giant tubeworm *Riftia pachyptila* was discovered more than 30 years ago at an eastern Pacific hydrothermal vent (see Cavanaugh et al. (2006) for details). Subsequently, chemosynthetic symbioses have been found worldwide in a large variety of marine environments including organic rich coastal sediments, Whale and Wood Falls, mud volcanoes, and Cold Seeps (Figure 1; Dubilier et al., 2008). Chemosynthetic symbioses have independently evolved multiple times, and involve at least seven different animal phyla and nine phylogenetically distinct clades of bacteria. Recently, the first case of a freshwater chemosynthetic symbiosis was discovered in a sulfur-rich limestone cave in central Italy, expanding the diversity of environments where these symbioses are found (Dattagupta et al., 2009). Since chemosynthetic microbes need to oxidize reduced compounds to derive energy, animals with chemosynthetic symbionts invariably need to bridge oxic–anoxic interfaces (Stewart et al., 2005; Cavanaugh et al., 2006). Thus, the biology and ecology of chemosynthetic symbioses are strongly interrelated with environmental geochemistry.

Summary

Symbiosis structures and influences many aspects of life on Earth. It is an important driving force in evolution, and it impacts not only the biology and ecology of organisms but also the biogeochemical processes of global importance.

Bibliography

Banfield, J. F., Barker, W. W., Welch, S. A., and Taunton, A., 1999. Biological impact on mineral dissolution: application of the

lichen model to understanding mineral weathering in the rhizosphere. *Proceedings of the National Academy of Sciences, USA*, **96**, 3404–3411.

- Brune, A., 2010. Methanogenesis in the digestive tracts of insects. In Timmis, K. N., (ed.), *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin, Heidelberg: Springer-Verlag, pp. 707–728.
- Cavanaugh, C. M., McKiness, Z. P., Newton, I. L. G., and Stewart, F. J., 2006. Marine chemosynthetic symbioses. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., and Stackebrandt, E. (eds.), *The Prokaryotes*, 3rd edn. Berlin, Heidelberg: Springer-Verlag, pp. 473–507.
- Chen, J., and Blume, H.-P., 2002. Rock-weathering by lichens in Antarctic: patterns and mechanisms. *Journal of Geographical Sciences*, **12**, 387–396.
- Chen, J., Blume, H.-P., and Beyer, L., 2000. Weathering of rocks induced by lichen colonization: a review. *Catena*, **39**, 121–146.
- Dattagupta, S., Schaperdorth, I., Montanari, A., Mariani, S., Kita, N., Valley, J. W., and Macalady, J. L., 2009. A novel symbiosis between chemolithoautotrophic bacteria and a freshwater cave amphipod. *The ISME Journal*, **3**, 935–943.
- Dixon, R., and Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology*, **2**, 621–631.
- Dubilier, N., Bergin, C., and Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology*, **6**, 725–740.
- Engelstädter, J., and Hurst, G. D. D., 2009. The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 127–149.
- Gorbushina, A. A., 2007. Life on the rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Hentschel, U., Steinert, M., and Hacker, J., 2000. Common molecular mechanisms of symbiosis and pathogenesis. *Trends in Microbiology*, **8**, 226–231.
- Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., et al., 2007. Coral reefs under rapid climate change and ocean acidification. *Science*, **318**, 1737–1742.
- Hoffmeister, M., and Martin, W., 2003. Interspecific evolution: microbial symbiosis, endosymbiosis and gene transfer. *Environmental Microbiology*, **5**, 641–649.
- Jackson, L. E., Burger, M., and Cavagnaro, T. R., 2008. Roots, nitrogen transformations, and ecosystem services. *Annual Review of Plant Biology*, **59**, 341–363.
- Jahren, A. H., Porter, S., and Kuglitsch, J. J., 2003. Lichen metabolism identified in early Devonian terrestrial organisms. *Geology*, **31**, 99–102.
- Johnson, N. C., Graham, J. H., and Smith, F. A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–586.
- Kutschera, U., and Niklas, K. J., 2005. Endosymbiosis, cell evolution, and speciation. *Theory in Biosciences*, **124**, 1–24.
- Margulis, L., 1970. *Origin of Eukaryotic Cells*. New Haven: Yale University Press.
- McCutcheon, J. P., 2010. The bacterial essence of tiny symbiont genomes. *Current Opinion in Microbiology*, **13**, 73–78.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., et al., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Moran, N. A., McCutcheon, J. P., and Nakabachi, A., 2008. Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics*, **42**, 165–190.
- Petersen, J. M., and Dubilier, N., 2009. Methanotrophic symbioses in marine invertebrates. *Environmental Microbiology Reports*, **1**, 319–335.

- Ridgwell, A., and Zeebe, R. E., 2005. The role of the global carbon cycle in the regulation and evolution of the Earth system. *Earth and Planetary Science Letters*, **234**, 299–315.
- Sachs, J. L., and Simms, E. L., 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution*, **21**, 585–592.
- Sapp, J., 2004. The dynamics of symbiosis: an historical overview. *Canadian Journal of Botany*, **82**, 1046–1056.
- Smith, D. C., 2001. Symbiosis research at the end of the millennium. *Hydrobiologia*, **461**, 49–54.
- Smith, S. E., and Read, D. J., 2008. *Mycorrhizal Symbiosis*, 3rd edn. London: Academic Press.
- Stewart, F. J., Newton, I. L. G., and Cavanaugh, C. M., 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. *Trends in Microbiology*, **13**, 439–448.
- Taylor, L. L., Leake, J. R., Quirk, J., Hardy, K., Banwart, S. A., and Beerling, D. J., 2009. Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology*, **7**, 171–191.
- Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W., and Hedderich, R., 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology*, **6**, 579–591.
- Uroz, S., Calvaruso, C., Turpault, M.-P., and Frey-Klett, P., 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology*, **17**, 378–387.
- van Schöll, L., Kuyper, T. W., Smits, M. M., Landeweert, R., Hoffland, E. and van Breemen, N., 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil*, **303**, 35–47.
- Wilkinson, D. M., 2001. At cross purposes – how do we cope with scientific terms that have two different definitions? *Nature*, **412**, 485 p.

Cross-references

[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Dinoflagellates](#)
[Fungi and Lichens](#)
[Hydrothermal Environments](#)
[Methanogens](#)
[Reefs](#)
[Whale and Wood Falls](#)

SYNTROPHY

Syntrophy is a symbiotic association, where metabolite exchange occurs between the organisms involved. See entry “[Symbiosis](#)” for further reading.

- Desulfovibrio desulfuricans*. *FEMS Microbiology Letters*, **166**, 181.
- Farquhar, J., Johnston, D. T., Wing, B. A., Habicht, K. S., Canfield, D. E., Airieau, S., and Thiemens, M. H., 2003. Multiple sulphur isotopic interpretations of biosynthetic pathways: implications for biological signatures in the sulphur isotope record. *Geobiology*, **1**, 27.
- Fry, B., 2006. *Stable Isotope Ecology*. Berlin: Springer.
- Habicht, K. S., Canfield, D. E., and Rethmeier, J., 1998. Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite. *Geochimica et Cosmochimica Acta*, **62**, 2585.
- Hoefs, J., 2008. *Stable Isotope Geochemistry*, 6th edn. Berlin: Springer.
- Kallmeyer, J., Ferdelman, T. G., Weber, A., Fossing, H., and Jørgensen, B. B., 2004. A cold chromium distillation procedure for radiolabelled sulfide applied to sulfate reduction measurements. *Limnology and Oceanography: Methods*, **2**, 171.
- Kaplan, I. R., and Rittenberg, S. C., 1964. Microbiological fractionation of sulphur isotopes. *Journal of General Microbiology*, **34**, 195.
- Wortmann, U. G., Bernasconi, S. M., and Böttcher, M. E., 2001. Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step bacterial sulfate reduction. *Geology*, **29**, 647.

Cross-references

[Iron Sulfide Formation](#)
[Isotopes and Geobiology](#)
[Manganese \(Sedimentary Carbonates and Sulfides\)](#)
[Sulfur Cycle](#)

SYMBIOSIS

Sharmishtha Dattagupta¹, Frank Zielinski²

¹University of Göttingen, Göttingen, Germany

²Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany

Definition

A close, prolonged physical and/or metabolic association between two or more distinct organisms.

Introduction

Most living organisms interact intimately with one or more symbiotic partners that are often vital for their health and survival. Symbiotic organisms are important ecosystem engineers (e.g., corals); they significantly impact biogeochemical cycles (e.g., legumes), and they contribute substantially to geological processes such as rock weathering (e.g., lichens and mycorrhizal fungi). Moreover, symbioses have played a crucial role in the origin and diversification of eukaryotic life.

Important terms and concepts

Symbioses are categorized as being mutualisms, where both organisms involved benefit, commensalisms, where

one organism benefits and the other remains unaffected, or parasitisms, where one organism benefits but the other is harmed. When metabolite exchange occurs between symbiotic organisms, it is called syntrophy. Syntrophic interactions among microorganisms can drive otherwise thermodynamically unfavorable reactions forward due to the removal of metabolic end products.

In symbiosis terminology, the smaller symbiotic partner (often a microbe) is referred to as the “symbiont,” whereas the larger partner is called the “host.” In ectosymbioses, the symbiont is located on the external surface of the body of its host, whereas in endosymbioses, the symbiont lives inside the body of its host, either within its cells or in extracellular spaces. Symbioses vary from being *obligate*, where the interaction is critical for the survival of at least one organism involved, or *facultative*, where the organisms involved can also survive independently of each other. The method by which the host acquires its symbionts is called the mode of transmission. In *vertical transmission*, symbionts are inherited from the previous generation, as they are passed from the parent to the egg or embryo. In *horizontal transmission*, symbionts are acquired anew in each generation either from co-occurring hosts or from the environment.

History

Symbiosis is derived from the Greek words, *syn* (together) and *bio* (life). German biologist Anton de Bary is usually credited with coining the term in the mid-nineteenth century, though an equally important contribution was made by his contemporary, Albert Bernhard Frank (who instead used the term *symbiotism*) (Sapp, 2004). De Bary introduced the term to discuss lichens, which he discovered were composites of algae and fungi. Frank, on the other hand, reported on the intimate association between fungi and roots of forest trees, which he named “mykorrhiza” (fungus root). De Bary defined symbiosis as the phenomenon in which “unlike organisms live together” and explicitly included parasitism in his definition (Kutschera and Niklas, 2005). Therefore, the original definition of symbiosis was meant to include the entire gamut of close and long-term associations between two distinct organisms, irrespective of the benefit or harm caused by the interaction. The historical definition of symbiosis was eventually replaced in many modern textbooks with a more restricted definition that included only mutually beneficial associations, making the meaning of symbiosis synonymous with “mutualism” (Wilkinson, 2001). Unfortunately, research on symbioses has been fraught with confusion and controversies, in part due to the two coexisting definitions of the term (Smith, 2001; Sapp, 2004).

Conceptually, the original definition of symbiosis makes more sense in light of the fact that “benefit” is difficult to quantify in studies of symbioses (Smith, 2001). Cost-benefit ratios of symbioses can change with environmental conditions. For example, symbiotic mycorrhizal

fungi benefit their host plants through nutrient uptake, but represent a metabolic cost to the plant under high nutrient conditions (Johnson et al., 1997). Moreover, the molecular mechanisms of communication underlying mutualistic and parasitic interactions are rather similar (Hentschel et al., 2000), and the nature of the interaction can switch during the course of evolution (Sachs and Simms, 2006). Thus, a natural continuum exists among mutualism, commensalism, and parasitism, and arguments about restricting the definition of symbiosis only serve to obscure a true understanding of the phenomenon.

Symbiosis and evolution

Symbioses can induce diverse physiological, morphological, and developmental modifications and thereby strongly influence the evolution of the organisms involved (Sapp, 2004). Microbial symbionts often confer novel metabolic capabilities to their hosts, allowing them to occupy new ecological niches. They can even alter the reproductive behavior of their hosts, and induce speciation through reproductive isolation of host populations (Engelstädter and Hurst, 2009). Conversely, the mode of transmission has a strong impact on symbiont evolution. Many vertically transmitted symbionts have severely reduced genomes, some of them retaining only limited genes corresponding to biosynthetic pathways for nutrients they provide to their host (Moran et al., 2008; McCutcheon, 2010).

Moreover, it is now commonly accepted that the rise of eukaryotes was intimately tied to symbiotic interactions. According to the “endosymbiosis hypothesis” popularized by Lynn Margulis (Margulis, 1970), mitochondria and plastids of present-day eukaryotes evolved from bacterial endosymbionts. Although the nature of the original host cell is still debated, molecular analyses have revealed that present-day mitochondria share a common ancestor with extant alpha-proteobacteria, while chloroplasts evolved from an ancient cyanobacterium (Kutschera and Niklas, 2005).

Symbiosis and its relevance to geobiology

Symbiosis research is inherently interdisciplinary in nature (Sapp, 2004), and in many cases lies at the interface between geology and biology. A few examples are given below to underscore the importance that symbioses hold for geobiologists.

Mineral weathering by symbiotic organisms

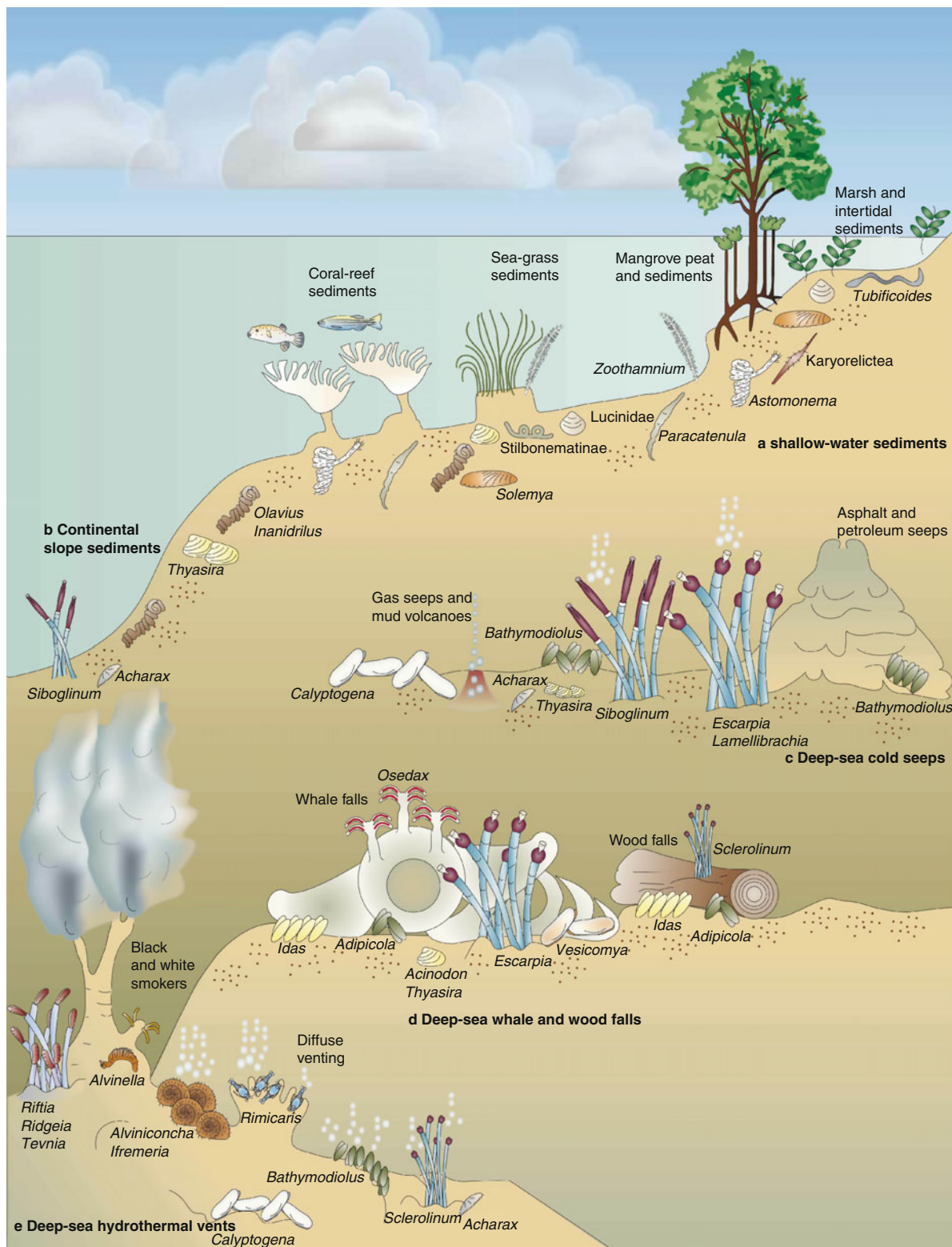
Bare rock surfaces are harsh environments for biological organisms, as they experience intense solar radiation, and strong fluctuations in temperature and humidity. Hence, cooperation rather than competition is favored, and many mutualistic or commensalistic interactions are observed in communities colonizing rock surfaces (Gorbushina, 2007). Perhaps the most well known of rock weathering agents are lichens (Fungi and Lichens). Lichens are usually considered to be symbiotic

associations of fungi and photosynthetic algae and/or cyanobacteria, but in reality they comprise complex communities of several types of microbes (Banfield et al., 1999). Lichens contribute to rock weathering through both physical and chemical processes (Chen et al., 2000). The fungal partners produce hyphae (long, branching filamentous cells) that penetrate minerals, and in combination with freeze-thaw cycles cause mineral breakdown. In addition, lichens dissolve minerals by exuding organic acids, the production of which is enhanced by symbiotic bacteria (Uroz et al., 2009). Lichens have colonized terrestrial habitats since the early Devonian (Jahren et al., 2003), and are widely distributed, occurring even in the southernmost parts of Antarctica (Chen and Blume, 2002). They can colonize and weather the same location on a rock for decades (Uroz et al., 2009), and therefore play a profound role in rock weathering.

In soil environments, mycorrhizal fungi dominate mineral weathering processes. Mycorrhizae (from the Greek *mycos* = fungus, and *rhiza* = root), which are plant-fungal associations, are the most widespread type of terrestrial symbiosis (Smith and Read, 2008). They are broadly of two types: ectomycorrhizal fungi (EMF), in which the fungal partner remains outside the plant cells, and endomycorrhiza (which includes arbuscular mycorrhizal fungi; AMF), in which part of the fungal hyphae penetrates inside the cell. EMF aggressively weather minerals by acidifying the surrounding soil microenvironment, and by exuding organic molecules that chelate metal ions (Taylor et al., 2009). They are referred to as “rock-eating fungi” as they not only dissolve surfaces of minerals but also form tunnels inside mineral grains (van Schöll et al., 2008). On the other hand, AMF indirectly enhance weathering by their plant hosts by supporting increased plant biomass and more extensive rooting systems (Taylor et al., 2009).

The role of symbiotic organisms in biogeochemical cycles

Symbiotic organisms influence biogeochemical cycles of global importance. Methane, an important green house gas, is produced in copious amounts by methanogenic symbionts of ruminants and insects (Thauer et al., 2008; Brune, 2010). In turn, Anaerobic Oxidation of Methane (AOM) in marine sediments, mediated by microbial consortia comprising intimate syntrophic associations between archaea and eubacteria, is a major biological sink of methane (Hoffmeister and Martin, 2003). AOM consortia are responsible for building massive carbonate reefs in present-day anaerobic environments such as the Black Sea and were likely important players in carbon cycling during anaerobic periods of Earth’s history (Michaelis et al., 2002). Carbonate accretion in the ocean is an important part of the global carbon cycle (Ridgwell and Zeebe, 2005), and can be enhanced by reef-building corals that depend on nutrients from their endosymbiotic dinoflagellates to maintain high calcification rates (Hoegh-Guldberg



Symbiosis, Figure 1 A wide variety of animals with chemosynthetic symbioses occur in diverse marine habitats. (From Dubilier et al., 2008, reprinted with permission from Macmillan Publishers Ltd.)

et al., 2007). Carbon cycling in Earth's history was also strongly influenced by mycorrhizal symbioses, which were intimately linked to the advent of land plants over 400 million years ago, an event that caused dramatic declines in atmospheric carbon dioxide levels (Taylor et al., 2009). Mycorrhizal symbioses also impact the global nitrogen cycle, but not as directly as nitrogen-fixing plant symbionts (Jackson et al., 2008; see entry "*Algae (Eukaryotic)*"). Bacteria belonging to the *Rhizobium* genera are often symbionts of leguminous plants, and can convert atmospheric nitrogen into ammonia, a process that has wide impact on the environment and on agriculture (Dixon and Kahn, 2004).

Geochemical influence on distribution of symbiotic organisms

Symbiotic organisms impact geological processes, and conversely, environmental geochemistry can influence the distribution and abundance of symbiotic fauna. Prime examples are chemosynthetic symbioses, which involve chemolithoautotrophic or methanotrophic microbes and invertebrate hosts (reviewed in Stewart et al., 2005; Cavanaugh et al., 2006; Dubilier et al., 2008; Petersen and Dubilier, 2009). Chemosynthetic microbes use chemical energy derived from reduced compounds such as sulfide and methane to fix inorganic carbon. The first case of a chemosynthetic symbiosis involving the giant tubeworm *Riftia pachyptila* was discovered more than 30 years ago at an eastern Pacific hydrothermal vent (see Cavanaugh et al. (2006) for details). Subsequently, chemosynthetic symbioses have been found worldwide in a large variety of marine environments including organic rich coastal sediments, Whale and Wood Falls, mud volcanoes, and Cold Seeps (Figure 1; Dubilier et al., 2008). Chemosynthetic symbioses have independently evolved multiple times, and involve at least seven different animal phyla and nine phylogenetically distinct clades of bacteria. Recently, the first case of a freshwater chemosynthetic symbiosis was discovered in a sulfur-rich limestone cave in central Italy, expanding the diversity of environments where these symbioses are found (Dattagupta et al., 2009). Since chemosynthetic microbes need to oxidize reduced compounds to derive energy, animals with chemosynthetic symbionts invariably need to bridge oxic–anoxic interfaces (Stewart et al., 2005; Cavanaugh et al., 2006). Thus, the biology and ecology of chemosynthetic symbioses are strongly interrelated with environmental geochemistry.

Summary

Symbiosis structures and influences many aspects of life on Earth. It is an important driving force in evolution, and it impacts not only the biology and ecology of organisms but also the biogeochemical processes of global importance.

Bibliography

Banfield, J. F., Barker, W. W., Welch, S. A., and Taunton, A., 1999. Biological impact on mineral dissolution: application of the

lichen model to understanding mineral weathering in the rhizosphere. *Proceedings of the National Academy of Sciences, USA*, **96**, 3404–3411.

- Brune, A., 2010. Methanogenesis in the digestive tracts of insects. In Timmis, K. N., (ed.), *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin, Heidelberg: Springer-Verlag, pp. 707–728.
- Cavanaugh, C. M., McKiness, Z. P., Newton, I. L. G., and Stewart, F. J., 2006. Marine chemosynthetic symbioses. In Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K. H., and Stackebrandt, E. (eds.), *The Prokaryotes*, 3rd edn. Berlin, Heidelberg: Springer-Verlag, pp. 473–507.
- Chen, J., and Blume, H.-P., 2002. Rock-weathering by lichens in Antarctic: patterns and mechanisms. *Journal of Geographical Sciences*, **12**, 387–396.
- Chen, J., Blume, H.-P., and Beyer, L., 2000. Weathering of rocks induced by lichen colonization: a review. *Catena*, **39**, 121–146.
- Dattagupta, S., Schaperdorth, I., Montanari, A., Mariani, S., Kita, N., Valley, J. W., and Macalady, J. L., 2009. A novel symbiosis between chemolithoautotrophic bacteria and a freshwater cave amphipod. *The ISME Journal*, **3**, 935–943.
- Dixon, R., and Kahn, D., 2004. Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology*, **2**, 621–631.
- Dubilier, N., Bergin, C., and Lott, C., 2008. Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. *Nature Reviews Microbiology*, **6**, 725–740.
- Engelstädter, J., and Hurst, G. D. D., 2009. The ecology and evolution of microbes that manipulate host reproduction. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 127–149.
- Gorbushina, A. A., 2007. Life on the rocks. *Environmental Microbiology*, **9**, 1613–1631.
- Hentschel, U., Steinert, M., and Hacker, J., 2000. Common molecular mechanisms of symbiosis and pathogenesis. *Trends in Microbiology*, **8**, 226–231.
- Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., et al., 2007. Coral reefs under rapid climate change and ocean acidification. *Science*, **318**, 1737–1742.
- Hoffmeister, M., and Martin, W., 2003. Interspecific evolution: microbial symbiosis, endosymbiosis and gene transfer. *Environmental Microbiology*, **5**, 641–649.
- Jackson, L. E., Burger, M., and Cavagnaro, T. R., 2008. Roots, nitrogen transformations, and ecosystem services. *Annual Review of Plant Biology*, **59**, 341–363.
- Jahren, A. H., Porter, S., and Kuglitsch, J. J., 2003. Lichen metabolism identified in early Devonian terrestrial organisms. *Geology*, **31**, 99–102.
- Johnson, N. C., Graham, J. H., and Smith, F. A., 1997. Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytologist*, **135**, 575–586.
- Kutschera, U., and Niklas, K. J., 2005. Endosymbiosis, cell evolution, and speciation. *Theory in Biosciences*, **124**, 1–24.
- Margulis, L., 1970. *Origin of Eukaryotic Cells*. New Haven: Yale University Press.
- McCutcheon, J. P., 2010. The bacterial essence of tiny symbiont genomes. *Current Opinion in Microbiology*, **13**, 73–78.
- Michaelis, W., Seifert, R., Nauhaus, K., Treude, T., Thiel, V., Blumenberg, M., et al., 2002. Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane. *Science*, **297**, 1013–1015.
- Moran, N. A., McCutcheon, J. P., and Nakabachi, A., 2008. Genomics and evolution of heritable bacterial symbionts. *Annual Review of Genetics*, **42**, 165–190.
- Petersen, J. M., and Dubilier, N., 2009. Methanotrophic symbioses in marine invertebrates. *Environmental Microbiology Reports*, **1**, 319–335.

- Ridgwell, A., and Zeebe, R. E., 2005. The role of the global carbon cycle in the regulation and evolution of the Earth system. *Earth and Planetary Science Letters*, **234**, 299–315.
- Sachs, J. L., and Simms, E. L., 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution*, **21**, 585–592.
- Sapp, J., 2004. The dynamics of symbiosis: an historical overview. *Canadian Journal of Botany*, **82**, 1046–1056.
- Smith, D. C., 2001. Symbiosis research at the end of the millennium. *Hydrobiologia*, **461**, 49–54.
- Smith, S. E., and Read, D. J., 2008. *Mycorrhizal Symbiosis*, 3rd edn. London: Academic Press.
- Stewart, F. J., Newton, I. L. G., and Cavanaugh, C. M., 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. *Trends in Microbiology*, **13**, 439–448.
- Taylor, L. L., Leake, J. R., Quirk, J., Hardy, K., Banwart, S. A., and Beerling, D. J., 2009. Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology*, **7**, 171–191.
- Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W., and Hedderich, R., 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology*, **6**, 579–591.
- Uroz, S., Calvaruso, C., Turpault, M.-P., and Frey-Klett, P., 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology*, **17**, 378–387.
- van Schöll, L., Kuyper, T. W., Smits, M. M., Landeweert, R., Hoffland, E. and van Breemen, N., 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil*, **303**, 35–47.
- Wilkinson, D. M., 2001. At cross purposes – how do we cope with scientific terms that have two different definitions? *Nature*, **412**, 485 p.

Cross-references

[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Dinoflagellates](#)
[Fungi and Lichens](#)
[Hydrothermal Environments](#)
[Methanogens](#)
[Reefs](#)
[Whale and Wood Falls](#)

SYNTROPHY

Syntrophy is a symbiotic association, where metabolite exchange occurs between the organisms involved. See entry “[Symbiosis](#)” for further reading.

DINOFLAGELLATES

Dinoflagellates are a highly diverse group of flagellated protists consisting of both photosynthetic and non-photosynthetic (heterotrophic) taxa in equal proportions (Taylor et al., 2008). Dinoflagellates are an important component of the modern plankton and have been common to abundant in marine and freshwater environments since the Mesozoic. More than 2,000 extant and 2,500 fossil species have been described. Being mostly biflagellated, swimming unicells of typically 10–100 μm in diameter, they can position themselves in the water column and take full advantage of the available light and nutrients. Many genera are indicative of (paleo-) environmental conditions such as water salinity, nutrient levels, and oceanic temperature zones. In modern and ancient sediments, dinoflagellate-derived organic matter contributions can be specified by distinctive steroid biomarkers (dinosterane and related compounds, see entry “*Biomarkers (Molecular Fossils)*”). Some dinoflagellates, called zooxanthellae, are capable of forming symbioses with a phylogenetically wide range of marine protists and invertebrate animals. For further information, see entries “*Algae (Eukaryotic)*” and “*Protozoa (Heterotroph, Eukaryotic)*.”

Bibliography

Taylor, F. J. R., Hoppenrath, M., and Saldarriaga, J. F., 2008. Dinoflagellate diversity and distribution. *Biodiversity and Conservation*, 17, 407–418.

DIVALENT EARTH ALKALINE CATIONS IN SEAWATER

Anton Eisenhauer
IFM-GEOMAR, Christian Albrechts Universität zu Kiel,
Kiel, Germany

Synonyms

Divalent cations in seawater

Definition

The most abundant divalent earth alkaline cations in seawater are Mg^{2+} , Ca^{2+} , and Sr^{2+} are simply termed the “divalent cations.” These ions and their dynamic change play a major role for the modern oceans. On long geological time scales dynamic changes of divalent cation concentrations in the oceans influenced the evolution of life and the climate evolution in the past.

Divalent cations in modern ocean water

Magnesium

Magnesium is a chemical element with the symbol *Mg*, the atomic number 12, and an atomic mass of 24.31.

Magnesium is the ninth most abundant element in the universe by mass. It constitutes about 2% of the Earth’s crust by mass, and it is the third most abundant element dissolved in seawater and it is the most abundant divalent cation followed by Ca and Sr in seawater (Brown et al., 1992). Mg ions are essential to all living cells, and is the 11th most abundant element by mass in the human body. In seawater ($S = 35$ psu), Mg is present at approximately 53 mM (mM = millimolar) (Brown et al., 1992). Only the monovalent sodium (Na^+ , 469 mM) is present at higher concentration. Magnesium is about five times more abundant than the third most abundant cation Ca (10 mM). Magnesium concentration is significantly lighter than calcium, so when compared on a weight basis, it is only about 3 times as concentrated (1285 vs. 420 ppm). Magnesium is present in seawater as a free divalent cation with water and other molecules attached to it (Brown et al., 1992; Kastner, 1999). It is estimated that each Mg^{2+} ion has approximately eight water molecules (ligands) tightly bound to it. A small portion (about 10%) of the magnesium is present as a soluble ion pair with sulfate (MgSO_4), and much smaller portions are paired with bicarbonate (MgHCO_3^+), carbonate (MgCO_3), fluoride (MgF^+), borate ($\text{MgB}(\text{OH})_4^+$), and hydroxide (MgOH^+).

While these ion pairs comprise only a small portion of the total magnesium concentration, they influence seawater chemistry to a large extent. In the case of carbonate (CO_3^{2-}), for example, the ion pairing to magnesium stabilizes the carbonate that it is present in far higher concentrations than it would be present in the absence of magnesium. This effect makes seawater a much better buffer in the pH range of 8.0 than it otherwise would be. Without this ion pairing, seawater pH might be significantly higher, and more susceptible to dynamic changes. The average residence time for a magnesium ion in seawater is on the order of several tens of millions of years. That time is substantially longer than that for Ca (~one million years) and aluminum (Al, 100 years), but less than Na (~250 million years). The long seawater residence time of Mg in seawater is a measure of its fairly unreactive chemical behavior indicating that it does get taken out of solution through various biological and chemical processes.

Sources and sinks for magnesium in the oceans

In the present-day ocean, the two most important calciumcarbonate (CaCO_3) polymorphs – low Mg-calcite and aragonite – are the most prevalent carbonates and major sinks for Mg replacing Ca to some extent in the crystal lattice. Mg also precipitates together with Ca directly from the water in some evaporitic settings, as well as by the dissolution and reprecipitation of calcite with magnesium replacing calcium in the crystal structure. Dolomite, a high magnesium type of calciumcarbonate usually does not form in the well-oxygenated modern oceans, although bacterial alteration of seawater is reported to induce dolomite precipitation (Kastner, 1999; McKenzie, 1981) in certain marine anoxic environments.

shortly after the extreme glaciations during the late Neoproterozoic (Hoffman et al., 1998) and was nearly synchronous with the rapid evolutionary pulse that coincides with the Ediacaran and Cambrian radiations. Final assembly of Gondwanaland and a circum-Gondwanaland mountain chain is thought to have provided nutrients to the oceanic realm and also resulted in a large $^{87}\text{Sr}/^{86}\text{Sr}$ spike due to the erosion of the mountains (Squire et al., 2006). Therefore, links between the assembly of the Gondwanaland supercontinent and biological evolution are forwarded by numerous studies (Meert and Lieberman, 2008).

Bibliography

- Collins, A. S., and Pisarevsky, S. A., 2005. Amalgamating eastern Gondwana: the evolution of the circum-Indian orogens. *Earth Science Reviews*, **71**, 229–270.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Fitzsimons, I. C. W., 2000. A review of tectonic events in the East Antarctic Shield, and their implications for three separate collisional orogens. *Journal of African Earth Sciences*, **31**, 3–23.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside out? *Science*, **252**, 1409–1412.
- Hoffman, P. F., Kaufman, A. J., Halverson, G. P., and Schrag, D. P., 1998. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Kelsey, D. E., Wade, D. P., Collins, A. S., Hand, M., Sealing, C. R., and Netting, A., 2008. Discovery of a Neoproterozoic basin in the Prydz Belt in East Antarctica and its implications for Gondwana assembly and ultrahigh temperature metamorphism. *Pre-cambrian Research*, **161**, 355–388.
- Meert, J. G., 2003. A synopsis of events related to the assembly of eastern Gondwana. *Tectonophysics*, **362**, 1–40.
- Meert, J. G., and Lieberman, B. S., 2008. The Neoproterozoic assembly of Gondwana and its relationship to the Ediacaran–Cambrian radiation. *Gondwana Research*, in press.
- Meert, J. G., and Torsvik, T. H., 2003. The making and unmaking of a supercontinent: Rodinia revisited. *Tectonophysics*, **375**, 261–288.
- Meert, J. G., Van der Voo, R., and Ayub, S., 1995. Paleomagnetic investigation of the neoproterozoic Gagwe lavas and Mbozi Complex, Tanzania and the assembly of Gondwana. *Precambrian Research*, **74**, 225–244.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Squire, R. J., Campbell, I. H., Allen, C. M., and Wilson, C. J. L., 2006. Did the Transgondwanan supermountain trigger the explosive radiation of animals on Earth? *Earth and Planetary Science Letters*, **250**, 116–133.
- Stern, R. J., 1994. Arc assembly and continental collision in the Neoproterozoic East Africa Orogen: implications for the consolidation of Gondwanaland. *Annual Reviews of Earth and Planetary Science*, **22**, 319–351.
- Yoshida, M., 2007. Geochronological data evaluation: implications for the Proterozoic tectonics of East Gondwana. *Gondwana Research*, **12**, 228–241.

Cross-references

[Breakup of Rodinia](#)
[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Origins of the Metazoa](#)
[Snowball Earth](#)
[Trace Fossils: Neoproterozoic](#)

GREAT OXYGENATION EVENT (GOE)

The term “Great Oxygenation Event” (GOE, also known as “Great Oxidation Event,” “oxygen catastrophe,” or “oxygen crisis”) describes a critical environmental change in Earth history that resulted from the appearance of diatomic oxygen (O_2), a waste product of oxygenic photosynthesis, in the atmosphere. The GOE occurred around 2.4 billion years (Ga) before present, close to the boundary between the Archean and the Proterozoic (2.5 Ga). For details, please refer to “[Critical Intervals in Earth History](#).”

GREEN ALGAE

Green algae are photosynthetic eukaryotes with simple plastids (derived from primary endosymbiosis) that are mostly microscopic and rarely more than a meter in greatest dimension. The approximate 6,000 extant species show an enormous diversity of growth habit and fine details of their cellular architecture. Green algae occur as unicellular or colonial, microscopic or macroscopic, motile planktonic, as well as benthic attached forms. Some (e.g., Dasycladales) are important carbonate producers in aquatic environments, others live in symbiotic associations with fungi to form lichens in terrestrial settings. Green algae living as endosymbionts inside heterotrophic organisms are a common phenomenon. The order Charales, in which full tissue differentiation occurs, is considered to include the closest relative of higher plants. For details, please refer to entry “[Algae \(Eukaryotic\)](#).”

GUILD

Metabolically related populations, e.g., sulfate-reducing microorganisms. See entry “[Microbial Communities, Structure, and Function](#)” for further reading.

GEYSERITE

A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some hot springs. See entries "[Sinter](#)" and "[Hot Springs and Geysers](#)" for further reading.

GLASS

See entry "[Basalt \(Glass, Endoliths\)](#)."

GLAUCOPHYTES

Glaucoephytes (glaucoecystophytes or glaucoecystids) are colorless freshwater protists containing plastids derived from primary endosymbiosis (see also entry [Symbiosis](#)) that is, they are still surrounded by a peptidoglycane wall, which may be viewed as reminiscent of their cyanobacterial origin. Previously, these primitive plastids were also called "cyanelles." For details, please refer to entry "[Algae \(Eukaryotic\)](#)."

Cross-references

[Algae \(Eukaryotic\)](#)

GOLD

Veit-Enno Hoffmann
University of Göttingen, Göttingen, Germany

Synonyms

Aurum (Latin)

Definition

Precipitation/Bioaccumulation of gold is the precipitation of fine-grained gold colloids by microbial activities.

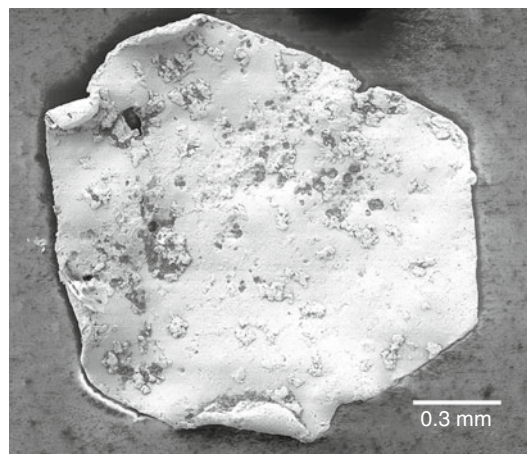
Nugget. A natural gold grain (>5 mm, >200 mg).

The impact of the use of microbial activity for the solution and precipitation of gold was undervalued for a long period, thus affording the opportunity for economic and environmentally friendly gold bio-processing. Furthermore, the current high price of gold has led to intensive research into the precipitation of gold.

Some iron and sulfur oxidizers (e.g., *Acidothiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) contribute to the disintegration of important gold ores (Nordstrom and Southam, 1997). In an environment of gold-bearing rocks, the dissolved gold content is 40 times higher than in comparable non-gold-bearing areas (Benedetti and Boulègue,

1991). It has been shown that the dissolution of gold is dominated by the gold thiosulfate complex $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ which is composed of the above-mentioned bacteria and Actinomycetes (e.g., *Streptomyces fradiae*) (Nordstrom and Southam, 1997; Sand et al., 2001). However, $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ is only stable until the sulfur-oxidation is complete (Lengke and Southam, 2005). Subsequently the gold thiosulfate complex is degraded by *A. thiooxidans*. Thereby gold accumulates in the form of fine-grained colloids inside the bacterial cell and on the surface of the cell membrane. Comparable with the results of Southam and Beveridge (1996) with *Bacillus subtilis* 168, hexagonal-octahedral gold crystals are generated out of the colloids after some months (Figure 1).

As a result of the toxicity of an increasing content of ionized gold, most of the microorganisms that can generate gold die on accumulation of gold. However, various microorganisms, such as bacteria (e.g., *Escherichia coli*, *Pseudomonas maltophilia*, *Plectonema boryanum*), Archaea (e.g., *Pyrococcus furiosus*), Actinomycetes (e.g., *Streptomyces cinamonensis*), fungi (e.g., *Neurospora sitophila*, *Fusarium oxysporum*) and yeasts (e.g., *Endomycopsis fibuligera*, *Sporobolomyces salmonicolor*) from different environments (e.g., hydrothermal, supergene), are partially resistant to the toxicity induced by the accumulation of gold (Kashefi et al., 2001; Nakajima, 2003; Lengke et al., 2006). These bioaccumulation processes are related to various microbial activities (e.g., detoxification, energy generation, passive adsorption). In the laboratory, *Pseudomonas aeruginosa* biofilms demonstrate a higher resistance to the toxicity of gold than planktonic *P. aeruginosa* cells and form solid gold at the base of the biofilms (Karthikeyan and Beveridge, 2002). Reith et al. (2006) isolated a 16SrDNA sequence from natural samples. This sequence was only found on gold grains



Gold, Figure 1 A secondary electron (SE) image of a hexagonal gold crystal from the secondary gold deposit Silberkuhle near Korbach, Germany.

REMINERALIZATION (OF ORGANIC MATTER)

Degradation of organic matter that results in net conversion of organic carbon to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- . See entries "[Carbon \(Organic, Cycling\)](#)" and "[Carbon \(Organic, Degradation\)](#)" for further reading.

RHODOPHYTA

The Rhodophyta, also referred to as "red algae," are a distinct eukaryotic lineage of algae with simple plastids (two membranes, derived from primary endosymbiosis). Rhodophytes have a long geological record since the Mesoproterozoic (1.2 Ga). Today, they encompass about 670 largely marine genera with up to 4,500 species predominating the coastal and continental shelf areas of tropical, temperate, and cold-water regions. Red algae are significant as primary producers and providers of structural habitat for other marine organisms. Calcifying (coralline) red algae play an important role in the primary establishment and maintenance of limestone reefs. For further details, see entry "[Algae \(Eukaryotic\)](#)."

Cross-references

[Algae \(Eukaryotic\)](#)
[Carbonate Environments](#)
[Symbiosis](#)

RNA-WORLD

The term "RNA-World," first suggested by Walter Gilbert in 1986, describes a popular concept on a hypothetical early stage in the origin of life on Earth. Based on discoveries of enzymatic activities in microbial ribonucleic acids (RNA), a system was suggested where primordial RNA molecules accounted for both, the storage of genetic information and catalytic functions necessary to assemble themselves in a primitive self-replicating system. The RNA molecules evolved in self-replicating patterns, using recombination and mutation to explore new functions and to adapt to new niches (Gilbert, 1986). Such a system would not have required the presence of DNA and/or protein enzymes for biochemical reactions. For further reading, please refer to "[Origin of Life](#)."

Bibliography

Gilbert, W., 1986. The RNA world. *Nature*, **319**, 618.

van der Wielen, P. W., Bolhuis, H., Borin, S., Daffonchio, D., Corselli, C., Giuliano, L., D'Auria, G., de Lange, G. J., Huebner, A., Varnavas, S. P., Thomson, J., Tamburini, C., Marty, D., McGenity, T. J., and Timmis, K. N., and BioDeep Scientific Party, 2005. The enigma of prokaryotic life in deep hypersaline anoxic basins. *Science*, **307**, 121–123.

Velieux, F., Madern, D., Zaccai, G., and Ebel, C., 2007. Molecular adaptation to high salt. In Gerday, C., and Glansdorff, N. (eds.), *Physiology and Biochemistry of Extremophiles*. Washington: ASM, pp. 240–253.

Cross-references

Archaea
Astrobiology
Deep Biosphere of Salt Deposits
Extreme Environments
Hypersaline Environments, Terrestrial
Saline Lakes
Soda Lakes

HAPTOPHYTES

Haptophytes (Haptophyta, Prymnesiophyta) are unicellular chlorophyll a and c containing algae with complex plastids derived from secondary endosymbiosis (“red lineage”) (see entry “*Symbiosis*”). They occur principally as solitary free-living motile cells that possess two smooth flagella, unequal in length in the Pavlovophyceae and more or less equal in the Prymnesiophyceae. Haptophytes inhabit littoral, coastal, and oceanic waters and are important primary producers in many aquatic environments. Within the haptophytes, the so-called coccolithophores (coccolithophorids) evolved the ability to control the intracellular calcification onto organic plates and the assembly of the mature calcium carbonate (calcite) scales (coccoliths) at the cell surface. These forms have been significantly contributing to the deposition of calcium carbonate in marine waters since the Mesozoic, today accounting for about a third of the total marine CaCO₃ production. In modern and ancient sediments, haptophyte-derived organic matter contributions can be specified by distinctive lipid biomarkers (alkenones), whose unsaturation patterns also provide a paleo-thermometer for past surface water temperatures (see entry “*Biomarkers (Molecular Fossils)*”). For further details, please see entry “*Algae (Eukaryotic)*.”

HEAVY METALS

Please refer to entries “*Acid Rock Drainage*,” “*Arsenic*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Copper*,” “*Geomycology*,” “*Metalloenzymes*,” “*Metals, Acquisition by Marine Bacteria*,” “*Ores, Microbial Precipitation and Oxidation*,” “*Zinc*.”

HISTOLOGY

Michael Gudo¹, Gerta Fleissner², Guenther Fleissner²

¹Morphisto Evolutionsforschung und Anwendung GmbH, Institut für Evolutionswissenschaften, Frankfurt am Main, Germany

²Goethe-University Frankfurt, Frankfurt a. M., Germany

Definition

Histology is the biological science concerned with the minute or microscopic structures of cells, tissues, and organs of animals, plants, and fungi, in relation to their function, and any organismic constructions produced by living individuals or symbiotic assemblies of living organisms.

Biopsy is the sample of an organism or part of a tissue that is processed for histological investigations.

Fixation serves the inhibition of all vital processes by special chemicals that also preserve and prepare organic matter for further histological or morphological investigations.

Mineralization is the process by which organic components of organisms are impregnated or replaced by inorganic material and converted to mineral matter.

Staining is the chemical reaction of particular laboratory chemicals (stains and dyes) with tissue components and materials of the samples.

Introduction

The term histology was coined by C. Mayer in 1819 as a part of the scientific discipline comparative anatomy (Mayer, 1819). Histological investigations, concerned with the minute structures of cells, cell organelles, intra- and extracellular fiber arrangements, and tissue components and organs in relation to their function, are usually done by light microscopy. Since the invention of the microscope by Leeuwenhoek in 1668 (Palm and Snelders, 1983), the early histological technology (Gerlach, 1998) has experienced major improvement by the refinement of microscopes (Bozzola and Russell, 1999) and new methods of tissue preservation, sectioning devices, stains, and staining methods. Histochemical methods now permit to identify and localize intracellular components and enzymes within distinct and isolated samples (e.g., Bancroft and Gamble, 2007; Böck, 1989; Horobin and Kiernan, 2002; Mulisch and Welsch, 2010). Therefore, the studied structures, tissues, complete organisms, or any organismic compound have to be preserved from enzymatic decay, and then cut into sections of just a few microns thickness. The histological sections can vary between ultra thin sections (40–80 nm), semi thin sections (0.1–3.0 µm), thin sections (3–20 µm), and thick sections (more than 20 µm). While ultra thin sections could be investigated only by transmission or scanning electron microscopy (TEM), the others can be investigated with any light microscope. Afterward, these sections are

Cross-references

Biomining (Mineral Bioleaching, Mineral Biooxidation)
Copper
Extreme Environments
Fe(II)-Oxidizing Prokaryotes
Iron Sulfide Formation
Ores, Microbial Precipitation and Oxidation
Sulfide Mineral Oxidation

ACIDOPHILES

“Acidophiles” are organisms thriving in environments below pH 5. For details, see entries “*Extreme Environments*,” “*Biomining (Mineral Bioleaching, Mineral Biooxidation)*,” “*Hot Springs and Geysers*,” *Hydrothermal Environments (Marine)* and “*Acid Rock Drainage*.”

ACRITARCHS

Acritarchs are organic-walled acid-resistant microfossils known from the Proterozoic and throughout the Phanerozoic. The position of these microfossils is still uncertain. A number of acritarch genera have been assigned to Green Algae; others are considered to bear some resemblance to the cysts of dinoflagellates. For more information, please refer to entries “Algae, Eukaryotic” and “Protozoa.”

AEROBIC METABOLISM

Heribert Cypionka
University of Oldenburg, Oldenburg, Germany

Synonyms

Aerobic respiration; Oxygen metabolism

Definition

Aerobic metabolism comprises the reduction of molecular oxygen as electron acceptor of aerobic respiration, its use as cosubstrate in the degradation of certain compounds, and reactions leading to the detoxification of partially reduced oxygen species.

Introduction

Life has evolved in the absence of molecular oxygen (see Chapter *Early Earth*). Therefore, it is not surprising that basic metabolic pathways involved in growth and cell division (like DNA replication or protein synthesis) do not depend on the presence of molecular O₂. The evolution of the oxygen-producing (oxygenic) phototrophs dramatically changed the situation of living organisms. Molecular oxygen raised the redox potential of the environment and enabled microorganisms to respire with a much higher energy yield than before. Although oxygen is involved in

a limited number of reactions only, its presence or absence has fundamental impact on biogeochemical processes.

Aerobic degradation of organic matter

Aerobic respiration consumes a major part of the photosynthetically produced O₂ and about 90% of the organic matter (see Chapter *Carbon Cycle*). Aerobic (oxygen-metabolizing) organisms normally oxidize their substrates completely to CO₂. In most cases, O₂ is not directly involved in this process. Substrate oxidation generates reduced electron carriers by means of the respiratory chain. Oxygen is reduced as the last step of the respiration process.

Respiratory chain

Respiration is a membrane-bound process that generates a chemiosmotic proton gradient across the membrane (Mitchell, 1966). Respiratory chains are composed of series of enzymes, which are organized in multienzyme complexes and use cofactors that allow the stepwise transport of reducing equivalents to oxygen (Figure 1). In eukaryotes (plants, animals, fungi) it takes place within the mitochondria, which are assumed to have evolved from endosymbiotic bacteria. In prokaryotes (Bacteria, Archaea), the respiratory chain is located in the cytoplasmic membrane. The respiratory chain of the bacterium *Paracoccus denitrificans* appears to have many similarities to the mitochondrial type (Harold, 1986). However, many bacteria have variations compared to the mitochondrial type presented in Figure 1.

Energetics and ATP conservation

Most reducing equivalents are fed into the respiration process via NADH₂, which has a standard redox potential (E_0') of -0.32 V. The terminal electron-accepting redox pair O₂/H₂O has redox potential of $+0.82$ V. Thus, the usable redox potential is about 1.14 V. From this redox potential one can calculate a standard $\Delta G_0'$ of -440 kJ per mol of O₂. Assuming that ATP conservation requires about -75 kJ per mol, the conservation of 5 to 6 ATP per O₂ reduced is possible. As a consequence of the high ATP yield, aerobic organisms can form more biomass from the same amount of substrate than anaerobic organisms.

Use of oxygen as cosubstrate

Molecular oxygen is used as a cosubstrate in some catabolic and anabolic reactions (Lengeler et al., 1999). The enzymes involved are called mono- or di-oxygenases, depending on the number of oxygen atoms transferred to their substrate. Oxygenases are used by aerobes to activate certain substrates that do not have functional groups that allow a simple degradation. They catalyze the initial hydroxylation of hydrocarbons to yield alcohols that can easily be metabolized further. They are also involved in the ring cleavage of aromatic compounds and in the first oxidation step of ammonia oxydation. Anaerobic organisms are either unable to oxidize the substrates activated

C

CALCAREOUS ALGAE

The term “calcareous algae” refers to various kinds of benthic and planktonic algae whose thalli contain biochemically precipitated calcium carbonate (CaCO_3) as skeletal material (Wray, 1977; Braga and Riding, 2005). Precipitation of CaCO_3 (as calcite and/or aragonite) may occur within or on the algal bodies. The term may also include mechanically accreted deposits of calcium carbonate caused by algae, usually as an interaction of biological and physical processes. Calcareous algae are a highly artificial group that constitutes calcifying members of the Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) and is sometimes also used for Cyanobacteria. At present, calcareous algae are one of the most important reef builders (see “[Carbonate Environments](#)”). For a detailed reading, please refer to “[Algae \(Eukaryotic\)](#).”

Bibliography

- Braga, J. C., and Riding, R., 2005. Calcareous algae. In Selley, R., Cocks, L. R. M., and Plimer, I. R. (eds.), *Encyclopedia of Geology*. Amsterdam: Elsevier, pp. 428–436.
- Wray, J. L., 1977. *Calcareous Algae. Developments in Palaeontology and Stratigraphy*. Amsterdam: Elsevier, Vol. 4, 185 pp.

CALCIFICATION

See entries “[Animal Biocalcification, Evolution](#),” “[Biofilms and Fossilization](#),” “[Calcified Cyanobacteria](#),” “[Calcite Precipitation, Microbially Induced](#),” “[Calcium Biogeochemistry](#),” “[Carbonate Environments](#),” “[Dolomite, Microbial](#),” “[Microbialites, Modern](#),” “[Microbialites, Stromatolites, and Thrombolites](#),” and “[Pedogenic Carbonates](#).”

CALCIFIED CYANOBACTERIA

Robert Riding
University of Tennessee, Knoxville, TN, USA

Definition

Cyanobacteria are alga-like bacteria that can perform oxygenic photosynthesis and nitrogen fixation (Whitton and Potts, 2000; Herrero and Flores, 2008). They have a long history and are diverse and widespread in marine, freshwater, and terrestrial environments at the present-day, where they are key primary producers in both microbial mat and planktic ecosystems. As the initiators of plastids, they have played a fundamental role in algal and plant evolution (Raven, 2002). Cyanobacteria occupy benthic substrates and can also drift in the water column. Their photosynthetic uptake of inorganic carbon can stimulate CaCO_3 precipitation. This calcification can produce filamentous microfossils in benthic mats that are preserved as stromatolites and thrombolites, and can also cause water column precipitation of carbonate mud that settles to lake and sea floors.

Introduction

However, cyanobacterial calcification is not obligatory and is directly dependent on environmental conditions. This accounts for apparent discrepancies between the geological ranges of organic-walled and calcified cyanobacterial fossils. Calcified cyanobacteria have not been recognized in marine sediments older than ~1200 Ma ago (Kah and Riding, 2007), whereas there is evidence that cyanobacteria appeared in the late Archaean or Palaeoproterozoic, in the range ~2900–2150 Ma ago (Cavalier-Smith et al., 2006; Hofmann, 1976). This mid-Proterozoic appearance of sheath-calcified cyanobacteria is thought to reflect the development of CO_2 -concentrating

- spicules in the marine demosponge *Suberites domuncula*. *Journal of Biological Chemistry*, **281**(17), 12001–12009.
- Schulze, F. E., 1886. *Über den Bau und das System der Hexactinelliden*. Berlin: Royal Prussian Academy of Sciences, pp. 3–97.
- Schulze, F. E., 1904. Hexactinellida. *Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898–1899*, **4**, 1–266.
- Schwartz, K., 1973. A bound form of silicon in glucosaminoglycans and polyuronides. *Proceedings of National Academy of Sciences of the United States of America*, **70**, 1608–1612.
- Shimizu, K., and Morse, D. E., 2000. The biological and biomimetic synthesis of silica and other polysiloxanes. In Baeuerlein, E. (ed.), *Biomimetalization: From Biology to Biotechnology and Medical Application*. Wiley, Weinheim, pp. 207–219.
- Shimizu, K., Cha, J. N., Stucky, G. D., and Morse, D. E., 1998. Silicatein a: cathepsin L-like protein in sponge biosilica. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6234–6238.
- Simpson, T. L., 1984. *The Cell Biology of Sponges*. New York: Springer, pp. 1–660.
- Simpson, T. L., 1989. Silicification processes in sponges: *Geodia asters* and the problem of morphogenesis of spicule shape. In Crick, R. E. (ed.), *Origin, Evolution and Modern Aspects of Biomimetalization in Plants and Animals*. New York: Kluwer, pp. 125–136.
- Simpson, T. L., 1990. Recent data on pattern of silicification and the origin of monaxons from tetraxons. In Rützler, K. (ed.), *New Perspectives in Sponge Biology. 3rd International Conference on the Biology of Sponges*, 1985. Washington: Smithsonian Institution Press, pp. 264–272.
- Skinner, H. C. W., 2005. Biominerals. *Mineralogical Magazine*, **69**(5), 621–641.
- Sollas, W. J., 1888. Report on Tetractinellida collected by H.M.S. challenger during the years 1873–1876. *Report of the Scientific Results of the Voyage of the H.M.S. Challenger. Zoology*, **5**, 1–458.
- Tabachnick, K. R., 1991. Adaptation of the Hexactinellid sponges to deep-sea life. In Reitner, J., and Keupp, H. (eds.), *Fossil and Recent Sponges*. Berlin: Springer, pp. 378–386.
- Tabachnick, K. R., 2002. Family Monorhaphididae Ijima, 1927. In Hooper, N. A. V., and Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1264–1266.
- Tabachnick, K. R., and Reisswig, H. M., 2002. Dictionary of Hexactinellida. In Hooper, J. N. A., and van Soest, R. W. M. (eds.), *Systema Porifera: A Guide to the Classification of Sponges*. New York: Kluwer, pp. 1224–1229.
- Uriz, M.-J., 2003. Biology of silica deposition of sponges. *Microscopical Research and Technology*, Wiley-Liss. California, **62**(4), 277–381.
- Uriz, M.-J., 2006. Mineral skeletogenesis in sponges. *Canadian Journal of Zoology*, **84**, 322–356.
- Uriz, M.-J., Turon, X., Becero, M. A., and Agell, G., 2003. Siliceous spicules and skeletal frameworks in sponges: origin, diversità, ultrastructural patterns, and biological functions. *Microscopical Research and Technology*, **62**, 279–299.
- Vosmaer, G. C. J., and Wijsman, H. P., 1905. On the structure of some Siliceous Spicules of Sponges. I. The styli of *Tethya lyncurium*. *Koninklijke Nederlandsche Akademie van Wetenschappen Proceedings*, **8**, 15–28.
- Weaver, J. C., and Morse, D. E., 2003. Molecular biology of demosponge axial filaments and their roles in biosilicification. *Microscopical Research and Technology*, **62**(4), 356–367.
- Weaver, J. C., Pietrasanta, I., Hedin, N., Chmelka, B. F., Hansma, P. K., and Morse, D. E., 2003. Nanostructural features of demosponge biosilica. *Journal of Structural Biology*, **144**, 271–281.
- Weiner, S., and Dove, P. M., 2003. An overview of biomineralization processes and the problem of the vital effect. *Reviews in Mineralogy and Geochemistry*, **54**, 1–27.
- Weissenfels, N., 1989. *Biologie und Mikroskopische Anatomie der Süßwasserschwämme (Spongillidae)*. Stuttgart: Fischer.
- Weissenfels, N., and Landschoff, H. W., 1977. Bau und Funktion des Süßwasserschwamms *Ephydatia fluviatilis* L. (Porifera). IV. Die Entwicklung der monaxialen SiO₂-Nadeln in Sandwich-Kulturen, *Zoologische Jahrbücher Abteilung für Anatomie und Ontogenese der Tiere*, **98**, 355–371.

Cross-references

[Biogeochemical Cycles](#)
[Critical Intervals in Earth History](#)
[Deep Biosphere of the Oceanic Deep Sea](#)
[Origins of the Metazoa](#)
[Reefs](#)
[Sponges \(Porifera\) and Sponge Microbes](#)

SILICOFLAGELLATES

Silicoflagellates are planktonic, microscopic algae with siliceous skeletons, characterized by their simple geometries and remarkable variability. Taxonomically, they belong to different classes of the Stramenopiles and possess complex plastids derived from secondary endosymbiosis (see entry [Symbiosis](#)). Silicoflagellate skeletons may have considerable potential as environmental indicators and are common in fossil deposits, dating back to the middle Cretaceous (ca. 120 Ma), with species diversity reaching a peak in the Miocene (ca. 23–25 Ma). Today, only a single genus *Dictyocha* with approximately five recognized species exists. For more details, please refer to entry ["Algae \(Eukaryotic\)"](#).

SINTER

Robin W. Renaut¹, Brian Jones²
¹University of Saskatchewan, Saskatoon, SK, Canada
²University of Alberta, Edmonton, Alberta, Canada

Synonyms

Silica sinter; Siliceous sinter

Definition

Sinter: A sedimentary rock primarily composed of silica that is precipitated from hot waters at the vents of high-temperature (high-enthalpy) hot springs and geysers, and from cooled waters on their surrounding discharge aprons.

- Tsien, H. H., 1985. Algal-bacterial origin of micrites in mud mounds. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 290–296.
- Webb, G. E., 1996. Was Phanerozoic reef history controlled by the distribution of non-enzymatically secreted reef carbonates (microbial carbonate and biologically induced cement)? *Sedimentology*, **43**, 947–971.
- Wilson, J. L., 1975. *Carbonate Facies in Geologic History*. Berlin: Springer, 471 p.

Cross-references

[Bacteria](#)
[Biofilms](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Reefs](#)
[Waulsortian Mud Mounds](#)

MUTUALISM

Mutualism is a form of a symbiosis, in which both organisms involved benefit. See entry “[Symbiosis](#)” for further reading.

MYCORRHIZAE

A mycorrhiza (pl mycorrhizae, from the Greek “*mycos*” = fungus and “*rhiza*” = root) is a plant-fungal association that comprises the most widespread type of terrestrial symbiosis. See entry “[Symbiosis](#)” for further reading.

- Tsien, H. H., 1985. Algal-bacterial origin of micrites in mud mounds. In Toomey, D. F., and Nitecki, M. H. (eds.), *Paleoalgology*. Berlin: Springer, pp. 290–296.
- Webb, G. E., 1996. Was Phanerozoic reef history controlled by the distribution of non-enzymatically secreted reef carbonates (microbial carbonate and biologically induced cement)? *Sedimentology*, **43**, 947–971.
- Wilson, J. L., 1975. *Carbonate Facies in Geologic History*. Berlin: Springer, 471 p.

Cross-references

[Bacteria](#)
[Biofilms](#)
[Calcite Precipitation, Microbially Induced](#)
[Cyanobacteria](#)
[Extracellular Polymeric Substances \(EPS\)](#)
[Microbial Communities, Structure, and Function](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Reefs](#)
[Waulsortian Mud Mounds](#)

MUTUALISM

Mutualism is a form of a symbiosis, in which both organisms involved benefit. See entry “[Symbiosis](#)” for further reading.

MYCORRHIZAE

A mycorrhiza (pl mycorrhizae, from the Greek “*mycos*” = fungus and “*rhiza*” = root) is a plant-fungal association that comprises the most widespread type of terrestrial symbiosis. See entry “[Symbiosis](#)” for further reading.

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of micro-organisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

P

PARASITISM

Parasitism is a form of symbiosis, in which one organism benefits but the other is harmed. See entry “*Symbiosis*” for further reading.

PEDOGENIC CARBONATES

Eric P. Verrecchia
Université de Lausanne, Lausanne, Switzerland

Synonyms

Secondary terrestrial carbonates; Soil carbonates; Vadose zone carbonates

Definition

Definition and mineralogy

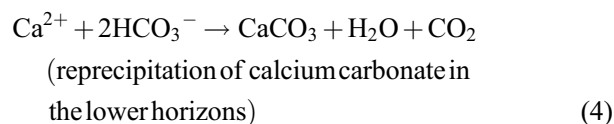
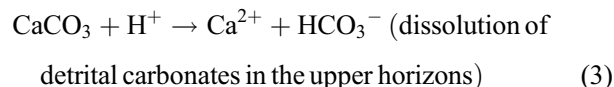
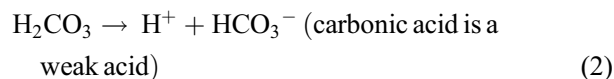
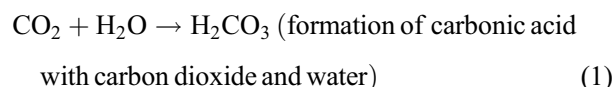
Pedogenic carbonates are authigenic (or secondary) carbonate deposits precipitated in soils (Lal et al., 2000). When found in the soil but directly inherited from parent materials, carbonates are defined as detrital or lithogenic (Kraimer et al., 2005). Pedogenic carbonates are characterized by a wide diversity of shapes, from secondary diffuse micritic (micron-size) crystals inside the soil matrix to coalescent hardened layers, sometimes capping whole landscapes. They are mainly composed of calcium carbonate as calcite and rarely as aragonite (Schaetzl and Anderson, 2005), of magnesian calcite, dolomite (Whipkey et al., 2002), and occasionally of iron-rich carbonate such as ferroan calcite, siderite, and ankerite (Table 1). Iron-rich carbonates are generally found in organic-rich soils and soils under the influence of

water-table fluctuations with active oxidation–reduction processes.

Origin

Carbonate physicochemical equilibria

Various processes are involved in pedogenic carbonate formation. The first significant process is related to the leaching of carbonate through the soil profile. In the upper horizons, inherited calcium carbonate is dissolved by carbon dioxide enriched waters and displaced in solution toward deeper horizons where it precipitates. This precipitation is supposed to occur if the concentration in Ca^{2+} increases, the pCO_2 decreases, or if soil solutions concentrate due to water loss (evaporation). In other words, all these processes are driven by the following general equations when lithogenic (detrital) carbonates are involved:



A second process is related to the transformation, in surficial environments, of primary minerals which can also lead to the precipitation of pedogenic carbonates. For

- Ridgwell, A., and Zeebe, R. E., 2005. The role of the global carbon cycle in the regulation and evolution of the Earth system. *Earth and Planetary Science Letters*, **234**, 299–315.
- Sachs, J. L., and Simms, E. L., 2006. Pathways to mutualism breakdown. *Trends in Ecology and Evolution*, **21**, 585–592.
- Sapp, J., 2004. The dynamics of symbiosis: an historical overview. *Canadian Journal of Botany*, **82**, 1046–1056.
- Smith, D. C., 2001. Symbiosis research at the end of the millennium. *Hydrobiologia*, **461**, 49–54.
- Smith, S. E., and Read, D. J., 2008. *Mycorrhizal Symbiosis*, 3rd edn. London: Academic Press.
- Stewart, F. J., Newton, I. L. G., and Cavanaugh, C. M., 2005. Chemosynthetic endosymbioses: adaptations to oxic-anoxic interfaces. *Trends in Microbiology*, **13**, 439–448.
- Taylor, L. L., Leake, J. R., Quirk, J., Hardy, K., Banwart, S. A., and Beerling, D. J., 2009. Biological weathering and the long-term carbon cycle: integrating mycorrhizal evolution and function into the current paradigm. *Geobiology*, **7**, 171–191.
- Thauer, R. K., Kaster, A.-K., Seedorf, H., Buckel, W., and Hedderich, R., 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology*, **6**, 579–591.
- Uroz, S., Calvaruso, C., Turpault, M.-P., and Frey-Klett, P., 2009. Mineral weathering by bacteria: ecology, actors and mechanisms. *Trends in Microbiology*, **17**, 378–387.
- van Schöll, L., Kuyper, T. W., Smits, M. M., Landeweert, R., Hoffland, E. and van Breemen, N., 2008. Rock-eating mycorrhizas: their role in plant nutrition and biogeochemical cycles. *Plant Soil*, **303**, 35–47.
- Wilkinson, D. M., 2001. At cross purposes – how do we cope with scientific terms that have two different definitions? *Nature*, **412**, 485 p.

Cross-references

[Algae \(Eukaryotic\)](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Biogeochemical Cycles](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Dinoflagellates](#)
[Fungi and Lichens](#)
[Hydrothermal Environments](#)
[Methanogens](#)
[Reefs](#)
[Whale and Wood Falls](#)

SYNTROPHY

Syntrophy is a symbiotic association, where metabolite exchange occurs between the organisms involved. See entry “[Symbiosis](#)” for further reading.

COSMIC MOLECULAR CLOUDS

Joachim Reitner

University of Göttingen, Göttingen, Germany

Synonyms

Complex molecular clouds; Interstellar clouds; Interstellar dust and molecules; Molecular clouds

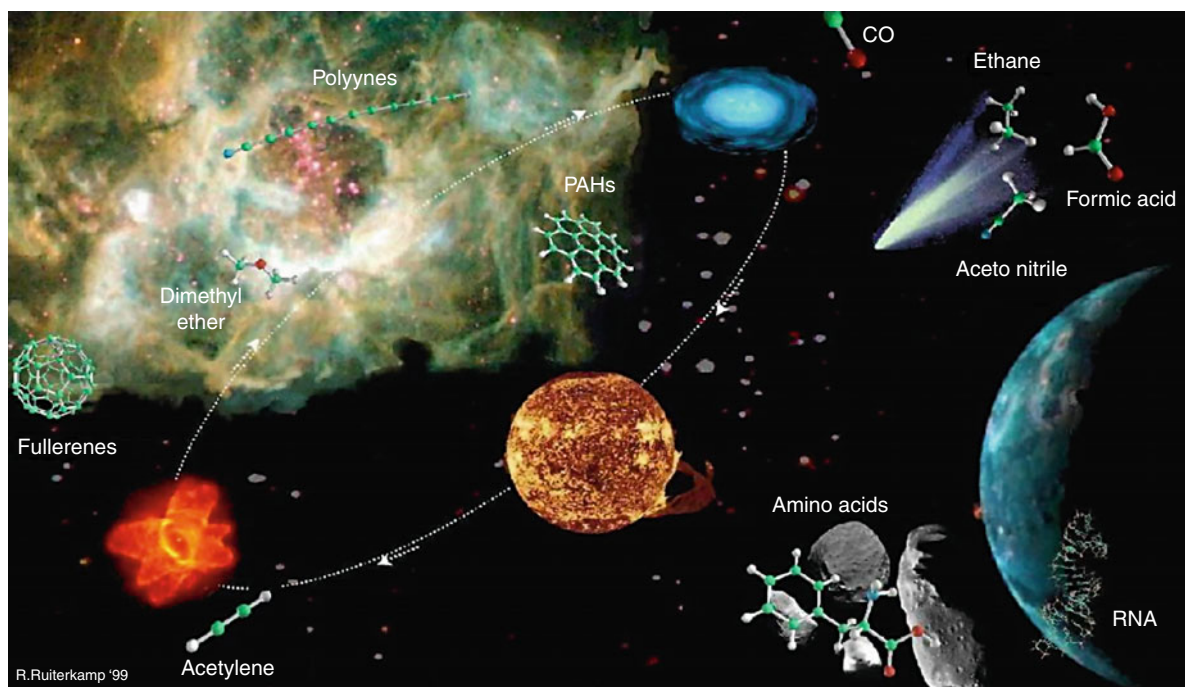
Definition

A cosmic molecular cloud is an accumulation of cosmic matter that is composed of organic and inorganic molecules. The dust grains in a cosmic molecular cloud are mainly composed of crystalline silicates (Watson et al., 2009) covered with ice of H_2O , NH_3 , and CO_2 . The main molecule constituent is H_2 -He, but other molecules also occur, e.g., NH_3 , CO , and complex organic molecules.

Cosmic molecular clouds are denser than the interstellar medium, with gas densities ranging from about 10 molecules per cm^3 on large scales to over 10^6 molecules per cm^3 in cloud cores. These cores are very cold, with temperatures ranging from 8 to 20 K. New stars and protoplanetary discs are formed, when it comes to a gravitational collapse of cosmic molecular clouds. Via nucleosynthesis, these stars produce elements, which may constitute organic molecules (e.g., H, C, N, O, S, P). When

stars burn out, they collapse either via catastrophic explosions or by forming expanding shells, thereby recycling modified material back into the interstellar space and, thus, forming new cosmic molecular clouds (Boss, 2004). Some of the interstellar clouds are enriched in prebiotic organic molecules. These molecules are found in cosmic matter, chondritic asteroids/meteorites, and cosmic dust particles.

Based on spectroscopic wavelength analyses, more than 120 different complex organic molecules could be detected within cosmic molecular clouds, including common PAHs (polycyclic aromatic hydrocarbons), amino acids, sugars ("polyols"), hydrogen cyanide (HCN), and formic acid (Figure 1) (Ehrenfreund and Menten, 2001). The largest organic molecules so far reported from cosmic molecular clouds are cyanodecapentayne (HC_{11}N) and diethyl ether ($\text{C}_2\text{H}_5)_2\text{O}$. Not all of these molecules are part of the gaseous phase, but the more complex ones are related with cosmic dust particles. A well studied cosmic molecular cloud is the Sagittarius B cloud, where the most complex organic molecules like ethyl cyanide, acetone, glycine, acetic acid, and glycolaldehyde were detected (Hollis et al., 2000; Snyder, 1997). Glycolaldehyde may react with ribose to form a molecule, which is a major constituent of RNA. It was hypothesized that organic molecules within a cosmic molecular cloud are formed on 1–2 μm -sized dust grains, e.g., via photochemical reactions. These dust grains have a distinctive shell-like structure. An inner core of silicates is covered by an inner rim



Cosmic Molecular Clouds, Figure 1 Interstellar space with the occurrence of different organic molecules. (From Ehrenfreund and Charnley, 2000.)

containing complex organic compounds like amino acids, “polyols,” and carbonaceous matter such as hydrogenated amorphous carbon or kerogen-like material. The inner rim is covered by an outer rim consisting of “dirty” ice. In that icy outer rim, due to the heavy ultraviolet radiation, radicals are formed and H_2 is dissociated in monoatomic H that may react with other radicals to form organic molecules (Sorrell, 2001; Pearson et al., 2002). An important finding obtained with the Hubble Space Telescope was the observation of protoplanetary discs in the Orion cloud (Megeath et al., 2005). The size of these discs is more or less similar to our solar system. During solar system formation, an interstellar cloud becomes the building block, from which planets, asteroids, and comets form. Comets and carbonaceous chondrites are chemically very similar to star-forming interstellar clouds and are relics of them (Busemann et al., 2006; Wickramasinghe et al., 2009). They carry pristine primitive organic matter and represent a plausible source of prebiotic organic matter on the late Hadean early Earth. The stable isotope compositions of organic matter, e.g., from the Murchison carbonaceous chondrite (CM2), provide clear evidence that the organic matter was formed under conditions in stellar clouds and not on Earth (Sephton et al., 2003, see entry “*Meteoritics*” for further reading). External delivery of water and organic compounds is widely accepted as the origin of pristine earth-related organic matter (Wickramasinghe, 2010) and may have pushed the start of early life with the first microbial organism – *LUCA* – *Last Universal Common Ancestor* (Hoenigsberg, 2003).

Bibliography

- Boss, A. P., 2004. From molecular clouds to circumstellar disks. In Festou, M. C., Keller, H. U., and Weaver, H. A. (eds.), *Comets II*. Tucson: University of Arizona Press, pp. 67–80, 745 pp.
- Busemann, H., Young, A. F., Alexander, C. M. O. D., Hoppe, P., Mukhopadhyay, S., and Nittler, L. R., 2006. Interstellar chemistry recorded in organic matter from primitive meteorites. *Science*, **312**, 727–730.
- Ehrenfreund, P., and Charnley, S. B., 2000. Organic molecules in the interstellar medium, comets and meteorites: a voyage from dark clouds to the early earth. *Annual Review of Astronomy & Astrophysics*, **38**, 427–483.
- Ehrenfreund, P., and Menten, K., 2001. From molecular clouds to the origin of life. In Horneck, G., and Baumstark-Khan, C. (eds.), *Lecture Notes in Physics: Astrobiology: The Quest for the Conditions of Life*. Berlin: Springer, pp. 7–23.
- Hoenigsberg, H., 2003. Evolution without speciation but with selection: *LUCA*, the last universal common ancestor in Gilbert’s RNA world. *Genetics and Molecular Research*, **2**, 366–375.
- Hollis, J. M., Lovas, F. J., and Jewell, P. R., 2000. Interstellar glycolaldehyde: the first sugar. *The Astrophysical Journal*, **540**, L107–L110.
- Megeath, S. T., Flaherty, K. M., Hora, J., Allen, L. E., Fazio, G. G., Hartmann, L., Myers, P. C., Muzerolle, J., Pipher, J. L., Siegler, N., Stauffer, J. R., and Young, E., 2005. A spitzer/IRAC survey of the Orion molecular clouds. In Cesaroni, R., Felli, M., Churchwell, E., and Walmsley, C. M. (eds.), *Massive Star Birth: A Crossroads of Astrophysics, Proceedings IAU Symposium No. 227*, pp. 383–388.
- Pearson, V. K., Sephton, M. A., Kearsley, A. T., Bland, P. A., Franchi, I. A., and Gilmour, I., 2002. Clay mineral-organic matter relationships in the early Solar System. *Meteoritics and Planetary Science*, **37**, 1829–1833.
- Sephton, M. A., Verchovsky, A. B., Bland, P. A., Gilmour, I., Grady, M. M., and Wright, I. P., 2003. Investigating the variations in carbon and nitrogen isotopes in carbonaceous chondrites. *Geochimica et Cosmochimica Acta*, **67**, 2093–2108.
- Snyder, L. E., 1997. The search for interstellar glycine. *Origins of Life and Evolution of Biospheres*, **27**, 115–133.
- Sorrell, W. H., 2001. Origin of amino acids and organic sugars in interstellar clouds. *The Astrophysical Journal*, **555**, L129–L132.
- Watson, D. M., Leisenring, J. M., Furlan, E., Bohac, C. J., Sargent, B., Forrest, W. J., Calvet, N., Hartmann, L., Nordhaus, J. T., Green, J. D., Kim, K. H., Sloan, G. C., Chen, C. H., Keller, L. D., d’Alessio, P., Najita, J., Uchida, K. I., and Houck, J. R., 2009. Crystalline silicates and dust processing in the protoplanetary disks of the Taurus young cluster. *The Astrophysical Journal Supplement Series*, **180**, 84–101.
- Wickramasinghe, J. T., Wickramasinghe, N. C., and Wallis, M. K., 2009. Liquid water and organics in comets: implications for exobiology. *International Journal of Astrobiology*, **8**, 281–290.
- Wickramasinghe, N. C., 2010. The astrobiological case for our cosmic ancestry. *International Journal of Astrobiology*, **9**, 119–129.

Cross-references

[Asteroid and Comet Impacts](#)
[Astrobiology](#)
[Chondrites](#)
[Meteoritics](#)
[Origin of Life](#)

CRITICAL INTERVALS IN EARTH HISTORY

Frank Wiese, Joachim Reitner
 University of Göttingen, Göttingen, Germany

Definition

In our human understandings of time, Darwinian evolution is something, which happens very slowly. Consulting the (biogeochemical) fossil record, we notice periods in life’s history, which are characterized by slow and continuous evolution, successive background extinction, and equally successive new speciations. On the other hand, we see also periods, during which a sudden occurrence of new groups and its rapid radiation and dispersal can be seen in the fossil record. *Vice versa*, we also recognize catastrophic extinction events (mass extinctions; see below), reducing the number of known taxa dramatically within a very short time of only thousands of years or even much less.

Among these intervals were phases, in which comparatively short-termed (in a geological sense) pulses of evolution resulted in significant and irreversible evolutionary innovations, which opened new paleobiological pathways, affecting the abiotic and biotic evolutionary landscape likewise. These intervals are what we understand as main critical intervals in earth’s history here, and we believe that three periods merit this designation. These are the

(Dobbek et al., 2001; Voordouw, 2002). The Ni-containing CODH is involved in many reactions in anaerobic microorganisms: it catalyzes the oxidation of CO with H₂O to CO₂ and H₂ in carboxydophilic bacteria, and assists the oxidation of acetate to CO₂ in sulfate-reducing bacteria and archaea and the disproportionation of acetate to CH₄ and CO₂ in methanogenic archaea. In many groups of anaerobic microorganisms utilizing the reductive acetyl-CoA pathway, such as acetogenic bacteria or methanogenic archaea, CODH is often found in a complex with ACS (Strauss and Fuchs, 1993; Holo and Sirevag, 1986; Drennan et al., 2004). ACS catalyzes the formation of acetyl-CoA from CO, leading in combination with the CODH to the synthesis of acetate from two CO₂. These two enzymes play an important role in the global carbon cycle, since up to 10% of the organic carbon produced by photosynthesis is remineralized by anaerobic microorganisms involving CODH/ACS (Drake, 1994). MCR is the key enzyme of methanogenic archaea catalyzing the final step during the reduction of carbon dioxide to methane (Thauer, 1998). All biologically formed methane is produced by methanogenic organisms in the terminal step of anaerobic degradation. The nickel is located in a tetrapyrrole coenzyme named F₄₃₀, which is unique to methanogenic archaea. MCR catalyzes the reduction of methyl-coenzyme M with a reduced coenzyme B, leading to the release of methane and a heterodisulfide of the two coenzymes. The latter can be cleaved in an energy yielding reaction. Furthermore, in a process called “reverse methanogenesis,” methane is anaerobically oxidized with sulfate as electron acceptor. This process – known as AOM – is carried out by an uncultured group of archaea (ANME), which has been shown to contain high amounts of a modified MCR (Krüger et al., 2003; Hallam et al., 2003). Hydrogenases are a family of oxidoreductase enzymes that catalyze the reversible oxidation of molecular hydrogen. Nickel-containing hydrogenases have been isolated from bacteria and archaea (Voordouw, 1992). Finally, nickel is also present in a number of other enzymes, such as urease, a superoxide dismutase, and glyoxylases. The functions and biological roles of these metalloproteins have been described in detail (Sigel et al., 2006).

Recently, due to its strong involvement in geologically important microbial reactions, especially methanogenesis, AOM, and carbon fixation, nickel has raised interest as a potential biosignature for these processes and the involved microorganisms in recent or ancient environments (Reitner et al., 2005; Hausrath et al., 2007).

Bibliography

- Dobbek, H., Svetlitchnyi, V., Gremer, L., Huber, R., and Meyer, O., 2001. Crystal structure of a carbon monoxide dehydrogenase reveals a [Ni-4Fe-5S] cluster. *Science*, **293**, 1281.
- Drake, H. L., 1994. *Acetogenesis*. New York: Chapman & Hall, pp. 273–504.

- Drennan, C. L., Doukov, T. I., and Ragsdale, S. W., 2004. The metallocusters of carbon monoxide dehydrogenase/acetyl-CoA synthase: a story in pictures. *Journal of Biological Inorganic Chemistry*, **9**, 511–515.
- Ermiler, U., Grabarse, W., Shima, S., Goubeaud, M., and Thauer, R. K., 1998. Active sites of transition metal enzymes with focus on nickel. *Current Opinion Structural Biology*, **8**, 749–756.
- Hallam, S. J., Girguis, P. R., Preston, C. M., Richardson, P. M., and DeLong, E. F., 2003. Identification of methyl coenzyme M reductase A (mcrA) genes associated with methane-oxidizing Archaea. *Applied and Environmental Microbiology*, **69**, 5483–5491.
- Hausrath, E. M., Liermann, L. J., House, C. H., Ferry, J. G., and Brantley, S. L., 2007. The effect of methanogen growth on mineral substrates: will Ni markers of methanogen-based communities be detectable in the rock record? *Geobiology*, **5**, 49–61.
- Holo, H., and Sirevag, R., 1986. Autotrophic growth and CO₂ fixation of *Chloroflexus aurantiacus*. *Archives Microbiology*, **145**, 173–180.
- Krüger, M., Meyerdierks, A., Glöckner, F. O., Amann, R., Widdel, F., et al., 2003. A conspicuous nickel protein in microbial mats that oxidize methane anaerobically. *Nature*, **426**, 878–881.
- Ragsdale, S. W., 2007. Nickel and the carbon cycle. *Journal of Inorganic Biochemistry*, **101**, 1657–1666.
- Reitner, J., Peckmann, J., Reimer, A., Schumann, G., and Thiel, V., 2005. Methane-derived carbonate build-ups and associated microbial communities at cold seeps on the lower Crimean shelf (Black Sea). *Facies*, **51**, 71–84.
- Sigel, A., Sigel, H., and Sigel, R. K. O. (eds.), 2006. *Nickel and its Surprising Role in Nature*. Weinheim, Germany: Wiley HCH.
- Strauss, G., and Fuchs, G., 1993. Enzymes of a novel autotrophic CO₂ fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. *European Journal Biochemistry*, **215**, 633–643.
- Thauer, R. K., 1998. Biochemistry of methanogenesis, a tribute to Marjory Stephenson. *Microbiology*, **144**, 2377–2406.
- Voordouw, G., 1992. Evolution of hydrogenase genes. In Cammack, R., and Sykes, A. (eds.), *Advances in Inorganic Chemistry*. London: Academic Press, Vol. 38, pp. 397–422.
- Voordouw, G., 2002. Carbon monoxide cycling by *Desulfovibrio vulgaris* Hildenborough. *Journal Bacteriology*, **184**, 5903–5911.

Cross-references

[Acetogens](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Biosignatures in Rocks](#)
[Carbon Cycle](#)
[Hydrogen](#)
[Metalloenzymes](#)
[Methanogens](#)
[Sulfate-Reducing Bacteria](#)

NITRIFICATION

Nitrification is the two-step microbial process by which ammonia (NH₃) is first oxidized to nitrite (NO₂[−]) which is, in a second reaction, converted to nitrate (NO₃[−]). See entry “[Nitrogen](#)” for detailed reading.

- Thauer, R. K., Jungermann, K., and Decker, K., 1977. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriological Reviews*, **41**, 100.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578.
- Widdel, F., Schnell, S., Heising, S., Ehrenreich, A., Aßmus, B., and Schink B., 1993. Anaerobic ferrous iron oxidation by anoxygenic phototrophs. *Nature (London)*, **362**, 834.
- Wolfe, R. S., 1992. Biochemistry of methanogenesis. *Biochemical Society Symposia*, **58**, 41.
- Zehnder, A. J. B., and Stumm, W., 1988. Geochemistry and biogeochemistry of anaerobic habitats. In Zehnder, A. J. B. (ed.), *Biology of anaerobic microorganisms*. New York: John Wiley & Sons, pp. 1–38.

Cross-references

[Aerobic Metabolism](#)
[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Carbon \(Organic, Degradation\)](#)
[Fe\(III\)-Reducing Prokaryotes](#)
[Fermentation](#)
[Geobacter](#)
[Hydrogen](#)
[Iron Sulfide Formation](#)
[Methane, Origin](#)
[Microbial Communities, Structure, and Function](#)
[Nitrogen](#)
[Photosynthesis](#)
[Shewanella](#)
[Sulfate-Reducing Bacteria](#)
[Terrestrial Deep Biosphere](#)

ANAMMOX

Anammox refers to the microbial **anaerobic oxidation** of **ammonium** by nitrite (NO_2^-), which forms N_2 . See entry “[Nitrogen](#)” for further reading.

ANIMAL BIOCALCIFICATION, EVOLUTION

Gert Wörheide¹, Daniel J. Jackson²

¹Ludwig-Maximilians-Universität München, München, Germany

²University of Göttingen, Göttingen, Germany

Definition

SOM. Soluble organic matrix

IOM. Insoluble organic matrix

GRN. Gene regulatory network

Introduction

One of the major events in the evolution of multicellular animals was the transition from soft-bodied organisms to those that possessed mineralized hard parts for protection

and support. This major evolutionary hallmark supported the rapid diversification of animals and their occupation of a diverse range of novel ecological niches at the dawn of the Phanerozoic, between 560 and 530 million years ago, when mineralized skeletons appeared relatively synchronous in a variety of Phyla during the so-called Cambrian Explosion (Knoll, 2003; Conway Morris, 2006). However, it is still unclear exactly what drove this sudden capacity to construct mineralized structures, be it changes in ocean chemistry (Brennan et al., 2004) or the evolution of more diverse ecologies (Cohen, 2005), including predators (Vermeij, 1989). Investigation of biomineralization processes will provide insight into the molecular and genetic mechanisms that accompanied the appearance of mineralized body parts during the early Cambrian, shedding light on the processes that generate the diversity of life we see around us today.

Until recently, studies of biomineralization processes were mainly restricted to describing the (ultra)structure of the minerals and their interaction with the organic material provided by the organism (Lowenstam and Weiner, 1989; Mann and Webb, 1989). However, the generation and elaboration of biominerals is ultimately controlled by genes (Jackson et al., 2006, 2007a, b), which must in turn be expressed in the appropriate tissue and at an appropriate stage of development. Understanding the evolution of such biomineralizing genes and the gene regulatory networks (GRNs) in which they act is, therefore, vital to the understanding of how animals evolved and will also provide clues for the fabrication of biomimetic materials.

Biomineralization

Biomineralization refers to the processes by which organisms form minerals (e.g., calcium carbonate, calcium phosphate, silica, iron oxides) under strict biological control. These processes are, therefore, defined as *biologically controlled mineralization*, with the skeletons formed being integral, functional parts of the organisms (Lowenstam and Weiner, 1989). There are, however, several other serendipitous processes linked to living organisms (e.g., microbes) or organic substances that result in mineral (mainly calcium carbonate) formation that are not under strict control of organisms. These processes are termed either *biologically induced mineralization*, where inorganic minerals are precipitated by adventitious precipitation resulting from secondary interactions between various metabolic processes and the surrounding environment (Mann, 2001), or *organomineralization*, a term used to describe mineralization processes that involve organic molecules or particles, whether linked to living organisms or not (e.g., degrading organic matter) (Trichet and Defarge, 1995).

While both *biologically induced* and *organomineralization* play an important role in the formation of carbonate systems in the geological record (e.g., microbialites, stromatolites, ooids) (Reitner and Neuweiler, 1995; Arp,

- Takai, K., and Horikoshi, K., 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Brinkhoff, T., Muyzer, G., Moser, D. P., Rethmeier, J., and Jannasch, H. W., 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. *Applied and Environmental Microbiology*, **66**(8), 3125–3133.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daae, F. L., and Pedersen, R. B., Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**(10), 6375–6378.
- Turley, C. M., and Mackie, P. J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep-Sea Research I*, **42**, 1453–1474.
- Tyler, P. A., 2003. *Ecosystems of the Deep Oceans. Ecosystems of the World*, 28. Elsevier: Amsterdam.
- Vanucci, S., Dell'Anno, A., Pusceddu, A., Fabiano, M., Lampitt, R. S., and Danovaro, R., 2001. Microbial assemblages associated with sinking particles in the Porcupine Abyssal Plain (NE Atlantic Ocean). *Progress in Oceanography*, **50**, 105–121.
- Venter, A., 2001. Deep cold biosphere. *Trends in Microbiology*, **9**(7), 313–313.
- Vogt, P. R., Gardner, J., and Crane, K., 1999. The Norwegian-Barents-Svalbard (NBS) continental margin: Introducing a natural laboratory of mass wasting, hydrates, and ascent of sediment, pore water, and methane. *Geo-Marine Letters*, **19**, 2–21.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivatskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., Lee, C., 1986. Evidence for sulfate-reducing and methane-producing micro-organisms in sediments from Site 618, Site 619, and Site 622. *Initial Reports of the Deep Sea Drilling Project*, **96**, 767–775.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Basalt \(Glass, Endoliths\)](#)
[Beggiatoa](#)
[Biofilms](#)
[Carbon Cycle](#)
[Carbon \(Organic, Cycling\)](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Mud Mounds](#)

[Piezophilic Bacteria](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)

DEEP FLUIDS

See entries “[Terrestrial Deep Biosphere](#),” “[Deep Biosphere of Salt Deposits](#),” and “[Deep Biosphere of the Oceanic Deep Sea](#).”

DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed **biodegradation**. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#),” and “[Microbial degradation](#) (of organic matter)” for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO_3^-) to N_2 and, to a minor extent, other gaseous species such as N_2O . See entry “[Nitrogen](#)” for further reading.

DESERT VARNISH

Randall S. Perry
 Imperial College, London, UK

Synonyms

Rock Varnish, *Rock Glaze*, *Silica Glaze*, and *Schutzrinde* were introduced in 1891, from a translation of the French term *manteau protecteur*: “*Schutzrinde*” for all dark coatings, including “*Wustenlack*” a thin, polished coating that may be an eolian polished patina; “*Dunkle Rinden*” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “*Schweinfurth*,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish ([Figure 1](#)). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama

- Michalski, G., Böhlke, J. K., and Thiemens, M., 2004. Long term atmospheric deposition as the source of nitrate and other salts in the Atacama Desert, Chile: new evidence from mass-independent oxygen isotopic compositions. *Geochimica et Cosmochimica Acta*, **68**, 4023–4038.
- Mingram, B., Hoth, P., Lüders, V., and Harlov, D., 2005. The significance of fixed ammonium in Palaeozoic sediments for the generation of nitrogen-rich natural gases in the North German Basin. *International Journal of Earth Science*, **94**, 1010–1022.
- Muehe, E. M., Gerhardt, S., Schink, B., and Kappler, A., 2009. Ecophysiology and the energetic benefit of mixotrophic Fe(II) oxidation by various strains of nitrate-reducing bacteria. *FEMS (Federation of European Microbiological Societies) Microbiology – Ecology*, **70**, 335–343.
- Oyarzun, J., and Oyarzun, R., 2007. Massive volcanism in the Altiplano-Puna volcanic plateau and formation of the huge atacama desert nitrate deposits: a case for thermal and electric fixation of atmospheric nitrogen. *International Geology Review*, **49**, 962–968.
- Purvaja, R., Ramesh, R., Ray, A. K., and Rixen, T., 2008. Nitrogen cycling: a review of the processes, transformations and fluxes in coastal ecosystems. *Current Science*, **94**, 1419–1438.
- Raghoebarsing, A. A., Pol, A., van de Pas-Schoonen, K. T., Smolders, A. J., Ettwig, K. F., Rijpstra, W. I., Schouten, S., Damsté, J. S., Op den Camp, H. J., Jetten, M. S., and Strous, M., 2006. A microbial consortium couples anaerobic methane oxidation to denitrification. *Nature*, **440**, 878–879.
- Reeburgh, W. S., 1997. Figures summarizing the global cycles of biogeochemically important elements. *Bulletin of the Ecological Society of America*, **78**, 260–267.
- Rosenfeld, J. K., 1979. Ammonium adsorption in nearshore anoxic sediments. *Limnology and Oceanography*, **24**, 356–364.
- Saito, M. A., Sigman, D. M., and Morel, F. M. M., 2003. The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean-Proterozoic boundary? *Inorganica Chimica Acta*, **356**, 308–318.
- Schmid, M., Maas, B., Dapena, A., van de Pas-Schoonen, K., van de Vossenberg, J., Kartal, B., van Niftrik, L., Schmidt, I., Cirpus, I., Kuenen, J. G., Wagner, M., Damsté, J. S. S., Kuypers, M., Revsbech, N. P., Mendez, R., Jetten, M. S. M., and Strous, M., 2005. Biomarkers for in situ detection of anaerobic ammonium-oxidizing (anammox) bacteria. *Applied and Environmental Microbiology*, **71**, 1677–1684.
- Scott, C., Lyons, T. W., Bekker, A., Shen, Y., Poulton, S. W., Chu, X., and Anbar, A. D., 2008. Tracing the stepwise oxygenation of the Proterozoic ocean. *Nature*, **452**, 456–459.
- Schott, J., Griffin, B. M., and Schink, B., 2010. Anaerobic phototrophic nitrite oxidation by *Thiocapsa* sp. strain KS1 and *Rhodopseudomonas* sp. strain LQ17. *Microbiology*, **156**, 2428–2437.
- Stal, L. J., and Zehr, J. P., 2008. Cyanobacterial nitrogen fixation in the ocean: diversity, regulation, and ecology. In Herrero, A., and Flores, E. (eds.), *The Cyanobacteria: Molecular Biology, Genomics and Evolution*. Norwich: Horizon Scientific Press, pp. 423–471.
- Ward, B. B., 2003. Significance of anaerobic ammonium oxidation in the ocean. *Trends in Microbiology*, **11**, 408–410.
- Weeks, M. E., 1934. Daniel Rutherford and the discovery of nitrogen. *Journal of Chemical Education*, **11**, 101–107.
- Zerle, A. L., House, C. H., Cox, R. P., and Canfield, D. E., 2006. Metal limitation of cyanobacterial N₂ fixation and implications for the Precambrian nitrogen cycle. *Geobiology*, **4**, 285–297.

Cross-references

[Archaea](#)
[Bacteria](#)
[Critical Intervals in Earth History](#)
[Cyanobacteria](#)
[Nitrogen Fixation](#)
[Thiotrophic Bacteria](#)

NITROGEN FIXATION

Nitrogen fixation is the conversion of diatomic nitrogen (N₂) into ammonia (NH₃), which is then used for biosynthesis of organic compounds. See entry “[Nitrogen](#)” for further reading.

- Westall, F., 1997. The influence of cell wall composition on the fossilization of bacteria and the implications for the search for early life forms. In Cosmovici, C., Bowyer, S., and Werthimer, D. (eds.), *Astronomical and Biochemical Origins and the Search for Life in the Universe*. Bologna: Editori Compositrici, pp. 491–504.
- Westall, F., 2008. Morphological biosignatures in terrestrial and extraterrestrial materials. *Space Science Reviews*, **135**, 95–114.
- Westall, F., 2009. Life on an anaerobic planet. *Science*, **232**, 471–472.
- Westall, F., and Folk, R. L., 2003. Exogenous carbonaceous microstructures in Early Archaean cherts and BIFs from the Isua Greenstone Belt: implications for the search for life in ancient rocks. *Precambrian Research*, **126**, 313–330.
- Westall, F., and Southam, G., 2006. Early life on Earth. In Benn, K., Mareschal, J. C., and Condie, K. (eds.), *Archean Geodynamics and Environments*. AGU Geophysical Monographs No 164, pp. 283–304.
- Westall, F., Boni, L., and Guerzoni, E., 1995. The experimental silicification of microorganisms. *Palaeontology*, **38**, 495–528.
- Westall, F., Steele, A., Toporski, J., Walsh, M., Allen, C., Guidry, S., Gibson, E., McKay, D., and Chafetz, H., 2000. Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *Journal of Geophysical Research*, **105**, 24,511–524,527.
- Westall, F., De Wit, M. J., Dann, J., Van Der Gaast, S., De Ronde, C., and Gerneke, D., 2001. Early Archaean fossil bacteria and biofilms in hydrothermally influenced, shallow water sediments, Barberton greenstone belt, South Africa. *Precambrian Research*, **106**, 91–112.
- Westall, F., de Ronde, C. E. J., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006a. Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth. *Philosophical Transactions of the Royal Society B-Biological Sciences*, **361**, 1857–1875.
- Westall, F., de Vries, S. T., Nijman, W., Rouchon, V., Orberger, B., Pearson, V., Watson, J., Verchovsky, A., Wright, I., Rouzaud, J.-N., Marchesini, D., and Anne, S., 2006b. The 3.466 Ga Kitty's Gap Chert, an Early Archaean microbial ecosystem. In Reimold, W. U., and Gibson, R. (eds.), *Processes on the Early Earth*. GSA Special Publications No 405, pp. 105–131.
- White, B., 1974. Microfossils from the Late Precambrian Altyn Formation of Montana. *Nature*, **247**, 452–453.

Cross-references

Archaea
 Astrobiology
 Bacteria
 Biofilms and Fossilization
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Biomarkers (Organic, Compound-Specific Isotopes)
 Cold Seeps
 Critical Intervals in Earth History
 Cyanobacteria
 Deep Biosphere of Salt Deposits
 Dolomite, Microbial
 Geochronology
 Isotope Fractionation (Metal)
 Isotopes and Geobiology
 Mat-Related Sedimentary Structures
 Microbialites, Modern
 Microbialites, Stromatolites, and Thrombolites

Origins of the Metazoa
 Sediment Diagenesis - Biologically Controlled
 Stromatactis
 Stromatolites
 Sulfate-Reducing Bacteria
 Sulfur Cycle

BIOSILICIFICATION

The biological formation of opal-like amorphous hydrated silica. This phenomenon occurs on a globally vast scale in a wide variety of organisms, including protists, radiolarian, foraminifera, sponges, molluscs, brachiopods, copepods, ascidians, diatoms, and higher plants. See entries “*Silica Biomineralization, Sponges*”, “*Microbial Silicification – Bacteria (or Passive)*” and “*Microbial Biomineralization*” for further reading.

BLACK SHALES

Wolfgang Oschmann
 University of Frankfurt, Frankfurt, Germany

Definition

Most modern and ancient aquatic (marine, brackish, and lacustrine) environments were sufficiently oxygenated to permit colonization by numerous benthic, planktic, and nectic organisms. Due to excess oxygen the biologically produced organic matter is rapidly degraded. The sediments formed thereby exhibit only very low amounts of organic matter.

In contrast, very high rates of primary production and/or stagnant water bodies result in black shale sedimentation, which is associated with oxygen depletion or even lack of oxygen within the substrate and to some extent also within the water column. Degradation of organic matter is incomplete, partly by anaerobic bacteria, causing strong enrichment of organic matter in sediments. Black shales therefore commonly exhibit excellent preservation of organic molecules and mineralized soft tissues and hard parts of organisms.

The importance of black shales for scientists and the society are twofold. Black shales serve as source rocks for fossil fuels (oil and gas), which cover most of the energy needs of modern societies, and due to excellent preservation they provide particular sites for palaeontological studies.

Sedimentary characteristics of black shales

Black shales sediments in common are dark gray to black in color and are characterized by microlaminations, which may vary from distinct to blurred. The lamination is caused by quiet depositional conditions and the scarcity or lack of bioturbation. In many cases, the lamina were formed by alignments of fecal pellets ([Figure 1a](#)), which rained down in great numbers from surface waters. The

- Sahling, H., Rickert, D., Lee, R. W., Linke, P., and Suess, E., 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series*, **231**, 121–138.
- Schoell, M., 1988. Multiple origins of methane in the earth. *Chemical Geology*, **71**, 1–10.
- Sibuet, M., and Olu, K., 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold seep communities at active and passive margin. *Deep-Sea Research Part*, **2**(45), 517–567.
- Smith, C. R., and Baco, A. R., 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology*, **41**, 311–354.
- Takeuchi, R., Matsumoto, R., Ogihara, S., and Machiyama, H., 2007. Methane-induced dolomite “chimneys” on the Kuroshima Knoll, Ryukyu islands, Japan. *Journal of Geochemical Exploration*, **95**, 16–28.
- Tappan, H., and Loeblich, A. R. Jr., 1973. Evolution of the oceanic plankton. *Earth-Science Reviews*, **9**, 207–240.
- Tomaru, H., Lu, Z., Fehn, U., Muramatsu, Y., and Matsumoto, R., 2007. Age variation of pore water iodine in the eastern Nankai Trough, Japan: evidence for different methane sources in a large gas hydrate field. *Geology*, **35**, 1015–1018.
- Tryon, M. D., and Brown, K. M., 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters*, **28**, 2863–2866.
- Tryon, M. D., and Brown, K. M., 2004. Fluid and chemical cycling at Bush Hill: implications for gas- and hydrate-rich environments. *Geochemistry Geophysics Geosystems*, **5**, 1–7.
- Tunnicliffe, V., Juniper, S. K., and Sibuet, M., 2003. Reducing environments of the deep-sea floor. In Tyler, P. A. (ed.), *Ecosystems of the Deep Oceans*. Amsterdam: Elsevier, pp. 81–110.
- Ueno, Y., Yamada, K., Yoshida, N., Maruyama, S., and Isozaki, Y., 2006. Evidence from fluid inclusions for microbial methanogenesis in the early Archaean era. *Nature*, **440**, 516–519.
- Valentine, D. L., 2002. Biogeochemistry and microbial ecology of methane oxidation in anoxic environments: a review. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, **81**, 271–282.
- Whiticar, M., 1999. Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chemical Geology*, **161**, 291–314.
- Zhang, Y., 2003. Methane escape from gas hydrate systems in marine environment, and methane-driven oceanic eruptions. *Geophysical Research Letters*, **30**, 1398.

Cross-references

Alkalinity
 Anaerobic Oxidation of Methane with Sulfate
 Archaea
 Biomarkers (Molecular Fossils)
 Biomarkers (Organic, Compound-Specific Isotopes)
 Biosignatures in Rocks
 Calcite Precipitation, Microbially Induced
 Cap Carbonates
 Carbon Isotopes
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Hydrogen
 Hydrothermal Environments, Terrestrial
 Iron Isotopes
 Iron Sulfide Formation
 Isotopes and Geobiology

Methane, Origin
 Methane Oxidation (Aerobic)
 Microbialites, Stromatolites, and Thrombolites
 Mud Mounds
 Pore Water
 Pyrite Oxidation
 Sulfate-Reducing Bacteria
 Sulfide Mineral Oxidation
 Thiotrophic Bacteria
 Whale and Wood Falls

COMETS

Comets are primitive conglomerates of the solar system containing a mixture of frozen gases, refractory grains, and carbonaceous particles rich in biogenic elements (Llorca, 2005). The great variety of organic molecules carried by comets could have played a major role in prebiotic processes prior to the appearance of microorganisms. Please refer to entries “*Astrobiology*,” “*Asteroid and Comet Impacts*,” “*Meteoritics*,” and “*Origin of Life*.”

Bibliography

- Llorca, J., 2005. Organic matter in comets and cometary dust. *International Microbiology*, **8**, 5–12.

COMMENSALISM

Commensalism is a form of a symbiosis, in which one organism benefits and the other remains unaffected. See entry “*Symbiosis*” for further reading.

COMMUNITY

All populations in a habitat. The composition of a community and the abundance of its members is referred to as community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

COPPER

Stephan M. Kraemer
 University of Vienna, Vienna, Austria

Definition

Copper (Cu) is a transition metal that serves as an essential nutrient to eukaryotes and many prokaryotes due to its many functions in copper enzymes. At elevated concentrations, Cu is toxic and the many industrial uses of copper

- Takai, K., and Horikoshi, K., 1999. Genetic diversity of Archaea in deep-sea hydrothermal vent environments. *Genetics*, **152**, 1285–1297.
- Teske, A., Brinkhoff, T., Muyzer, G., Moser, D. P., Rethmeier, J., and Jannasch, H. W., 2000. Diversity of thiosulfate-oxidizing bacteria from marine sediments and hydrothermal vents. *Applied and Environmental Microbiology*, **66**(8), 3125–3133.
- Thorseth, I. H., Torsvik, T., Torsvik, V., Daae, F. L., and Pedersen, R. B., Keldysh-98 Scientific Party, 2001. Diversity of life in ocean floor basalt. *Earth and Planetary Science Letters*, **194**, 31–37.
- Treude, T., Knittel, K., Blumenberg, M., Seifert, R., and Boetius, A., 2005. Subsurface microbial methanotrophic mats in the Black Sea. *Applied and Environmental Microbiology*, **71**(10), 6375–6378.
- Turley, C. M., and Mackie, P. J., 1995. Bacterial and cyanobacterial flux to the deep NE Atlantic on sedimenting particles. *Deep-Sea Research I*, **42**, 1453–1474.
- Tyler, P. A., 2003. *Ecosystems of the Deep Oceans. Ecosystems of the World*, 28. Elsevier: Amsterdam.
- Vanucci, S., Dell'Anno, A., Pusceddu, A., Fabiano, M., Lampitt, R. S., and Danovaro, R., 2001. Microbial assemblages associated with sinking particles in the Porcupine Abyssal Plain (NE Atlantic Ocean). *Progress in Oceanography*, **50**, 105–121.
- Venter, A., 2001. Deep cold biosphere. *Trends in Microbiology*, **9**(7), 313–313.
- Vogt, P. R., Gardner, J., and Crane, K., 1999. The Norwegian-Barents-Svalbard (NBS) continental margin: Introducing a natural laboratory of mass wasting, hydrates, and ascent of sediment, pore water, and methane. *Geo-Marine Letters*, **19**, 2–21.
- Vorobyova, E., Soina, V., Gorlenko, M., Minkovskaya, N., Zalinova, N., Mamukelashvili, A., Gilichinsky, D., Rivkina, E., and Vishnivatskaya, T., 1997. The deep cold biosphere: facts and hypothesis. *FEMS Microbiology Reviews*, **20**, 277–290.
- Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R., Lee, C., 1986. Evidence for sulfate-reducing and methane-producing micro-organisms in sediments from Site 618, Site 619, and Site 622. *Initial Reports of the Deep Sea Drilling Project*, **96**, 767–775.
- Whitman, W. B., Coleman, D. C., and Wiebe, W. J., 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences of the United States of America*, **95**, 6578–6583.

Cross-references

[Anaerobic Oxidation of Methane with Sulfate](#)
[Archaea](#)
[Bacteria](#)
[Basalt \(Glass, Endoliths\)](#)
[Beggiatoa](#)
[Biofilms](#)
[Carbon Cycle](#)
[Carbon \(Organic, Cycling\)](#)
[Chemolithotrophy](#)
[Cold Seeps](#)
[Deep Biosphere of Salt Deposits](#)
[Extreme Environments](#)
[Hydrothermal Environments, Marine](#)
[Methane Oxidation \(Aerobic\)](#)
[Microbial Biomineralization](#)
[Microbial Degradation](#)
[Microbial Mats](#)
[Microbialites, Modern](#)
[Microbialites, Stromatolites, and Thrombolites](#)
[Mud Mounds](#)

[Piezophilic Bacteria](#)
[Sulfide Mineral Oxidation](#)
[Sulfur Cycle](#)
[Terrestrial Deep Biosphere](#)

DEEP FLUIDS

See entries “[Terrestrial Deep Biosphere](#),” “[Deep Biosphere of Salt Deposits](#),” and “[Deep Biosphere of the Oceanic Deep Sea](#).”

DEGRADATION (OF ORGANIC MATTER)

Transformations of organic matter within the range of temperatures, pressures and environmental conditions found at or near earth surface environments. If biological activity is confirmed to cause the transformations, termed **biodegradation**. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#),” and “[Microbial degradation](#) (of organic matter)” for further reading.

DENITRIFICATION

Denitrification is the biological reduction of nitrate (NO_3^-) to N_2 and, to a minor extent, other gaseous species such as N_2O . See entry “[Nitrogen](#)” for further reading.

DESERT VARNISH

Randall S. Perry
 Imperial College, London, UK

Synonyms

Rock Varnish, *Rock Glaze*, *Silica Glaze*, and *Schutzrinde* were introduced in 1891, from a translation of the French term *manteau protecteur*. “*Schutzrinde*” for all dark coatings, including “*Wustenlack*” a thin, polished coating that may be an eolian polished patina; “*Dunkle Rinden*” as a dark brown to black coating, possibly more similar to Sonoran and Mojave desert varnish; and “*Schweinfurth*,” which describes black coatings on rocks in Egypt that have spread on surrounding sands.

Definition

Desert Varnish. In arid environments such as Death Valley (California), rocks are covered with black opalescent desert varnish ([Figure 1](#)). Desert varnishes have been found in all continents, in locations such as the Gobi, Sonoran, Mojave, Namibian, Victorian, and Atacama

- Fietzke, J., and Eisenhauer, A., 2006. Determination of temperature-dependent stable strontium isotope ($^{88}\text{Sr}/^{86}\text{Sr}$) fractionation via bracketing standard MC-ICP-MS. *Geochemistry, Geophysics, Geosystems*, **7**(8), doi:10.1029/2006GC001243.
- Gussone, N., Eisenhauer, A., Heuser, A., Dietzel, M., Bock, B., Böhm, F., Spero, H., Lea, D. W., Bijma, J., and Nägler, T. F., 2003. Model for kinetic effects on calcium isotope fractionation ($\delta^{44}\text{Ca}$) in inorganic aragonite and cultured planktonic foraminifera. *Geochimica et Cosmochimica Acta*, **67**(7), 1375–1382.
- Hastings, D. W., Russell, A. D., and Emerson, S. R., 1998. Foraminiferal magnesium in *G. sacculifer* as a paleotemperature proxy in the equatorial Atlantic and Caribbean surface oceans. *Paleoceanography*, **13**(2), 161–169.
- Hippler, D., Eisenhauer, A., et al. 2006. “Tropical Atlantic SST history inferred from Ca isotope thermometry over the last 140ka.” *Geochim Cosmochim Acta*, **70**, 90–100, doi:10.1016/j.gca.2005.07.022.
- Holland, H. D., 1984. *The Chemical Evolution of the Atmosphere and Oceans*. Princeton: Princeton University Press.
- Kastner, M., 1999. Oceanic minerals: their origin, nature of their environment, and significance. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 3380–3387.
- Katz, A., 1973. The interaction of magnesium with calcite during crystal growth at 25–90°C and one atmosphere. *Geochimica et Cosmochimica Acta*, **37**(6), 1563–1578, IN3, 1579–1586.
- Kinsman, D. J. J., and Holland, H. D., 1969. The co-precipitation of cations with CaCO_3 . The co-precipitation of Sr^{2+} with aragonite between 16° and 96°C. *Geochimica et Cosmochimica Acta*, **33**, 1–17.
- Kisakürek, B., Eisenhauer, A., et al. 2008. Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminifera, Globigerinoides ruber (white). *Earth and Planetary Science Letters*, **273**(3–4), 260–269.
- Lea, D. W., 2003. Elemental and isotopic proxies of past ocean temperatures. In Holland, H. D., and Turekian K. K. (eds.), *Treatise on Geochemistry*, Oxford: Elsevier, Vol. 6, pp. 365–390.
- Lea, D. W., Mashiotta, T. A., and Spero, H. J., 1999. Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing. *Geochimica et Cosmochimica Acta*, **63**(16), 2369–2379.
- Lea, D. W., Pak, D. K., and Spero, H. J., 2000. Climate impact of late quaternary equatorial Pacific sea surface temperature variations. *Science*, **289**, 1719–1724.
- Linsley, B. K., Wellington, G. M., and Schrag, D. P., 2000. Decadal sea surface temperature variability in the subtropical South Pacific from 1726 to 1997 A.D. *Science*, **290**, 1145–1148.
- Mantua, J. N., Hare, S. R., Zhang, Y., Wallace, J. M., and Francis, R. C., 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. *American Meteorological Society*, **78**(6), 1069–1079.
- Mashiotta, T. M., Lea, D. W., and Spero, H. J., 1999. Glacial-interglacial changes in Subantarctic sea surface temperature and $\delta^{18}\text{O}$ -water using foraminiferal Mg. *Earth and Planetary Science Letters*, **170**, 417–432.
- McKenzie, J. A., 1981. Holocene dolomitization of calcium carbonate sediments from the coastal sabkhas of Abu Dhabi, UAE: a stable isotope study. *Journal of Geology*, **89**, 185–198.
- Millero, F. J., 1995. Thermodynamics of the carbon dioxide system in the oceans. *Geochimica et Cosmochimica Acta*, **59**(4), 661–677.
- Mottl, M. J., and Holland, H. D., 1978. Chemical exchange during hydrothermal alteration of basalt by seawater-I. Experimental results for major and minor components of seawater. *Geochimica et Cosmochimica Acta*, **42**(8), 1103–1115.
- Mottl, M. J., and Wheat, C. G., 1993. Hydrothermal circulation through mid-ocean ridge flanks: Fluxes of heat and magnesium. *Geochim Cosmochim Acta*, **58**, 2225–2237.
- Nägler, T., Eisenhauer, A., Müller, A., Hemleben, C., and Kramers, J., 2000. The $\delta^{44}\text{Ca}$ -isotopes: new powerful tool for reconstruction of past sea surface temperatures. *Geochemistry, Geophysics, Geosystems*, **1**(2000GC000091).
- Nürnberg, D., Bijma, J., et al. 1996. Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures. *Geochim Cosmochim Acta*, **60**, 803–814.
- Nürnberg, D., Müller, A., and Schneider, R. R., 2000. Paleo-sea surface temperature calculations in the equatorial east Atlantic from Mg/Ca ratios in planktic foraminifera: a comparison to sea surface temperature estimates from Uk^{37} , oxygen isotopes, and foraminiferal transfer function. *Paleoceanography*, **15**(1), 124–134.
- Savin, S. M., and Douglas, R. G., 1973. Stable isotope and magnesium geochemistry of recent planktonic foraminifera from South-Pacific. *Geological Society of America Bulletin*, **84**(7), 2327–2342.
- Wilkinson, B. H., and Algeo, T. J., 1989. Sedimentary carbonate record of calcium-magnesium cycling. *American Journal of Science*, **289**, 1158–1194.

Cross-references

Alkalinity
 Biological Control on Diagenesis: Influence of Bacteria and Relevance to Ocean Acidification
 Calcite Precipitation, Microbially Induced
 Calcium Biogeochemistry
 Carbon Cycle
 Carbonate Environments
 Carbonates
 Dolomite, Microbial
 Isotopes and Geobiology
 Salinity History of the Earth's Ocean
 Soda Ocean Hypothesis

DIVERSITY

The number of different species in a habitat; sometimes (falsely) used as a synonym for →community structure. See entry “*Microbial Communities, Structure, and Function*” for further reading.

DOLOMITE, MICROBIAL

Jennifer A. Roberts, Paul A. Kenward
 University of Kansas, Lawrence, KS, USA

Synonyms

Bacterial dolomite; Organogenic dolomite

Definition

Dolomite. Widely distributed rock-forming mineral, $\text{CaMg}(\text{CO}_3)_2$. Usually, white or colorless, but can be yellowish and brown with rhombohedral crystals exhibiting curved, composite faces. It also occurs with massive and granular habit and has rhombohedral {1011} cleavage.
Dolomitization. The process of converting calcite in limestones to dolomite by the action of magnesium-bearing solutions.

is related with the Snowball Earth scenarios and increase of oxygen in the atmosphere.

Bibliography

- Awramik, S. M., and Barghoorn, E. S., 1997. The Gunflint microbiota. *Precambrian Research*, **5**, 121–142.
- Brocks, J. J., Summons, R. E., Logan, G. A., and Buick, R., 2003a. Molecular fossils in Archean rocks II: composition, thermal maturity and integrity of hydrocarbons extracted from sedimentary rocks of the 2.78–2.45 billion years old Mount Bruce Supergroup, Pilbara Craton, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4289–4319.
- Brocks, J. J., Buick, R., Summons, R. E., and Logan, G. A., 2003b. A reconstruction of Archean biological diversity based on molecular fossils from the 2.78–2.45 billion year old Mount Bruce Supergroup, Hamersley Basin, Western Australia. *Geochimica et Cosmochimica Acta*, **67**, 4321–4335.
- Brocks, J. J., Logan, G. A., Buick, R., and Summons, R. E., 1999. Archean molecular fossils and the early rise of Eukaryotes. *Science*, **285**, 1033–1036.
- Fralick, P., Davis, D. W., and Kissin, S. A., 2002. The age of the Gunflint Formation, Ontario, Canada: single zircon U–Pb age determinations from reworked volcanic ash. *Canadian Journal of Earth Science*, **39**, 1085–1091.
- George, S. C., Volk, H., Dutkiewicz, A., Ridley, J., and Buick, R., 2008. Preservation of hydrocarbons and biomarkers in oil trapped inside fluid inclusions for >2 billion years. *Geochimica et Cosmochimica Acta*, **72**, 844–870.
- Graczyk, T. K., 2005. Is Giardia a living fossil? *Trends in Parasitology*, **21**, 104–107.
- Javaux, E., Marshall, C. P., and Bekker, A., 2010. Organic-walled microfossils in 3.2-billion-year-old shallow-marine siliciclastic deposits. *Nature*, **463**, 934–938.
- Kazmierczak, J., 1979. The eukaryotic nature of Eosphaera-like feriferous structures from the Precambrian Gunflint Iron Formation, Canada: a comparative study. *Precambrian Research*, **9**, 1–22.
- Knoll, A. H., Javaux, E. J., Hewitt, D. and Cohen, P., 2006. Eukaryotic organisms in Proterozoic oceans. Philosophical Transactions of the Royal Society of London B, **361**, 1023–1038.
- Love, G. D., Grosjean, E., Stalvies, C., Fike, D. A., Grotzinger, J. P., Bradley, A. S., Kelly, A. E., Bhatia, M., Meredith, W., Snape, C. E., Bowring, S. A., Condon, D. J., and Summons, R. E., 2009. Fossil steroids record the appearance of Demospongiae during the Cryogenian period. *Nature*, **457**, 718–721.
- MaCaffrey, M. A., Moldowan, J. M., Lipton, P. A., Summons, R. E., Peters, K. E., Jeganathan, A., and Watt, D. S., 1994. Palaeoenvironmental implications of novel C30 steranes in Precambrian to Cenozoic age petroleum and bitumen. *Geochimica et Cosmochimica Acta*, **58**, 529–532.
- Mereschkowski, C., 1905. Über Natur und Ursprung der Chromatophoren im Pflanzenreiche. *Biologisches Centralblatt*, **25**, 593–604.
- Rasmussen, B., Fletcher, I. R., Brocks, J. J., and Kilburn, M. R., 2008. Reassessing the first appearance of eukaryotes and cyanobacteria. *Nature*, **455**, 1101–1104.
- Sagan, L. (Margulis, L.) 1967. On the origin of mitosing cells. *Journal of Theoretical Biology*, **14**(3), 255–274.
- Wallin, I. E., 1923. The Mitochondria Problem. *The American Naturalist*, **57**(650), 255–261.
- Waldbauer, J. R., Sherman, L. S., Sumner, D. Y., and Summons, R. E., 2009. Late Archean molecular fossils from the Transvaal Supergroup record the antiquity of microbial diversity and aerobiosis. *Precambrian Research*, **169**, 28–47.

Cross-references

Algae (Eukaryotic)
 Biomarkers (Molecular Fossils)
 Critical Intervals in Earth History
 Ediacara Biota
 Geomycology
 Lateral Gene Transfer
 Origin of Metazoa
 Protozoa (Heterotroph, Eukaryotic)
 Sponges (Porifera) and Sponge Microbes
 Symbiosis

ECOLOGICAL NICHE

Different from the habitat (see entry *Habitat*) (spatial), the niche defines the function of an organism. See entry “*Microbial Communities, Structure, and Function*” for further reading.

EDIACARAN BIOTA

Dmitriy Grazhdankin
 Russian Academy of Sciences, Novosibirsk, Russia

Definition

Ediacaran period: An interval in the history of earth after the Marinoan/Varanger glaciation of the Neoproterozoic Era (see Chapter *Snowball Earth*), but before the Cambrian radiations. This interval marks the introduction of complex macroscopic organisms, leading to a revolution in the structure and evolution of marine paleocommunities, including the establishment of multi-level trophic structures, coevolutionary predator–prey interactions and infauunal activity.

Ediacaran biota: A highly distinctive assemblage of Ediacaran-age macroscopic organisms preserved as casts and moulds in siliciclastic, volcanoclastic, and carbonate sediments.

Introduction

Ediacaran biota is a heterogeneous assemblage representing the earliest known communities of macroscopic organisms. Diverse Ediacaran fossil assemblages are coeval with the earliest phosphatized metazoan embryos (Xiao et al., 1998; but see Bailey et al., 2007; Xiao et al., 2007), the earliest undisputed traces of metazoan activities (Jensen, 2003), and the time of eumetazoan crown-group divergence estimated by some (but not all) molecular clocks (Peterson and Butterfield, 2005). Nonetheless, placing the Ediacaran morphologies into a metazoan phylogeny has been an enduring challenge to paleobiologists (Gehling, 1991; Fedonkin, 1992; Jenkins,

GEYSERITE

A dense, banded or laminated variety of sinter that forms at and near the vents of geysers and some hot springs. See entries “*Sinter*” and “*Hot Springs and Geysers*” for further reading.

GLASS

See entry “*Basalt (Glass, Endoliths)*.”

GLAUCOPHYTES

Glaucoephytes (glaucoecystophytes or glaucoecystids) are colorless freshwater protists containing plastids derived from primary endosymbiosis (see also entry *Symbiosis*) that is, they are still surrounded by a peptidoglycane wall, which may be viewed as reminiscent of their cyanobacterial origin. Previously, these primitive plastids were also called “cyanelles.” For details, please refer to entry “*Algae (Eukaryotic)*.”

Cross-references

Algae (Eukaryotic)

GOLD

Veit-Enno Hoffmann
University of Göttingen, Göttingen, Germany

Synonyms

Aurum (Latin)

Definition

Precipitation/Bioaccumulation of gold is the precipitation of fine-grained gold colloids by microbial activities.

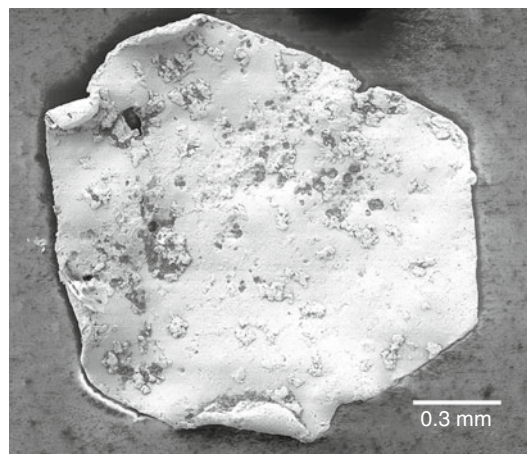
Nugget. A natural gold grain (>5 mm, >200 mg).

The impact of the use of microbial activity for the solution and precipitation of gold was undervalued for a long period, thus affording the opportunity for economic and environmentally friendly gold bio-processing. Furthermore, the current high price of gold has led to intensive research into the precipitation of gold.

Some iron and sulfur oxidizers (e.g., *Acidothiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) contribute to the disintegration of important gold ores (Nordstrom and Southam, 1997). In an environment of gold-bearing rocks, the dissolved gold content is 40 times higher than in comparable non-gold-bearing areas (Benedetti and Boulègue,

1991). It has been shown that the dissolution of gold is dominated by the gold thiosulfate complex $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ which is composed of the above-mentioned bacteria and Actinomycetes (e.g., *Streptomyces fradiae*) (Nordstrom and Southam, 1997; Sand et al., 2001). However, $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ is only stable until the sulfur-oxidation is complete (Lengke and Southam, 2005). Subsequently the gold thiosulfate complex is degraded by *A. thiooxidans*. Thereby gold accumulates in the form of fine-grained colloids inside the bacterial cell and on the surface of the cell membrane. Comparable with the results of Southam and Beveridge (1996) with *Bacillus subtilis* 168, hexagonal-octahedral gold crystals are generated out of the colloids after some months (Figure 1).

As a result of the toxicity of an increasing content of ionized gold, most of the microorganisms that can generate gold die on accumulation of gold. However, various microorganisms, such as bacteria (e.g., *Escherichia coli*, *Pseudomonas maltophilia*, *Plectonema boryanum*), Archaea (e.g., *Pyrococcus furiosus*), Actinomycetes (e.g., *Streptomyces cinamonensis*), fungi (e.g., *Neurospora sitophila*, *Fusarium oxysporum*) and yeasts (e.g., *Endomycopsis fibuligera*, *Sporobolomyces salmonicolor*) from different environments (e.g., hydrothermal, supergene), are partially resistant to the toxicity induced by the accumulation of gold (Kashefi et al., 2001; Nakajima, 2003; Lengke et al., 2006). These bioaccumulation processes are related to various microbial activities (e.g., detoxification, energy generation, passive adsorption). In the laboratory, *Pseudomonas aeruginosa* biofilms demonstrate a higher resistance to the toxicity of gold than planktonic *P. aeruginosa* cells and form solid gold at the base of the biofilms (Karthikeyan and Beveridge, 2002). Reith et al. (2006) isolated a 16SrDNA sequence from natural samples. This sequence was only found on gold grains



Gold, Figure 1 A secondary electron (SE) image of a hexagonal gold crystal from the secondary gold deposit Silberkuhle near Korbach, Germany.

shortly after the extreme glaciations during the late Neoproterozoic (Hoffman et al., 1998) and was nearly synchronous with the rapid evolutionary pulse that coincides with the Ediacaran and Cambrian radiations. Final assembly of Gondwanaland and a circum-Gondwanaland mountain chain is thought to have provided nutrients to the oceanic realm and also resulted in a large $^{87}\text{Sr}/^{86}\text{Sr}$ spike due to the erosion of the mountains (Squire et al., 2006). Therefore, links between the assembly of the Gondwanaland supercontinent and biological evolution are forwarded by numerous studies (Meert and Lieberman, 2008).

Bibliography

- Collins, A. S., and Pisarevsky, S. A., 2005. Amalgamating eastern Gondwana: the evolution of the circum-Indian orogens. *Earth Science Reviews*, **71**, 229–270.
- Dalziel, I. W. D., 1991. Pacific margins of Laurentia and East Antarctica as a conjugate rift pair: evidence and implications for an Eocambrian supercontinent. *Geology*, **19**, 598–601.
- Fitzsimons, I. C. W., 2000. A review of tectonic events in the East Antarctic Shield, and their implications for three separate collisional orogens. *Journal of African Earth Sciences*, **31**, 3–23.
- Hoffman, P. F., 1991. Did the breakout of Laurentia turn Gondwanaland inside out? *Science*, **252**, 1409–1412.
- Hoffman, P. F., Kaufman, A. J., Halverson, G. P., and Schrag, D. P., 1998. A Neoproterozoic snowball Earth. *Science*, **281**, 1342–1346.
- Kelsey, D. E., Wade, D. P., Collins, A. S., Hand, M., Sealing, C. R., and Netting, A., 2008. Discovery of a Neoproterozoic basin in the Prydz Belt in East Antarctica and its implications for Gondwana assembly and ultrahigh temperature metamorphism. *Precambrian Research*, **161**, 355–388.
- Meert, J. G., 2003. A synopsis of events related to the assembly of eastern Gondwana. *Tectonophysics*, **362**, 1–40.
- Meert, J. G., and Lieberman, B. S., 2008. The Neoproterozoic assembly of Gondwana and its relationship to the Ediacaran–Cambrian radiation. *Gondwana Research*, in press.
- Meert, J. G., and Torsvik, T. H., 2003. The making and unmaking of a supercontinent: Rodinia revisited. *Tectonophysics*, **375**, 261–288.
- Meert, J. G., Van der Voo, R., and Ayub, S., 1995. Paleomagnetic investigation of the neoproterozoic Gagwe lavas and Mbozi Complex, Tanzania and the assembly of Gondwana. *Precambrian Research*, **74**, 225–244.
- Moores, E. M., 1991. Southwest U.S.-East Antarctic (SWEAT) connection: a hypothesis. *Geology*, **19**, 425–428.
- Squire, R. J., Campbell, I. H., Allen, C. M., and Wilson, C. J. L., 2006. Did the Transgondwanan supermountain trigger the explosive radiation of animals on Earth? *Earth and Planetary Science Letters*, **250**, 116–133.
- Stern, R. J., 1994. Arc assembly and continental collision in the Neoproterozoic East Africa Orogen: implications for the consolidation of Gondwanaland. *Annual Reviews of Earth and Planetary Science*, **22**, 319–351.
- Yoshida, M., 2007. Geochronological data evaluation: implications for the Proterozoic tectonics of East Gondwana. *Gondwana Research*, **12**, 228–241.

Cross-references

[Breakup of Rodinia](#)
[Critical Intervals in Earth History](#)
[Ediacaran Biota](#)
[Origins of the Metazoa](#)
[Snowball Earth](#)
[Trace Fossils: Neoproterozoic](#)

GREAT OXYGENATION EVENT (GOE)

The term “Great Oxygenation Event” (GOE, also known as “Great Oxidation Event,” “oxygen catastrophe,” or “oxygen crisis”) describes a critical environmental change in Earth history that resulted from the appearance of diatomic oxygen (O_2), a waste product of oxygenic photosynthesis, in the atmosphere. The GOE occurred around 2.4 billion years (Ga) before present, close to the boundary between the Archean and the Proterozoic (2.5 Ga). For details, please refer to “[Critical Intervals in Earth History](#).”

GREEN ALGAE

Green algae are photosynthetic eukaryotes with simple plastids (derived from primary endosymbiosis) that are mostly microscopic and rarely more than a meter in greatest dimension. The approximate 6,000 extant species show an enormous diversity of growth habit and fine details of their cellular architecture. Green algae occur as unicellular or colonial, microscopic or macroscopic, motile planktonic, as well as benthic attached forms. Some (e.g., Dasycladales) are important carbonate producers in aquatic environments, others live in symbiotic associations with fungi to form lichens in terrestrial settings. Green algae living as endosymbionts inside heterotrophic organisms are a common phenomenon. The order Charales, in which full tissue differentiation occurs, is considered to include the closest relative of higher plants. For details, please refer to entry “[Algae \(Eukaryotic\)](#).”

GUILD

Metabolically related populations, e.g., sulfate-reducing microorganisms. See entry “[Microbial Communities, Structure, and Function](#)” for further reading.

H

HABITAT

The physical location or dwelling place of a particular organism, where an individual ecotype can be found or isolated from. See entry “*Microbial Communities, Structure, and Function*” for further reading.

HALOBACTERIA – HALOPHILES

Helga Stan-Lotter
University of Salzburg, Salzburg, Austria

Definition

Halobacteria (plural), *halobacterium* (singular). Colloquial for halophilic archaeobacteria (or archaea), which are single-celled life forms. Synonyms: Haloarchaea (plural), haloarchaeon (singular).

Halobacterium. The name of a genus of the archaeal family *Halobacteriaceae* (Grant, 2001).

Halophilic. Requires a salt-rich environment for growth and survival.

Introduction

Saline waters dominate the earth, with the oceans holding 97% of the planet's water (a total of 1.338×10^9 km³; <http://ga.water.usgs.gov/edu/earthwherewater.html>). Saline inland seas, groundwater, and saltwater lakes hold another 0.9–0.94%, which exceeds the volume of the world's available freshwater ($\sim 1.1 \times 10^7$ km³). The definition of a hypersaline environment is one that possesses a salt concentration greater than that of seawater (>3.5% w/v). Water-containing environments are usually described as thalassohaline or athalassohaline (see

Chapters “*Saline Lakes*,” “*Soda Lakes*”). Thalassohaline waters are marine-derived and therefore contain, at least initially, the seawater composition; with increasing evaporation, the concentration of various salts is changing. Athalassohaline waters may also receive influx of seawater; however, the chemical composition is mainly determined by geological, geographical, and topographical parameters.

Interactions between halophilic microorganisms and geological materials have been recognized early in the history of mankind and continue to impact human endeavors, including the exploration of energy sources, the storage of waste, the search for extraterrestrial life (see Chapter “*Deep Biosphere of Salt Deposits*”). For example, the evaporation of seawater for the purpose of making salt and the concomitant development of red color of brines, which is due to pigmented microorganisms, have been described already 2,700 years ago (Oren, 2002). It was also observed early that the presence of microorganisms in solar salterns enhances the precipitation and the yield of salt (Oren, 2002). Halite, the mineral of sodium chloride, primarily forms from the evaporation of seawater. Subsurface deposits of halite occur in many areas and are far more abundant than was previously recognized. The advent of widespread oil drilling revealed a frequent association of ancient salt deposits and the presence of the biogenic energy sources, oil and methane (Sassen et al., 1994). The potential impact of viable halophilic microorganisms in salt deposits (see Chapter “*Deep Biosphere of Salt Deposits*”), some of which are being used as storage sites for long-lived radioactive isotopes such as transuranic wastes, has been the focus of much research. The striking discovery of extraterrestrial halite in meteorites (see Chapter “*Deep Biosphere of Salt Deposits*”) and recently, in salt pools on Mars (Osterloo et al., 2008) make a search for halophilic microorganisms on other planets or moons plausible.

- Mohagheghi, A., Updegraff, D. M., and Goldhaber, M. B., 1985. The role of sulfate-reducing bacteria in the deposition of sedimentary uranium ores. *Geomicrobiology Journal*, **4**(2), 153–173.
- Moreau, J. W., Webb, R. I., and Banfield, J. F., 2004. Ultrastructure, aggregation state, and crystal growth of biogenic nanocrystalline sphalerite and wurtzite. *American Mineralogist*, **89**, 950–960.
- Nordstrom, D. K., and Southam, G., 1997. Geomicrobiology of sulfide mineral oxidation. In Nealson, K. H. (ed.), *Geomicrobiology. Reviews in Mineralogy*. Washington: Mineralogical Society of America, pp. 361–390.
- Oremland, R. S. et al., 2005. A microbial arsenic cycle in a salt-saturated extreme environment. *Science*, **308**, 1305–1308.
- Rackley, R. L., 1972. Environment of Wyoming tertiary uranium deposits. *American Association of Petroleum Geologists Bulletin*, **56**, 755–774.
- Rainbow, A., Kyser, T. K., and Clark, A. H., 2006. Isotopic evidence for microbial activity during supergene oxidation of a high-sulfidation epithermal Au-Ag deposit. *Geology*, **34**(4), 269–272.
- Reitner, J., 2004. Fossile tiefe Biosphäre in Klüften des Triberg Granits (Moosengrund, Schwarzwald). In Schmidt, G. (ed.), *Geobiologie. 74. Jahrestagung der Paläontologischen Gesellschaft, Göttingen, 02. bis 08. Oktober 2004. Kurzfassungen der Vorträge und Poster*. Universitätsdruckerei Göttingen, Göttingen.
- Reynolds, R. L., Goldhaber, M. B., and Carpenter, D. J., 1982. Biogenic and nonbiogenic ore-forming processes in the South Texas uranium district: Evidence from the Panna Maria deposit. *Economic Geology*, **77**, 541–556.
- Saunders, J. A., and Swann, C. T., 1994. Mineralogy and geochemistry of a cap-rock Zn-Pb-Sr-Ba occurrence at the Hazlehurst salt dome, Mississippi. *Economic Geology*, **89**(2), 381–390.
- Schaefer, M. O., Gutzmer, J., and Beukes, N. J., 2001. Late proterozoic Mn-rich oncoids: earliest evidence for microbially mediated Mn precipitation. *Geology*, **29**(9), 835–838.
- Schroll, E., 1996. The Triassic carbonate-hosted Pb-Zn mineralization in the Alps (Europe): the genetic position of Bleiberg Type deposits. In Sangster, D. F. (ed.), *Carbonate-Hosted Lead-Zinc Deposits*. Michigan: Society of Economic Geologists, Special Publication 4.
- Sillitoe, R. H., Folk, R. L., and Saric, N., 1996. Bacteria as mediators of copper sulfide enrichment during weathering. *Science*, **272**, 1153–1155.
- Southam, G., and Saunders, J. A., 2005. The Geomicrobiology of ore deposits. *Economic Geology*, **100**(6), 1067–1084.
- Stolz, J. S., and Oremland, R. S., 1999. Bacterial respiration of arsenic and selenium. *FEMS Microbiology Reviews*, **23**(5), 615–627.
- Strubny, B., and Puchelt, H., 1991. Geochemical and metallogenic aspects of organic carbon-rich pelitic sediments in Germany. In Pagel, M., and Leroy, J. L. (eds.), *Source, Transport and Deposition of Metals*. Rotterdam: Balkema, pp. 593–598.
- Suzuki, Y., and Banfield, J. F., 1999. Geomicrobiology of uranium. In Burns, P. C., and Finch, R. (eds.), *Uranium: Mineralogy, Geochemistry and the Environment*. Washington: Mineralogical Society of America, pp. 393–432.
- Taylor, B. E., 2004. Biogenic and thermogenic sulfate reduction in the Sullivan Pb-Zn-Ag deposit, British Columbia (Canada): Evidence from micro-isotopic analysis of carbonate and sulfide in bedded ores. *Chemical Geology*, **204**, 215–236.
- Tebo, B. M., Ghiorse, W. C., van Waasbergen, L. G., Siering, P. L., and Caspi, R., 1997. Bacterially mediated mineral formation: Insights into manganese(II) oxidation from molecular genetic and biochemical studies. In Banfield, J. F., and Nealson, K. H. (eds.), *Geomicrobiology: Interactions between microbes and minerals. Reviews in Mineralogy*, No. 35. Washington: Mineralogical Society of America.
- Waber, N., Schorscher, H. D., and Peters, T., 1992. Hydrothermal and supergene uranium mineralization at the Osamu Utsumi mine, Poços de Caldas, Minas Gerais, Brazil. *Journal of Geochemical Exploration*, **45**, 53–112.
- Wanty, R. B., Goldhaber, M. B., and Northrop, H. R., 1990. Geochemistry of vanadium in an epigenetic, sandstone-hosted vanadium-uranium deposit, Henry Basin, Utah. *Economic Geology*, **85**, 270–284.
- Warren, C. G., 1971. A method for discriminating between biogenic and chemical origins of the ore-stage pyrite in a roll-type uranium deposit. *Economic Geology*, **66**, 919–928.
- Warren, C. G., 1972. Sulfur isotopes as a clue to the genetic geochemistry of a roll-type uranium deposit. *Economic Geology*, **67**, 759–767.
- Widdel, F. et al., 1993. Ferrous iron oxidation by anoxygenic phototropic bacteria. *Nature*, **362**, 834–836.
- Wilkinson, J. J., Eyre, S. L., and Boyce, A. J., 2005. Ore-forming processes in Irish-type carbonate-hosted Zn-Pb deposits: evidence from mineralogy, chemistry, and isotopic composition of sulfides at the Lisheen mine. *Economic Geology*, **100**(1), 63–86.

Cross-references

[Acid Rock Drainage](#)
[Banded Iron Formations](#)
[Biomining \(Mineral Bioleaching, Mineral Biooxidation\)](#)
[Biosignatures in Rocks](#)
[Black Shales](#)
[Carbon \(Organic, Degradation\)](#)
[Extreme Environments](#)
[Hydrogen](#)
[Hydrothermal Environments, Fossil](#)
[Nan\(n\)obacteria](#)
[Origin of Life](#)
[Radioactivity \(Natural\)](#)
[Reduction Spheroids](#)
[Subsurface Filamentous Fabrics](#)
[Sulfate-Reducing Bacteria](#)
[Sulfide Mineral Oxidation](#)

ORGANIC CARBON

Carbon that occurs in materials of ultimately biological origin that can be oxidized. Organic matter is material that contains organic carbon. See entries “[Carbon \(Organic, Cycling\)](#)” and “[Carbon \(Organic, Degradation\)](#)” for further reading.

ORGANOMINERALIZATION

Christian Défarge
 Université d'Orléans and Université François-Rabelais de Tours, Orléans, France

Synonyms

Organomineral formation

REMINERALIZATION (OF ORGANIC MATTER)

Degradation of organic matter that results in net conversion of organic carbon to inorganic (oxidized) carbon species such as CO_2 or HCO_3^- . See entries "[Carbon \(Organic, Cycling\)](#)" and "[Carbon \(Organic, Degradation\)](#)" for further reading.

RHODOPHYTA

The Rhodophyta, also referred to as "red algae," are a distinct eukaryotic lineage of algae with simple plastids (two membranes, derived from primary endosymbiosis). Rhodophytes have a long geological record since the Mesoproterozoic (1.2 Ga). Today, they encompass about 670 largely marine genera with up to 4,500 species predominating the coastal and continental shelf areas of tropical, temperate, and cold-water regions. Red algae are significant as primary producers and providers of structural habitat for other marine organisms. Calcifying (coralline) red algae play an important role in the primary establishment and maintenance of limestone reefs. For further details, see entry "[Algae \(Eukaryotic\)](#)."

Cross-references

[Algae \(Eukaryotic\)](#)
[Carbonate Environments](#)
[Symbiosis](#)

RNA-WORLD

The term "RNA-World," first suggested by Walter Gilbert in 1986, describes a popular concept on a hypothetical early stage in the origin of life on Earth. Based on discoveries of enzymatic activities in microbial ribonucleic acids (RNA), a system was suggested where primordial RNA molecules accounted for both, the storage of genetic information and catalytic functions necessary to assemble themselves in a primitive self-replicating system. The RNA molecules evolved in self-replicating patterns, using recombination and mutation to explore new functions and to adapt to new niches (Gilbert, 1986). Such a system would not have required the presence of DNA and/or protein enzymes for biochemical reactions. For further reading, please refer to "[Origin of Life](#)."

Bibliography

Gilbert, W., 1986. The RNA world. *Nature*, **319**, 618.

SKELETON

Biologically, a skeleton is a hard or rigid framework that could provide protection and support in many types of organisms. Most of skeletons are mineralized, but some may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, not including hydroskeletons. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites. Endoskeletons are internal hard parts (typical of many vertebrates). Most of endoskeletons are generally surrounded by skin and musculature. See entry "[Animal Skeletons, Advent](#)" for further reading.

SMALL SHELLY FOSSILS

Small shelly fossils (SSF, also "Small Shelly Fauna") is a non-taxonomic term, first used by Matthews and Missarzhevsky in the title of their review paper in 1975, to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites. See entries "[Animal Skeletons, Advent](#)" and "[Critical Intervals in Earth History](#)" for further reading.

SNOWBALL EARTH

Paul F. Hoffman
Harvard University, Cambridge, MA, USA

Synonyms

Cryochron; Global glaciation; Hard snowball; Ice-albedo catastrophe; Snowball (in geology); White Earth

Definition

Snowball Earth is a climate in which the oceans and most land areas are permanently covered by glacial ice. As less sunlight is absorbed by ice than by water or land, a critical area of ice will cause surface temperatures to fall below freezing everywhere, resulting in a snowball Earth. This arguably occurred near the beginning and end of the Proterozoic eon. Snowball Earths self-destruct after millions of years due to the buildup of atmospheric carbon dioxide of volcanic and metamorphic origin, which could not be converted into organic matter or limestone. Biological evolution is a postulated cause and consequence of snowball episodes.

History of the concept

Sedimentary deposits of glacial origin were first described from the late Proterozoic (Neoproterozoic) of Scotland in 1871 and from the early Proterozoic (Paleoproterozoic) of Canada in 1907. By 1940, it was apparent that the Neoproterozoic glaciation(s) had been more extreme than those of the Pleistocene and at least equal in severity to the late Paleozoic glaciations of Gondwanaland. In the 1960s, the occurrence of glacial deposits within thick successions of nearly pure carbonate strata suggested that Neoproterozoic ice sheets had entered what were normally the warmest parts of the globe. This was confirmed in the 1980–1990s by reliable paleomagnetic data showing that ice sheets had reached the ocean close to the paleoequator in the Neoproterozoic of Australia and Paleoproterozoic of South Africa (Evans, 2003). Moreover, evidence of marine geochemical anomalies concurrent with glaciation came increasingly to light in the form of sedimentary Fe_2O_3 and MnO_2 ore deposits, large excursions in stable carbon and sulfur isotopic compositions of minerals precipitated from seawater, and distinctive post-glacial "cap" carbonate sediments that rest directly on the terminal glacial deposit at all latitudes (Hoffman and Schrag, 2002). Neoproterozoic glacial deposits are now known from 22 different paleocontinents and microcontinents, and Paleoproterozoic deposits from 6.

The fraction of incoming solar radiation absorbed by ocean water under cloudless skies is 0.9, but for continuous ice it is only 0.4–0.6 and as low as 0.1 if the ice is covered by fresh snow. In the 1950–1960s, calculations by meteorologists of zonal average surface temperatures at different latitudes, assuming radiative energy balance and incorporating crude parameterizations of the radiative effects of clouds, meridional heat transport, and positive feedbacks due to the reflectivity (albedo) of ice, suggested that a lowering of solar luminosity by a few percent would result in a globe-encircling glaciation, or "white" Earth (North, 1990). If the luminosity is incrementally lowered, polar ice caps expand until their edges reach a critical latitude, beyond which ice advances rapidly to the equator without any additional luminosity reduction, or even with increased luminosity. This is due to runaway ice-albedo feedback – more ice reflects more radiation, creating more ice. (The same calculations indicated that a small increase in radiative forcing, due to anthropogenic CO_2 emissions for example, could cause a sudden loss of polar sea ice at a critical $p\text{CO}_2$ threshold.) These sensational findings greatly stimulated the nascent field of climate modeling, but proponents were puzzled how a "white" Earth disaster had been averted given that solar luminosity has increased by 25–30% over geological time (the so-called faint young Sun paradox). They assumed (incorrectly, as it turned out) that such an occurrence would extinguish all life – deep-sea chemoautotrophs and polar extremophiles were then unknown – and would be permanent because of the very large increase (~25%) in radiative forcing required to overwhelm a "white" Earth's high albedo.

SKELETON

Biologically, a skeleton is a hard or rigid framework that could provide protection and support in many types of organisms. Most of skeletons are mineralized, but some may not be mineralized (e.g., some annelid tubes being agglutinated). They could be subdivided into exoskeletons and endoskeletons, not including hydroskeletons. Exoskeletons are external hard parts. They may enclose the soft tissues and organs of the body, or may be external sclerites. Endoskeletons are internal hard parts (typical of many vertebrates). Most of endoskeletons are generally surrounded by skin and musculature. See entry "[Animal Skeletons, Advent](#)" for further reading.

SMALL SHELLY FOSSILS

Small shelly fossils (SSF, also "Small Shelly Fauna") is a non-taxonomic term, first used by Matthews and Missarzhevsky in the title of their review paper in 1975, to denote the earliest skeletal fossils, although some of these fossils may not be small and most of them are actually not shells. It has been popularly used in literature somewhat as a catchall term for the early Cambrian skeletal fossils, including spicules, shells, tubes, and diverse disarticulated sclerites. See entries "[Animal Skeletons, Advent](#)" and "[Critical Intervals in Earth History](#)" for further reading.

SNOWBALL EARTH

Paul F. Hoffman
Harvard University, Cambridge, MA, USA

Synonyms

Cryochron; Global glaciation; Hard snowball; Ice-albedo catastrophe; Snowball (in geology); White Earth

Definition

Snowball Earth is a climate in which the oceans and most land areas are permanently covered by glacial ice. As less sunlight is absorbed by ice than by water or land, a critical area of ice will cause surface temperatures to fall below freezing everywhere, resulting in a snowball Earth. This arguably occurred near the beginning and end of the Proterozoic eon. Snowball Earths self-destruct after millions of years due to the buildup of atmospheric carbon dioxide of volcanic and metamorphic origin, which could not be converted into organic matter or limestone. Biological evolution is a postulated cause and consequence of snowball episodes.

History of the concept

Sedimentary deposits of glacial origin were first described from the late Proterozoic (Neoproterozoic) of Scotland in 1871 and from the early Proterozoic (Paleoproterozoic) of Canada in 1907. By 1940, it was apparent that the Neoproterozoic glaciation(s) had been more extreme than those of the Pleistocene and at least equal in severity to the late Paleozoic glaciations of Gondwanaland. In the 1960s, the occurrence of glacial deposits within thick successions of nearly pure carbonate strata suggested that Neoproterozoic ice sheets had entered what were normally the warmest parts of the globe. This was confirmed in the 1980–1990s by reliable paleomagnetic data showing that ice sheets had reached the ocean close to the paleoequator in the Neoproterozoic of Australia and Paleoproterozoic of South Africa (Evans, 2003). Moreover, evidence of marine geochemical anomalies concurrent with glaciation came increasingly to light in the form of sedimentary Fe_2O_3 and MnO_2 ore deposits, large excursions in stable carbon and sulfur isotopic compositions of minerals precipitated from seawater, and distinctive post-glacial "cap" carbonate sediments that rest directly on the terminal glacial deposit at all latitudes (Hoffman and Schrag, 2002). Neoproterozoic glacial deposits are now known from 22 different paleocontinents and microcontinents, and Paleoproterozoic deposits from 6.

The fraction of incoming solar radiation absorbed by ocean water under cloudless skies is 0.9, but for continuous ice it is only 0.4–0.6 and as low as 0.1 if the ice is covered by fresh snow. In the 1950–1960s, calculations by meteorologists of zonal average surface temperatures at different latitudes, assuming radiative energy balance and incorporating crude parameterizations of the radiative effects of clouds, meridional heat transport, and positive feedbacks due to the reflectivity (albedo) of ice, suggested that a lowering of solar luminosity by a few percent would result in a globe-encircling glaciation, or "white" Earth (North, 1990). If the luminosity is incrementally lowered, polar ice caps expand until their edges reach a critical latitude, beyond which ice advances rapidly to the equator without any additional luminosity reduction, or even with increased luminosity. This is due to runaway ice-albedo feedback – more ice reflects more radiation, creating more ice. (The same calculations indicated that a small increase in radiative forcing, due to anthropogenic CO_2 emissions for example, could cause a sudden loss of polar sea ice at a critical $p\text{CO}_2$ threshold.) These sensational findings greatly stimulated the nascent field of climate modeling, but proponents were puzzled how a "white" Earth disaster had been averted given that solar luminosity has increased by 25–30% over geological time (the so-called faint young Sun paradox). They assumed (incorrectly, as it turned out) that such an occurrence would extinguish all life – deep-sea chemoautotrophs and polar extremophiles were then unknown – and would be permanent because of the very large increase (~25%) in radiative forcing required to overwhelm a "white" Earth's high albedo.

Bibliography

- Crawford, J. W., Harris, J. A., Ritz, K., and Young, J. M., 2005. Towards an evolutionary ecology of life in soil. *Trends in Ecology and Evolution*, **20**, 81–87.
- Driessen, P., Deckers, J., Spaargaren, O., and Nachtergaele, F., 2001. *Lecture Notes on the Major Soils of the World*. World Soil Resources Reports 94, Rome: FAO.
- FAO-UNESCO, 2002. *Digital Soil Map of the World and Derived Soil Properties*. Land and Water Digital Media Series No. 1 Rev. 1. Rome: FAO.
- Fiedler, S. H., Jungkunst, P. F., Kleber, M., Sommer, M., Jahn, R., and Stahr, K., 2002. Linking soil classification and soil dynamics – pedological and ecological perspectives. *Journal of Plant Nutrition and Soil Science*, **165**, 517–529.
- IUSS Working Group WRB, 2006. *World Reference Base for Soil Resources: Atlas*. Leuven, The Netherlands: ISRIC-FAO-ISSS-Acco, p. 63.
- Jones, D. L., and Bassington, D. S., 1998. Sorption of organic acids in acid soils and its implication in the rhizosphere. *European Journal of Soil Science*, **49**, 447–455.
- Kögel-Knabner, I., Hatcher, P. G., and Zech, W., 1990. *Decomposition and Humification Processes in Forest Soils: Implications from Structural Characterization of Forest Soil Organic Matter*. Kyoto, Japan: Trans. 14th Int. Congr. Soil Sci., pp. 218–223.
- Meir, P., Cox, P., and Grace, J., 2006. The influence of terrestrial ecosystems on climate. *Trends in Ecology and Evolution*, **21**, 254–260.
- O'Donnell, A. G., Young, J. M., Rushton, S. P., Shirley, M. D., and Crawford, J. W., 2007. Visualization, modeling and prediction in soil microbiology. *Nature Reviews Microbiology*, **5**, 689–699.
- Rillig, M. C., and Mummey, D. L., 2006. Mycorrhizas and soil structure. *New Phytologist*, **171**, 41–53.
- Schaumann, G. E., 2006. Soil organic matter beyond molecular structure, part I: macromolecular and supramolecular characteristics. *Journal of Plant Nutrition and Soil Science*, **169**, 145–156.
- Stevenson, F. J., 1994. *Humus Chemistry*. New York: Wiley.
- van der Heijden, M. G., Bardgett, R. D., and van Straalen, N. M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, **11**, 296–310.
- Wardle, D. A., 2006. The influence of biotic interactions on soil biodiversity. *Ecology Letters*, **9**, 870–886.
- Watt, M., Silk, W. K., and Passioura, J. B., 2006. Rates of root and organism growth, soil conditions, and temporal and spatial development of the rhizosphere. *Annals of Botany*, **97**, 839–855.
- Watteau, F., and Berthelin, J., 1994. Microbial dissolution of iron and aluminum from soil minerals: efficiency and specificity of hydroxamate siderophores compared to aliphatic acids. *European Journal of Soil Biology*, **30**, 1–9.
- Weihermüller, L., Siemens, J., Deurer, M., Knoblauch, S., Rupp, H., Göttlein, A., and Pütz, T., 2007. In situ soil water extraction: a review. *Journal Of Environmental Quality*, **36**, 1735–1748.

Cross-references

Bacterial Clay Autigenesis
 Biogeochemical Cycles
 Carbon Cycle
 Denitrification
 Microbial Degradation
 Microbial-Metal Binding
 Mycorrhizae
 Nitrogen Fixation
 Siderophores

SPECIES (MICROBIAL)

Microorganisms exhibiting a high degree of phenotypic and genetic similarity; a pragmatic approach to demarcating “microbial species” using molecular biology tools has been 97% 16S rRNA sequence identity or 70% genome identity based on DNA-DNA hybridization; thus, microbial species are often defined by operational characteristics (operational taxonomic unit). The microbial species concept is still a matter of controversy. See entry “*Microbial Communities, Structure, and Function*” for further reading.

SPELEOTHEMS

Roman Aubrecht
 Comenius University, Bratislava, Slovakia

Synonyms

Cave deposits; Cave sinters

Definition

The word “speleothem” is from Greek and means “cave deposit.” According to its origin, this term can be used for any cave deposits, but it is used mostly for secondary mineral deposits formed (precipitated) in caves. In this entry, speleothem is understood as constructively formed, new cave material. Materials originated by destructive phenomena, such as erosion, weathering, etching, biocorrosion, or bioerosion are not subjects of this entry.

Biogenic and abiogenic speleothems

Most of the speleothems originate inorganically by precipitation of various minerals from solutions. The minerals are dissolved by undersaturated fluids and precipitate when the solutions become supersaturated, e.g., by evaporation, outgassing, or pH changes. Mineralogical compositions of speleothems vary depending on the environment in which the caves are formed. The most common speleothem-forming mineral is calcite (as most of the caves are formed of limestones), forming well-known sinter forms, such as dripstones (stalactites, stalagmites) and flowstones. Locally, mostly in hydrothermally influenced cavern systems, speleothems may be formed by its rhombic modification – aragonite. Gypsum and other sulfate minerals commonly form speleothems in gypsum karst but are widespread also in other types of caves. Speleothems formed in silicate rocks (e.g., sandstones, quartzites, granites, etc., [Figure 1](#)) are relatively rare, composed mostly of various forms of silica (from opal-A to microquartz depending on its recrystallization). Other speleothems can be represented by ferroan and manganese oxides and hydroxides forming cave flowstones.